Integrated Fish-Shellfish Mariculture in Puget Sound

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Final Report

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Principal Investigator: Jack Rensel Ph. D.[1]

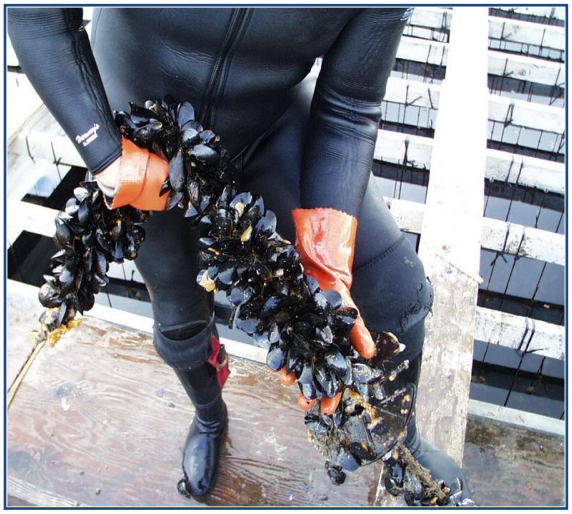
Associate Investigators: Kevin Bright[2], Zach Siegrist[3]

[1/] Research Scientist
Rensel Associates Aquatic Sciences
4209 234th Street N.E.
Arlington, WA 98223

[2/] Biologist and Institutional Contact
American Gold Seafoods LLC
P.O. Box 669
Anacortes, WA 98221

[3/] Aquatic Scientist
Rensel Associates Aquatic Sciences
4209 234th Street N.E.
Arlington, WA 98223

(Fish in net pens being crowded for harvest)



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EXECUTIVE SUMMARY

We evaluated the efficacy of waste transfer from Atlantic salmon aquaculture pens to shellfish cultured immediately downstream and at different depths and distances in Puget Sound, Washington. Studies occurred concurrently in central Puget Sound near Clam Bay and in north Puget Sound at Cypress Island. Fish have been cultivated continuously since 1969 at the former site and since 1980 at the latter site. Both of these areas are moderately enriched with nutrients and phytoplankton due to the naturally occurring upwelling of nutrient-rich deep-water along the west coast U.S.

The null hypothesis for this work was that growth of shellfish would not be enhanced or tracing of stable isotopes of carbon and nitrogen would not show spatial effect of being near the fish farm. The alternative hypothesis was that one or both would demonstrate an effect. We suspended Pacific oysters (*Crassostrea gigas*) and "gallo" mussels (*Mytilus galloprovincialis*) in plastic Aquapurse culture units at several sites at varying distances and depths relative to fish farm net pens for about 9 months (fall to spring) in Experiment one and for mussels only in Experiment two for April through March of the following year. As fish farms in this region are dependent on tidal currents to supply oxygen to the cages, we placed several treatments of shellfish below the surface layer to assess their growth at strata that would not interfere with surface currents. Prior studies have shown these areas to be well vertically mixed, so we hypothesized and indeed confirmed that there was no measureable difference between near surface and subsurface concentrations of phytoplankton measured as chlorophyll *a*.

Oysters at Cypress Island received significant nutritional and growth benefits from placement near the salmon net pens. Oysters closer to the net pens experienced consistently higher growth and were able to take advantage of fish feces produced by the site. Increased oyster growth nearer the Clam Bay net pens was measured in the first fall and early winter, but by the completion of grow out in spring, there were statistical differences among treatments and no evidence of a stable isotope signature in the tissue of the oysters indicative of fish farm wastes. Comparison of TVS, TSS, phytoplankton organics, non-phytoplankton organics, chlorophyll a and seston stable isotope data collected concurrently do not fully explain the differences between growth and stable isotope signatures at the two sites. No significant differences in these water quality parameters were noted up or downstream of the fish farms or at different depths. Sampling was conducted at random times, and had we sampled during feeding would undoubtedly have measured greater concentrations of TSS and TVS downstream. Lack of differences among sites could be due to fast changing phytoplankton and seston composition over time scales of days to weeks, where our sampling for these factors was through economic necessity, monthly.

In contrast, mussels grown at Cypress Island and at Clam Bay did not experience any significant growth or stable isotope composition effects due to proximity to fish farms. It is probable that oysters and mussels have different feeding and pseudofeces production behaviors that apparently allowed the oysters to take better advantage of fish farm-origin wastes.

In addition, the growth results matched up very well with our mixing model analysis, which indicated this benefit as originating at least partially from waste fish feces. The probability distribution outputs from the IsoSource mixing model showed that oysters growing at the reference site relied primarily on phytoplankton during the entire experiment, while the 30m distant and deep treatment oysters also fed on phytoplankton in the fall, but switched to fish feces in the winter and spring. This is both reasonable and logical but cannot be explained by simple, infrequent measures such as chlorophyll and total suspended or volatile solids concentration. It is probable that this extra food source, available in quantity to the 30m oysters but not to the reference oysters, supported the 30m distance oysters' significantly greater growth performance.

For Puget Sound waters, we recommend further investigation and scaled-up trials of the efficacy of oyster culture as a companion crop to fish aquaculture. The trials to date with oysters have been promising and we can envision several benefits from such systems. In the relatively cool waters of Puget Sound main basins and channels where fish farms are located, oysters rarely reproduce naturally thus would not add to the biofouling load on the nets and floats that could occur with mussels when then reproduce. They could also be used in some cases to provide current flow diversion at sites with overly strong currents or temporarily at some sites where spring tides produce currents that exceed optimum velocities. Oysters will not replace fish as a primary cash crop at fish farm sites due to space limitations, but subsurface growing systems would benefit not only from the slowly settling organic particulate matter but would be less subject to algal biofouling compared to surface raft culture of shellfish.

In other regions of the world, seaweed culture is also being practiced as a means to reduce dissolved nitrogen loading. Puget Sound net pen sites are by design located in non-nutrient sensitive areas but background levels of nitrogen are naturally high in main basins of Puget Sound. The pen origin nitrogen plus the natural background flux of nitrogen could help insure a desirable and continuous supply to insure sustained growth of seaweeds near the net pens. However, combined shellfish plus seaweed culture at fish farm sites in Puget Sound may not be technically feasible because of space limitations and the fact that seaweeds must be grown near the surface to allow photosynthesis, whereas the shellfish are not subject to this limitation.

INTRODUCTION

The purpose of this study was to assess the use of integrated multitrophic aquaculture (IMTA) using coupled fish and shellfish aquaculture in differing regions of Puget Sound, Washington where existing net pen salmon aquaculture occurs. IMTA offers a possibility to capture valuable organic wastes from fish farms to be incorporated into "companion crops" to help diversify production and reduce organic waste loading in the vicinity of the culture area. The hypothesis for this work is that growth of shellfish will be enhanced or stable isotope tracing of carbon and possibly nitrogen will show an effect. The alternative hypothesis is that no growth or stable isotope effect will occur.

We selected Pacific oysters (*Crassostrea gigas*) and "gallo" mussels (*Mytilus galloprovincialis*) as our target shellfish species. Pacific oysters are a hearty species and grow well throughout Puget Sound and gallo mussels generally grow better than native mussels (*Mytilus trossulus*) in areas like Southern Puget Sound and near Whidbey Island in Saratoga Passage, and were available as seed stock from local hatcheries. The salmon farms culture Atlantic salmon (*Salmo salar*) as there is a high demand for the species and very low or no risk of escaped Atlantic salmon colonizing or establishing populations in the Pacific Northwest in the studied opinion of experts at the National Oceanic and Atmospheric Administration (e.g., Nash and Waknitz 2003).

This research took place at two net pen sites in Puget Sound, Clam Bay in central Puget Sound and Cypress Island Site 3 in North Puget Sound (Figure 1). These sites have differing hydrographic characteristics and seasonal differences but both experience moderately abundant phytoplankton density during the growing season and modest occurrence of seston.

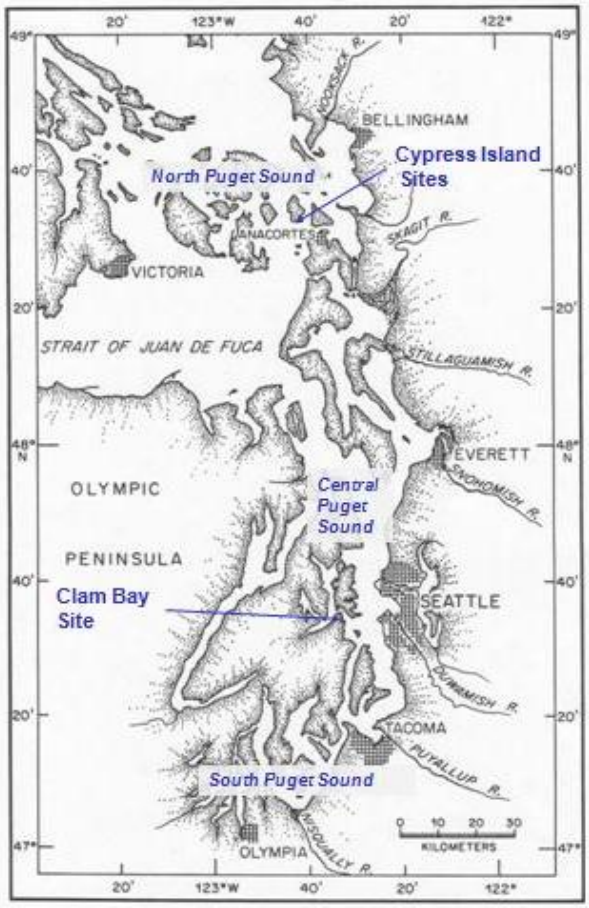

Figure 1. Vicinity map and locations

Two experiments were conducted: Experiment 1 commenced in fall and was designed to assess efficacy of IMTA during winter, the period of lowest phytoplankton abundance for both oysters and mussels. Experiment 2 began in late spring and ended in early spring to assess different culture period results for mussels, and to further investigate the results of Experiment 1 with mussels only.

Water temperatures are moderate in Puget Sound and adjacent marine waters compared to Northeast United States coastal areas and there are no problems with sea lice infection due to the prevalence of lower salinity water than in many other salmon farming areas of the world. As explained in this report, however, coastal and local upwelling of deep oceanic water that is low in dissolved oxygen does occur in late spring through fall in some areas and years, so placement of shellfish upstream of current flows would have to be carefully considered with regard to further interference with oxygen supply as explained in the next section of this report.

EXPERIMENTAL LAYOUT

Location of stations for culture of the shellfish was based on knowledge of persistent tidal current velocity and direction at both sites. Data sources for Clam Bay were dual recording current meter records of Weston and Gowen (unpublished, cited in WDF 1990) and for Cypress Island the current meter results of Rensel (1996) plus local knowledge of the fish farming staff were used in this regard.

Table 1 summarizes the layout of treatments for Experiments 1 and 2. In each experiment, four spatial pattern treatments were established: two treatments were placed adjacent to the net pens, one at a shallow depth and one deeper but both within 1 horizontal meter and downstream of active salmon pens. Another treatment was placed an intermediate distance (30 meters) away from the pens, at much greater depth to intercept sinking particulate waste matter. A fourth treatment was placed approximately 150 m from the pens at a deeper depth. The two treatments closest to the net pens are referred to as “farm shallow” and “farm deep,” the intermediate distance treatment is called the “30m distant deep” treatment, and the farthest treatment is referred to as “reference deep”.

Table 1. Sampling design spatial layout of Experiment 1 (September 2008 – June 2009) and Experiment 2 (April 2010 – 10 March 2011). Depth in meters to the center of each string of Aquapurses.

Study Area	Treatment Site	Experiment 1 Depth (m)	Experiment 2 Depth (m)	Distance from cages
Cypress Island, North Puget Sound	Farm shallow	5	5	Immediately adjacent to cage array, east end
Same	Farm deep	20	15	Immediately adjacent to cage array, east end
Same	30m distant deep	10	15	30 m from above strings
Same	Reference deep	10	15	Reference area 200 m from nearest pens but in same bay
Clam Bay, Central Puget Sound	Farm shallow	5	10	Immediately adjacent to cage array, South side
Same	Farm deep	25	25	Immediately adjacent to cage array, South side
Same	30m distant deep	15	25	30 m further south of pens and above arrays
Same	Reference deep	15	25	150 m ESE of pens ESE in same bay

Experiment 1: 03 September 2008 – 10 June 2009 at Clam Bay; 05 September 2008 – 12 June 2009 at Cypress Island
Experiment 2: 13 April 2010 – 10 March 2011 at Clam Bay; 14 April 2010 – 11 March 2011 at Cypress Island

The emphasis on deep treatments relates to the need to grow shellfish in a manner that would not interfere with currents and oxygen flux into the pens. Since these sites are relatively well mixed without significant vertical stratification at any time of year, it was hypothesized that sufficient plankton would be available as feed at depth and that much of the particulate waste stream from the cages could be intercepted at such depths. Figure 2 shows aerial photo images with the stations superimposed at each site.

When designing Experiment 2, treatment depths were slightly changed for Clam Bay, but kept the same for Cypress Island. The Clam Bay farm shallow treatment depth was increased from 5 to 10 meters, and the 30m distant deep and reference group depths were increased from 15 to 25

meters. The farm deep treatment was kept the same, at 25 meters deep. These changes were made because the Clam Bay site is significantly deeper in general than the Cypress Island site and we wished to further assess shellfish culture at these depths. A diagram of the treatment and reference locations relative to each site is shown in Figure 3. Because depth at the selected Deepwater Bay site was shallower than the Clam Bay site, only one depth was used for each of the three strings of deep shellfish cages. At Clam Bay, a deep set of cages was installed beneath the set that was adjacent to the pens. Waste fecal matter of salmonids, particularly larger ongrowing fish, sinks relatively rapidly and it was thought that we may see a difference among the shallower and deeper strings.

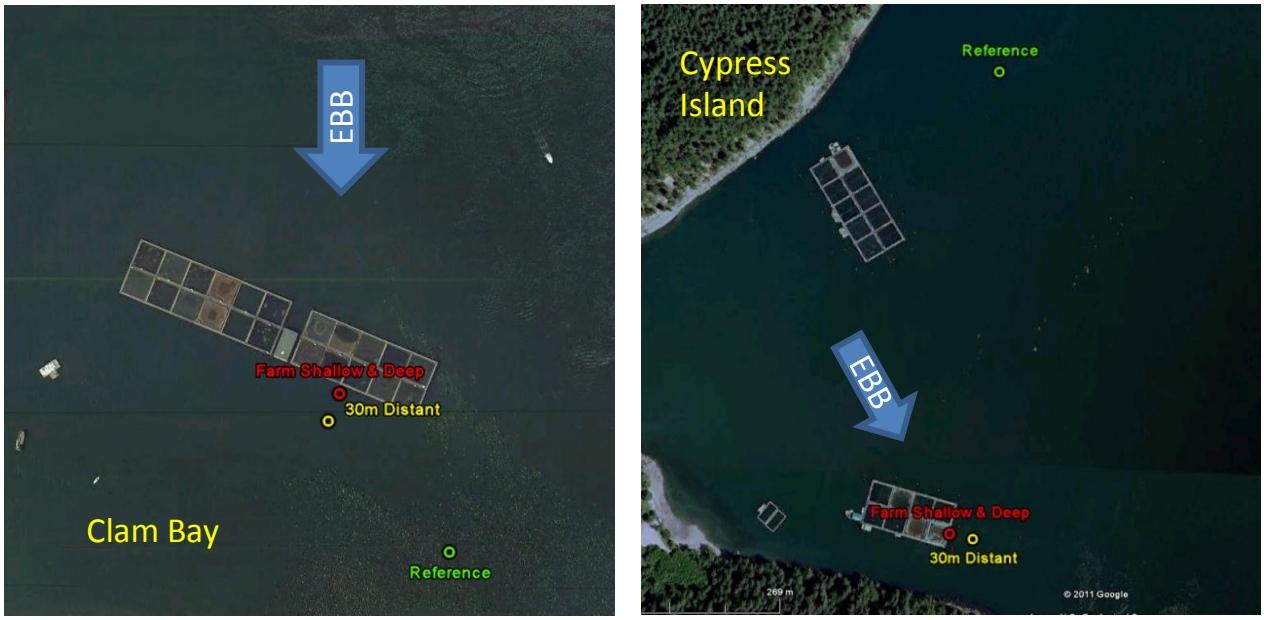

Figure 2. Google map images of the two study sites at Clam Bay and Cypress Island.

The other net pen shown in Figure 2 at Cypress Island was not occupied during these experiments.

Current velocity at the Cypress Island site averaged about 15.5 cm/s during current meter monitoring of a mean tidal exchange day at this site (Rensel 1996). Currents were strongly bidirectional with the ebb tide flowing through the pens from west to east into the location of the IMTA trial Aquapurse units. No current meter information was available from the Clam Bay site but judging from the coarse sand bottom and large carrying capacity of the site that routinely meets NPDES performance standards, currents are equally as strong on average. Previously, Weston and Gowen (unpublished, cited in WDF 1990) had a recording of Aanderaa Vane style

current meters on both ends of this site, but that location was slightly shallower than the present day site location.

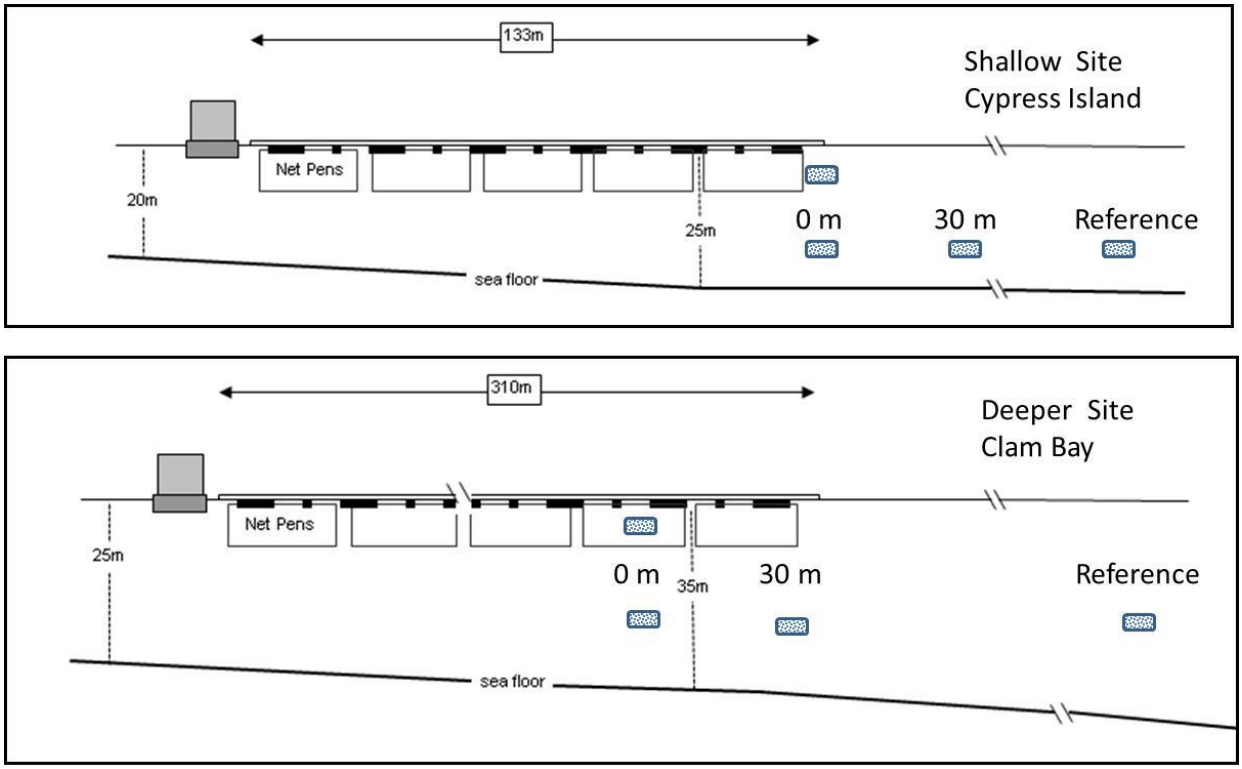

Figure 3. Diagrams of the experimental layouts at each study site. Treatment and reference groups represented by blue rectangles. For Clam Bay, flow was tangential through the side of the cages and the 2D picture does not fully represent the arrays properly. See prior aerial image for spatial relationships.

METHODS

Juvenile shellfish used in both experiments were produced by Taylor shellfish and originated in Southern Puget Sound. Experiment 1 used both *Crassostrea gigas* (triploid Pacific oysters) and *Mytilus galloprovincialis* (blue mussels) while Experiment 2 only used *M. galloprovincialis*. For both experiments, mussels were hatched in Dabob Bay and oysters in Quilcene Bay. They were later moved to nearby nursery grounds: Totten Inlet for the mussels, Oakland Bay for the oysters. At the beginning of Experiment 1, shellfish were approximately 10 months old at time of stocking; juvenile mussels used in Experiment 2 were younger (7 months).

All shellfish were cultured in replicate Aquapurse trays (Figure 4), reusable plastic purses that were set in between perimeter lines with spreader bars at the top and bottom. The strings were set at

different depths and locations as specified in Table 1. At both sites, the net pens are galvanized steel cage systems with all rearing pens held within the same array. The cages move very little during tidal cycles, but there is some slight shifting according to GPS measurements made during routine permit compliance monitoring. In both cases, the immediate downstream Aquapurse trays were placed in one of two dominant tidal current directions. A commercial project for mussels would use suspended strings of mussels, but the Aquapurses offered the advantage of predator avoidance without the use of predator deterrent nets and ease of sampling access.

Figure 4. String of 4 oyster and 4 mussel stocked Aquapurses being placed at the Clam Bay site.

On each scheduled sampling date, Aquapurse units were removed from the water and all shellfish lengths were measured. Length was recorded in millimeters using specialized metric rulers; in the case of broken shells, length was visually estimated. Shellfish tissue volume was not measured. Total count of all living specimens and empty shells was also tallied, and lengths of all empty shells were also measured and recorded in Experiment 2. In addition, during each measurement period, several shellfish from each Aquapurse were selected at random and removed for later stable isotope analysis. After all shellfish length measurement was completed, shellfish were placed back into their respective Aquapurses, which were then re-suspended in the water.

Shellfish mortalities were calculated by subtracting the total amount of live specimens found during each collection date from the number of live specimens found during the previous collection date. For example, if there were 50 live mussels measured in September 2010, and 30 in March 2011, there would be 20 mortalities recorded for the September-March period. This was deemed a more accurate method than simply recording the number of dead specimens and empty shells found at each collection date, because often-empty shells may break up or otherwise disappear for a variety of reasons. Percentage of total mortalities per day was also calculated in order to observe any differences of mortality rate at different sites or treatments. To determine percentage of total mortalities per day, percentage of total mortality was first calculated for each measurement period (e.g., April-September and September-March, for Experiment 2, and then divided by the amount of days of each measurement period.

Experiment 1 also involved collection of a variety of other water quality data, including dissolved inorganic nitrogen (DIN), chlorophyll *a*, total volatile solids (TVS) and total suspended solids (TSS). These were sampled at different depths (2m and 20m), at upstream and downstream locations, and at different times during the year in order to develop a more complete representation of site conditions at Clam Bay and Cypress Island. Vertical profile measurements of salinity, temperature, *in vivo* chlorophyll *a*, dissolved oxygen, pH and turbidity have also been collected periodically at both study sites. These data were collected on some shellfish sampling dates, as well as in between those periods. Water temperature data and fish farm biomass was also obtained from the fish farms' records. In addition, seston and phytoplankton analysis were conducted periodically at the IMTA sites by analyzing TVS, TSS and chlorophyll *a* content of the water as described below, some of which uses calculations based on Hawkins et al. (2002). Analysis of nutrients was performed by the University of Washington Oceanography Routine Chemistry Laboratory using state of the art autoanalyzer techniques. TSS, TVS and chlorophyll analyses were conducted at Aquatic Research Inc. laboratory in Seattle using Washington State Dept. of Ecology and EPA approved methodologies.

TVS was measured as an estimate of particulate organic matter (POM) which in turn is a crude surrogate for total available shellfish food. TVS was measured by weighing a GF/F filter before and after suctioning sample water through it and after combusting the filter to calculate the portion which ignites.

Likewise, TSS was measured as a representation of the total pool of total particulate matter (TPM) using standard methods of filtering water and drying the filtrate. TSS was measured by filtering a water sample on a dry, pre-weighed filter, drying the sample/filter and weighing again.

Chlorophyll *a* (µg/l units) was measured in the field with a CTD probe (Turner Co. SCUFA) and discrete samples collected in 1 liter bottles and analyzed at a certified laboratory (Aquatic Research Incorporated, Seattle, WA) using spectrophotometer based methods. Chlorophyll *a* is a surrogate measure of phytoplankton abundance that can be factored below into comparable units when calculating phytoplankton organics. It serves as a measure of food for shellfish.

Total particulate matter (TPM) was estimated by analysis of total suspended solids (TSS) in mg/L.

Particulate inorganic matter (PIM) was estimated by subtraction of chlorophyll *a* minus POM.

Phytoplankton organics (abbreviated PHYORG in units of mg/l) was estimated by the method of Grant and Bacher (1998), where measured chlorophyll *a* concentration (mg/l) is multiplied by the coefficient value 50 (for relatively rich growing waters such as Puget Sound, see Welschmeyer and Lorenzen (1984) and Taylor et al. (1997)). This is the so-called carbon to chlorophyll *a* ratio and varies considerably. That value of total phytoplankton organic carbon is then divided by the factor of 0.38 (a conversion factor for algae in nearshore waters) to achieve an estimate of PHYORG.

Non-phytoplankton organics (DETORG in mg/l) was then calculated as POM minus PHYORG. This is the material not useful for phytoplankton growth (i.e., detritus) although it may be cycled into phytoplankton through mineralization. Few estimates of PHYORG and DETORG are available for Puget Sound, although basic POM and related estimates from North Totten Inlet in Southern Puget Sound have been described in unpublished reports by Brooks (2008). Measured TVS (e.g., POM) of surface waters of North Totten Inlet averaged 13.5 mg/L (95% CI of +14.6/-12.5) and TSS averaged 51.8 (95% CI of +55.0/-48.5) in this study. South Puget Sound has modest phytoplankton abundance during the growing season like central and north Puget Sound; however, it is rich in seston year round compared to these other areas. The actual cause of this situation is unknown, but circulation with the ocean is restricted and turbidity relatively high compared to the other basins of Puget Sound.

The above calculations allow insight into the source of diet for the shellfish, which includes a large, often major portion of the diet due to non-phytoplankton organics. Unfortunately, this varies from place to place as well as seasonally, so it has to be determined locally. In these calculations, we must acknowledge that not all phytoplankton is equally useful for shellfish growth, but this approach is currently considered one of the few reasonable approaches to measure available shellfish food supply (e.g., Hawkins et al. 2002).

