

SALINITY VARIATION, TROPHIC INTERACTIONS
AND COMMUNITY STRUCTURE ON ROCKY SHORES

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ABSTRACT

Variation in physical environmental conditions may affect species directly based on physiological tolerance or indirectly via changes in interspecific interactions. We investigated the direct and indirect effects of spatiotemporal variation in salinity on intertidal community structure in the Strait of Georgia, British Columbia. Richness and abundance of herbivores, barnacles and red algae were significantly greater at sites with consistently high salinity and lower at sites near the mouth of the Fraser River where salinity was seasonally variable. Conversely, abundance of green and brown algae and mussels was greater at low salinity sites. Based on these patterns, we predicted that 1) the herbivore distributional pattern is determined by hypo-osmotic stress, and 2) salinity driven variation in herbivore abundance would lead to differences in community structure. Laboratory trials confirmed that salinity conditions at low salinity sites exceeds the tolerance of limpets, *Lottia* spp., despite a capacity for local adaptation, but not that of the green alga, *Ulva* sp. Field based herbivore exclusion experiments demonstrated that the effect of salinity on abundance is indirect and driven by a reduction in limpets in *Ulva* spp., and by an increase in limpets in *Chthamalus dalli*. Our results emphasize the importance of community level interactions in determining spatial and temporal patterns of distribution and abundance across environmental gradients.

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INTRODUCTION

Intertidal communities vary spatially and temporally in response to both small and large scale environmental gradients (Schoch *et al.* 2006; Blanchette *et al.* 2008). Variations in community development and structure result from the direct effects of environmental stressors on the physiology of organisms and indirect effects on the interactions between species (Underwood 1999; Dahlhoff *et al.* 2002; Longtin *et al.* 2009). These effects may influence the recruitment, settlement or survival of intertidal species, thereby altering patterns of succession (Farrell 1991; Connolly *et al.* 2001). Distributional patterns are further complicated by the dynamic nature of abiotic conditions, which can vary daily, seasonally and long term in response to large scale geographical and climatological processes (Helmuth *et al.* 2002; Hsieh *et al.* 2005; Menge *et al.* 2011). This effect is illustrated in coastal estuarine communities, where sea surface salinity varies spatially, with salinity increasing with distance from the source of freshwater runoff, as well as temporally, in response to periods of peak outflow (Zacharias and Roff 2001; Ysebaert and Herman 2002). Understanding the effects of such fluctuations in environmental conditions is integral to understanding the mechanisms that drive patterns of species composition in intertidal systems.

Salinity changes can have profound influences on the survival and function of intertidal organisms. Exposure to fresh water for as little as one hour has been shown to induce physiological stress in limpets, a dominant group of intertidal herbivores, with responses including decreased heart rate, reduced haemolymph osmolality and mortality (De Pirro *et al.* 1999; Chelazzi *et al.* 2001; Firth and Williams 2009). Similarly, hypersaline conditions have been shown to reduce feeding, activity and reproduction (Cheung 1997), reduce larval development rate (Zimmerman and Pechenik 1991), and increase risk of heavy metal toxicity (De Wolf *et al.* 2004) in other marine gastropods. Many bivalve species are able to tolerate salinities as low as 2psu (Angonesi *et al.* 2008; Yuan *et al.* 2010; Ridgway and Naevdal 2004), although physiological stress responses can still be detected during acclimation periods (Hamer *et al.* 2008). While many adult barnacle species are also tolerant of a wide range of salinities, levels of less than 10psu have been found to reduce the survival, development and settlement of larvae, and subsequently influence adult distribution (Qiu and Qian 1999; Dineen and Hines 1994;

Starczak *et al.* 2011). Hyposaline conditions can induce stress responses in algal species, including reduced pigment production (Karsten and Kirst 1989; Chakraborty *et al.* 2010; Krabs and Buchel 2011), increased concentration of antioxidative enzymes (Chakraborty *et al.* 2010; Kumar *et al.* 2010), decreased growth rate (Thessen *et al.* 2005; Kumar *et al.* 2010; Spurkland and Iken 2011; Krabs and Buchel 2011) and decreased photosynthetic rate (Karsten and Kirst 1989; Fleming *et al.* 2007). However, many of these species have demonstrated a wide salinity tolerance range (Chang *et al.* 1999; Rath and Adhikary 2005), as well as a high capacity for recovery from (Kim and Garbary 2007) and local adaptation to extreme salinities (Nygard and Ekelund 1999, 2006; Nygard and Dring 2008).