Shellfish removed for stable isotope analysis were packed on ice and brought back to Arlington, Washington. Within 24 hours, shellfish tissue consisting of entire soft body viscera was removed

and packaged in Whirl-Pak bags, frozen and shipped to the University of Idaho Stable Isotopes Laboratory for carbon and nitrogen stable isotope analysis. At the laboratory, samples were preprocessed by homogenizing the tissue in a mortar and pestle in liquid nitrogen before analysis. An elemental analyzer (NC2500, CE Instruments, Milan, Italy) was used to liberate $N_2(g)$ and $CO_2(g)$ from solid samples by flash combustion, and subsequent oxidation and reduction reactions. A gas chromatographic column in the elemental analyzer separates the two gas species which are vented to a mass spectrometer (Delta+, ThermoElectron Corp., Bremen, Germany) via a continuous flow interface (ConFlo II, ThermoElectron Corp., Bremen, Germany) for isotope ratio analysis. Standardized acetanilide are analyzed every 11 samples for assurance of stability, drift correction, and elemental mass fractions. Acetanilide working standards were calibrated against an acetanilide primary standard and reported as a relative ratio to Peedee belemnite (PDB) for carbon and as a relative ratio to atmospheric nitrogen for nitrogen. The precision of the specific analysis was calculated from the standard deviation of the four secondary standard replicates. The mean of the four acetanilide working standards were used to do a 1-point correction estimate of %C and %N. For quality control, a tertiary standard was used to verify the quality of the normalization applied.

Spatial oxygen consumption of Pacific oysters was estimated from Ren et al. (2000) and used to calibrate model simulations of AquaModel 3D-GIS based aquaculture monitoring software for a site with characteristics similar to the Cypress Island sites.

All data was reviewed for QAQC concerns and grouped by species and time period for computation of basic statistics involving ANOVA and Tukey's post hoc testing. Microsoft Excel and Statistix statistical analysis software were used to process and analyze data. In addition, the number of shellfish mortalities per sampling period was calculated by subtracting the total amount of live specimens found during each collection date from the number of live specimens found during the previous collection date. This was deemed a more accurate method than simply recording the number of dead specimens and empty shells found on each collection date, because often empty shells may break up or otherwise disappear for a variety of reasons.

To evaluate the significance of IMTA in terms of removing fish farm wastes we constructed a matrix of possible effects including:

1) Growth in treatments near the farm versus the reference areas, noting that increased growth near the farms should have been higher if food was a limiting factor.

2) Stable isotope effect, in terms of mixing model results.

3) Survival, in terms of significantly higher shellfish survival at treatment versus reference locations.

Single stable isotope results were also analyzed, but did not correlate well with growth and mixing model results and were judged less reliable as a suitable index of performance of the IMTA system. Because fish produce mostly soluble nitrogen wastes but carbon as particulate wastes (and as respiration) we would have expected a possible single stable isotope result for carbon, not nitrogen, but that was not what we found as discussed later.

WATER QUALITY RESULTS

A range of water quality measurements were taken over the course of the experiment in order to provide supplemental information and potential explanations for growth and stable isotope results. The majority of water quality data, including chlorophyll *a*, dissolved inorganic nitrogen (abbreviated DIN, which includes most of the common nitrogen plant nutrients of nitrate, nitrite and ammonium), total volatile solids (TVS) and total suspended solids (TSS) were only sampled during Experiment 1 but still gives an idea of environmental conditions present during Experiment 2. Water temperature, another important factor that may well influence shellfish growth, survival and feeding habits, was recorded daily by the fish farmers and during our routine sampling sessions for the majority of both experiments.

WATER TEMPERATURE

Between September 2008 and June 2009, the time period of Experiment 1, Cypress Island water temperature averaged 8.9 °C. At Clam Bay, water temperature was significantly higher, at 9.8 °C. Likewise, water temperatures in 2010 were significantly lower at Cypress Island than at Clam Bay, averaging 9.7 °C and 10.6°C, respectively. In addition to having a higher overall water temperature at Clam Bay, there were also no individual months in which average water temperatures were higher at Cypress Island. As an example, Figure 5 shows average monthly water temperature in 2010 for both Clam Bay and Cypress Island.

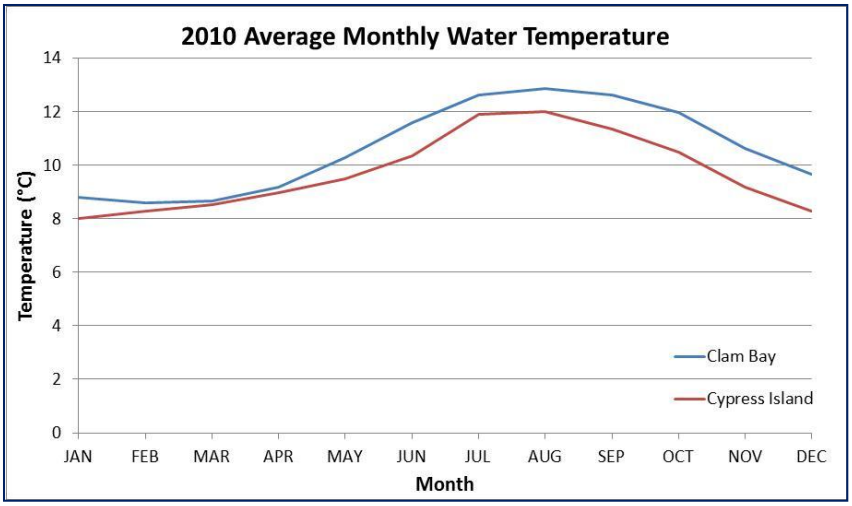

Figure 5. Average monthly water temperatures measured in 2010 at Clam Bay and Cypress Island.

Because of the modest temperatures that rarely become much colder, aquaculture in Puget Sound (and British Columbia) is advantageous for continual growth of many cultured species compared to other locations in temperate North America. As shown later in this report, however, winter temperatures (and/or food supply) was low enough to result in no growth of gallo mussels during the January to early March period and was nominally lower at Cypress Island.

SALINITY

Typically, surface salinity in Puget Sound averages about 28.5 psu (practical seawater units ~ to parts per thousand) and increases slightly further out into the Strait of Juan de Fuca based on decades of observations following initial studies by American and Canadian scientists (e.g, Herlinveaux and Tully 1961; Collias et al. 1974). In the present study salinity was only occasionally monitored (e.g., Appendix 1) but varied from ~ 30 to ~32.8 psu at both stations. Higher salinity is sometimes associated with upwelling, cold, deep water in this region and although nutrient replete, not necessarily more productive compared to more brackish water stemming from the numerous large local rivers and the largest of them all, the Fraser River that flows in the South Strait of Georgia just north of the US-Canada border.

DISSOLVED OXYGEN

Sampling locations for dissolved oxygen sampling followed the prescribed pattern required in the Washington State NPDES permits, represented here by Figure 6. Net pen facilities at both sites consist of a set of contiguous cages surrounded by steel walkways forming a rectangular pattern as seen from an aerial or plan view. Although the dissolved oxygen sampling pattern (points A-E in Figure 6, explained below figure) refers to specific measurement points at different current

locations, both Clam Bay and Cypress Island are subject to oscillating tidal flows and the "down current" end is not in every case strictly defined. Therefore, in Table 2, we present a collection of dissolved oxygen data taken at various locations around Clam Bay and Cypress Island net pens from the National Pollutant Discharge Elimination System (NPDES). These data were taken without regard to time of tide or direction and show no spatial or temporal trends whatsoever, but were purposely taken during a period of low dissolved oxygen (Rensel 2010).

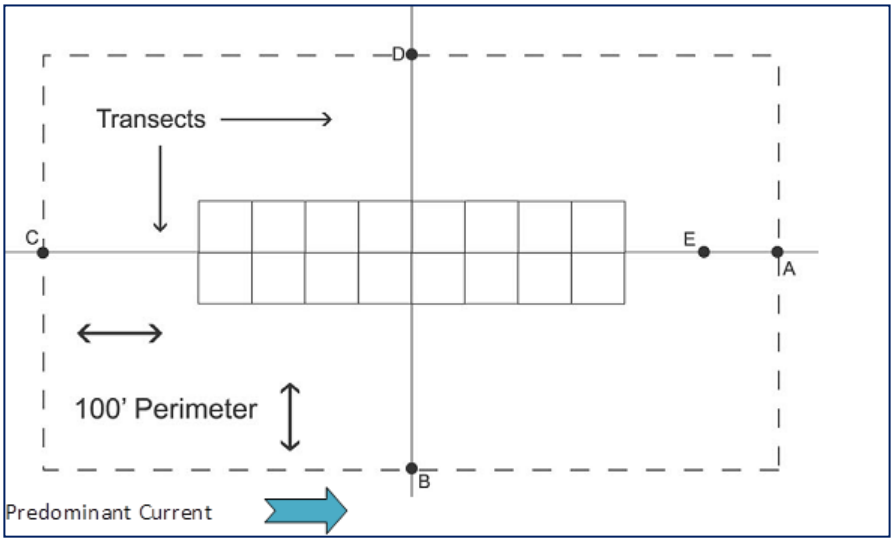

Figure 6. Layout of allowable impact zone around net pens in Washington State and sampling stations for dissolved oxygen in this project.

See below for station codes.

A) 100' (~30m) from "down current" (dominant current or suitable sea bottom) end

B) 100' from seaward end

C) 100' from "up current" (less dominant current or unsuitable rocky bottom) end

D) 100' from shoreward end

E) 50' (~15m) from "down current" end

Table 2. Results of dissolved oxygen monitoring at sampling stations around Cypress Island (Site 1) and Clam Bay net pens with reference minus mean pen values plus grand average difference shown in right column. Ref. = Reference location. From Rensel 2010.

Site & Water Temp.	Station Code Direction and distance from pens	Dissolved Oxygen mg/L at three different depths			Mean Difference (mg/L)	Pen vs. Ref. Difference
Cypress Island 11.1°C		**1m Deep**	**Mid Water**	**1m Above Bottom**		
	S1N100	5.20	5.16	5.23		
	S1S100	5.20	5.20	5.19		
	S1W100	5.14	5.19	5.00		
	S1W50	5.12	5.15	5.02		
	S1E100	5.12	5.22	5.32		
	Mean	5.16	5.18	5.15	5.16	
	Ref 200' East	5.08	5.12	5.11	5.10	0.06
Clam Bay 12.4°C						
	CBSE100	7.30	6.96	6.91		
	CBSW100	7.25	7.12	7.18		
	CBNW100	7.13	7.09	7.08		
	CBNW50	7.12	7.09	7.04		
	CBNE100	7.38	7.22	6.82		
	Mean	7.24	7.10	7.01	7.11	
	Ref 200' East	7.25	7.01	6.81	7.02	0.09

In order to quantify dissolved oxygen effects of shellfish farms placed by fish farms, we initially thought we would have to measure dissolved oxygen consumption at a representative mussel farm. The senior author is highly experienced at measuring flux rates around fish farms but knew that such field-based estimates pale in accuracy compared to laboratory and model based results. Therefore instead, we based our estimates on published Pacific oyster respiration rates of Ren et al. (2000) to calibrate the 3D-GIS aquaculture simulation software AquaModel by substituting in oysters for fish (see Rensel et al. 2007, Kiefer et al. 2008, Kiefer et al. 2011 for details of the model construction and operation). The above cited oyster respiration rates were altered by the dry weight-wet weight conversion factors found in Ricciardi and Bourget (1998).

We estimated that an average 70mm shell length oyster at harvest is respiring at approximately 0.00896 ml O2/hr. Compared to salmon, which have a much higher basal and active metabolism

or approximately 300 mg O^2/kg/hr (wet weigh and for normal activity levels), oysters respire much less, at 8.27 mg O^2/kg/hr (wet weight) or 36 times less per unit weight. We applied that correction factor to the standing stock of fish in the pens to adjust the standing stock of oysters being tested during the simulation.

AquaModel evaluates sediment and water column effects of floating aquaculture systems and has not been fully configured for use with shellfish so here we only used the respiration rate of oysters at 12° C and in a simulation of twelve each 90 m^2 rafts with a total estimated annual production of 84 metric tons. This is small compared to a full sized floating shellfish operation, but reasonably sized given the relatively narrow downstream plume and cross section of a net pen farm with the current running through them longitudinally, as often occurs in Puget Sound with the strong current velocities. The shellfish are being held at 12 meter deep and deeper, not at the surface as per the approximate depth of many of our treatments in this study.

Figure 7 is a screen-print snapshot of one hourly time step in a model run showing the each of the twelve cages (represented as green dots, not to scale of the outline of the rafts). A vector arrow in the center of the array indicates (in this case) the near surface current vector (velocity & direction). The current velocity at this time was a moderate 6 cm/s (0.12 knots) and the oxygen deficit relative to ambient is shown as a vertical profile taken at the location of the most northwesterly (reddish) raft and as a small red dot superimposed on that location. Inset charts show a vertical "drill" profile in the worst-case position (top center/left) and a longitudinal transect through the cages (bottom right) derived from a moveable red longitudinal line was drawn through the array to produce a section profile of oxygen in the water column. Surface and near bottom current velocity is shown as the plot in the lower left. These are but 3 of about 45 different plots available in AquaModel for water column and benthic parameters. Other features of this plot are the simulation time control that allows the user to play the simulation forwards or backwards and the main image oxygen concentration scale (upper right) and water current vector scale (middle right).

In Figure 7 we notice that the oxygen deficit plume is mostly restricted to the water column below the downstream rafts but is quite small as indicated in the vertical profile and limited to about 0.6 mg/L. Further downstream, by 30 meters distance (first brown dot in a row of three) the reduction is only 0.3 mg/L less than ambient as shown in Figure 8. The final screen-print of this series illustrates conditions when flows are near neap, at 3 cm/s and direction of flow has shifted from north to south as it does at the subject site during the changing of the tidal phase (Figure 9). This condition represents worst-case oxygen use but of course is restricted to within the culture area and not being advected downstream any great distance.

We could show many more images at different stages of the tide but the results are generally similar. For the production unit of this size the dissolved oxygen effect of the oysters is relatively insignificant compared to what a comparable biomass of fish would produce. This is not to say that deflection of water flow around the salmon farm from the shielding of the oyster rafts would not be a problem if the currents were weak, but again, here we are evaluating effects of a "sunken" oyster raft array.

By way of comparison to real field data, an unpublished study reported by NewFields Northwest (2008) of shellfish induced oxygen deficit plumes measured around a large mussel farm in Totten Inlet, South Puget Sound, appeared to affect conditions only a relatively short distance downstream, often just a few meters downstream. Although the results were variable, in no case was an effect measurable by 70m away from the mussel rafts. Unfortunately, there were no measurement locations between a few meters downstream and 70 m downstream. Also for comparison, Parametrix et al. (1991) reported the results of several dissolved oxygen flux studies (not just concentrations) at fish farms in Puget Sound that showed no measurable effect downstream of the farms at a distance of 30 meters. Fish farms were generally smaller then than they are presently, but given the much greater respiration rates of salmon versus oysters, these observations show how the modeled estimates may be approximately correct. The 1991 study was performed by the senior author of this report as a subcontract to Parametrix.

Diversion of water current remains an issue, because it is dissolved oxygen flux – not just concentrations – that are important to maintain the fish. Fortunately, it appears that at our selected sites, shellfish grown at 10-15m deep were able to grow at similar rates to surface-cultured shellfish as discussed later in this report.

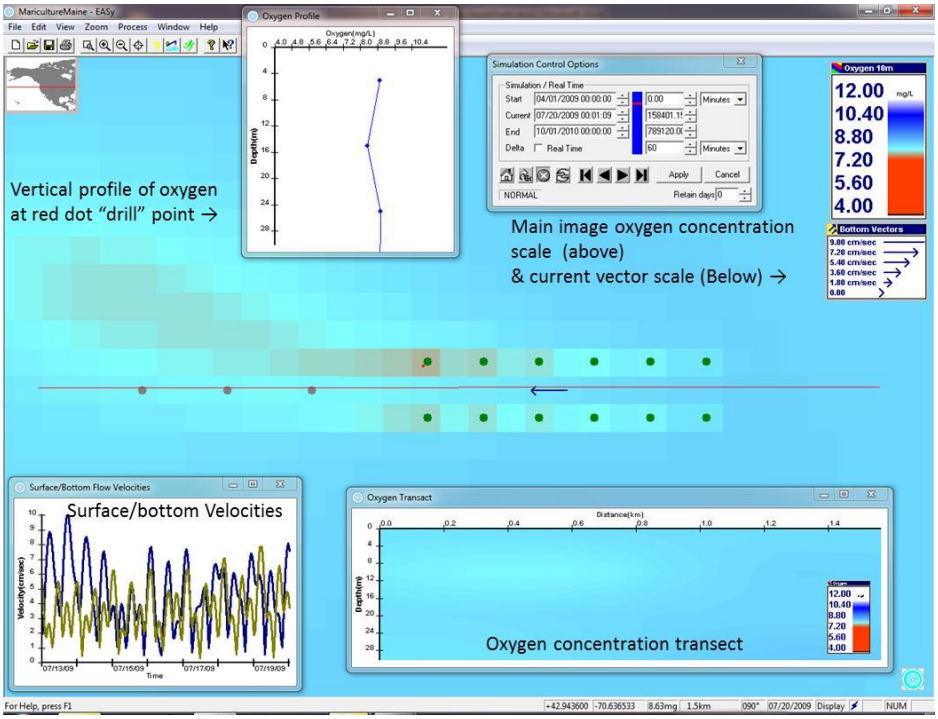

Figure 7. Screen print of AquaModel simulation of oyster rafts. See text for explanation.

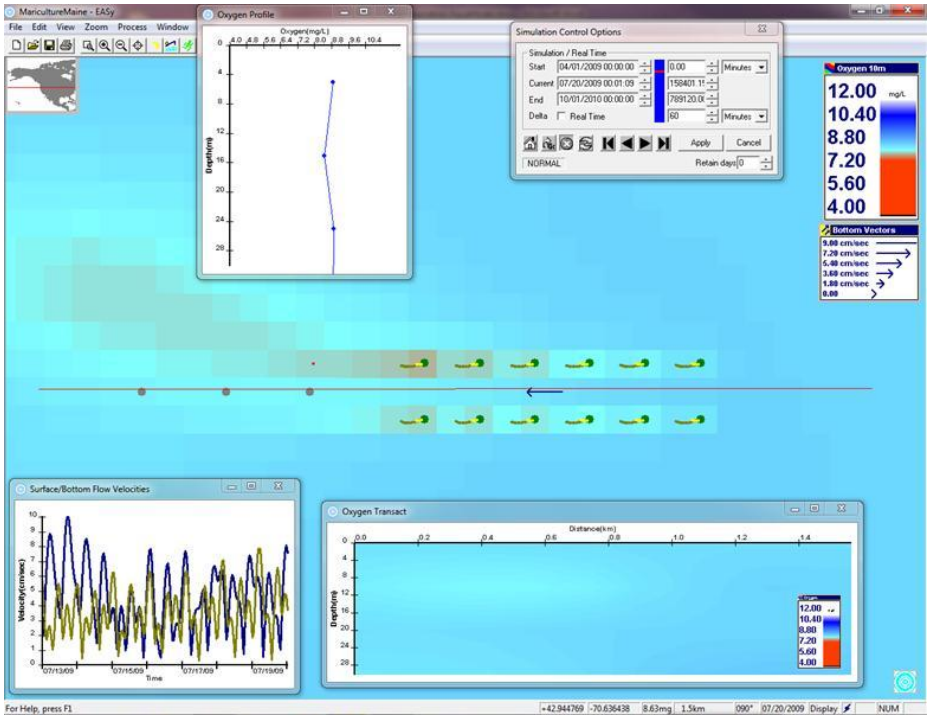

Figure 8. Screen print of AquaModel simulation of oyster rafts current velocity of 6 cm/s (0.12 knots) at the same time as above but with the vertical profile point moved 30m downstream.

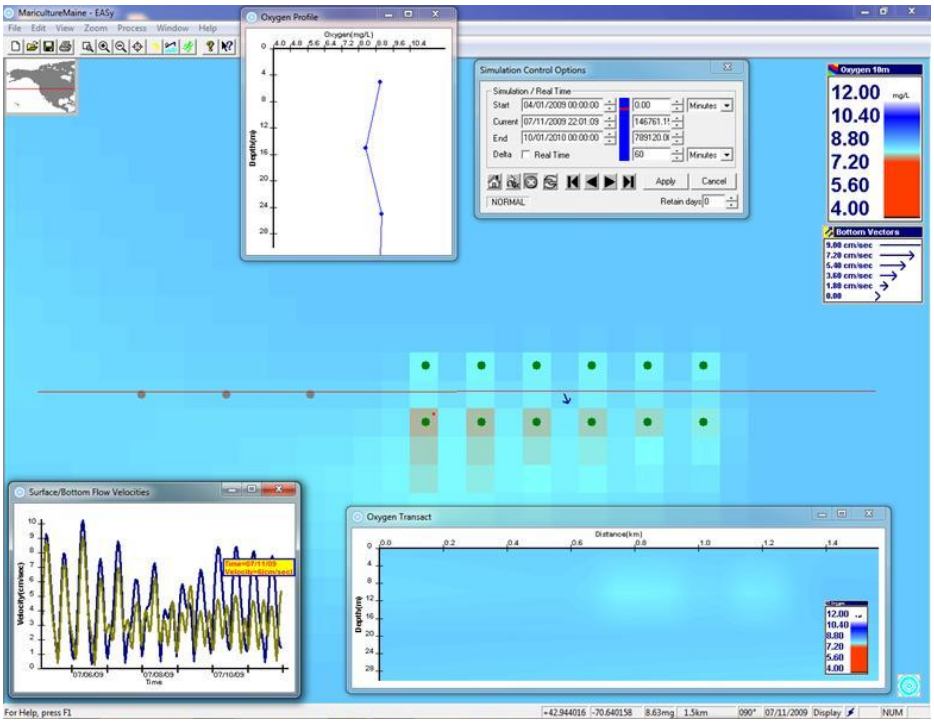

Figure 9. Screen print of AquaModel simulation of oyster rafts current velocity of 1.5 cm/s (0.03 knots) shortly after the previous time period and with the vertical profile point placed through the lower left raft location.

Based on the foregoing, fish farmers in moderate current areas in Puget Sound would have a choice between 1) placing shellfish at the surface but keeping the density reasonably low; or 2) placing shellfish subsurface at any desired density that does not invoke a "density dependency" growth reduction.

CHLOROPHYLL AND NUTRIENT RESULTS

Chlorophyll *a* and dissolved inorganic nitrogen sampling results are shown below in Figure 10 for both net-pen sites. Chlorophyll profiles were similar at both upstream and downstream locations within sites and the general seasonal expected shape was similar, but greater levels of chlorophyll were observed at Clam Bay in spring and summer. However, since the shellfish were harvested in June, we note that chlorophyll was somewhat lower on average in Clam Bay versus Cypress Island during the growth experiment and both sites exhibited low winter values.

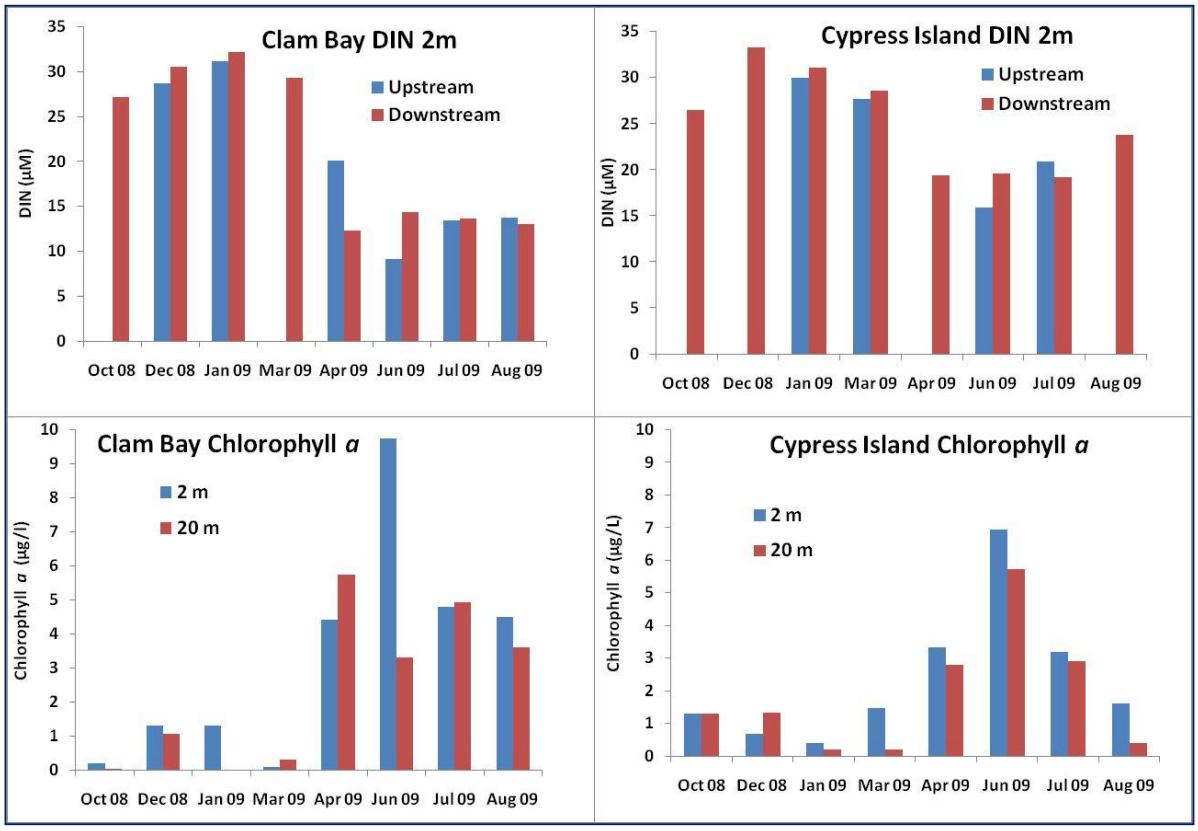

Figure 10. Clam Bay and Cypress Island dissolved inorganic nitrogen (DIN) and Chlorophyll *a* sampled during Experiment 1.

Increased chlorophyll at Clam Bay in summer is understandable as the bay itself is rapidly flushed and source waters during the ebb include the Port Orchard (bay) area waters that are often enriched with phytoplankton due to more ideal algal growing conditions. Source waters for Cypress Island, really more of bight than a bay, are the relatively open, deep waters of Bellingham Channel and North Puget Sound.

Fish farms in Puget Sound do not directly affect chlorophyll content of the water as it takes a day or longer for cells to divide and nutrients are not limiting at farm sites to the growth of phytoplankton in the main basins of Puget Sound (Rensel Associates and PTI Environmental Services 1991). Background concentrations of dissolved inorganic nitrogen are naturally high throughout these areas and note that there was no large or consistent upstream to downstream nitrogen differences in these measurements. When dissolved nitrogen availability far surpasses the phytoplankton or seaweed demands in an area, other factors, such as sunlight or vertical mixing and advection control algal populations. Fish farms produce dissolved nitrogen wastes and eventually some of that waste is sequestered by phytoplankton or seaweed even if background

concentrations are large. However, the literature clearly demonstrates that other factors, including sunlight availability and advection of cells to the deep layer are factors considered to limit primary productivity in the main basins of Puget Sound, and not nutrient supply.

Not shown above in Figure 10 are the 20 m depth DIN results which mirror the 2 m results almost exactly. This is what is expected in well-mixed or actively mixing areas that represent optimum fish farm siting. Data tables for all of the above are found in Appendix 2.

TOTAL VOLATILE SOLIDS AND TOTAL SUSPENDED SOLIDS RESULTS

Total volatile solids (TVS) and total suspended solids (TSS) were measured in order to analyze available shellfish food. TVS can act as an estimate of particulate organic matter (POM) which is a surrogate for total available shellfish food; TSS represents a measure of the total pool of particulate matter and can be used in conjunction with other measurements to calculate phytoplankton organic matter and non-phytoplankton organics.

In Figure 11 we show TVS and TSS results for Clam Bay and Cypress Island. Error bars are not included as often single samples were collected, although we collected at least one duplicate sample daily as a quality assessment measure and results indicate that the duplicates in all cases closely matched the companion sample. We sampled upstream and downstream or the pens, and compared to see if there was a difference, but *t*-tests of the data indicate no significant differences overall. This is a bit surprising, especially for the 20 m depths, as the fish farms have large amounts of fish biomass on hand (Figure 12).

Immediately apparent in Figure 11 are the seasonal and total variation between sites. Clam Bay had lower TVS in fall through spring, but higher concentrations in summer than Cypress Island. For TSS, Clam Bay was consistently lower throughout the year with the exception of the final sample in August 2009. Just based on these results, we would have expected Deepwater Bay to produce larger oysters and mussels. However, the opposite occurred, which may be due to the confounding effect of colder average temperatures at Cypress Island, and/or differences in food quality. Phytoplankton species composition was not assessed, as funds were limited for this project. However, routine sampling for harmful algae during the growing season has been conducted for decades at these sites by the fish farmer technicians who are trained in this matter and no overtly different species composition differences have been noted between these sites.

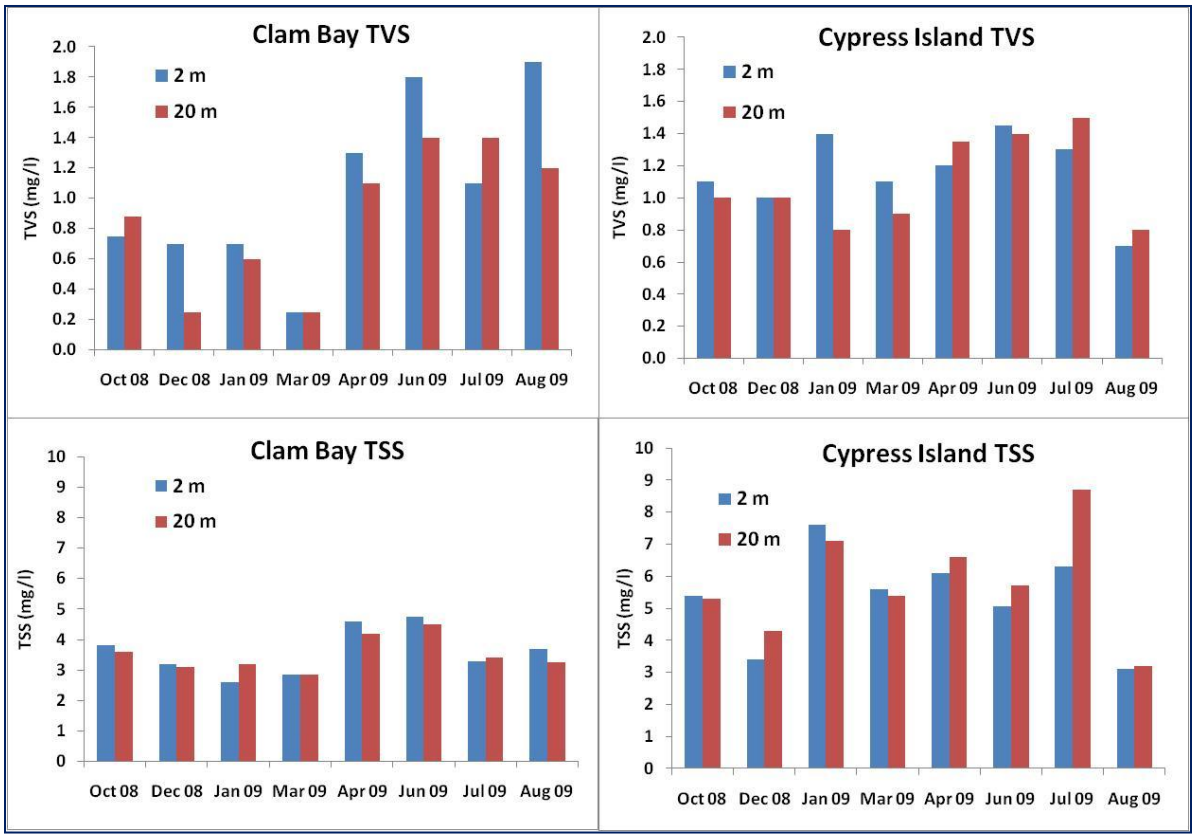

Figure 11. Clam Bay and Cypress Island monthly total volatile solids (TVS) and total suspended solids (TSS) at 2 and 20 m depths.

Sutherland et al. (2001) found an increase in suspended particulate matter (SPM) immediately next to a salmon net pen in the Broughton Archipelago of British Columbia of 0.6 mg/L but that was within the middle of the fish cages. Only 30% of that was seen immediately adjacent to the pens at 5 m distance. About 80% of the inside SPM pen load was measured vertically below the pens. Sampling was conducted when the tide was running at reasonable strength of 10 cm/s below the pens (which is sufficient for sea bottom resuspension of wastes). Samples were collected on a transect up to 30 m from the farm but results were not reported. A reference station 500 m distant had lower levels of particulate organic matter than within and immediately adjacent to the farm site. Dr. Sutherland is an experienced worker in the field and no doubt these measurements were made to the highest level of accuracy. The authors' measurements were during feeding periods only and were not put into context of background variation in other regional areas, and we believe the maximum results (0.6 mg/L) represent a very small effect.

We cannot directly compare results with the Sutherland et al. study as we used TSS rather than SPM, a somewhat different methodology, but nevertheless another measure of particulate matter

in the water column that should have produced similar results. Our reference samples (not upstream, but remote upstream) were similar to the upstream samples (Appendix 2). Overall, both Puget Sound sites had significantly higher particulate matter results(~3 to 8 mg/L) compared to the B.C. site (maximum of 0.6 mg/L) and we present multiple day results whereas the B.C. study represented single feeding events (of unknown number) over a three day period in March. We can say that background levels of particulates are much higher at the Puget Sound sites and that there was no consistent production of particulate matter measured downstream.

In addition, our measured TSS and TVS values are much lower than those measured by Brooks (2008); however, Brooks' data are from southern Puget Sound in North Totten Inlet, which often experiences much higher TVS and TSS than the waters of northern Puget Sound. Totten Inlet is a prime oyster and mussel growing area in Puget Sound.

FISH FARM BIOMASS

Fish farm biomass was consistently greater at Clam Bay than at Cypress Island (Figure 12). Clam Bay operations ranged from approximately 725 to 2300 metric tons; Cypress Island net pens contained standing stock of between ~400 and 860 metric tons.

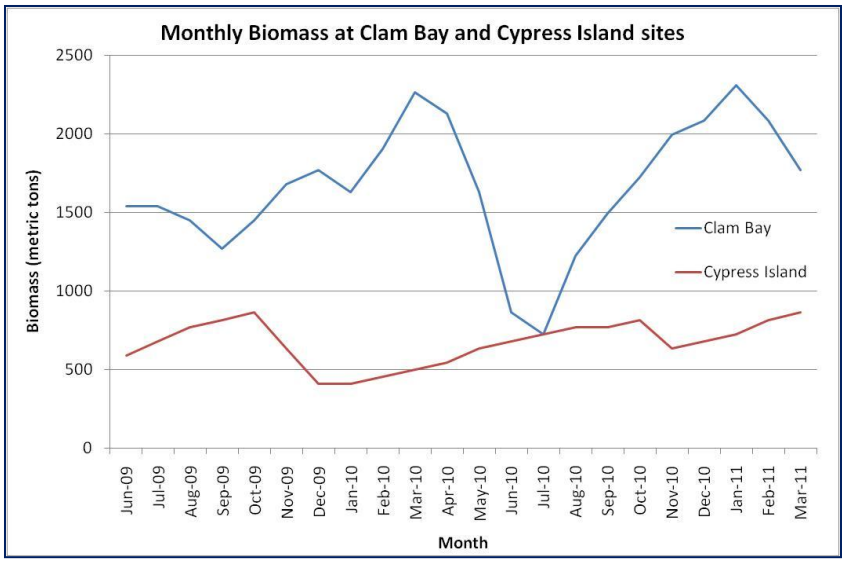

Figure 12. Monthly biomass at Clam Bay and Cypress Island Sites from June 2009 to March 2011. Biomass units in metric tons.

PHYTOPLANKTON ORGANICS AND NON-PHYTOPLANKTON ORGANICS RESULTS

As explained in the methods, phytoplankton organics (abbreviated PHYORG in units of mg/l) and non-phytoplankton organics (DETORG in mg/l) were calculated using TSS and chlorophyll *a* data, as well as several coefficient and conversion factors found in the literature. These calculations allow insight into the source of diet for the shellfish, which includes a large, often major portion of the diet due to non-phytoplankton organics. Unfortunately, this varies from place to place as well as seasonally, so it has to be determined locally. In these calculations, we must acknowledge that not all phytoplankton is equally useful for shellfish growth, but this approach is currently considered one of the few reasonable approaches to measure available shellfish food supply (e.g., Hawkins et al. 2002).

Figure 13 shows calculated PHYORG and DETORG for both Clam Bay and Cypress Island. Similar to other water quality results, all data are from sampling between October 2008 and August 2009. Overall, PHYORG at Clam Bay was slightly greater than at Cypress Island, notably during the spring and summer months. Without exception, non-phytoplankton organics were greater than phytoplankton organics at both sites. The contrast between DETORG and PHYORG is especially strong during the winter months, which is supported by the fact that phytoplankton are much more abundant during the spring and summer. During the winter, shellfish must rely on other diet sources, which in the present treatment cases may include fish feed and feces. Indeed, this was supported with our stable isotope mixing results, which are presented later in this report.

To summarize, the most important aspect of this analysis is the consistently higher winter non-phytoplankton organics at the Cypress Island site (Figure 13 lower right). This result stems from the also consistent and higher TVS and TSS in winter as previously discussed (Figure 11 right panels). These higher levels in winter at Cypress Island are apparently not due to the fish farms, as both upstream and downstream measurements around the farms were similar.

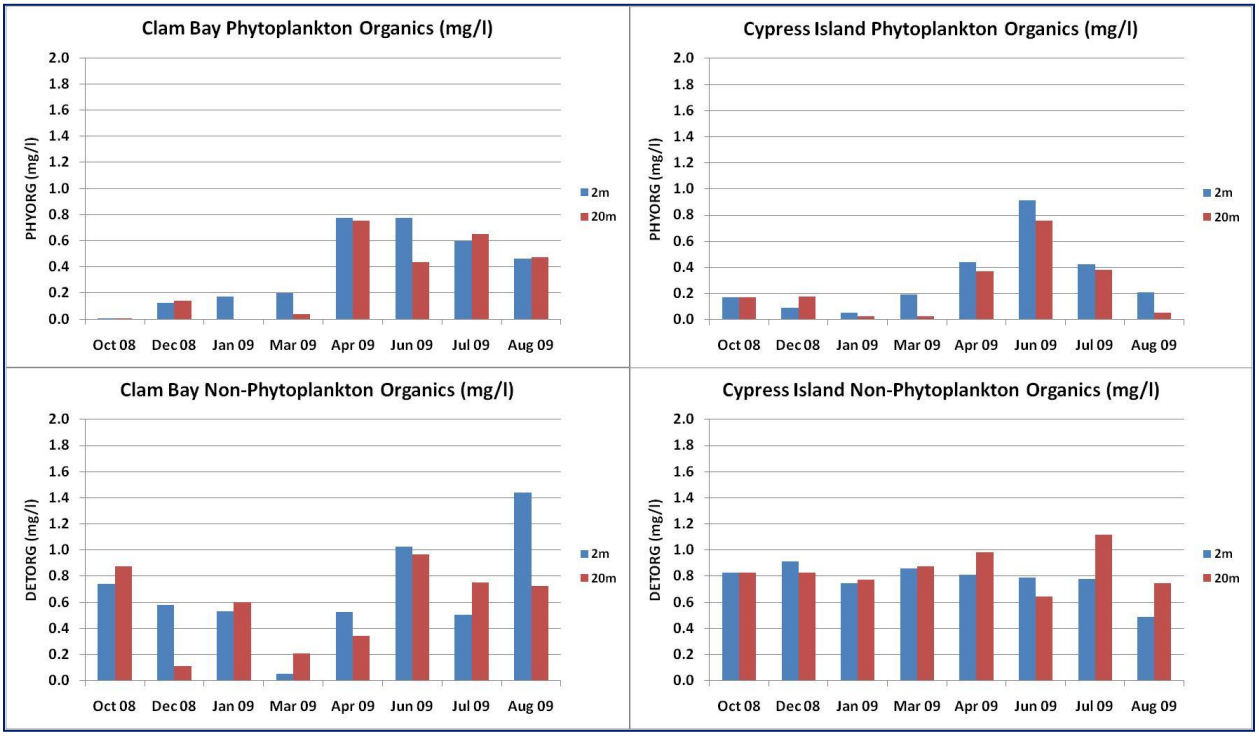

Figure 13. Phytoplankton Organics and Non-Phytoplankton Organics for Clam Bay and Cypress Island, calculated from water quality samples taken between October 2008 and August 2009.

GROWTH

EXPERIMENT 1 GROWTH

In Experiment 1 where mussels and oysters were cultured concurrently, oyster growth far exceeded that of mussels. For example, Figure 14 illustrates the results from Clam Bay for the entire experiment. In Figure 14, as in all following figures, error bars represent ± one standard deviation.

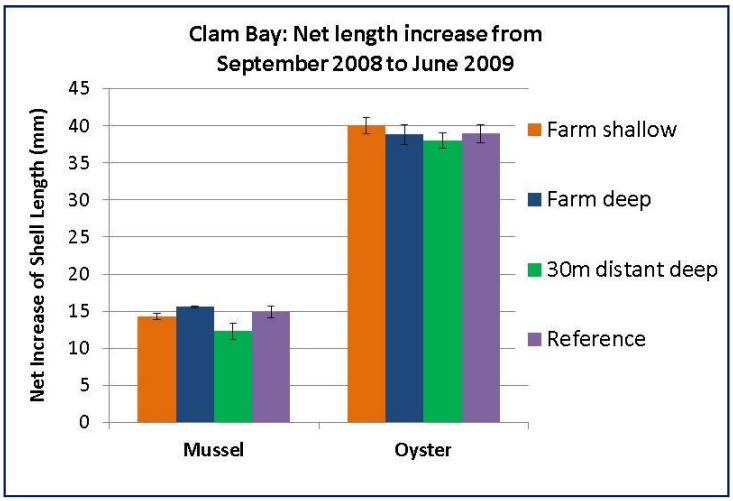

Figure 14. Total length increase of Clam Bay mussels and oysters cultured during Experiment 1.

Comparison of shell length between species is not an accurate measure, as oysters can grow in different shapes versus mussels, but the better growth of oysters accompanied some increase in growth nearer the farms and significant stable isotope effect as discussed below. Figure 14 is given for general reference, as there were slight, but significant size differences in initial shell length among Experiment 1 shellfish. For analysis, we used net growth by measurement intervals to deal with the unequal initial length issues. Mean and standard deviation of shellfish data are found in Appendix 3.

EXPERIMENT 1 OYSTERS

Clam Bay oysters outperformed mussels and showed growth enhancement near the net pens and in a stepwise spatial fashion away for the net pens during the fall and early winter period (Figure 15). However, in the remaining period until harvest the differences diminished to insignificant. These data suggest at least a seasonal effect of particulate waste reduction from the fish farm and utilization by the oysters.

Over the course of the entire Experiment 1 (September 2008 – June 2009), oysters outperformed mussels at Clam Bay across all treatments: oysters gained 38-40 mm of total growth while mussels gained 12.3-15.6 mm of total growth (Figure 14).

During the first measurement period (September 2008 – January 2009), Clam Bay oysters displayed a clear stepwise spatial pattern of growth, with farm shallow treatments experiencing the greatest net growth, followed by farm deep, 30m distant deep, and finally reference deep with the lowest growth (Figure 15, top). This pattern suggested a growth enhancement effect could have been occurring. Growth was excellent in all treatments but statistically greater near the farm.

The significant growth and spatial pattern seen in the oyster treatments was not present in the winter growth period and in all treatments net growth was slow. Between January 2009 and March 2009, the Clam Bay reference oysters had the greatest incremental growth, while farm shallow and farm deep oysters still had greater growth than the 30m distant deep treatment (Figure 15, middle). Finally, the spring growth period yielded the greatest incremental net growth at the 30m distant deep treatment. The farm shallow treatment had the least growth (Figure 15, bottom).

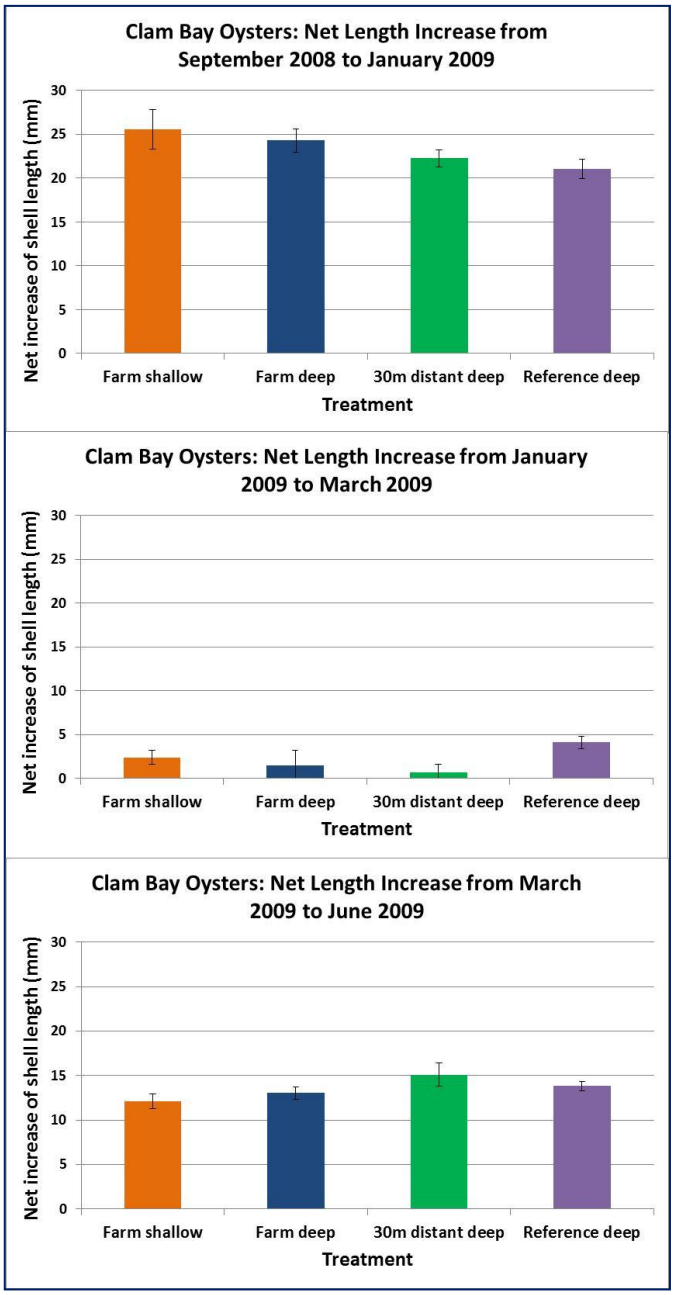

Figure 15. Net length increase of Clam Bay oysters for all three-measurement periods of Experiment 1. Top panel = first growth period, middle panel = second growth period, bottom panel = final growth period

By the end of the experiment in June, all treatments did not statistically differ; the farm shallow treatment had a higher net growth than the reference treatment but with only a length difference of 1.0 mm for the entire experiment combined, and not statistically different from any other treatment.

Overall, Clam Bay oysters in the farm shallow treatment had the greatest total growth (40.0 mm), followed by the reference oysters (39.0 mm), farm deep oysters (38.8 mm) and finally the 30m distant deep oysters had the lowest net growth at 38.0 mm.

At Cypress Island, significant growth increases were seen in each time interval (Figure 16) and for the total experiment for oysters near the farm (30 m distant deep) compared to the reference area with the exception of January through March when neither treatment experienced any measurable growth (Figure 16).

Cypress Island farm shallow and farm deep treatments of Experiment 1 were lost due to fish farm worker errors, so these treatment data were not recorded. However, we were still able to observe overall significantly higher net growth in the 30m distant deep treatment than in the reference group (Figure 17). These results were, despite the unfortunate loss, encouraging.

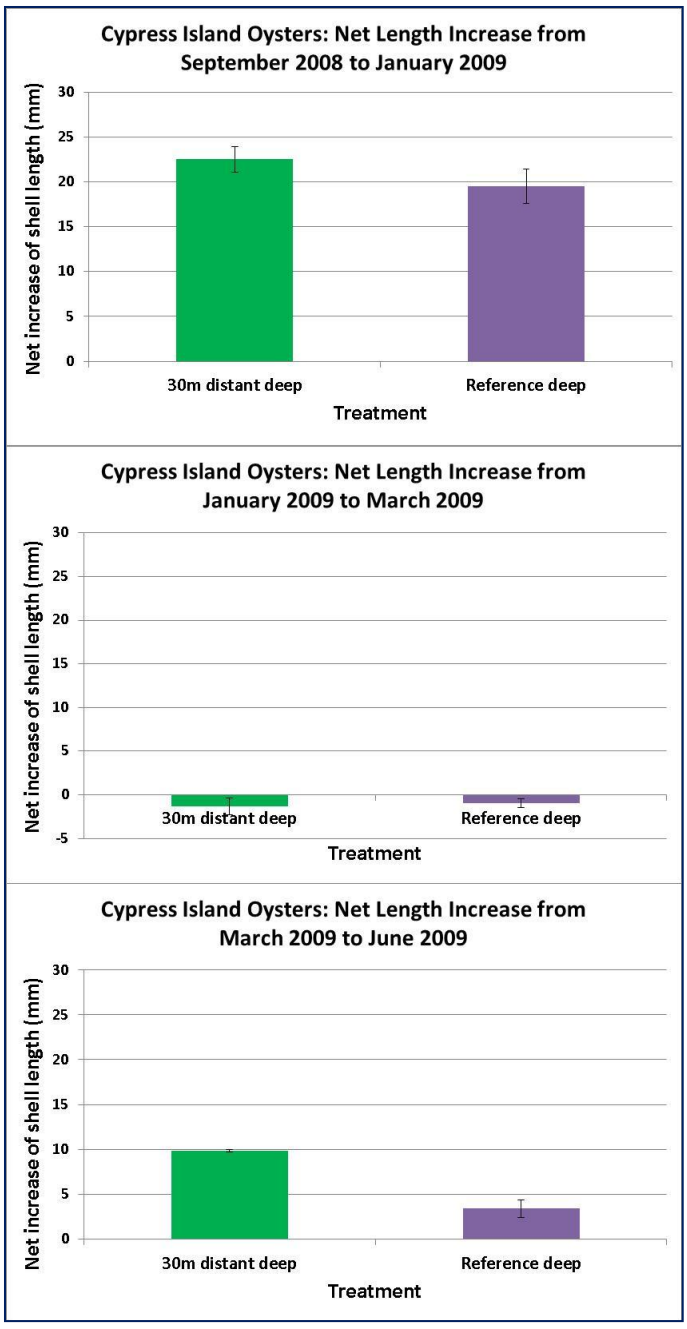

Figure 16. Net length increase of Cypress Island oysters during incremental periods of Experiment 1.

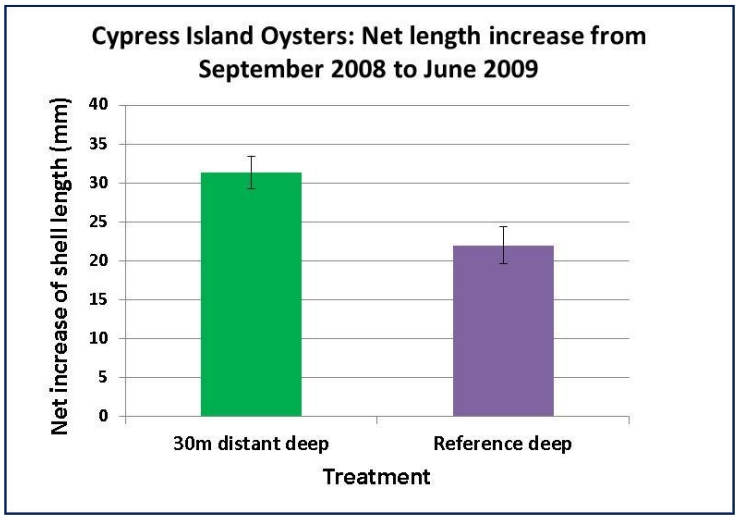

Figure 17. Cypress Island oyster net length increase during Experiment 1.

Total net growth for oysters at Clam Bay was greater than growth at Cypress Island. Net growth of Clam Bay oysters in the 30m distant deep treatment was 38.0±1.1 mm; 30m distant deep oysters at Cypress Island grew 31.3±2.0 mm. Total net growth of Clam Bay and Cypress Island oysters in the reference group was 39.0±1.2 mm and 22.0±2.4 mm, respectively. Much of this difference is likely explained by the year-round cooler temperatures at Cypress Island compared to Clam Bay, which may have reduced metabolism and growth for our study shellfish.

EXPERIMENT 1 MUSSELS

Mussel growth in Experiment 1 from September 2008 to January 2009 did not display the growth and spatial trend of the oysters, nor were there any significant differences between treatments at either site (Figure 18). For Experiment 1 in total, Clam Bay mussels, farm deep treatment grew slightly better than the reference (15.6 mm net growth versus 14.9 mm respectively), while farm shallow (14.3 mm) and 30m distant deep (12.3 mm) grew slower than the reference. At Cypress Island, the total net growth of mussels in the 30m distant deep treatment was nominally greater than those in the reference group, but the differences were not significant.