In addition to direct physiological impacts, variation in salinity can have indirect effects on species distribution and abundance. Intertidal species diversity has been shown to correlate with natural salinity gradients (Zacharias and Roff 2001; Hampel *et al.* 2009; Rubal *et al.* 2012). Witman and Grange (1998) showed that rainfall induced salinity gradients in Doubtful Sound, New Zealand, influenced the spatial distribution of invertebrate species both directly and indirectly through physiological stress and changes in predator abundance, respectively. Nielsen and Gosselin (2011) showed that periodic salinity stress could indirectly benefit scavenger species by causing high mortality in prey species. Salinity induced effects on coastal species have also been shown to result in changes in patterns of succession (Ritter *et al.* 2005) and community structure (Schoch *et al.* 2006).

The Strait of Georgia, British Columbia, presents a unique and ideal environment for studying the effects of salinity on coastal communities. The 220km strait is located between Vancouver Island and mainland British Columbia, and is partially isolated from the Pacific Ocean by restricted flow through narrow channels around the northern and southern tips of the island (Figure 1). Seasonal variation in freshwater influx via the Fraser River, regularly reaching a mean of more than 7000m³/s in summer months (Masson 2006; Halverson and Pawlowicz 2008), causes a corresponding variation in sea surface salinity near the Fraser plume, with an annual drop from approximately 25psu to less than 15psu during peak discharge (Halverson and Pawlowicz 2011). This effect, however, declines with increasing distance from the estuary, with waters southeast of the Southern Gulf Islands maintaining salinities of 23psu to 30psu year round (Tully and Dodimead 1957; Halverson and Pawlowicz 2011).

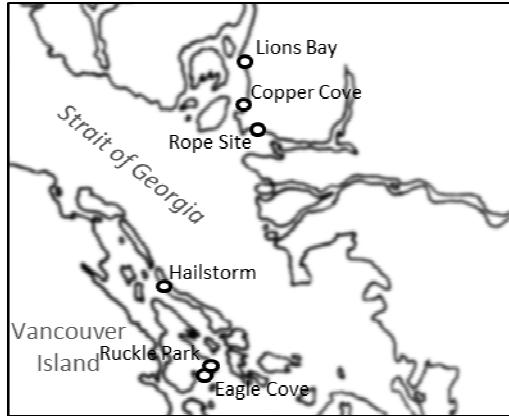


Figure 1. Map of the study region. Low salinity sites are located in West Vancouver and include Lions Bay, Copper Cove and Rope Site. High Salinity sites are located in the Gulf Islands and include Hailstorm, Ruckle Park and Eagle Cove.

Because limpet species have been shown to be highly susceptible to low salinity stress compared to algae and other invertebrates, and because limpet density has been shown to impact algal abundance (Branch 1981; Cubit 1984), we predicted that observed differences in community structure between consistently high salinity environments and periodically low salinity environments would be driven by trophic effects as well as physiological stress. We hypothesized that limpets and other marine gastropods are disproportionately affected by the annual decrease in surface salinity experienced in the coastal areas near the mouth of the Fraser River. We predicted that the abundance of gastropods in periodically low salinity environments would be lower than that of consistently high salinity areas around the Southern Gulf Islands, and that this difference would increase with observed differences in salinity. This reduction in gastropod grazers would lead to a reduction in grazing pressure on palatable green algae, biofilms and algal spores, resulting in greater algal cover in low salinity environments. Such a change in algal abundance could alter patterns of succession and community development, and we therefore predicted that high and low salinity environments would also differ in diversity and abundance of red and brown algae and sessile invertebrates. To test these predictions, we combined laboratory salinity tolerance trials on limpets and green algae with transect surveys and limpet exclusion/inclusion experiments at three sites within each of the two salinity regions.