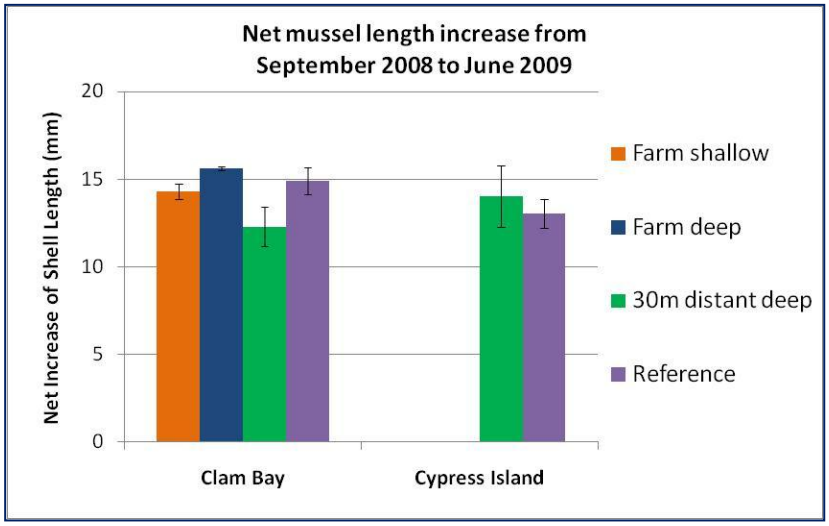

Figure 18. Clam Bay and Cypress Island mussel net length change September 2008 to June 2009.

EXPERIMENT 2 MUSSELS

Prior results focused on the initial experiment that ran from September 2008 to June 2009. Experiment 2 consists of a second, follow-up experiment that was conducted from April 2010 to March 2011, with a midpoint measurement taken in September 2010. Unlike the first experiment, only mussels were used. The mussels used in the follow-up experiment were also much younger at the beginning of the experiment, ranging from 17-26 mm in length. Mussels were stocked on April 13th (Clam Bay) and April 14th (Cypress Island) 2010. Elapsed time of culture at the sites until sampling on September 27th and September 29th 2010 was 167 days for the former and 168 for the latter. Elapsed time of total culture was 331 days for both sites (to March 10, 2011 for Clam Bay and to March 11, 2011 for Cypress Island). Throughout the rest of this report, the first half of the experiment (April 2010 – September 2010) will be referred to as the "growing season" and the second half of the experiment (September 2010 – March 2011) will be referred to as "fall-winter".

Due to fish farm staff error, all replicates of the 30m distant deep treatment mussels in Experiment 2 were lost at the Clam Bay site. Despite this setback, the farm shallow and farm deep treatments can still be compared to the reference treatments for useful information. Cypress Island, on the other hand, had no losses and we were successful in maintaining all replicates of all treatments.

In Experiment 2, Clam Bay reference mussels grew significantly better (39.2 mm net growth over the entire experiment) than the other two remaining groups of farm shallow (35.6 mm) and farm deep (34.5 mm, Figure 19 left). Farm shallow and farm deep treatments did not significantly differ

from each other. In contrast, Experiment 2 Cypress Island mussel treatments were not significantly different, while all mussels from Cypress Island experienced significantly lower net growth than Clam Bay mussels (Figure 19 right).

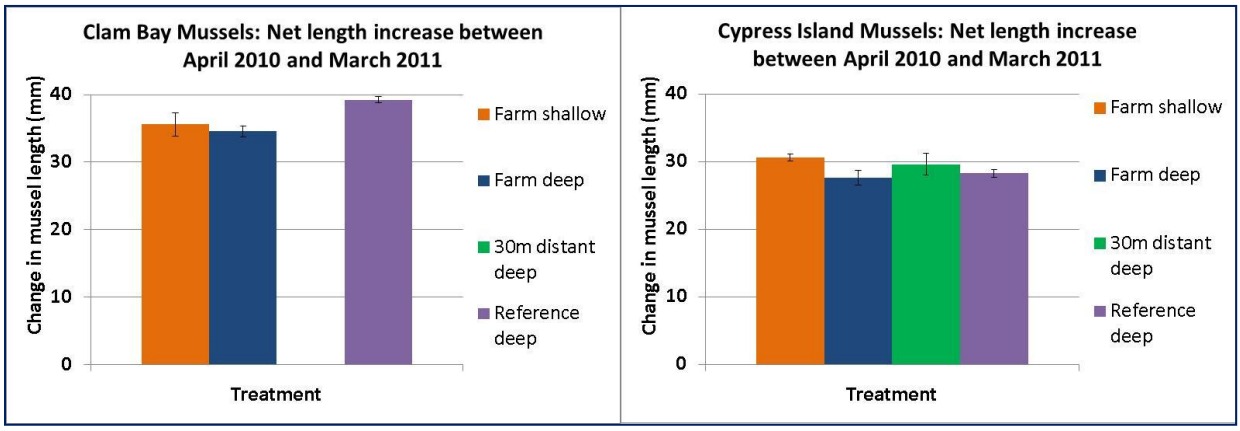

Figure 19. Net growth of mussels for the entire culture period during Experiment 2.

Figure 20 shows net increase of length among treatments for mussels at Clam Bay and Cypress Island during each measurement period. It is obvious that mussels at both sites grew significantly more during the growing season than in the fall–winter months. During the first portion of the experiment (Figure 20, upper half), Clam Bay mussels had a greater net growth than those at Cypress Island for all treatments [$p<.05$ $F(6, 14) = 37.2$, $p = 0.0000$]. This can be attributed to warmer water temperatures and typically higher primary productivity in central Puget Sound versus north Puget Sound, locations of Clam Bay and Cypress Island, respectively.

For the fall-winter period (Figure 20, lower half), growth declined greatly at both locations but relatively better growth again occurred at Clam Bay compared to Cypress Island for all treatments [$p<.05$ $F(6, 14) = 45.3$, $p = 0.00001$].

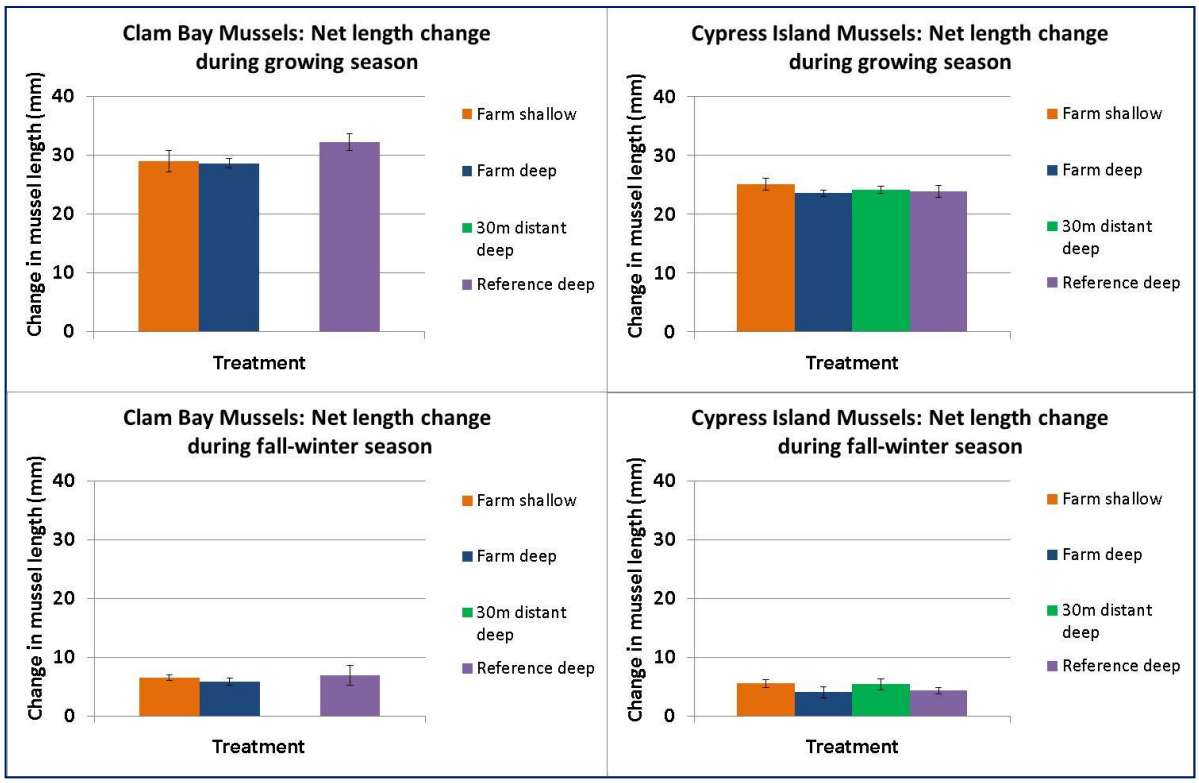

Figure 20. Incremental net increases in length of mussels at Clam Bay (left) and Cypress Island (right) during the algal growing season and fall-winter season.

Analysis of net growth for the entire experimental period of April 2010 to March 2011 shows that the pattern observed after the midpoint measurements, where Clam Bay mussels were growing significantly more than the Cypress Island mussels, holds true for the rest of the experiment. Interestingly, incremental mussel growth at the Clam Bay reference location (39.2 mm) statistically exceeded all other treatment and locations, followed by the other Clam Bay treatments (~35 mm each), far surpassing Cypress Island treatments that ranged from 28.2 to 30.6 mm. There is no plausible explanation for this other than the possibility that the mussels near the farm were energetically at a disadvantage by having to filter more particulate wastes from the farm while possibly not selecting these wastes as food (but rather rejecting them as pseudofeces). MacDonald et al. (2011) measured increased rates of mussel filtration near fish farms in New Brunswick compared to reference sites, suggesting that the mussels have the capability to capture fish farm material. However, the ability to capture fish feed and waste does not necessarily amount to assimilation, especially if other food sources like phytoplankton are available.

As the seed stock length for all treatments for both sites were statistically the same for Experiment 2, total growth plots are informative too. Figure 21 reinforces the conclusion of significantly better growth at the Clam Bay site. However, within each site, the significant differences within growth periods discussed above were not apparent.

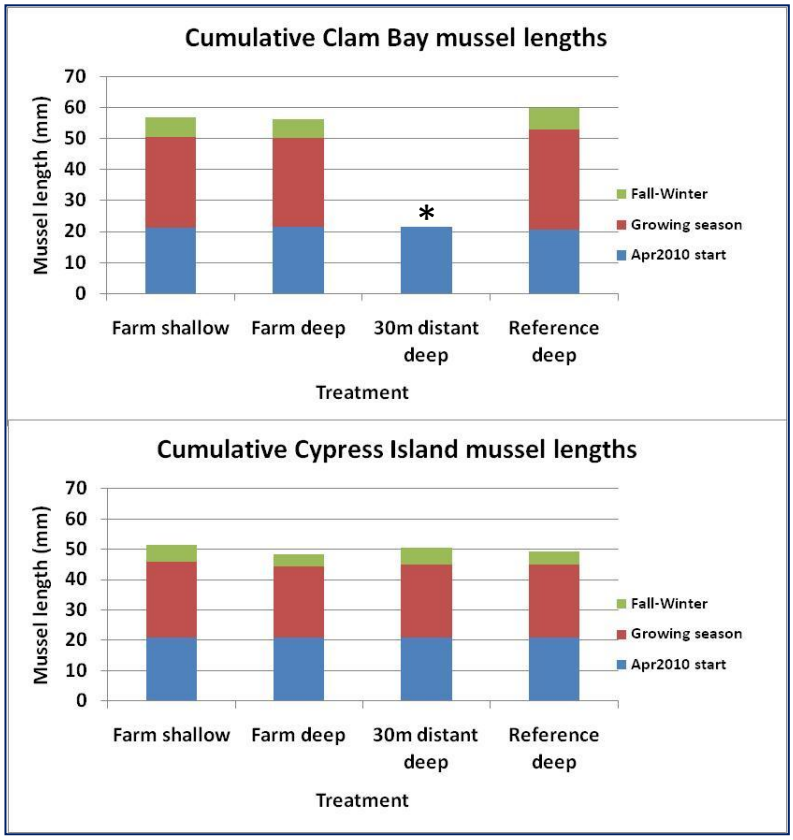

Figure 21. Total mussel growth measured during Experiment 2, including measured mussel lengths at beginning of experiment.

An asterisk in this figure indicates that the Clam Bay 30m distant deep treatment was lost during the growing season.

SURVIVAL

PERCENTAGE MORTALITY PER DAY

As previously discussed in the Methods section, shellfish mortalities were calculated by subtracting the total amount of live specimens found during each collection date from the number of live specimens found during the previous collection date. Appendix 4 shows all averaged shellfish mortality data. In order to compare mortalities between Experiments 1 and 2, percentage of total mortalities per day was calculated in order to observe any differences of mortality rate at different sites or treatments (Figure 22).

EXPERIMENT 1 OYSTERS

Oysters displayed lower survival rates earlier in the experiment (fall and winter, at 99.5% and 99.4% survival per day respectively), with few if any mortalities reported during the spring season (100.0% survival per day). Total survival percentage per day of oysters was approximately equal to that of mussels (99.6% for both species)

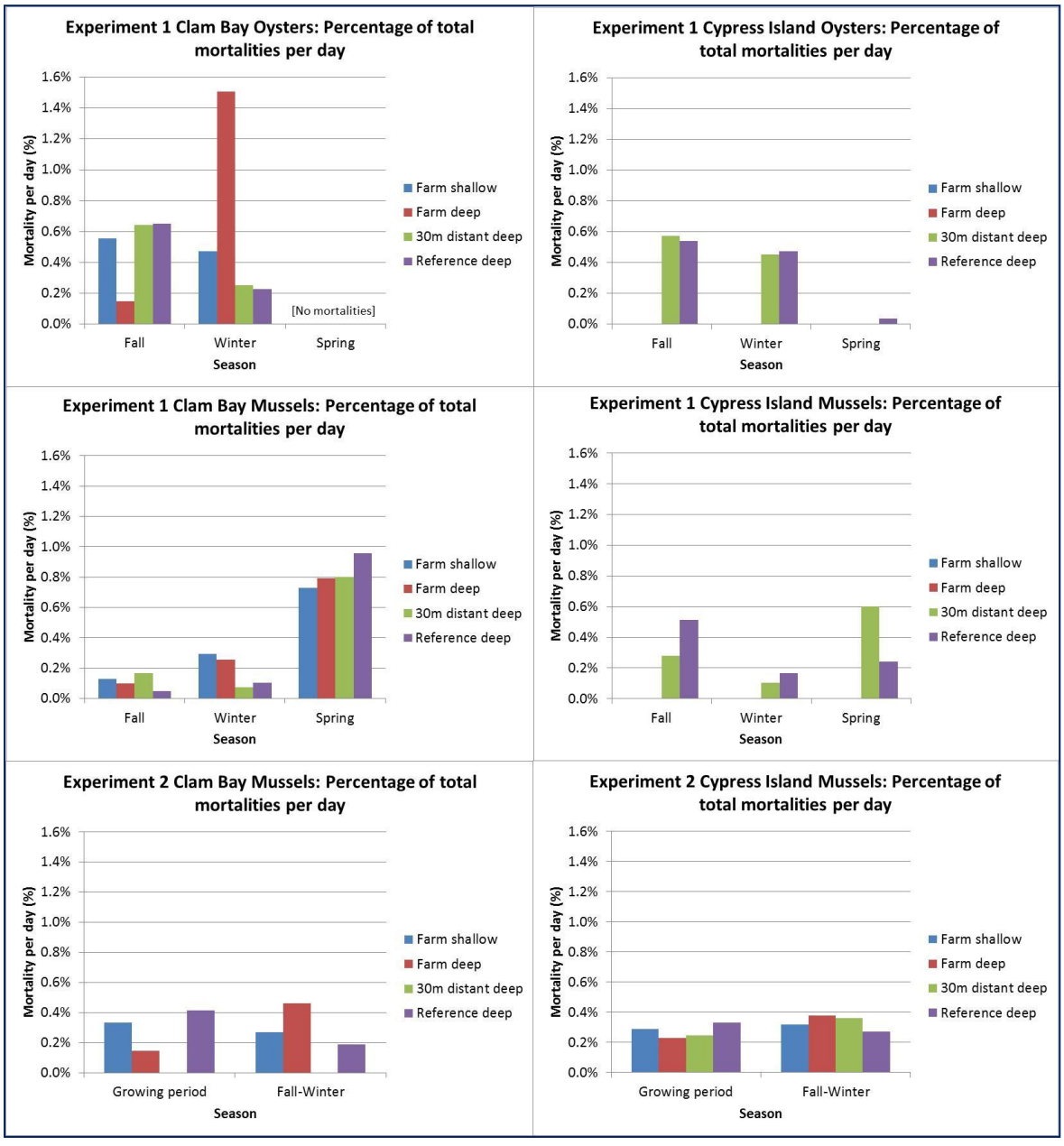

Figure 22. Percentage of total mortalities per day for Experiment 1 and Experiment 2.

EXPERIMENT 1 MUSSELS

Mussel survival was reasonably good at Clam Bay in Experiment 1 until near the end of the grow-out during spring season. Mid to late spring mortality is common among both native and gallo mussels commonly cultured in some regions of Puget Sound, likely due to pre-spawning or spawning stress. In the case of gallo mussels, which originate from the warmer Mediterranean region, less than optimum water temperatures combined with energy shunting to gonad development may act as serious stressors. However, mussels grown at Cypress Island were subjected to colder water temperatures than at Clam Bay, and Cypress Island mortality rate was lower. We hypothesize that colder temperatures may have delayed the onset of spawning and the associated stress. In addition, survival rates observed here were similar to those observed in South Puget Sound mussel culture (Gordon King, pers. comm. March 2011). In an attempt to avoid the pre-spawning (post March period) mortality issue, Experiment 2 was completed in March 2011.

EXPERIMENT 2 MUSSELS

Experiment 2 mussels at Clam Bay displayed no overall survival trends among treatments. During the growing period (April-September 2010), Clam Bay mussels in the farm shallow and reference groups displayed higher percent mortality rates during the growing period, while the farm deep mussels had higher percent mortality during the fall-winter period (Figure 23, upper right). The latter is more evident when looking at total mortalities (Figure 23, upper left). However, there were no significant differences among treatment groups during each time period. At Cypress Island, mussels generally showed slightly higher mortality during the fall-winter period than during the growing period (Figure 23, bottom), but again, there were no significant differences among treatments. In general, Cypress Island mussels had a higher overall survival compared to Clam Bay mussels, which may be explained by the fact that Cypress Island has colder average water temperatures, which, as explained previously, may have delayed sexual maturation of the mussels and reduced or eliminated stress due to pre-spawning metabolic processes.

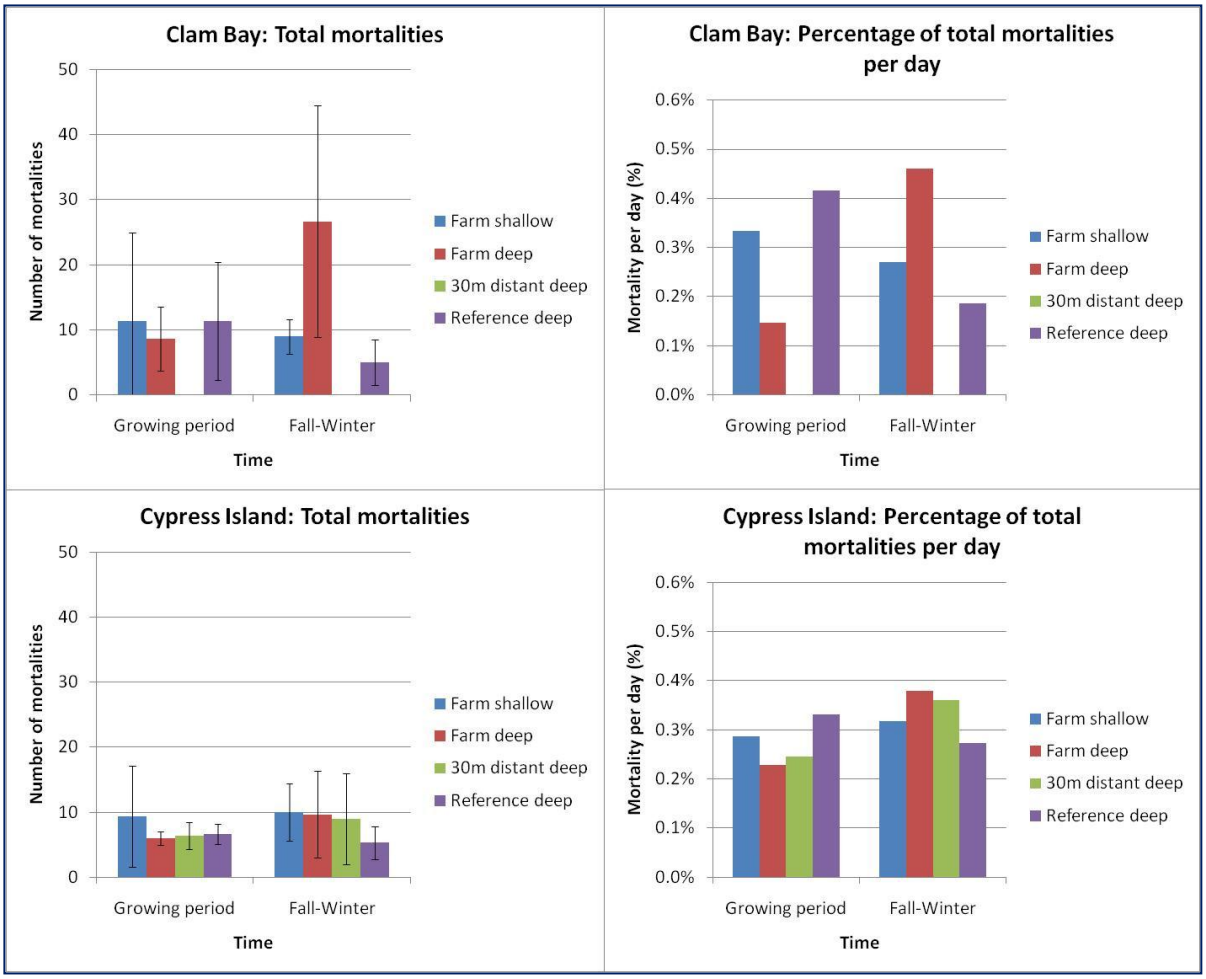

Figure 23. Total mussel mortalities and percent total mussel mortalities per day for Experiment 2.

SIZE AND TIMING OF MUSSEL MORTALITY

In Experiment 2, mussel mortality lengths were also measured by recording the lengths of all empty shells found inside the mussel cages (Figure 24, Appendix 5). Mortality length measurements may not be representative of all total mortalities, since some empty shells are lost in between collection periods; however, we can still observe patterns of mortality length between different sites, treatments and dates. Both Clam Bay and Cypress Island mortalities were larger during the second half of Experiment 2 than during the first half; this is likely due to the simple fact that the mussels were growing during the course of the experiment, so mortalities later on would naturally be larger than those mussels which died earlier.

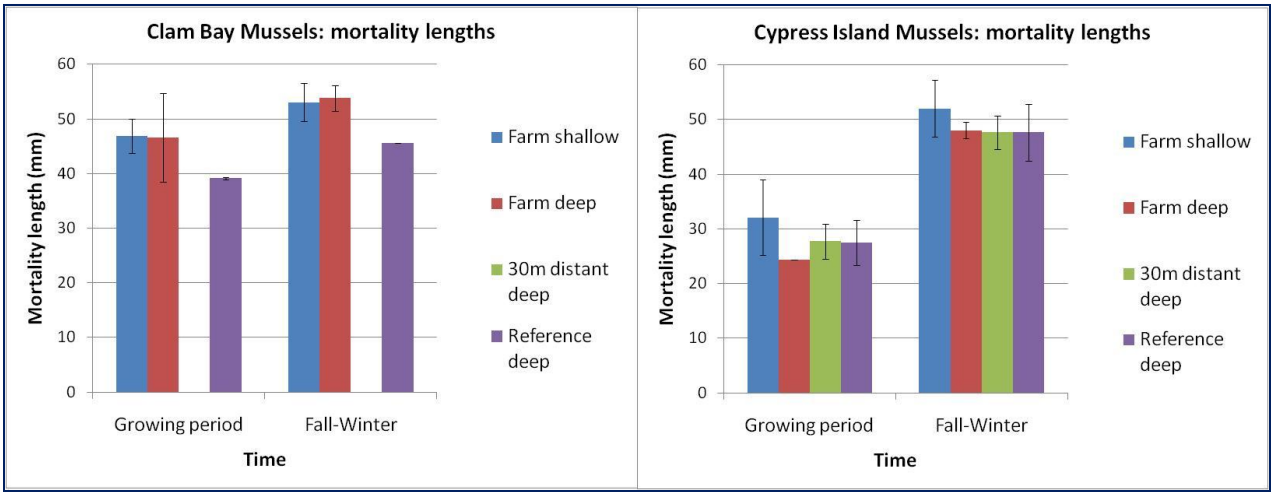

Figure 24. Observed average mussel mortality lengths and SD in Experiment 2.

Interestingly, Cypress Island experienced a much greater difference between earlier and later mortalities than those from Clam Bay, suggesting some event at Cypress Island that occurred early in the growing season and caused the majority of that period's mortalities. Cypress Island mortality lengths averaged 27.9 mm during the growing period, and 48.8 mm during the fall-winter seasons. Chlorophyll *a* concentrations from Experiment 1 indicated similar wintertime concentrations between the sites (refer back to Figure 10). But for summer, the concentrations were significantly higher at Clam Bay.

There were also some differences in mortality length across different treatments. In Clam Bay, farm shallow and farm deep mussel mortalities were larger than those of the reference treatment. This is especially noteworthy because live Clam Bay mussels in Experiment 2 actually grew significantly more in the reference treatment than in the other treatments (refer back to Figure 19). This may suggest that the farm shallow and farm deep treatments were dying more frequently at large sizes than were the reference mussels. Some of the larger mussels were getting ready to spawn, which acts as a stressor on the organism. In addition, the mussel species used in this experiment, *Mytilus galloprovincialis*, probably does not perform as well in the cooler waters of central and northern Puget Sound as in southern Puget Sound. The combination of spawning stress and temperature stress from cold waters may have led to the larger, faster-growing individuals dying before the end of the experiment in March 2011.

In contrast, Cypress Island mussels experienced little or no significant differences in mortality lengths across the four different treatments, as well as less overall mortality than Clam Bay. Indeed, Experiment 2 average mortality counts at Clam Bay were 54% higher than at Cypress Island. One possible explanation why Cypress Island mussels had lower total mortalities is the temperature difference between Cypress Island and Clam Bay, discussed earlier in this report.