METHODS

Study Sites

We conducted field studies in three sites within each of two regions with contrasting salinity regimes: West Vancouver and the Southern Gulf Islands. West Vancouver is located approximately 30km north of the Fraser River outflow. Sites include Lions Bay, Copper Cove and Rope Site. The Southern Gulf Islands are located southwest of the Fraser River outflow. Sites include Hailstorm on Galiano Island, and Ruckle Park and Eagle Cove on Salt Spring Island (Figure 1). The two areas are similar in terms of climate and topography. Sea surface temperature in the two regions is comparable during the spring and summer months, but tends to reach lower minimums in West Vancouver in the winter (Fisheries and Oceans Canada 2009). Tidal heights are higher in West Vancouver, ranging from 0.5m to 4.7m, compared to 0.3m to 3.4m in the Gulf Islands, relative to Canadian chart datum approximated as the lowest astronomical tide, or lower low water large tide (Fisheries and Oceans Canada 2008). Sites in West Vancouver are composed of granite rock and were fairly open and exposed. Rope Site and Copper Cove were prone to large boat and ferry traffic. Sites in the Gulf Islands are less exposed, but Ruckle Park and Eagle Cove were also prone to ferry traffic. Eagle Cove and Ruckle Park are composed of granite rock, while Hailstorm is composed of sandstone. The slope of the rock face was more variable in the Gulf Islands, though more consistent in aspect. Gulf Island plots were generally northeast to south facing, while those in West Vancouver were generally northwest to southwest (Table 1).

Site	Region	Salinity Category	Latitude	Longitude	Vertical Height (m)	Slope (°)	Aspect (°)
Lions Bay	WV	Low	49°28'14"N	123°14'39"W	3.3	10-22	210-280
Copper Cove	WV	Low	49°22'43"N	123°16'49"W	3.8	19-37	250-320
Rope Site	WV	Low	49°20'18"N	123°13'14"W	3.3	9-16	80-210
Hailstorm	SGI	High	48°59'25"N	123°34'52"W	2.3	10-33	40-60
Ruckle Park	SGI	High	48°46'24"N	123°21'59"W	2.0	4-16	85-120
Eagle Cove	SGI	High	48°45'23"N	123°23'49"W	2.4	1-26	150-230

Table 1. Physical characteristics of study sites. Regions include West Vancouver (WV) and the Southern Gulf Islands (SGI). Vertical height refers to height above Canadian chart datum. Slope refers to degrees above the horizontal. Aspect refers to degrees east of magnetic north.

Transect Surveys

Surveys were conducted once per month during low tide from May 2011 to August 2011, at each of the six study sites. Because the tidal range differs between the two areas, surveys were conducted at the vertical height corresponding to approximately 30% air exposure time. This occurs at 2.1m in the Southern Gulf Islands and 3.0m in West Vancouver. Ten meters of transect tape were laid across the selected area and eight randomly selected points were surveyed using 25x25cm quadrat. Mobile invertebrates were counted and sessile invertebrates and algae were estimated by percent cover.

Tolerance Experiments

Salinity Tolerance of Lottia pelta with tidal treatment

To determine whether or not the salinity tolerance of limpets is influenced by the periodic emersion from hyposaline conditions experienced during low tides, we conducted a salinity tolerance experiment which incorporated a treatment mimicking tidal exposure. Limpets (*L. pelta*) were collected from the Hailstorm site, Galiano Island from a salinity of 32psu. Four limpets were randomly assigned to one of twenty-four 1000cm³ Ziploc® containers with mesh walls and two containers were randomly assigned to each of twelve 20L aquaria containing seawater at 30psu. Aquaria were covered, provided compressed air and placed inside of a flow

through sea water system to maintain a water temperature of 12°C. Salinity treatments of 30psu, 20psu and 10psu were randomly assigned to each aquarium. Salinities were lowered by 2.5psu per day with chilled dechlorinated freshwater until the desired salinity was reached. To control for continued water replacements in the lowest salinity treatment, those treatments that had already reached their target salinity were subjected to daily water replacements of the same volume using filtered sea water of the target salinity in place of dechlorinated freshwater.

At approximately 10am every morning, one randomly selected container from within each aquarium, designated the “out” container, was removed from the water and placed on top of the aquarium. At approximately 6pm every evening, the container was placed back inside the aquarium to simulate exposure during low tide. Each day, limpets within each container were examined for signs of mortality, including tissue damage, discolouration and rigidity. Dead limpets were removed from the containers and measured before disposal. The experiment continued for twenty-eight days. Limpets were not fed during experiments.