Cypress Island is located in northern Puget Sound while Clam Bay is situated more centrally; consequently, Cypress Island experiences even cooler average water temperatures than Clam Bay. In 2010, average water temperatures were significantly lower at Cypress Island than at Clam Bay, averaging 9.7 °C and 10.6°C, respectively. Although *M. galloprovincialis* doesn't do well at lower temperatures, the cold water of Cypress Island may have delayed sexual maturation of the Cypress Island mussels, reducing the likelihood of spawning stress, as well as any potential additive stress from spawning and cold temperatures.

SUMMARY: SIZE AND TIMING OF MUSSEL MORTALITIES

As expected, mortality lengths were consistently lower than, or statistically similar to, live lengths, across all treatments, collection dates and sites (Figure 25). In no cases were mortality lengths significantly larger than those of live mussels. Figure 25 also displays the general trend of both live and mortalities increasing in size from the growing period to the fall-winter period. This is due to continued mussel growth throughout the experiment.

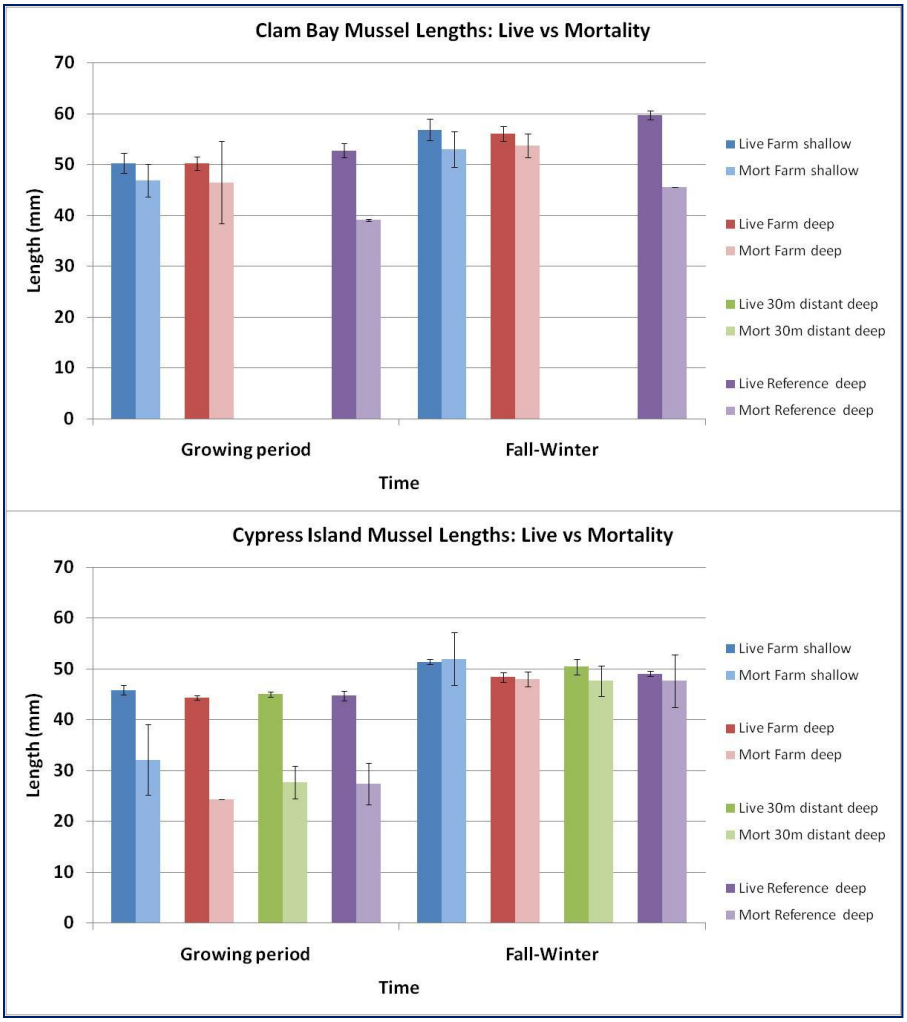

Figure 25. Comparison of live mussel lengths and mortality average lengths and SD.

STABLE ISOTOPE ANALYSES

STABLE ISOTOPE PRIMER

Here we provide a brief overview of the methodology, which appears on the surface complex, but is conceptually simple. However, the applications can be complex because of multiple food sources for tested animals, as explained below. This primer is designed to give reviewers the key concepts, and we borrow freely, but in our own words and images, from Brian Fry's (2006) volume "Stable Isotope Ecology", written by a world's leader in the field. The volume could be renamed "Stable Isotope Ecology for Idiots" as it clearly sets out the principals and problems in a very readable and humorous fashion but you need not be an idiot to read it. The following is a synthesis of that material, amended for the present context:

Elements exist in stable and some in unstable (radioactive) forms. Most elements of biological interest (including carbon, hydrogen, oxygen, nitrogen and sulfur) have two or more stable isotopes. Among stable isotopes, the most useful as biological tracers are the heavy isotopes of carbon and nitrogen, the focus of this work. C and N are found in the earth, the atmosphere, and all living things (carbon as the carbon skeleton of organic matter and nitrogen as protein for example). Each has a heavy isotope (^{13}C and ^{15}N) with a natural abundance of only 1% or less and a light isotope (^{12}C and ^{14}N) that makes up all of the remainder. Do not confuse these with radioactive isotopes such as carbon 14 (typically indicated as ^{14}C) used in dating artifacts and materials or for spiking (labeling) of primary productivity algal experiments as a means to measure rates of photosynthesis, for example. Stable isotopes are perfectly harmless!

Biologists often use carbon and nitrogen isotope tracing to estimate what organisms consume and where they fit in the food chain or food web. Isotopes of the same element take part in the same chemical reactions, but the lighter isotope acts just a wee bit faster or slower. This is the key to understanding the process, known as "fractionation". Physical processes such as evaporation discriminate against heavy isotopes; and enzymatic discrimination and differences in kinetic characteristics and equilibria can result in reaction products that are isotopically heavier or lighter than their precursor materials. When assimilating C and N from their food, consumer organisms preferentially respire the light C isotope (^{12}C) and preferentially excrete the light N isotope (^{14}N). As a result, consumers are usually enriched with heavier isotopes in relation to their food. This is the basis of the methodology.

An example of how this process occurs is shown diagrammatically in the cartoon Figure 26.

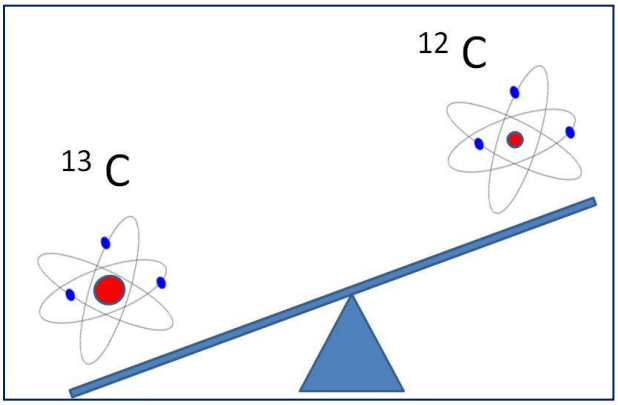

Figure 26. Cartoon of heavy ^{13}Carbon with one more proton than light ^{12}Carbon isotope, with red circle representing neutron mass of each and the teeter totter illustrating the relative weights (after Fry 2006, but drawn from scratch).

So what does this mean in practical terms? It means that the difference between the light and heavy isotope values can be used to measure and sometimes trace the flow of C and N from a source to the environment, in the sediments, and in food webs to a plant producer or animal consumer.

If there are multiple sources of nutrient or food, and we have enough information, we can create a "mixing model" to estimate the contribution of elements C and N to an organism's body from a source as discussed below. But often this is very difficult or impossible without extensive research or ends up being too complex to be of practical use. Often the best use of stable isotope methodology is lowest in the food web. Higher up there are complications as higher trophic level organisms feed on a variety of sources, from multiple trophic levels. Stable isotope studies are not cookbook science, every situation is unique and must be studied on its own and sometimes the results simply do not make sense for a variety of known or unknown reasons.

Isotope ratios are reported in "delta" (δ) notation that is defined as the "per mil (parts per thousand or 1/10 of a percent, the same units used in tax levies) deviation from the recognized isotope standard, atmospheric N_2 for $^{15}N/^{14}N$ and Peedee Belemnite (a limestone found in S. Carolina) for $^{13}C/^{12}C$ ratios. Herein we use the common notation for these units of "‰". These are internationally accepted standards that all laboratories use. So if there is no enrichment of heavy marine derived N (known as "MDN") for example, the δ values are low (for nitrogen). Values for C are often negative, only because it is measured relative to the standard, which can be confusing at first. Nitrogen isotopes are often more reliable indicators or predictors of the trophic level that an organism occupies in the food web because of the large ^{15}N enrichment from one trophic level to another (Owens 1987, Peterson and Fry 1987). But sometimes C isotope measurements are useful too, as in fish farms where most of the particulate waste has C but very little N (that is dissolved instead). We pay for both of them in the analysis and let the chips fall where they may! A helpful guideline is:

- Do not get hung up on the absolute values of these measurements, as you might be when observing basic water quality results. We are more concerned with the observed sample differences among treatment locations, with differences between treatment and reference sites, and with fluctuations across different time periods.

It is important to emphasize that $\delta^{15}N$ and $\delta^{13}C$ isotopes do not pass through the food web intact, except at the beginning in plant primary production (photosynthesis). As atmospheric N is fixed in primary production, there is no fractionation in that process, hence the $\delta^{15}N$ values begin low and increase as nitrogen passes up through progressively higher trophic levels.

Each time the element is digested and egested by a higher consumer, it is fractionated again; and by measuring the ratio of the either set of isotopes, i.e., "normal" or light isotopes of C and N, biologists may be able to detect who ate what. In the present case, we start with measured ratios of both carbon and nitrogen, heavy to light isotope ratios in several potential fish food sources, including fish feed, fish feces, phytoplankton, seston, etc. Some of these data come from measurements we took in the field; others are collected from the literature. For example, the feed, which is heavily influenced from fish meal and fish oil and other products that sometimes creates a distinctive ratio (or not, it depends on local conditions), can sometimes be distinguished from other food sources. Most fish waste N is excreted as ammonium and a little urea and about ½ of consumed C respired as carbon dioxide, and roughly equal amounts are retained as fish tissue or egested as feces. The C and N compounds and molecules released are then subject to uptake by primary producers (in the case of dissolved C and N) or consumers, who are further up the food web. The fractionation occurs again so if the tracing system is working properly we expect an enrichment or positive change in $\delta^{15}N$ and $\delta^{13}C$ each time the element goes through a trophic level of the food web, so:

- The higher the δ value, the greater the amount of heavy isotope. The lower the δ value, the lower the amount of heavy isotope, or as Fry (2006) says "higher heavier, lower lighter."

For carbon, the result values are reported as negative, and the less negative ones represent more marine origin isotope, i.e., they are enriched. The negative delta-C values result from the way delta notation is calculated ((Rsample/Rstandard -1)*1000, where R is the ratio of ^{13}C to ^{12}C). It just means that almost all samples have less ^{13}C than the PeeDee belemnite standard which is an unfortunate condition as the results are usually negative, but the same idea applies as with N, higher values indicate enrichment, so another quasi-rule:

- If there is a single food source flowing one step up a food web, except from macronutrient to plant, one may anticipate a fractionation result difference of ~ +3.5 $^{0/}_{00}$ (range 3 to 4) for nitrogen and +0.5 $^{0/}_{00}$ (range 0.1 to 1.0) for carbon stable isotopes (DeNiro and Epstein 1978, DeNiro and Epstein 1981, Peterson and Fry 1987). Results rarely match these stated means or ranges, so +/- 1$^{0/}_{00}$ in both cases is considered reasonable and some species, such as marine mussels, have less whole-body nitrogen fractionation, in the range of 1.2 to 2.5 $^{0/}_{00}$ (Hill et al. 2008).

For example, an increased δ^{15}N of 3.5 $^{0/}_{00}$ for nitrogen , e.g., from -18 to -14.5 δ^{15}N would constitute a single food web trophic level enrichment, as if a fish ate an insect larvae that was in turn feeding on periphyton or biofouling algae. Atmospheric N is isotopically lighter than plant tissues, and soil δ^{15}N values tend to be higher still, suggesting that microbes discriminate against the light isotope during decomposition. Non-nitrogen-fixing plants, which derive their entire N from the soil N pool, can thus be expected to be isotopically heavier than nitrogen-fixing plants, which derive some of their N directly from the atmosphere including the blue-green algae (cyanobacterial) group.

Biologists are able to determine if one or two sources of feed are involved in a food web study by preparing "dual isotope plots" which are little more than a scatter diagram with δ^{15}N on one axis and δ^{13}C on the other axis. Vertical and horizontal differences in distance in these plots can represent trophic level jumps, if the sources are initially different in profile. If not, sorry, the system doesn't work so well. If there are three or more sources, then complex mixing models are required, as are measurements for all the food sources likely contributing to the target organism that is the study focus. In this manner we could distinguish 5 sources if we had 4 actual sources characterized by baseline measures, with only one unknown remaining. These models are not, however, simple but there are published models and freely available spreadsheet models to accomplish this task. See EPA website for details.[1]

IsoSource, one such model, is a Microsoft Visual Basic software package which calculates ranges of stable isotope source proportional contributions to a mixture. We can use IsoSource to generate mixing models from our data. The program generates every possible combination of source proportions, compares these with the observed mixture isotopic signatures, and reports all combinations that successfully sum to the mixture signatures. These results are then presented as a histogram of the distribution of all possible combinations of source materials." These histograms

[1] http://www.epa.gov/wed/pages/models/stableIsotopes/isosource/isosource.htm)

can provide quantitative information that gives us an idea of the relative proportions of each food source ingested by the study shellfish.

If you are confused by the above, this primer has not served its purpose, but a quick search on line will yield a PDF copy of the Fry (2006) volume which really is very readable. Reading individual scientific papers will usually only confuse you more, unless you read Fry's (2006) volume or a similar volume to grasp the basics. So now we present the stable isotope results of the project, beginning with hypotheses.

HYPOTHESES AND APPROACH

This work was designed to look for spatial differences in stable isotope enrichment as a measure of how different food sources, potentially including fish farm feed and wastes, are incorporated into the shellfish in our experimental treatments. The primary null hypothesis is that there is no enrichment effect of the fish farm on the shellfish. This is determined simply by comparing results from our experimental treatments (grown close to the farm) to our reference group (grown far away from the farm). If we see significant statistical trends by regression analysis or other parametric analyses, we reject the null hypothesis.

Secondly, we examine the amount of variation between source of N and C from the pens compared to content of other food sources such as phytoplankton and seston. This gives us the possibility of preparing a "mixing model" using IsoSource that will guide us on quantifying the relative amount of the food web components that originate from the fish farm.

STABLE ISOTOPE ANALYSES

Initially, we theorized that $\delta^{13}C$ would be the best tracer in shellfish tissue near the fish farm, since fish feces are rich in carbon but relatively poor in nitrogen as most is excreted as dissolved nitrogen. Shellfish therefore would consume relatively less salmon waste nitrogen, so we would not expect as much $\delta^{15}N$ enrichment or in this case, depletion, from consuming them.

Turning to real data from this study, in Figure 27 we see that replicate samples of fish feces collected from fish at Clam Bay had a $\delta^{13}C$ content of -20.8‰. Fish feed was even more negative and seston, which includes phytoplankton, was found to have a $\delta^{13}C$ content of about -21.3‰ at Clam Bay and about -23.1‰ at Cypress Island, based on monthly samples in 2009. Puget Sound phytoplankton have a $\delta^{13}C$ content of approximately -20.3‰ (Carpenter and Peterson 1989). A figure illustrating the varying ranges of stable isotope signatures in different food sources can be seen in Figure 27. Appendix 6 contains all averaged replicate stable isotope data.

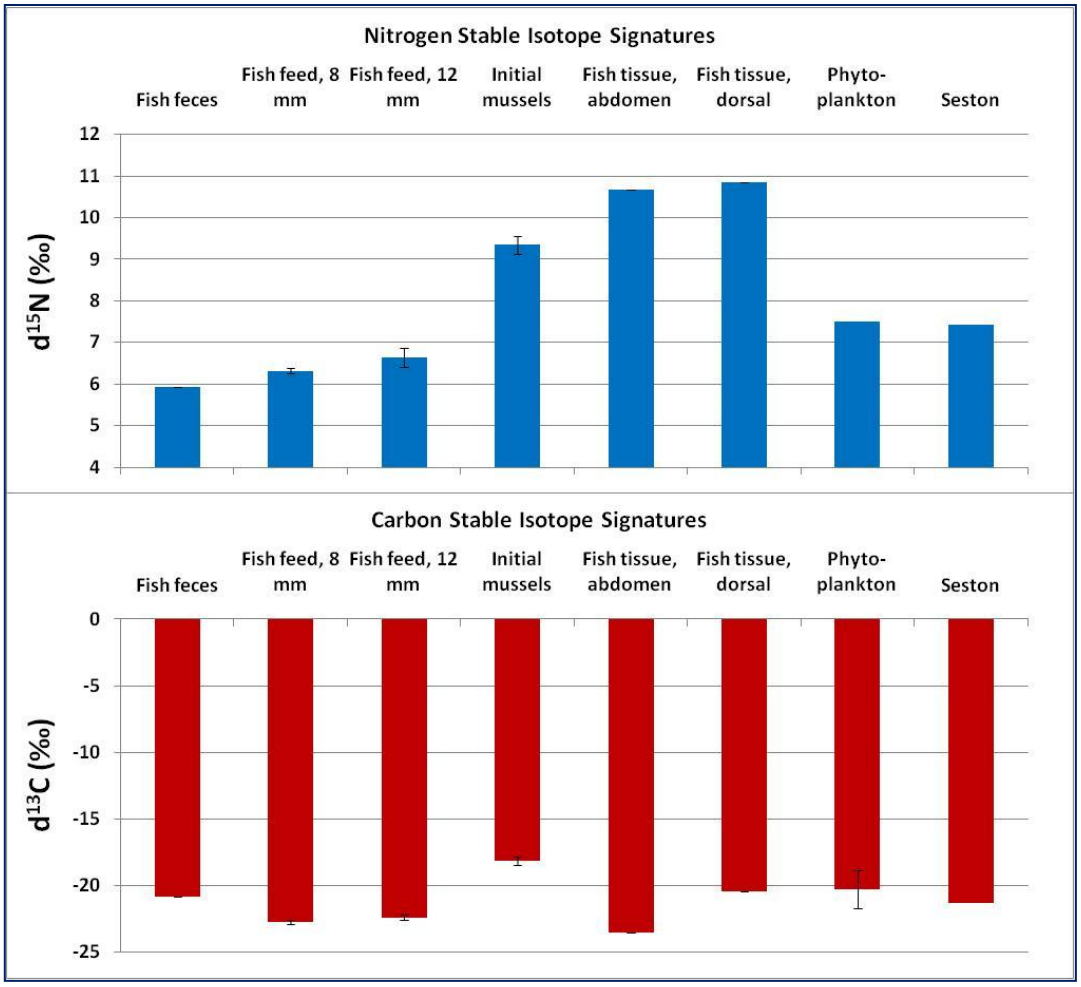

Figure 27. Nitrogen and Carbon stable isotope signatures of various food sources, wastes and initially stocked mussels for Experiments 1 and 2 combined. Seston pictured here is Clam Bay seston.

Since the initial stock of shellfish were measured to have a $\delta^{13}C$ content of about -18.1‰, consuming a $\delta^{13}C$ diet of -21‰ will result in a shift to -20.5‰ due to the approximate fractionation shift of 0.5‰, as explained above. All of the known or suspected sources of feed for our shellfish would have resulted in a depletion of their $\delta^{13}C$ content when assimilated into the tissues of the shellfish. As will be demonstrated below, we are seeing significant differences between mussels and oysters in their $\delta^{13}C$ content and for each type separately within seasons.

Likewise, we see similar trophic shifts in nitrogen; however, the nitrogen enrichment jump between trophic levels is often much greater than that of carbon. While carbon shifts are generally around 0.5‰, a wide range of different nitrogen jumps have been reported in the

literature. For our purposes, we assume a jump of 1.7‰ $\delta^{15}N$, which is a value determined and used in other stable isotope studies involving marine mollusks (Hill et al. 2008).

CLAM BAY

The set of four plots (Figure 28) below represent mean and SD of stable isotope $\delta^{15}N$ and $\delta^{13}C$ values for Clam Bay oysters from September to the following January or June 2009. These figures show consistent enrichment of $\delta^{13}C$ for all treatments compared to the initial measurement, and a continual $\delta^{13}C$ enrichment as the experiment progressed. This only reflects the fact that $\delta^{13}C$ was most likely lower for oysters' diet at the hatchery location than at Clam Bay, and is not directly relevant to our experiment. Similar to $\delta^{13}C$, there was enrichment of $\delta^{15}N$ in the fall and early winter of Experiment 1. But, by the experiment's end in June 2009, $\delta^{15}N$ levels in all of the treatments had dropped back down to initial levels or lower. Again, there is a possibility that this is biologically significant, but likely not.

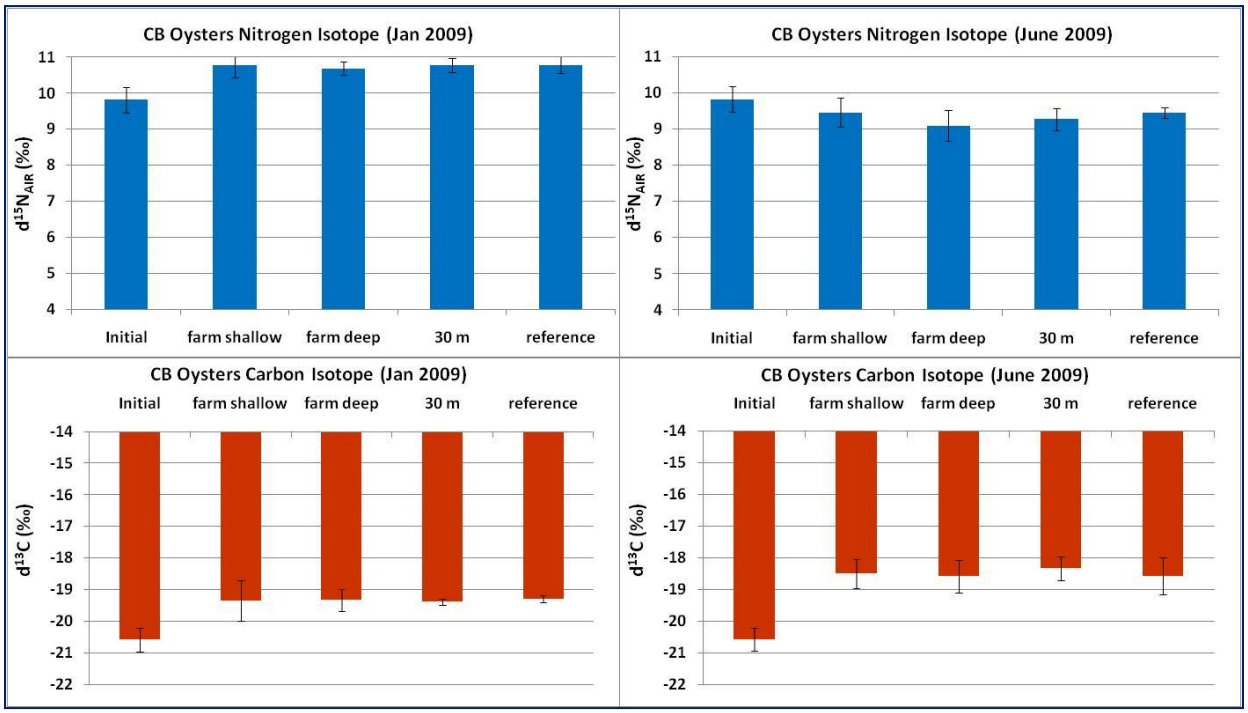

Figure 28. Clam Bay (CB) oyster carbon and nitrogen stable isotope results for January (mid experiment) and June (experiment end) 2009 in Experiment 1, compared to initial values.

Similar to oysters, fall period $\delta^{15}N$ enrichment of mussels was measured but by the end of the experiment in June, $\delta^{15}N$ levels declined (Figure 29). In these mussels, $\delta^{15}N$ levels declined to concentrations lower than initial values, suggesting an effect of feeding on the depleted $\delta^{15}N$ sources discussed in the introduction to this section. The fact that both oysters and mussels are responding similarly among seasons is evidence of a dietary shift that is at least in part, natural, not net pen waste driven.

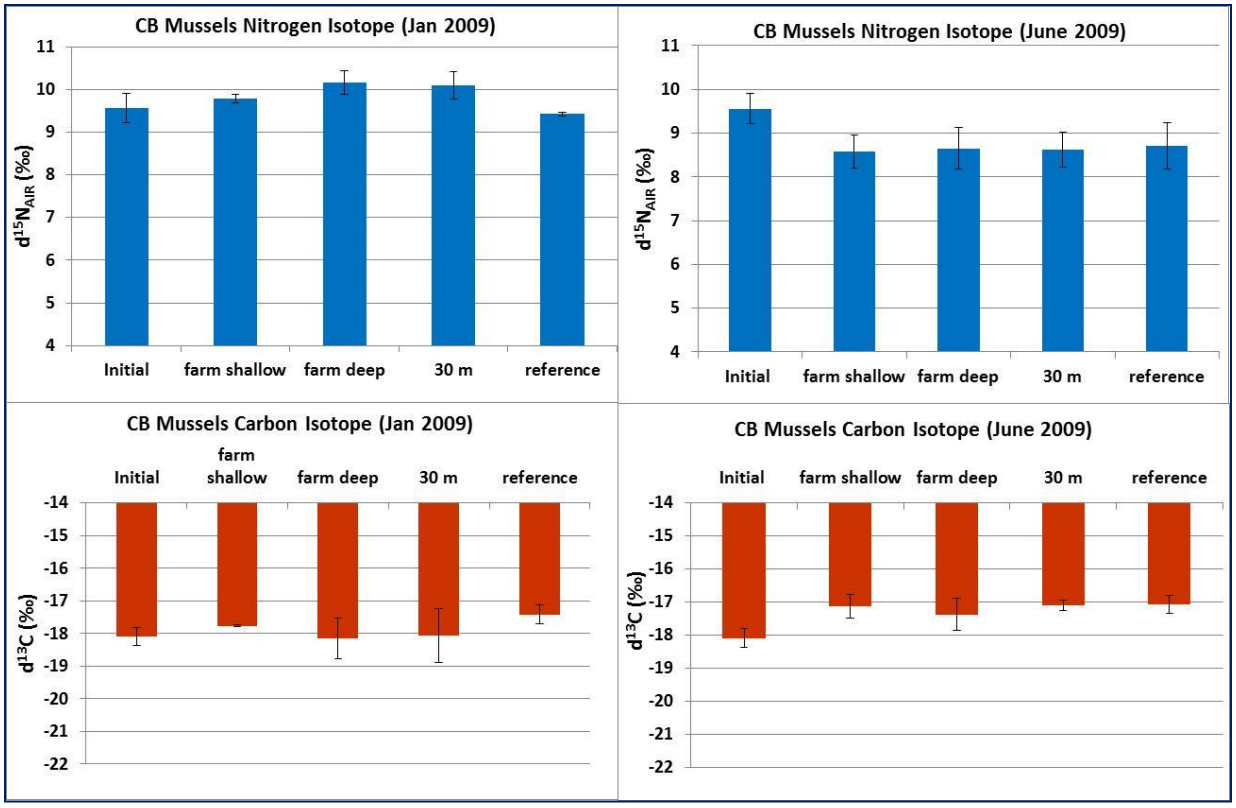

Figure 29. Clam Bay (CB) mussel C and N stable isotope Experiment 1 results, January and June 2009, compared to initial values.

Clam Bay mussel results for Experiment 2 are presented in Figure 30. During the growing period (April-September 2010, top left of Figure 30), nitrogen was depleted relative to initial values; it subsequently went back to initial values when measured in March 2011 at the conclusion of the experiment (top right of Figure 30). A much greater carbon enrichment was seen in the first half of Experiment 2; the second half saw $\delta^{13}C$ falling back towards initial values.

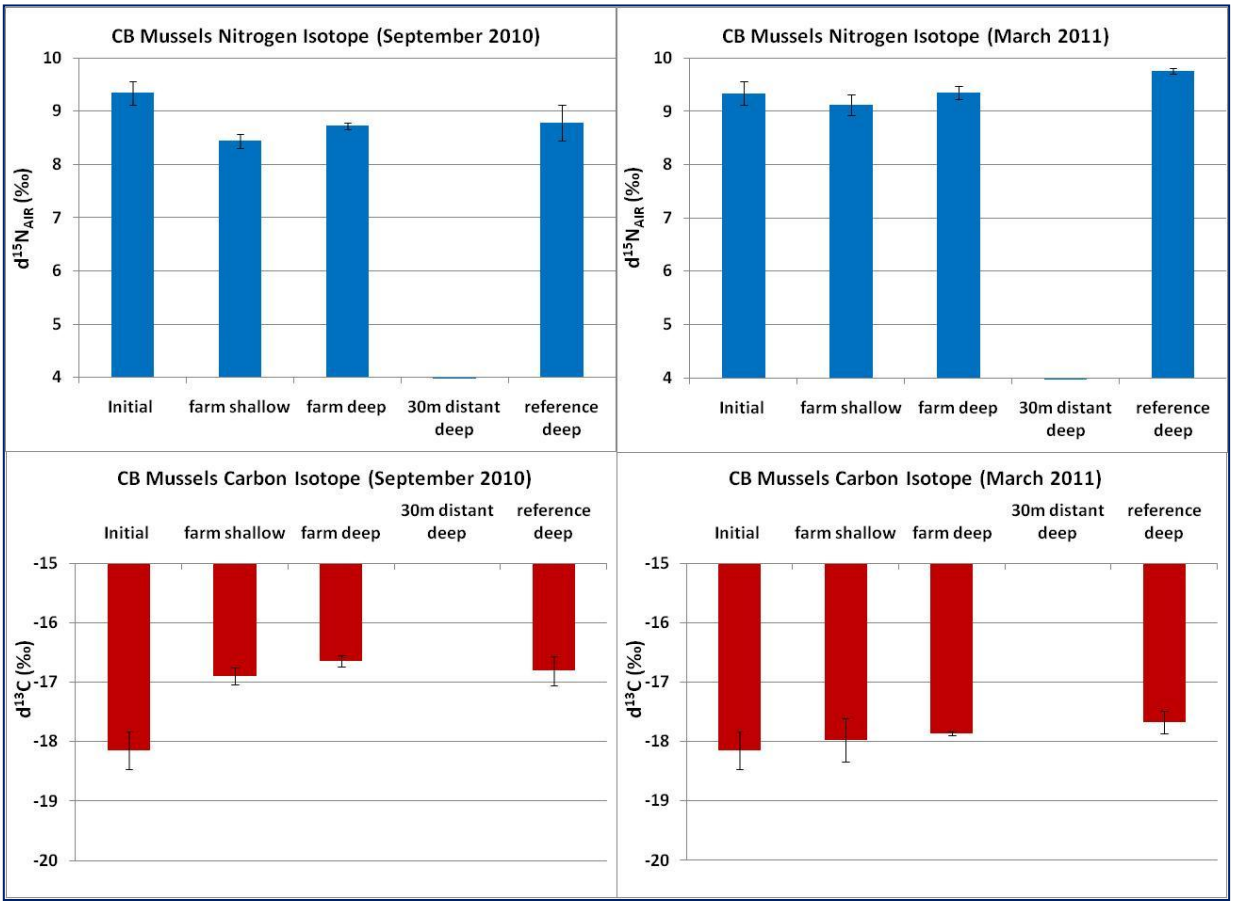

Figure 30. Clam Bay (CB) mussel C and N stable isotope Experiment 2 results, September 2010 and March 2011, compared to initial values.

CYPRESS ISLAND

At Cypress Island in Experiment 1 during January 2009, a significant increase in $\delta^{13}C$ enrichment was recorded for oysters cultivated near the farm (30m distant station) versus the reference and initial conditions the prior September (Figure 31 left side). Not only was this statistically significant but it meets the test of a different food source for carbon enrichment discussed above, i.e., an increase of about 0.5 $\delta^{13}C$. For the latter period of January through June, however, the differences had disappeared (Fig. 31 right side). This was due to the reference oysters attaining the same level of enrichment as the near farm oysters for that time period. Since growth of all shellfish in these areas in January through March was minimal, as previously discussed, it may have been a shift to reliance on fish feces at the near farm location, as is evident in our mixing model results discussed below. These results are especially important because Cypress Island oysters at the 30m distant deep treatment also had significantly stronger growth than at the reference site. On the other hand, oysters experienced progressively lower nitrogen enrichment levels as the experiment continued. Again, this is likely explained by the presence of food sources at the oysters' hatchery

sites that were much more enriched in nitrogen than food sources available at Cypress Island. Further investigation of Cypress Island oysters with IsoSource is discussed later in this report.

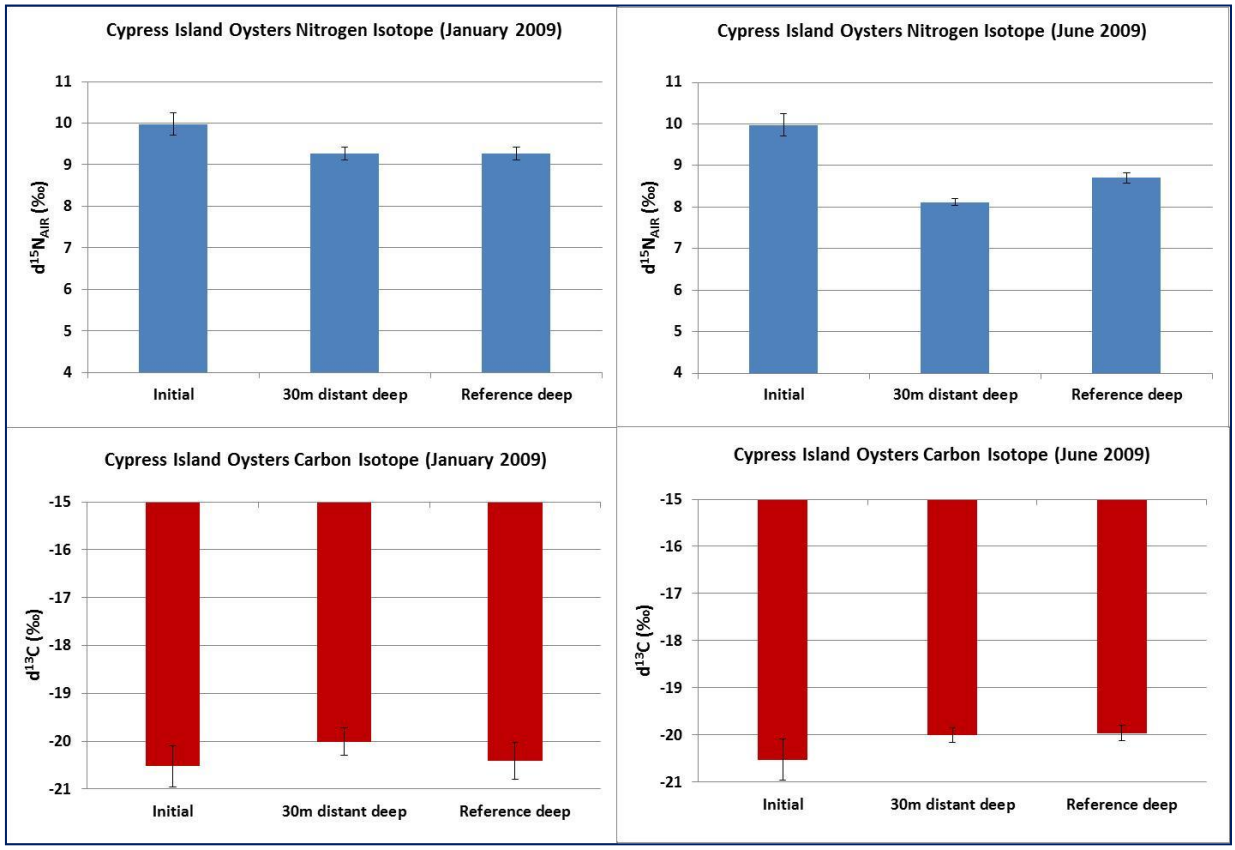

Figure 31. Cypress Island oyster stable isotope results for January (mid experiment) and June (experiment end) 2009 in Experiment 1.

Unlike oysters, mussels in Experiment 1 experienced no differences between treatment and reference $\delta^{13}C$ concentrations; therefore, no spatial effect of stable isotope enrichment from the fish farm was likely, unless the effects were equally present throughout the sampled area (Figure 32). Similarly, in Experiment 2, there were no instances in which treatment groups were significantly more enriched than the reference groups with respect to $\delta^{13}C$ and $\delta^{15}N$ (Figure 33).

Notably, Cypress Island mussels experienced the opposite from Clam Bay: all experimental $\delta^{13}C$ concentrations were less enriched than the initial measurements. This suggests the possibility of Cypress Island mussels sequestering significant amounts of farm wastes; which is supported by the IsoSource analysis presented later in this report.

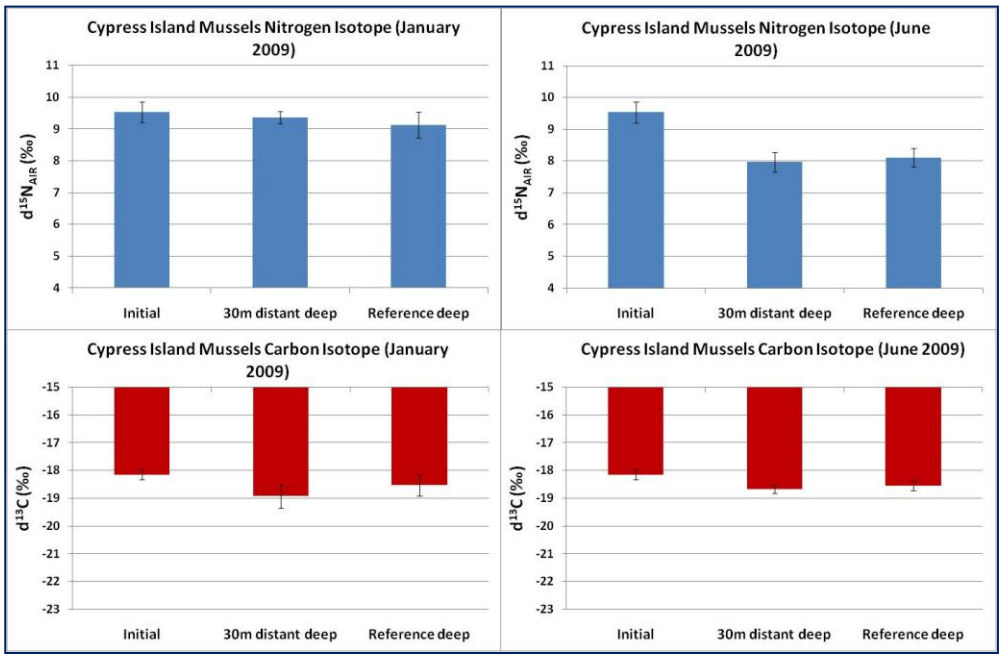

Figure 32. Cypress Island mussel C and N stable isotope Experiment 1 results, January and June 2009, compared to initial values.

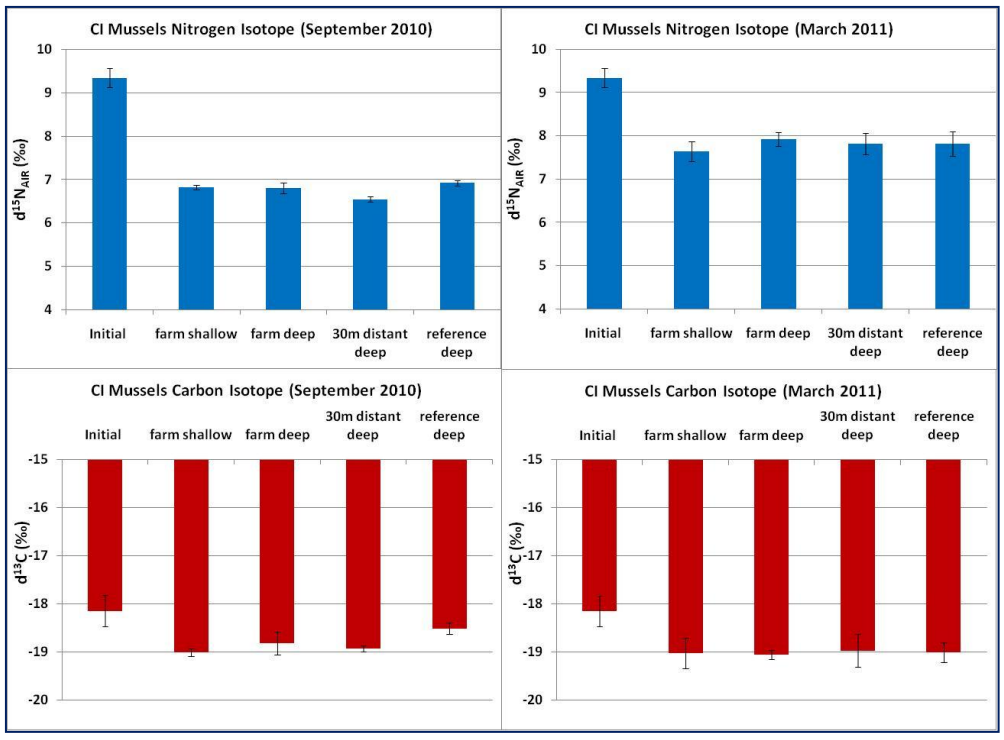

Figure 33. Cypress Island (CI) mussel C and N stable isotope Experiment 2 results, September 2010 and March 2011, compared to initial values.

Overall, seasonal differences had much more of an effect on stable isotope enrichment by separate ^{13}C or ^{15}N isotope than did treatment differences. With hindsight, even our reference groups were probably much too close to the net pens. It is very possible that due to the very high area currents, small dissolved particles and other material coming from the fish farms may be carried a long distance before settling. The reference treatment may have received the same or a similar amount of material as other treatments closer to the farms. However, our hypothesis may remain valid. Diffusion and horizontal mixing studies in Puget Sound and the Gulf of Maine indicate that a net pen "plume" can be initially quite narrow, so the 30m and reference locations should have been in and out of the particulate supply of the net pens, depending on current conditions.

In addition, seasonal differences are likely due to shifts in species composition of the phytoplankton (e.g., spring phytoplankton blooms of diatoms versus summer and fall flagellates). This was not an original focus of our study, and there is a surprising lack of literature that reports isotopic values for different phytoplankton species.

Even if we do not factor in seasonal changes, we often saw enriched $\delta^{13}C$ and depleted $\delta^{15}N$ compared to initial values (with the exception of Cypress Island mussels, which depleted $\delta^{13}C$ and depleted $\delta^{15}N$). It is most likely that $\delta^{13}C$ was lower in the shellfish's diets at the original hatchery locations, and that $\delta^{15}N$ was higher.

MIXING MODEL ANALYSES

The Visual Basic software package IsoSource was used for further analysis in an attempt to determine the percentages of different food sources that the study shellfish were ingesting during our experiments. By inputting known or estimated stable isotope signatures for a variety of food sources, IsoSource can generate all possible combinations of source proportions and compare these to the isotopic values of our sampled shellfish tissue, and then generate histograms of the probability distribution, as well as the mean percentages of each food source. Food source isotopic values, as well as shellfish isotopic values used in our IsoSource analysis, are found in Table 3. Trophic level jumps were taken into account by decreasing our shellfish carbon values by 0.5 and nitrogen by 1.7, following accepted isotopic trophic jumps (Fry 1988, Raikow and Hamilton 2001, Moore and Suthers 2005, Hill et al. 2008).

Food Source	Carbon	Nitrogen
Fish Feed	-22.3	6.5
Seston	-23.1	6.2
Phytoplankton	-20.3	7.5
Fish Feces	-20.8	5.9
Shellfish	**Carbon**	**Nitrogen**
30m Jan	-20.5	7.6
30m June	-20.5	6.4
Ref Jan	-20.9	7.6
Ref June	-20.5	7.0

Table 3. Food source isotopic values and shellfish isotopic values used in IsoSource. Shellfish values have been corrected for marine mussel only trophic level jumps (0.5 Carbon and 1.7 Nitrogen were subtracted from their original values).

IsoSource was very effective at analyzing Cypress Island oyster data, and was able to generate food source results for all four Cypress Island oyster conditions (30m January, 30m June, Reference January, Reference June, Figure 34). IsoSource was unable to process data for Clam Bay oysters, as well as all mussels in Experiments 1 and 2. This was likely because a) food source isotopic values – especially those of phytoplankton and seston – may vary dramatically at different times during the year and over shorter time periods than our sampling; b) our study species may selectively process carbon and nitrogen at different rates than the 0.5 C and 1.7 N as reported in the literature; and c) mussels in particular have very different feeding habits than oysters, as discussed later, which also may confound our results. Nonetheless, our Cypress Island oyster data yields significant information and contributes valuable information to this project.

The probability distributions shown in Figure 34 show clearly that Cypress Island oysters at both the 30m site and the reference site relied heavily on phytoplankton during the fall and early winter, as depicted by phytoplankton (in green) with a much higher average source proportion than the other three food sources. Mean percentages of each food source consumed were derived from the probability distribution data and are shown in Table 4. Mean values are less accurate and possibly misleading compared to the frequency distributions, unless the latter are normally distributed and not multimodal. An important difference is apparent during the late winter and spring as follows in both the mean values and the distributions as follows. While the reference oysters are still predominantly feeding on phytoplankton, the oysters from nearest the farm (30m distant) were more reliant on fish feces (in Figure 34, purple). This difference tells us several things.

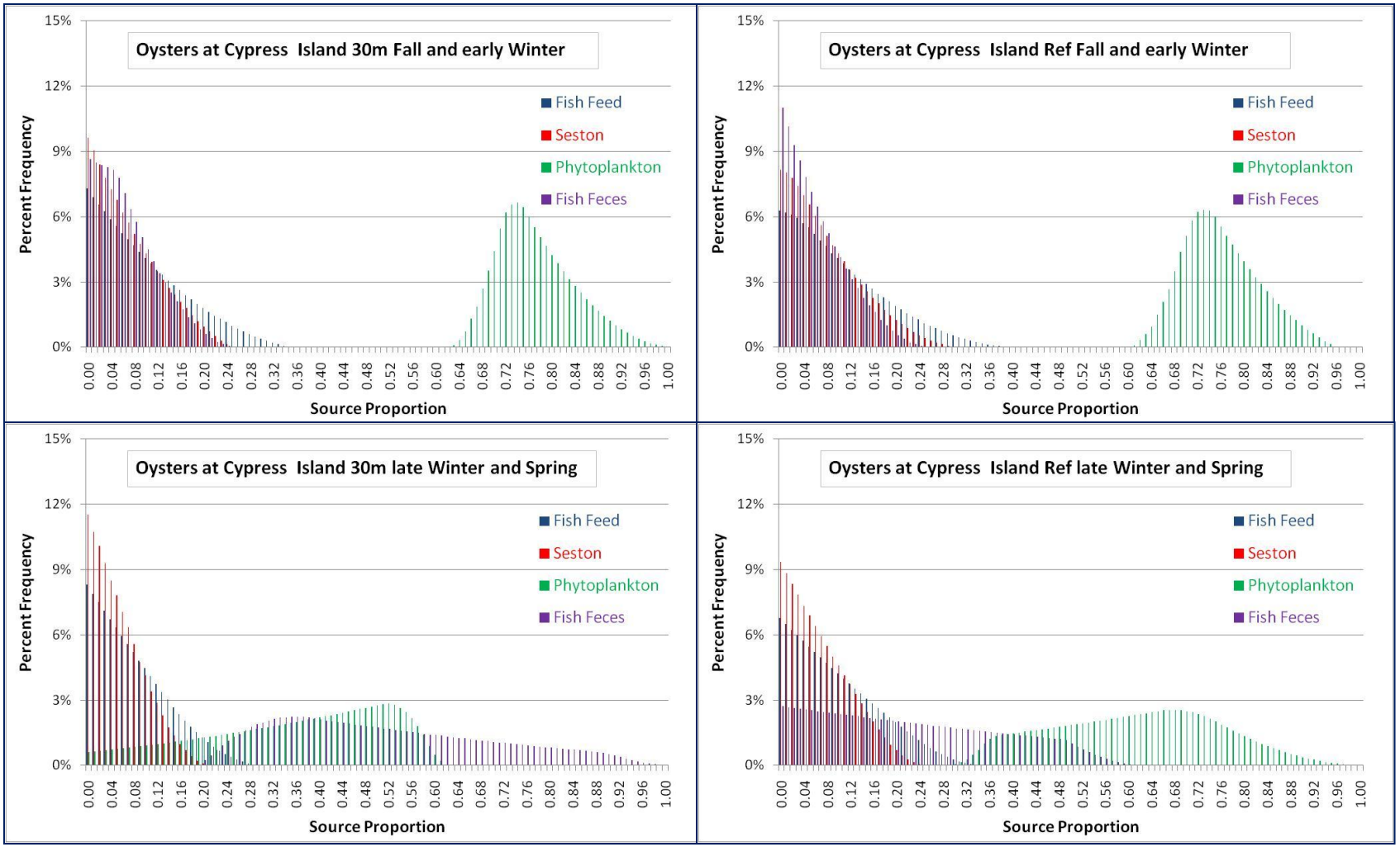

Figure 34. IsoSource mixing model probability distribution histograms for Cypress Island oysters.

Table 4. Mean percentages of each food source consumed by Cypress Island oysters, derived from IsoSource mixing model data for both treatments and sampling time period, January = Fall and early winter, June = Winter and Spring.

Treatment	Fish Feed	Seston	Phyto-plankton	Fish Feces
30m Jan	9%	7%	77%	7%
30m June	8%	5%	36%	51%
Ref Jan	10%	8%	76%	6%
Ref June	10%	7%	61%	23%

First, the 30m distant oysters were apparently receiving benefits from the net pens in the form of fish feces being assimilated into their tissue. The reference oysters were feeding on a small amount of fish feces as well, which supports to some extent our earlier speculation that the reference site was too close to the net pens, and that high currents were carrying some fish fecal material out to the reference location. However, it is probable that that oysters at the 30m distant site, being much closer to the pens, were able to take advantage of the increased organic particulates from the pen pens. This directly relates to our growth results, in which we found that Cypress Island 30m distant oysters grew significantly greater than the reference oysters. In addition, the seasonal growth differences are explained by the fact that non-phytoplankton organics were much more available in the first half of the experiment at Cypress Island (Figure 13) than at Clam Bay. This stems from the much higher winter concentrations of total volatile solids at Cypress Island than at Clam Bay (Figure 11). Despite this advantage, net growth of oysters at Clam Bay nominally exceeded that at Cypress Island (compare Figures 15 and 16), suggesting that non-phytoplankton organics are less useful than phytoplankton to support growth.

The stable isotope signatures may also be viewed in a dual isotope plot (Figure 35) with carbon isotopic values on the x-axis, and nitrogen isotopic values on the y-axis. This allows for a visual observation of food pathways from one trophic level to the next and qualitative evaluation of slopes between possible linked components. In Figure 35, we can see how Cypress Island oysters were primarily reliant on phytoplankton and fish feces. Considering that stable isotope content is generally enriched by ~0.5‰ carbon and ~1.7‰ nitrogen per trophic level jump, we would expect to find the major food sources of our oysters at approximately 0.5 C and 1.7 N less than the oysters. Figure 35 shows that it is only phytoplankton and fish feces that fall into this approximate range. The other potential food sources shown in Figure 35, i.e., fish feed and seston, have $\delta^{13}C$ contents that are much less enriched, and therefore not likely food sources for the oysters. This also directly correlates with our IsoSource mixing model results seen in Figure 34 and explained earlier.

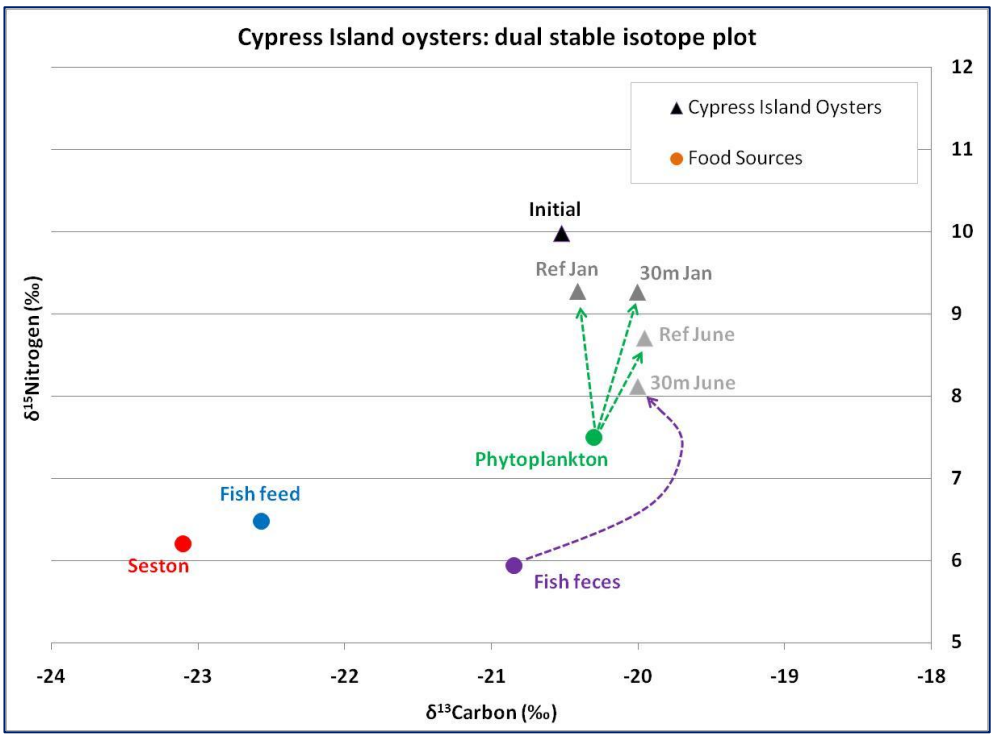

Figure 35. Dual isotope plot of Cypress Island oysters and their food sources. Fish feces data has a standard deviation of ±0.12 Nitrogen and ±0.3 Carbon; phytoplankton standard deviation is unknown.