*Salinity Tolerance of *L. pelta* from two populations*

Limpets (*L. pelta*) approximately 20mm in length, were collected from the Hailstorm site, Galiano Island, on June 2, 2011 from a salinity of 27psu and from the Rope Site, West Vancouver, on June 6, 2011 from a salinity of 10psu. Limpets from the high salinity site (Hailstorm) were randomly sorted into eighteen containers, for a total of six limpets in each. Each container was placed inside of an aquarium containing seawater at 30psu. The salinity of the water within these aquaria was lowered by 2.5psu per day until a salinity of 20psu was reached. Limpets were allowed to acclimatize to this salinity for ten days. Limpets from the low salinity site (Rope Site) were randomly sorted into an additional eighteen containers, with six limpets in each. All containers were placed into aquaria containing seawater at 10psu which was increased at increments of 2.5psu per day to 20psu. These limpets were allowed to acclimatize for six days. On June 16, containers were randomly arranged into eighteen aquaria, all containing water at 20psu, so that each aquarium contained one container of limpets originally from Hailstorm and one container of limpets originally from Rope Site. Aquaria were randomly assigned salinity treatments of 5, 8, 11, 14, 17 and 20psu. Salinities were lowered at a rate of 3psu every 30 minutes until the desired salinity was reached. The experiment continued for twenty eight days.

Salinity Tolerance of Ulva sp.

Ulva sp. was collected from Copper Cove, West Vancouver, on Dec. 7, 2011.

Approximately five grams of blot dried *Ulva* sp. was separated into each of sixty-four 1L plastic bottles. Each bottle was randomly assigned a salinity treatment of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5 or 30psu and provided with compressed air. The 0psu treatment contained only distilled water, while all other treatments contained combinations of filtered seawater at 31psu and dechlorinated freshwater at 0.5psu. Bottles were placed inside of a flow through sea water system to maintain a water temperature of 12°C and provided $25\pm5\mu\text{mol}$ of continuous light. After three weeks, all samples were blot dried and weighed. One sample from each treatment was randomly selected to be assessed for photosynthetic efficiency using a pulse amplitude modulation (PAM) fluorometer (Jr PAM, Heinz Walz GmbH). Light intensities were altered using a 240W Fiber Optic Illuminator (6000-1, Intralux®) and screening filters. Samples were dark acclimated for one hour before quantum yields were measured by applying a saturating light pulse after reaching a steady state. Photosynthesis vs. irradiance curves were used to determine maximum photosynthetic electron transport rate (ETRmax).

Field Exclusion Experiments

Experiments were conducted from May to August, 2011. Seven areas at each of the six sites were manually cleared of organisms. Each plot contained a limpet inclusion, exclusion and control. Inclusions and exclusions were formed by securing two copper fences, 2.5cm high and 28.5cm in diameter to the rock face within each plot with Quickcrete® quick drying cement. One circular area within each plot, 28.5cm in diameter, was marked with steel bolts as a control. The order of control and treatments was randomized within each plot. This methodology was adopted from Harley (2006). Copper controls were not included in this study, as previous work has shown that partial treatments lead to partial effects which are difficult to interpret (Johnson 1992).

Sampling occurred once per month during low tide from May 2011 to August 2011. A 10x10cm quadrat was used to count mobile invertebrates and barnacles and estimate percent cover of algae and mussels within each treatment *in situ*. Four limpets (*L. pelta*) collected from nearby shoreline within each site were placed into the rings designated as limpet inclusion

treatments. *L. pelta* were determined from previous transect data to be the largest grazer present in the mid-intertidal in both salinity regions, and body size of grazers has been shown to positively correlate with grazing pressure (Geller 1991). The average density of limpets per 100cm² was 3.65 in high salinity sites and 0.55 in low salinity sites. Any other grazers found inside rings were removed (Figure 2). Limpet treatment densities were maintained every two weeks by adding or removing limpets as necessary. Salinity samples were taken at each sampling event and measured using a refractometer (S/Mill-E, Atago Inc.).



Figure 2. Experimental design. Treatments (from left) include exclusion, control and inclusion.

Statistical Analyses

Transect data for each species were analyzed with repeated measures ANOVA to determine differences in abundance between salinity regions and over time. *Littorina* spp. were indistinguishable from one another and were analyzed together. Limpet and green and red alga species were combined into respective functional groups for statistical analyses. Mobile invertebrate count data were log transformed.

Limpet salinity tolerance was determined using fixed effects ANOVA to analyse differences in the proportion of limpets surviving in each treatment and from each population on the last day of the experiment. Net productivity of *Ulva* sp. was determined as the change in biomass before and after the salinity treatments. Change in biomass and ETRmax were analyzed using least-squares regression.