It is important to note that the lack of valid mixing model results for Clam Bay oysters and for all of our mussel treatments does not necessarily indicate a lack of fish farm effects. The mixing model analyzes two different isotopes simultaneously and is far more complicated than the results of either isotope taken individually. Indeed, when examining the data, it was sometimes clear that IsoSource was not able to yield valid results because trophic jumps were too high for either N or C isotope, but not the other. This suggests that the possibility that either our shellfish were selectively incorporating food enriched with one element much more than the other, or that one or more of our assumptions was inaccurate.

Different species of shellfish may selectively incorporate stable isotopes at different rates, and at different times of year. We used our limited food source stable isotope data, as well as stable isotope literature that is variable. Furthermore, Puget Sound is a highly complex body of water, and in order to get a truly accurate estimation of all food source stable isotope values, we would have to conduct frequent and detailed measurements throughout the course of the year, which was beyond the scope of our study and its modest budget. Stable isotope values for phytoplankton may have significant changes in spatial and temporal variation as species composition and abundance changes from season to season. Because of these reasons, it is quite

possible that proximity to fish net pens had some significant effect on our other shellfish groups, even though IsoSource was unable to identify results.

DECISION MATRIX

After review the data extensively, we decided to focus our decision about efficacy of fish-shellfish IMTA primarily on shellfish growth and stable isotope mixing model results. Other aspects of this experiment, such as survival and stable isotope analyses, were judged not as important because they showed few or no significant differences among treatments, but rather differences among sites. Differences between sites are indeed important to try to tease out forcing factors, but indicate that other factors – such as temperature, phytoplankton quality/quantity, etc. – were the primary drivers of any observed differences. As this project was modestly funded, we had no initial intention of attempting to measure all factors that would influence the above parameters. An example would be sampling for phytoplankton stable isotope composition and species composition on a weekly basis. This would be required to adequately describe average and variable conditions that fluctuate greatly no doubt. Instead, we opted to use literature values for phytoplankton but did collect extensive seston data.

Table 5 shows a matrix used to differentiate between significant and non-significant results of the IMTA experiment. For clarity, and to distinguish our stable isotope analysis from our stable isotope mixing model results, the single stable isotope analysis was not included in the matrix, i.e., we did not include separate analysis of ^{13}C and ^{15}N results. We conducted this analysis but found that the results would sometimes conflict with the dual isotope mixing model analysis, which we believe to be more powerful. However, a case could be made that proportionately much more carbon was flowing from the pens in particulate form than nitrogen, as waste feces and feed is rich in C but most N is excreted as dissolved form and therefore theoretically not as available to the adjacent shellfish. This remains a research topic to be explored.

Shellfish survival, while also not very significant compared to our mixing models and growth results, was included as a lower ranking factor in our final decision as to efficacy of the IMTA in Puget Sound. In Table 5, cells marked with a + indicate the presence of significant differences between experimental groups within a site and time period and those marked with a - indicate no significant differences.

Table 5 clearly indicates that most significant IMTA results stem from oysters in Experiment 1. Statistically significant growth was observed for oysters for both locations in the fall to early winter period, and for winter to spring at Cypress Island. Even at Clam Bay in winter to spring growth was

nominally greater, but not quite statistically significant for the 30 meter distant and deep oysters that did well in other time periods and at Cypress Island.

Table 5. Decision matrix that differentiates between significant and non-significant results.

Experiment 1: 2008-2009								
Ranking Factor	Oysters				Mussels			
	Clam Bay		Cypress Island		Clam Bay		Cypress Island	
	Fall	Winter - Spring	Fall	Winter - Spring	Fall	Winter - Spring	Fall	Winter - Spring
1) Growth	+	-	+	+	-	-	-	-
2) Mixing Model	-	-	+	+	-	-	-	-
3) Survival	-	-	-	-	+	+	-	-
Cummulative	Maybe	No	Yes	Yes	No	No	No	No

Experiment 2: 2010-2011				
Ranking Factor	Mussels			
	Clam Bay		Cypress Island	
	Spr-Sum	Fall-Wint	Spr-Sum	Fall-Wint
1) Growth	-	-	-	-
2) Mixing Model	-	-	-	-
3) Survival	-	-	-	-
Cummulative	No	No	No	No

Other notable results presented in Table 5 involve growth of Clam Bay oysters in the fall of Experiment 1, and the survival of Clam Bay mussels in Experiment 1. However, unlike our Cypress Island oyster results, multiple aspects of our experiment supported neither of these results. Despite this, we can speculate about these outcomes as follows:

As explained previously in the growth results section above, Clam Bay oysters experienced a significant stepwise growth trend during the beginning of Experiment 1, with progressively greater growth the closer the oysters were to the fish farms. However, this difference between treatments disappeared as Experiment 1 continued, and by the experiment's end, there was no significant difference between any of the Clam Bay oyster treatments. We may still tentatively

conclude that the significant growth present in the first part of the experiment is due to the fish farm – but we cannot be sure, since other results did not correlate with the growth results in this case except for nominally greater growth at the 30 m distant and deep station (again).

Likewise, Clam Bay mussels during Experiment 1 experienced some significant survival differences among treatments. During the fall of Experiment 1, reference mussels had a lower average mortality count compared to mussels closer to the farm, but in the following spring, the opposite occurred and reference mussels had significantly higher mortality counts than the other treatment groups. Because these results do not correlate with growth results or stable isotope analysis, however, it is unlikely that this was related to the net pens, and is more likely due to some other localized factor. In addition, when we transform the mortality count data into percentage of total mortalities per day, the differences between treatments become much less pronounced.

Despite the lack of significance differences among treatments for mussels, the fact that oysters at Cypress Island – and perhaps at Clam Bay as well – did experience greater growth at areas closer to the fish pens is an indication that IMTA may confer significant benefits to oyster production and particulate waste load reduction.

FURTHER INTERPRETATION

A unique aspect of Experiment 1 was the concurrent culture of mussels and oysters and the finding that oysters successfully used fish farm wastes and grew better at one of the sites. Here we compare our results to prior efforts to illustrate differences and parallels when possible.

OYSTERS

We tentatively conclude that oyster culture is technically feasible at representative net pen sites in Puget Sound and that some proportion of the particulate waste produced by the fish farms is captured and utilized by these shellfish. Oysters grew statistically faster and had stable isotope profiles nearer the net pens that indicated direct use of fish feces at Cypress Island all year and for part of the year at Clam Bay in terms of increased growth. Pacific oysters are readily available as seed stock from local hatcheries and are hearty and well suited for raft culture near fish farms in Washington State that are presently in protected, but very physically active sites exhibiting little or no measurable adverse water column or benthic effects as discussed above.

We are not the first to examine oyster growth at Pacific Northwest fish farms. Jones and Iwawa (1991) grew oysters at a fish farm in Jervis Inlet, British Columbia that is a fjord-like inlet on the east side of the central Strait of Georgia. Their report is sometimes cited as evidence that IMTA is

a successful method. One publication stated, "*Jones and Iwama (1991) found that oysters grew three times the amount in shell height and growth rate when integrated with salmon farms than at reference sites. This increase in weight and growth of the co-cultured species is a positive side effect and holds obvious economic benefit for farmers"* (Source intentionally not cited).

The subject inlet, like Sechelt Inlet of B.C. that was once the center of a thriving net pen industry in the 1980s, is subject to seasonally intensive vertical stratification that can lead to micro flagellate and dinoflagellate blooms. Since that time, all but one of the fish farming operations has moved out of the area. Upon closer examination, the paper clearly shows that phytoplankton (measured as chlorophyll *a*) concentrations were most strongly linked to growth of the different treatments of oysters (increase in shell height) but curiously, monthly growth rate was correlated strongly to particulate organic matter associated with each treatment of reference. These conflicting results are not rationalized in the paper. We highlight these results because the site characteristics are so different from Puget Sound sites, as discussed below.

MUSSELS

The use of gallo mussels to capture wastes at these same fish farms was apparently ineffective for sequestering particulate wastes from the fish farms in the present study, both in terms of growth (shell length) and for stable isotope effect. An assumption explicit in this and some prior studies has been that accelerated growth of shellfish nearer to fish farms is demonstrative of IMTA efficacy. Typically, the species of choice has been mussels of the genus *Mytilus*. Food quality and quantity and water temperature are key factors controlling shellfish growth rate, but the former (food quality) is difficult to assess.

We were unable to find more than a few published or unpublished cases of fish/mussel IMTA resulting in accelerated growth near farms as others have reported (see review portion of Troell and Norberg 1998). In some cases, positive results have been reported but no data or only limited hydrographic information was provided on the study site, which is not helpful in terms of understanding why the result occurred. There is a common thread explaining this variation and it has to do with the background trophic status of the culture and/or reference areas in such studies.

Sara et al. (2009) reported accelerated growth of mussels near cages in the Mediterranean Sea on the south coast of Sicily. This area is clearly oligotrophic with low concentrations of nutrients. It has a deep mixed layer and nutricline and no large rivers nearby to supply major inputs of nitrogen or phosphorus. Background chlorophyll *a* concentrations were near low at ~ 1 µg/L during their

study that occurred over one year. In contrast, average chlorophyll *a* concentrations at Clam Bay and Cypress Island were approximately 2-3 times greater in the present study and dissolved inorganic nitrogen was always >10 µM due to natural, oceanic sources as explained in this report.

Similarly, Peharda et al. (2007) measured growth and condition index of Gallo mussels grown near fish cages in the eastern Adriatic Sea, an arm of the Mediterranean Sea of reduced salinity and reportedly increased productivity (compared to the relatively barren Mediterranean Sea in most regions) but they did not measure TVS, TSS or chlorophyll *a*. They found that mussels nearest the pens grew slower than those 60m distant and not much different from reference mussels 600m away. Condition index results were better nearest the pens during September through April but other times exhibited mixed results.

Intuitively, one would expect shellfish grown in oligotrophic waters with low phytoplankton and seston concentrations to be poor. In such areas, placing shellfish near fish farms that produce particulate and in some cases dissolved inorganic or organic nutrients that could embellish phytoplankton would be logical. But in moderate to fully eutrophic conditions, shellfish are not necessarily reliant on fish farm wastes, particularly during the plankton growing season, so we should not *a priori* expect that the shellfish will perform the service of fish farm waste removal in such cases.

FEEDING SELECTIVITY AND OTHER EXPLANATIONS

Several factors may account for the lack of growth or stable isotope effect of cultured mussels in our study besides background phytoplankton and seston quantities. Selective feeding behavior of mussels may have been an issue where the oysters probably differ. Literature involving shellfish feeding ecology and selectivity is abundant and inconclusive in many cases, illustrating few constants among studies, locations and species. We note, however, that naturally occurring oysters have evolved and are often cultured extensively to be epibenthic organisms. Mussels may occur on any surface or submerged littoral zone but tend to occur (in Puget Sound) off bottom, on rocks, floating objects, and other non-benthic locations where they often grow in great abundance as a contiguous population. While this may be due to predation and survival, oysters normally ingest suspended and resuspended living and decaying organic material whereas mussels, being located higher in the water column on rocks or other natural structures, would be less likely to evolve feeding strategies that focus on epibenthic detritus. There are many exceptions to this in other regions.

It is likely that mussel are not selective “feeders” in terms of sorting particles upon ingestion but at high seston or phytoplankton availability, selection can occur in the form of excess pseudofeces

production, either because the food source is not suitable or simply that there is too much food available (see citations in Ren et al. 2000). Our study was not designed to evaluate this aspect of the feeding ecology of shellfish or to provide an extensive review of the literature that would be a major study unto itself.

Arifin and Bendell-Young (2000) present an alternative view stating that feeding behavior (from other cited studies) in many marine bivalves suggests that they possess a highly selective feeding strategy that allows for selection of organic over inorganic particles when high quality and quantity seston is available. When low quality/quantity of seston is available, feed shifts to include both organic and inorganic particles of lower quality for ingestion. In the case of fish farms and shellfish, this would be likely during the non-algal growing season in temperate waters.

We find attractive the conclusion of Troell and Norberg (1998) "… that environmental factors and design of cultivation technology are of importance in integrated cultivation systems. The availability of organic food particles have been mentioned as being the single most important factor determining growth rate of mussels (Seed and Suchanek, 1992), and maybe the existence of both temporal and spatial variation in food availability in natural water bodies can explain the degree of success". The lack of effect for stable isotope tracing of either mussels or oysters at Clam Bay is not surprising as no detectable differences occurred between upstream and downstream TSS, TVS or chlorophyll *a* measurements. But neither was a difference observed for the Cypress Island site, yet oysters apparently grew better near the farm there than at a reference location. We believe that is due to the higher level of solids in the water during the winter than at Clam Bay although we had no net pen site versus reference site water quality data to assess the differences.

We also believe that it is necessary to sample and analyze plankton very frequently to adequately describe their dynamics, but that was well beyond the scope of this preliminary study. The lack of more frequent than monthly quantification of the seston food sources that would be necessary in order for a more accurate mixing model calibration. If fish farmers decide to scale up oyster culture at fish farm sites, we recommend that a bimonthly sampling of water quality/stable isotope signatures and oyster growth be conducted. Only in this manner can the food source and stable isotope probabilities be quantified. Determining this for phytoplankton versus other seston remains a difficult issue potentially confounding the estimates but in our study seemed not to be a problem with adequate separation on the dual isotope plots

OPTIMUM SITING VERSUS IMTA?

A main point of the above review of Jones and Iwawa (1991) is during summer all of their treatment and reference locations were located in a typical Pacific Northwest embayment habitat with vertically stratification, water and nutrient poor surface water and "bust or boom" phytoplankton production patterns (Rensel Associates and PTI Environmental Sciences 1991). After spring diatom blooms, such waters have low phytoplankton biomass until dinoflagellate blooms prevail in calm days of summer. These highly mobile flagellates may not be measurable near the surface in many cases, as they vertically migrate to depth at night to obtain their nutrients and sometimes form thin layers at depth or remain near the nutricline for extended periods. We would expect increased shellfish growth near a fish farm in Jervis Inlet describe above compared to a reference location under these circumstances. By one informed estimate, about 50% of the fish farms in B.C. are located in such environments, but none are allowed (or preferred) in such areas in Puget Sound as previously described above. Puget Sound fish farm sites rarely have flagellate blooms, except in extreme river flow years when the weather is extremely mild (e.g., Rensel et al. 2010) and at such times large parts of the entire Salish Sea are subject to the same conditions, not just the poorly flushed backwater bays.

In Washington State, fish farms are purposely located in non-nutrient sensitive areas where their dissolved nitrogen wastes will not directly contribute to or initiate algal blooms as previously discussed as a requirement of aquatic lands leasing of the Washington Department of Natural Resources. These non-nutrient sensitive areas are, however, not the locations that shellfish growers often prefer, but they tend to be in the remote inlets and embayments where the shellfish growth is enhanced by recurring phytoplankton blooms throughout the growing season.

In the present experiment, we contrasted our TVS and TSS results to those in Totten Inlet, a renowned mussel, oyster and clam growing area of southern Puget Sound. The fish farms studied were in Central and North Puget Sound and tend to have lower phytoplankton production and much lower seston concentrations than Totten Inlet. That should be a positive factor for success of shellfish/fish IMTA at the fish farm sites but the evidence from Clam Bay for oysters and for both sites for mussel suggests otherwise, that either the rate of supply was not sufficient or that selective feeding and pseudofecal production resulted in no measurable gain versus reference specimens.

Many locations where IMTA has been advanced as a means to reduce fish farm wastes are likely comparable to the Jervis Inlet example above, e.g., Chilean fjords and bays, New Brunswick bays, Mediterranean Sea water, etc. While IMTA in these environments is an improvement over existing

fish monoculture at sites without adequate waste assimilatory ability, we argue that not all aquaculture venues are similar. We view this as an ill-informed one-size-fits-all-approach of calling for IMTA as a solution to a problem that, in the case of the advanced farm siting policies and procedures in Washington State, does not exist. See the Ocean Conservancy website and report[2] in this regard for an example of citing IMTA as a broad-brush solution to a perceived, universal problem.

Clearly there are tradeoffs in siting fish farms, but should not fish farms be located in areas suitable to assimilate their organic particulate or dissolved nutrient wastes without the need for additional strategies that may or may not be effective? From our review of the literature, extensive experience in siting of aquaculture facilities, impact assessment and environmental modeling of the same, it does appear that efficacy of IMTA varies inversely sustainability of fish farm siting, so this sets up a conundrum.

Should regulators continue, as they have for decades in Washington State, to promote fish farm location in areas where food web assimilation and adequate dispersion of wastes occur?

or

Should nutrient sensitive, poorly flushed areas be re-targeted as they were 30 years ago in the Pacific Northwest?

The answer is clear for Washington State: there is no turning back the clock to sites that have proven unsustainable in terms of benthic deposition and risky to the fish farmer for fish survival, even if IMTA methods were highly effective or predictable for waste removal, which presently by any measure, they are not. Other factors such as naturally occurring harmful algal blooms that often initiate in Pacific Northwest nutrient-sensitive backwater areas would deter fish farmers from the return to these areas in most cases regardless of the other considerations.

An analogy to this choice is as follows: automobiles once relied on a poorly conceived and executed emission control systems for cleanup of tailpipe emissions. The industry has evolved away from that approach into more efficient engines that are cleaner because they produce less harmful waste and more completely combust the fuel.

Accordingly, there is a danger in promoting IMTA as a cure all for the supposed ailments of aquaculture everywhere and in every case. Regulators and fish farmers in Puget Sound have worked for 40 years to establish policies that result in more optimum fish farm siting. This is not

[2] http://www.oceanconservancy.org/our-work/aquaculture/assets/pdf/oc_rfts_v11_single.pdf

the case in many major fish farm producing countries where many sites are located in nutrient-sensitive areas for whatever reason. Environmental NGOs often portray the fish farming industry with one broad brush (e.g., the Monterey Bay Seafood Program, a perfect example), but there is no monolithic, single industry to describe as such.

THE FUTURE FOR IMTA IN PUGET SOUND AND STRAIT OF JUAN DE FUCA

Despite the foregoing, IMTA could embellish fish farm environmental performance in Puget Sound or similar environments (e.g., Cobscook Bay in Maine) even if the rate of fish farm waste removal is less than optimum or as much as demonstrated herein for Cypress Island oysters. Even a low rate of assimilation of wastes could, when scaled up appropriately, translate into an equivalent increase in sustainable fish production. Although fish farmers will continue to rely on fish production as their primary crop, having shellfish production nearby could also help diversify their production as has been pointed out by IMTA advocates. IMTA could allow for additional expansion within existing salmon aquaculture lease sites in some cases, but in other cases, new leases with the State of Washington may have to be negotiated due to space limitation. The risk of expansion would be on the fish farmer, as in any case benthic performance standards must be met or the operation reduced in size, modified in configuration or relocated with new permits.

In open ocean aquaculture in some cases where current direction is highly variable, capture of fish farm wastes is logistically more difficult and may have to involve complex pivoting systems to keep the companion crops in line with the downstream currents. The open ocean is not a place for complex, potentially cumbersome or poorly designed and built systems. In Washington State, however, open ocean aquaculture means the oceanic conditions of the Strait of Juan de Fuca (Rensel et al. 2007) where currents are strongly bidirectional and tidally driven for the most part. In the very high current velocity Strait, oysters could potentially act as current deflectors up and downstream of cages as tidal current passes through fish net pens and retain some particulate waste production too.

In the relatively cool waters of Puget Sound main basins and channels where fish farms are located, Pacific oysters rarely reproduce naturally thus would not add to the biofouling load on the nets and floats that could occur with mussels when then reproduce. They could also be used in some cases to provide current flow diversion at sites with overly strong currents or temporarily at some sites where spring tides produce currents that exceed optimum velocities. Oysters will not replace fish as a primary cash crop at fish farm sites due to space limitations, but subsurface growing systems would benefit not only from the slowly settling organic particulate matter but would be less subject to algal biofouling compared to surface raft culture of shellfish.

In other regions of the world, seaweed culture is also being practiced as a means to reduce dissolved nitrogen loading. Puget Sound net pen sites are by design located in non-nutrient sensitive areas but background levels of nitrogen are naturally high in main basins of Puget Sound. The pen origin nitrogen plus the natural background flux of nitrogen could help insure a desirable and continuous supply to insure sustained growth of seaweeds near the net pens. However, combined shellfish plus seaweed culture at fish farm sites in Puget Sound may not be technically feasible because of space limitations and the fact that seaweeds must be grown near the surface to allow photosynthesis, whereas the shellfish are not subject to this limitation.

For Puget Sound waters, we recommend further investigation and scaled-up trials of the efficacy of oyster culture as a companion crop to fish aquaculture. The trials to date with oysters have been promising and we can envision several benefits from such systems as discussed herein.

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APPENDICES

Appendix 1. Vertical profile data from Experiment 1. Data collected with a Hydrolab 4a with SCUFA in vivo chlorophyll *a* sensor. December 2008 (lab samples taken for chlorophyll a at Clam Bay (coded as CB). S1 = the study site at Cypress Island (Site 1)

Date	Time	Temp	DO	Sal	pH	Chlorophyll	Turb	Dep100	DO%	BPSvr4a
MMDDYY	HHMMSS	C	mg/l	ppt	Units	ug/l	NTU	meters	Sat	mBar
Annotation at 120908 093444 : S1 (Site one East end, neap period or maybe very slight ebb, eastern motion)										
120908	94137	8.63	6.69	30.27	7.43	1.3	6.6	0.5	68.8	1041
120908	94148	8.62	6.60	30.24	7.43	1.2	6.8	0.4	67.8	1042
120908	94159	8.62	6.49	30.14	7.42	1.1	6.0	1.3	66.8	1041
120908	94211	8.63	6.43	30.20	7.42	1.0	6.5	2.3	66.4	1041
120908	94255	8.63	6.34	30.17	7.41	0.8	7.1	3.2	65.3	1042
120908	94302	8.63	6.30	30.17	7.41	0.7	5.1	4.0	65.0	1042
120908	94307	8.63	6.28	30.19	7.41	0.6	5.7	4.5	64.6	1042
120908	94318	8.63	6.15	30.15	7.40	0.6	5.2	5.2	63.4	1041
120908	94327	8.63	6.05	30.33	7.40	0.6	5.0	5.2	62.3	1041
Annotation at 121008 102221 : CB IMTA1 (Clam Bay South side of e biomass is approx 1300 tons on site										
121008	102348	10.22	6.42	30.15	7.35	0.0	4.6	0.2	67.2	1048
121008	102358	10.22	6.37	30.32	7.35	0.0	3.6	0.7	66.8	1048
121008	102409	10.23	6.36	30.35	7.35	0.0	3.3	1.4	66.7	1048
121008	102421	10.23	6.35	30.22	7.35	0.0	3.4	2.8	66.6	1048
121008	102429	10.23	6.34	30.18	7.35	0.0	3.1	3.5	66.5	1048
121008	102439	10.23	6.36	30.21	7.34	0.0	2.9	5.0	66.7	1047
121008	102447	10.23	6.35	30.13	7.35	0.0	3.0	5.0	66.6	1049
121008	102455	10.23	6.35	30.18	7.35	0.0	3.1	6.1	66.6	1049
121008	102512	10.23	6.32	30.27	7.34	0.0	3.3	6.9	66.2	1048
121008	102528	10.24	6.33	30.13	7.34	0.0	3.1	8.7	66.3	1048
121008	102538	10.24	6.31	30.13	7.34	0.0	3.1	9.4	66.1	1048
121008	102546	10.25	6.30	30.27	7.34	0.0	3.0	11.1	66.1	1048
121008	102554	10.24	6.27	30.29	7.34	0.0	3.1	12.1	65.9	1046
121008	102603	10.27	6.29	30.23	7.34	0.0	2.7	14.7	66.0	1048
121008	102610	10.27	6.24	30.18	7.34	0.0	4.4	15.1	65.4	1048
121008	102617	10.27	6.25	30.35	7.34	0.0	2.8	16.0	65.6	1048
121008	102625	10.27	6.22	30.24	7.34	0.0	3.2	17.7	65.3	1048
121008	102633	10.27	6.22	30.16	7.34	0.0	3.0	19.6	65.2	1048
121008	102640	10.27	6.19	30.18	7.34	0.0	3.0	20.0	65.0	1048
121008	102650	10.27	6.18	30.30	7.34	0.0	2.8	21.0	64.9	1048
121008	102658	10.27	6.16	30.21	7.34	0.0	3.0	22.4	64.6	1048
121008	102708	10.27	6.14	30.25	7.34	0.0	2.9	23.4	64.4	1049
121008	102726	10.27	6.12	30.18	7.33	0.0	3.2	24.5	64.2	1048

Date	Time	Temp	DO	Sal	pH	Chlorophyll	Turb	Dep100	DO%	BPSvr4a
MMDDYY	HHMMSS	C	mg/l	ppt	Units	ug/l	NTU	meters	Sat	mBar
Annotation at 121008 105722 : CB2 (5 meters Downstream of cages on West end, current running alongside and paralle										
121008	105745	10.21	6.09	30.30	7.27	0.0	3.2	0.2	63.8	1048
121008	105806	10.20	6.03	30.23	7.27	0.0	3.4	0.7	63.0	1048
121008	105822	10.18	6.02	30.10	7.27	0.0	2.8	3.1	62.7	1048
121008	105833	10.19	5.98	30.22	7.27	0.0	3.2	3.2	62.6	1048
121008	105844	10.23	5.91	30.15	7.28	0.0	3.1	5.2	61.9	1048
121008	105852	10.24	5.78	30.21	7.27	0.0	2.8	6.4	60.6	1048
121008	105901	10.23	5.70	30.23	7.28	0.0	3.1	7.0	59.8	1048
121008	105910	10.24	5.66	30.14	7.28	0.0	3.2	7.6	59.3	1049
121008	105919	10.24	5.62	30.15	7.28	0.0	2.9	9.8	58.9	1048
121008	105931	10.24	5.59	30.21	7.27	0.0	3.0	12.3	58.2	1048
121008	105939	10.24	5.53	30.26	7.27	0.1	2.6	13.4	58.1	1049
121008	105945	10.24	5.55	30.17	7.27	0.0	2.9	15.3	58.2	1048
121008	105953	10.24	5.58	30.24	7.27	0.0	2.7	16.1	58.6	1049
121008	110001	10.24	5.63	30.18	7.28	0.0	3.0	17.1	59.6	1048
121008	110009	10.25	5.77	30.15	7.28	0.0	2.7	18.8	60.4	1048
121008	110020	10.27	5.90	30.28	7.27	0.0	3.0	20.9	62.0	1048
121008	110028	10.27	5.97	30.25	7.27	0.0	3.0	22.3	62.6	1048
121008	110041	10.27	6.01	30.20	7.28	0.0	2.8	24.8	63.1	1048
121008	110051	10.27	6.02	30.35	7.28	0.0	3.1	25.5	63.2	1048
Annotation at 121008 113113 : CB3 Reference area nearer Orchard Point										
121008	113215	10.28	6.43	30.14	7.24	0.0	2.7	0.2	67.5	1048
121008	113225	10.29	6.38	30.19	7.24	0.0	2.8	1.2	66.9	1048
121008	113232	10.29	6.36	30.19	7.24	0.0	2.7	1.9	66.7	1049
121008	113239	10.29	6.34	30.24	7.24	0.0	2.7	3.6	66.5	1048
121008	113250	10.29	6.34	30.20	7.24	0.0	2.9	5.0	66.7	1048
121008	113300	10.29	6.35	30.18	7.24	0.0	2.7	6.3	66.6	1047
121008	113311	10.29	6.34	30.19	7.24	0.0	3.7	7.6	66.7	1048
121008	113319	10.29	6.37	30.19	7.24	0.0	2.8	8.7	66.8	1048
121008	113330	10.29	6.36	30.22	7.24	0.0	2.5	10.8	66.7	1046
121008	113341	10.29	6.35	30.40	7.24	0.0	2.8	12.4	66.7	1049
121008	113348	10.29	6.34	30.32	7.24	0.0	3.3	13.4	66.6	1048
121008	113359	10.29	6.33	30.22	7.24	0.0	2.5	15.5	66.5	1048
121008	113407	10.29	6.32	30.21	7.24	0.0	2.8	19.2	66.3	1048
121008	113418	10.29	6.32	30.22	7.24	0.0	3.0	20.0	66.4	1049
121008	113425	10.30	6.27	30.29	7.24	0.0	2.8	22.5	65.8	1048
121008	113432	10.30	6.27	30.31	7.25	0.0	3.3	23.5	65.5	1045
121008	113442	10.30	6.22	30.24	7.25	0.0	2.8	24.2	65.3	1049
121008	113457	10.30	6.17	30.22	7.25	0.0	2.6	25.2	64.7	1048
121008	113507	10.30	6.07	30.22	7.25	0.0	2.8	26.0	63.7	1046
121008	113541	10.30	6.04	30.16	7.25	0.0	3.0	27.5	63.4	1049
121008	113550	10.30	6.06	30.20	7.25	0.0	2.9	29.0	63.6	1046
121008	113636	10.30	6.01	30.33	7.25	0.0	1.9	30.7	63.2	1049
121008	113653	10.30	6.01	30.28	7.25	0.0	3.1	35.3	63.4	1046
121008	113702	10.30	6.04	30.17	7.25	0.0	2.8	38.8	63.3	1048

January 2009

Date	Time	IBVSvr4a	BPSvr4a	Temp	DO	Sal	pH	Turb	Dep100	DO%	BPSvr4a
MMDDYY	HHMMSS	Volts	inHg		mg/l	ppt	Units	NTU	meters	Sat	mBar
Annotation at 011509 104131 : Clam Bay: South side of cages immediately adjacent to pens, no other casts taken											
1/15/2009	104316	7.8	30.88	7.86	8.5	30.49	6.94	79.9	0.4	84.7	1046
1/15/2009	104323	7.8	30.88	7.89	8.35	30.53	6.94	118	1.3	83.3	1045
1/15/2009	104339	7.8	30.89	7.94	8.37	30.92	6.94	44.8	3.5	83.8	1046
1/15/2009	104350	7.8	30.89	7.96	8.5	30.88	6.94	37.9	4.7	85.2	1045
1/15/2009	104401	7.8	30.9	7.97	8.54	30.86	6.94	29	5.4	85.5	1046
1/15/2009	104418	7.8	30.89	7.99	8.48	30.99	6.94	5.8	8	85	1045
1/15/2009	104427	7.8	30.89	8	8.27	31.14	6.93	6.4	9.8	83	1046
1/15/2009	104441	7.8	30.88	8.01	8.1	31.2	6.94	1.5	11.7	81.4	1045
1/15/2009	104455	7.8	30.89	8.02	8.03	31.42	6.93	1.6	12.7	80.8	1043
1/15/2009	104554	7.7	30.9	8.03	8.77	31.32	6.95	1	16.4	88.9	1046
1/15/2009	104603	7.7	30.89	8.04	8.88	31.64	6.94	1.1	19.1	89.3	1046
1/15/2009	104611	7.7	30.9	8.09	8.83	31.89	6.94	0.6	21.7	89.3	1046
1/15/2009	104621	7.7	30.89	8.13	8.68	32.01	6.94	0.7	24.4	87.8	1045
1/15/2009	104631	7.7	30.89	8.14	8.48	32.07	6.95	1.1	26	85.7	1046
1/15/2009	104641	7.7	30.87	8.14	8.37	31.89	6.95	1	28.4	84.7	1046
1/15/2009	104650	7.7	30.88	8.18	8.25	32.37	6.95	1.1	30.4	83.6	1045
Annotation at 011609 124335 : Cypress Island S1 EAST end which was upstream with strong ebb at time of sampling											
1/16/2009	124406	7.8	30.96	7.07	8.1	32.88	7.05	4	0.2	80.6	1049
1/16/2009	124417	7.8	30.96	7.08	8.1	32.95	7.05	5.4	1.3	80.8	1049
1/16/2009	124425	7.8	30.98	7.08	8.2	32.84	7.05	3.7	3	81.4	1048
1/16/2009	124432	7.8	30.97	7.08	8.3	32.87	7.05	4.1	3.7	82.2	1048
1/16/2009	124442	7.8	30.96	7.08	8.3	32.86	7.05	4.4	4.9	82.8	1048
1/16/2009	124459	7.8	30.96	7.08	8.4	32.86	7.05	4.8	6.6	83.8	1048
1/16/2009	124505	7.8	30.96	7.08	8.4	32.87	7.05	4.9	7.1	83.9	1048
1/16/2009	124517	7.8	30.96	7.08	8.5	32.95	7.05	4.9	7.8	84.2	1048
1/16/2009	124528	7.8	30.96	7.08	8.5	32.87	7.05	4.7	8.8	84.6	1048
1/16/2009	124552	7.8	30.98	7.08	8.5	32.84	7.05	4.7	10	84.5	1048
1/16/2009	124619	7.7	30.97	7.08	8.4	32.93	7.05	3.9	11.3	84	1048
1/16/2009	124705	7.7	30.98	7.08	8.1	32.97	7.05	4.7	13	80.2	1048
1/16/2009	124739	7.7	30.96	7.09	8.0	32.96	7.05	5.3	13.9	79.3	1048
1/16/2009	124822	7.7	30.97	7.08	7.9	32.89	7.05	3.9	15	79.2	1048
1/16/2009	124838	7.7	30.96	7.09	7.9	32.9	7.05	5	17.1	78.8	1048
1/16/2009	124851	7.7	30.95	7.09	7.9	32.95	7.05	4	18.1	78.6	1048
Annotation at 011609 135 Cypress Island Site 1 west end which was downstream with relatively strong current at time of sampling											
1/16/2009	135406	7.6	30.92	7.03	7.8	32.82	6.94	6.9	0.3	77.2	1047
1/16/2009	135421	7.6	30.93	7.03	7.6	32.89	6.94	3.7	1.4	74.9	1047
1/16/2009	135437	7.6	30.88	7.03	7.4	32.78	6.94	3.8	2.6	73.5	1047
1/16/2009	135446	7.6	30.93	7.04	7.4	32.74	6.94	3.7	3.1	73.5	1047
1/16/2009	135454	7.6	30.95	7.04	7.4	32.75	6.94	4.2	4.9	73.3	1047
1/16/2009	135503	7.6	30.92	7.04	7.4	32.81	6.94	3.3	5.6	72.8	1047
1/16/2009	135511	7.6	30.93	7.04	7.3	32.74	6.93	3.4	4.5	72.5	1046
Annotation at 011609 141700 : Bellingham Channel "reference"											
1/16/2009	141923	7.6	30.94	6.97	8.6	32.66	6.94	3.1	0.3	83.8	1047
1/16/2009	141933	7.6	30.93	6.97	8.2	32.59	6.93	3.4	1.5	81.1	1047
1/16/2009	141939	7.6	30.93	6.97	8.1	32.61	6.93	3.6	2	80.3	1047
1/16/2009	141945	7.6	30.95	6.98	8.1	32.59	6.93	3.5	3.3	80	1047
1/16/2009	141954	7.6	30.93	6.98	8.1	32.63	6.93	4.2	3.4	80	1048
1/16/2009	142003	7.6	30.94	6.97	8.1	32.63	6.93	3.1	4.1	80	1047
1/16/2009	142013	7.6	30.93	6.98	8.1	32.59	6.93	3	4.3	79.8	1048
Recovery finished at 011709 120556											

March 2009 Clam Bay

Date	Time	IBVSvr4a	BPSvr4a	Temp	DO	Sal	pH	Chl	Turb	Dep100	DO%	BPSvr4a
MMDDYY	HHMMSS	Volts	inHg	?	mg/l	ppt	Units	?g/l	NTU	meters	Sat	mBar
Annotation at 031009 133843 : CB												
31009	133948	7.9	30.87	7.37	9.03	32.52	7.72	0.0	18.6	0.4	92.1	1044
31009	134000	7.9	30.87	7.35	8.96	32.48	7.72	0.1	18.1	0.7	91.7	1044
31009	134012	7.9	30.86	7.33	9.04	32.60	7.72	0.0	17.5	2.1	92.4	1044
31009	134041	7.9	30.84	7.32	9.26	32.54	7.73	0.0	19.1	3.0	94.6	1045
31009	134052	7.9	30.85	7.32	9.32	32.50	7.73	0.0	18.5	4.6	95.4	1045
31009	134105	7.9	30.86	7.32	9.50	32.55	7.73	0.2	16.8	7.1	97.8	1044
31009	134115	7.9	30.83	7.31	9.74	32.62	7.73	0.2	15.7	9.3	99.7	1044
31009	134124	7.9	30.86	7.31	9.94	32.64	7.73	0.2	11.8	11.2	102.4	1045
31009	134136	7.9	30.84	7.31	10.15	32.52	7.73	0.2	16.8	12.6	103.9	1044
31009	134225	7.9	30.85	7.31	9.71	32.60	7.74	0.3	16.0	11.7	99.3	1042
31009	134241	7.8	30.84	7.31	9.66	32.64	7.74	0.3	12.8	13.7	99.0	1044
31009	134256	7.8	30.84	7.30	9.69	32.51	7.74	0.3	10.0	16.3	99.1	1044
31009	134306	7.8	30.84	7.30	9.70	32.61	7.75	0.2	8.4	19.0	99.3	1043
31009	134314	7.8	30.78	7.30	9.70	32.57	7.75	0.6	7.2	20.3	99.3	1044
31009	134323	7.8	30.79	7.30	9.73	32.63	7.75	0.2	7.8	22.6	99.8	1044
31009	134334	7.8	30.84	7.30	9.80	32.56	7.75	0.1	6.5	24.7	100.3	1044
31009	134345	7.8	30.83	7.30	9.75	32.67	7.75	0.1	6.4	26.2	100.7	1044
Recovery finished at 032309 202338												

Appendix 2. Water quality data for Clam Bay and Cypress Island.

Clam Bay DIN (µM)

Date	Up 2m	Down 2m	Up 20m	Down 20m	Ref 2m	Ref 20m	Far Ref 2m	Far Ref 20m
Oct 08		27.2						
Dec 08	28.7	30.6	29.7		28.7	29.7		
Jan 09	31.2	32.2	31.2	30.9				
Mar 09		29.3		29.7				
Apr 09	20.1	12.3	20.5	17.8	18.8	19.5		
Jun 09	9.1	14.3	16.4	14.9	9.1	16.4		
Jul 09	13.5	13.7	14.4	13.0			13.5	14.4
Aug 09	13.7	13.0	19.0	20.9				

Cypress Island DIN (µM)

Date	Up 2m	Down 2m	Up 20m	Down 20m	Ref 2m	Ref 20m	Far Ref 2m	Far Ref 20m
Oct 08		26.4		26.4				
Dec 08		33.2		31.9		29.0		
Jan 09	30.0	31.0	30.1	31.2				30.3
Mar 09	27.7	28.6						
Apr 09		19.3		19.6			19.2	18.8
Jun 09	15.9	19.6	17.9	20.1			17.9	19.8
Jul 09	20.9	19.2	21.2	19.8			22.5	23.1
Aug 09		23.8		23.4			23.7	24.7

Clam Bay Chlorophyll-*a* (µg/l)

Date	Up 2m	Down 2m	Up 20m	Down 20m	Ref 2m	Ref 20m	Far Ref 2m	Far Ref 20m
Oct 08	0.2	0.1	0.1	0.1				
Dec 08	1.3	0.9		1.1	1.3			
Jan 09	1.3	1.3	0.1					
Mar 09	0.1	1.5	0.1	0.3				
Apr 09	4.4	5.9		5.7				
Jun 09	9.7	5.9		3.3	9.7			
Jul 09	4.8	4.5	3.7	4.9			4.8	3.7
Aug 09	4.5	3.5	2.9	3.6				

Cypress Island Chlorophyll-*a* (µg/l)

Date	Up 2m	Down 2m	Up 20m	Down 20m	Ref 2m	Ref 20m	Far Ref 2m	Far Ref 20m
Oct 08	1.3	1.3	1.3	1.3				
Dec 08	1.3	0.7		1.3				
Jan 09	0.1	0.4		0.2				
Mar 09	1.5	1.5		0.2				
Apr 09	2.8	3.3		2.8	2.8			
Jun 09		6.9		5.7				6.3
Jul 09	4.1	3.2		2.9				
Aug 09		1.6		0.4				

Clam Bay TVS (mg/l)

Date	Up 2m	Down 2m	Up 20m	Down 20m	Ref 2m	Ref 20m	Far Ref 2m	Far Ref 20m
Oct 08		0.8		0.9				
Dec 08	0.3	0.7	0.6	0.3	0.3	0.6		
Jan 09	0.6	0.7	0.9	0.6				
Mar 09		0.3		0.3				
Apr 09	1.1	1.3	1.2	1.1	1.2	1.1	1.1	
Jun 09	1.8	1.8		1.4	1.8	1.4		
Jul 09	1.1	1.1	0.9	1.4			1.1	0.9
Aug 09	1.6	1.9	0.9	1.2				

Cypress Island TVS (mg/l)

Date	Up 2m	Down 2m	Up 20m	Down 20m	Ref 2m	Ref 20m	Far Ref 2m	Far Ref 20m
Oct 08	1.1	1.0	1.1	1.0				
Dec 08	1.0	1.0		1.0	0.7			
Jan 09	1.4	0.8	0.9	0.8		0.7		0.6
Mar 09	1.1	1.1		0.9				
Apr 09	1.2	1.3	1.5	1.4	1.2	1.5	1.1	1.1
Jun 09	1.5	1.7	1.6	1.4			1.2	1.2
Jul 09	1.3	1.2	1.7	1.5				0.9
Aug 09	0.7	0.7		0.8			0.7	1.0

Clam Bay TSS (mg/l)

Date	Up 2m	Down 2m	Up 20m	Down 20m	Ref 2m	Ref 20m	Far Ref 2m	Far Ref 20m
Oct 08	3.6	3.8	3.2	3.6				
Dec 08	2.5	3.2	2.6	3.1	2.5	2.6		
Jan 09	3.8	2.6	3.8	3.2				
Mar 09	3.1	2.9	3.5	2.9				
Apr 09	4.1	4.6	4.4	4.2	4.3	4.3	4.5	
Jun 09	5.1	4.8	4.5	4.5	5.1	4.6		
Jul 09	3.3	3.3	3.1	3.4			3.3	3.1
Aug 09	3.8	3.7	2.4	3.3				

Cypress Island TSS (mg/l)

Date	Up 2m	Down 2m	Up 20m	Down 20m	Ref 2m	Ref 20m	Far Ref 2m	Far Ref 20m
Oct 08	5.4	5.3	5.4	5.3				
Dec 08	3.4	3.8	4.4	4.3	3.3			
Jan 09	7.6	5.4	7.6	7.1		5.5		5.1
Mar 09	5.6	5.6	7.1	5.4				
Apr 09	6.1	6.5	7.6	6.6	6.1	7.6	5.2	5.8
Jun 09	5.1	5.9	5.5	5.7			4.7	4.8
Jul 09	6.3	4.2	9.3	8.7				5.9
Aug 09	3.1	3.3	3.0	3.2			3.6	6.1

Appendix 3. Mean and standard deviation of replicate shellfish lengths during measurement intervals.

EXPERIMENT 1: Oyster growth over time (average length in mm)

Date	September 2008				January 2009				March 2009				June 2009			
Site	Clam Bay		Cypress Island		Clam Bay		Cypress Island		Clam Bay		Cypress Island		Clam Bay		Cypress Island	
treatment	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev
Farm shallow	37.4	0.8	39.6	2.7	63.0	1.8	N/A	N/A	65.4	1.4	N/A	N/A	77.5	0.6	N/A	N/A
Farm deep	35.9	1.4	38.9	1.2	60.2	2.4	N/A	N/A	61.7	1.2	N/A	N/A	74.7	1.8	N/A	N/A
30m distant deep	36.2	0.7	39.3	1.1	58.4	1.4	61.9	2.5	59.1	1.6	60.5	2.8	74.2	1.5	70.5	3.3
Reference deep	36.1	1.2	37.8	0.3	57.1	1.0	57.3	1.8	61.2	1.5	56.4	1.3	75.1	1.9	59.8	2.2

EXPERIMENT 1: Mussel growth over time (average length in mm)

Date	September 2008				January 2009				March 2009				June 2009			
Site	Clam Bay		Cypress Island		Clam Bay		Cypress Island		Clam Bay		Cypress Island		Clam Bay		Cypress Island	
treatment	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev
Farm shallow	50.5	0.6	47.4	1.1	62.9	0.5	N/A	N/A	61.8	0.6	N/A	N/A	64.8	0.4	N/A	N/A
Farm deep	50.1	0.7	46.4	0.5	62.4	0.7	N/A	N/A	61.9	0.8	N/A	N/A	65.7	0.7	N/A	N/A
30m distant deep	50.5	0.3	47.8	0.6	61.0	1.0	56.9	0.3	60.5	1.0	58.2	0.8	62.8	0.8	61.7	1.3
Reference deep	49.2	0.6	49.1	0.6	62.2	0.8	57.6	0.6	61.8	0.8	59.0	0.8	64.1	1.2	62.1	0.8

EXPERIMENT 2: Mussel growth over time (average length in mm)

Date	April 2010				September 2010				March 2011			
Site	Clam Bay		Cypress Island		Clam Bay		Cypress Island		Clam Bay		Cypress Island	
treatment	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev	length	stdev
Farm shallow	21.3	0.5	20.8	0.2	50.4	2.0	45.9	0.9	56.9	2.1	51.4	0.5
Farm deep	21.6	0.7	20.8	0.2	50.3	1.3	44.4	0.4	56.2	1.4	48.4	0.9
30m distant deep	21.5	0.4	20.9	0.2	N/A	N/A	45.1	0.6	N/A	N/A	50.4	1.5
Reference deep	20.5	0.4	20.8	0.1	52.8	1.4	44.8	1.0	59.7	0.9	49.1	0.5

Appendix 4. Mean and standard deviation of replicate shellfish mortality counts per each measurement interval.

EXPERIMENT 1: Average mussel mortality counts

Date	January 2009				March 2009				June 2009			
Site	Clam Bay		Cypress Island		Clam Bay		Cypress Island		Clam Bay		Cypress Island	
	count	stdev	count	stdev	count	stdev	count	stdev	count	stdev	count	stdev
Farm shallow	8.0	1.8	N/A	N/A	7.3	3.1	N/A	N/A	31.3	5.3	N/A	N/A
Farm deep	3.8	3.1	N/A	N/A	3.8	4.3	N/A	N/A	20.3	2.6	N/A	N/A
30m distant deep	11.8	7.5	1.8	1.7	2.0	3.4	0.3	0.5	38.8	9.4	2.7	3.1
Reference deep	3.3	1.7	6.0	4.2	2.8	1.3	0.8	1.5	44.5	5.9	2.0	1.4

EXPERIMENT 1: Average oyster mortality counts

Date	January 2009				March 2009				June 2009			
Site	Clam Bay		Cypress Island		Clam Bay		Cypress Island		Clam Bay		Cypress Island	
	count	stdev	count	stdev	count	stdev	count	stdev	count	stdev	count	stdev
Farm shallow	4.5	6.4	N/A	N/A	1.5	1.3	N/A	N/A	0.0	0.0	N/A	N/A
Farm deep	0.5	1.0	N/A	N/A	2.0	2.7	N/A	N/A	0.0	0.0	N/A	N/A
30m distant deep	6.5	3.8	2.5	1.0	1.0	2.0	0.8	0.5	0.0	0.0	0.0	0.0
Reference deep	5.5	2.4	5.3	2.6	0.8	1.5	1.8	1.5	0.0	0.0	0.3	0.5

EXPERIMENT 2: Average mussel mortality counts

Date	September 2010				March 2011			
Site	Clam Bay		Cypress Island		Clam Bay		Cypress Island	
	count	stdev	count	stdev	count	stdev	count	stdev
Farm shallow	11.3	13.7	9.3	7.8	9.0	2.6	10.0	4.4
Farm deep	8.7	4.9	6.0	1.0	26.7	17.8	9.7	6.7
30m distant deep	N/A	N/A	6.3	2.1	N/A	N/A	9.0	7.0
Reference deep	11.3	9.0	6.7	1.5	5.0	3.5	5.3	2.5

Appendix 5. Mean and standard deviation of replicate mussel mortality lengths for measurement intervals of Experiment 2.

EXPERIMENT 2: Average mussel mortality lengths								
Date	September 2010				March 2011			
Site	Clam Bay		Cypress Island		Clam Bay		Cypress Island	
	length	stdev	length	stdev	length	stdev	length	stdev
Farm shallow	46.9	3.2	32.1	6.9	53.1	3.5	52.0	5.2
Farm deep	46.6	8.1	24.3	0.0	53.8	2.4	48.0	1.5
30m distant deep	N/A	N/A	27.8	3.2	N/A	N/A	47.7	3.1
Reference deep	39.1	0.2	27.5	4.1	45.7	0.0	47.7	5.2

Appendix 6. Mean and standard deviations of replicate shellfish stable isotope results for each treatment within experiments.

EXPERIMENT 1: CLAM BAY OYSTERS

September 2008	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
initial	9.8	0.4	-20.6	0.4
January 2009	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	10.8	0.1	-19.3	0.0
farm deep	10.7	0.2	-19.3	0.8
30m distant deep	10.8	0.2	-19.4	2.6
reference deep	10.8	0.2	-19.3	1.1
June 2009	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	9.5	0.2	-18.5	0.4
farm deep	9.1	0.2	-18.6	0.3
30m distant deep	9.3	0.1	-18.3	0.1
reference deep	9.4	0.1	-18.6	0.3

EXPERIMENT 1: CYPRESS ISLAND OYSTERS

September 2008	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
initial	10.0	0.3	-20.5	0.4
January 2009	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	N/A	N/A	N/A	N/A
farm deep	N/A	N/A	N/A	N/A
30m distant deep	9.3	0.2	-20.0	0.3
reference deep	9.3	0.2	-20.4	0.4
June 2009	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	N/A	N/A	N/A	N/A
farm deep	N/A	N/A	N/A	N/A
30m distant deep	8.1	0.1	-20.0	0.2
reference deep	8.7	0.1	-20.0	0.2

EXPERIMENT 1: CYPRESS ISLAND MUSSELS

September 2008	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
initial	9.5	0.3	-18.1	0.2
January 2009	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	N/A	N/A	N/A	N/A
farm deep	N/A	N/A	N/A	N/A
30m distant deep	9.4	0.2	-18.9	0.4
reference deep	9.1	0.4	-18.5	0.4
June 2009	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	N/A	N/A	N/A	N/A
farm deep	N/A	N/A	N/A	N/A
30m distant deep	8.0	0.3	-18.7	0.1
reference deep	8.1	0.3	-18.5	0.2

EXPERIMENT 1: CLAM BAY MUSSELS

September 2008	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
initial	9.6	0.3	-18.1	0.3
January 2009	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	9.8	0.3	-17.8	0.6
farm deep	10.2	0.3	-18.2	3.0
30m distant deep	10.1	0.3	-18.1	2.5
reference deep	9.4	0.0	-17.4	0.8
June 2009	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	8.6	0.2	-17.1	0.2
farm deep	8.7	0.3	-17.4	0.3
30m distant deep	8.6	0.3	-17.1	0.1
reference deep	8.7	0.2	-17.1	0.2

Appendix 6, cont. Mean and standard deviations of replicate shellfish stable isotope results for each treatment within experiments.

EXPERIMENT 2: CLAM BAY MUSSELS				
April 2010	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
initial	9.3	0.2	-18.1	0.3
September 2010	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	8.4	0.1	-16.9	0.1
farm deep	8.7	0.1	-16.6	0.1
30m distant deep	N/A	N/A	N/A	N/A
reference deep	8.8	0.3	-16.8	0.2
March 2011	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	9.1	0.2	-18.0	0.4
farm deep	9.4	0.1	-17.9	0.0
30m distant deep	N/A	N/A	N/A	N/A
reference deep	9.8	0.1	-17.7	0.2

EXPERIMENT 2: CYPRESS ISLAND MUSSELS				
April 2010	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
initial	9.3	0.2	-18.1	0.3
September 2010	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	6.8	0.1	-19.0	0.1
farm deep	6.8	0.1	-18.8	0.2
30m distant deep	6.5	0.1	-18.9	0.1
reference deep	6.9	0.1	-18.5	0.1
March 2011	$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	
	average	stdev	average	stdev
farm shallow	7.6	0.2	-19.0	0.3
farm deep	7.9	0.2	-19.1	0.1
30m distant deep	7.8	0.2	-19.0	0.3
reference deep	7.8	0.3	-19.0	0.2