Field experiment data for each species were analysed by month using fixed effects ANOVA to determine differences in abundance between treatments and salinity regions. Limpet, periwinkle and green and red alga species were combined into their respective functional groups. *Porphyra perforata* was considered a palatable species and was therefore analyzed separate from the unpalatable red algae functional group. Unidentifiable barnacle recruits were not included in the analyses of *Balanus glandula* and *Chthamalus dalli*. Invertebrate count data were log transformed. *Post-hoc* Tukey HSD analyses were performed to determine the degree of difference between group means. Abundance of sessile organisms was low and similar across all treatments in May and June. Patterns of abundance of sessile organisms were most pronounced for the month of July and abundance of most sessile organisms decreased in August. Therefore, only July data are reported for sessile organisms.

RESULTS

Spatial and Temporal Variation in Salinity

Measured surface salinities showed a seasonal pattern across all sites, with highest values occurring in the winter and early spring months (October to April) and lowest values occurring in the summer and early autumn (June to September) (Figure 3). Salinity in the Gulf Islands was consistently higher than in West Vancouver (ANOVA, $p<0.0001$), and this difference was greatest in the summer months (May to August).

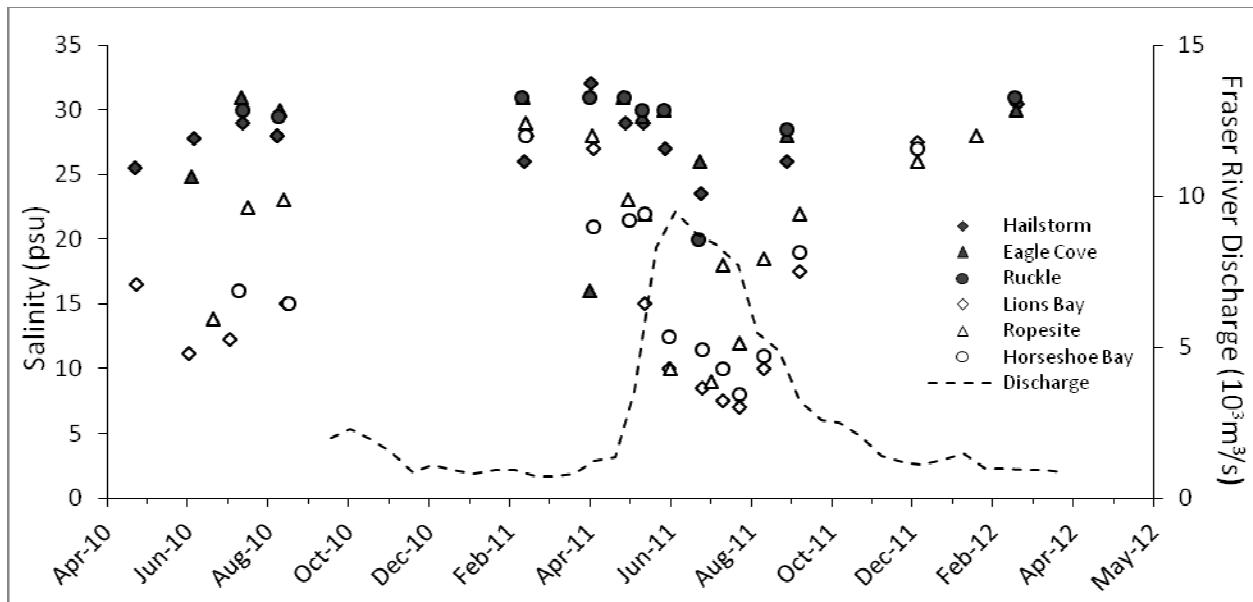


Figure 3. Measured surface salinity (psu) from sites in the Gulf Islands (shaded) and in West Vancouver (unshaded), British Columbia. Dashed line indicates Fraser River discharge rate ($10^3 \text{m}^3/\text{s}$) measured at Hope, British Columbia (Environment Canada, 2012). Surface salinity for Eagle Cove, April 7, 2011, was influenced by heavy rainfall.

Transect Surveys

Limpets were more abundant in the high salinity sites, and this difference increased throughout the summer (Figure 4A). *Lottia paradigitalis* was the numerically dominant limpet

species in both regions, followed by unknown juveniles. Both salinity and month were significantly correlated with limpet abundance, and there was a significant interaction between salinity and month (Repeated Measures MANOVA, $p<0.001$, $p=0.004$, $p<0.001$, respectively. See Table 2). Periwinkle (*Littorina* spp.) abundance varied throughout the summer in both regions, but was consistently greater in the high salinity sites (Figure 4B). Salinity had a significant effect on *Littorina* spp. abundance ($p<0.001$), while month showed a weak, non-significant effect ($p=0.069$), and the effect of salinity on periwinkles changed over time ($p<0.001$, Table 2).

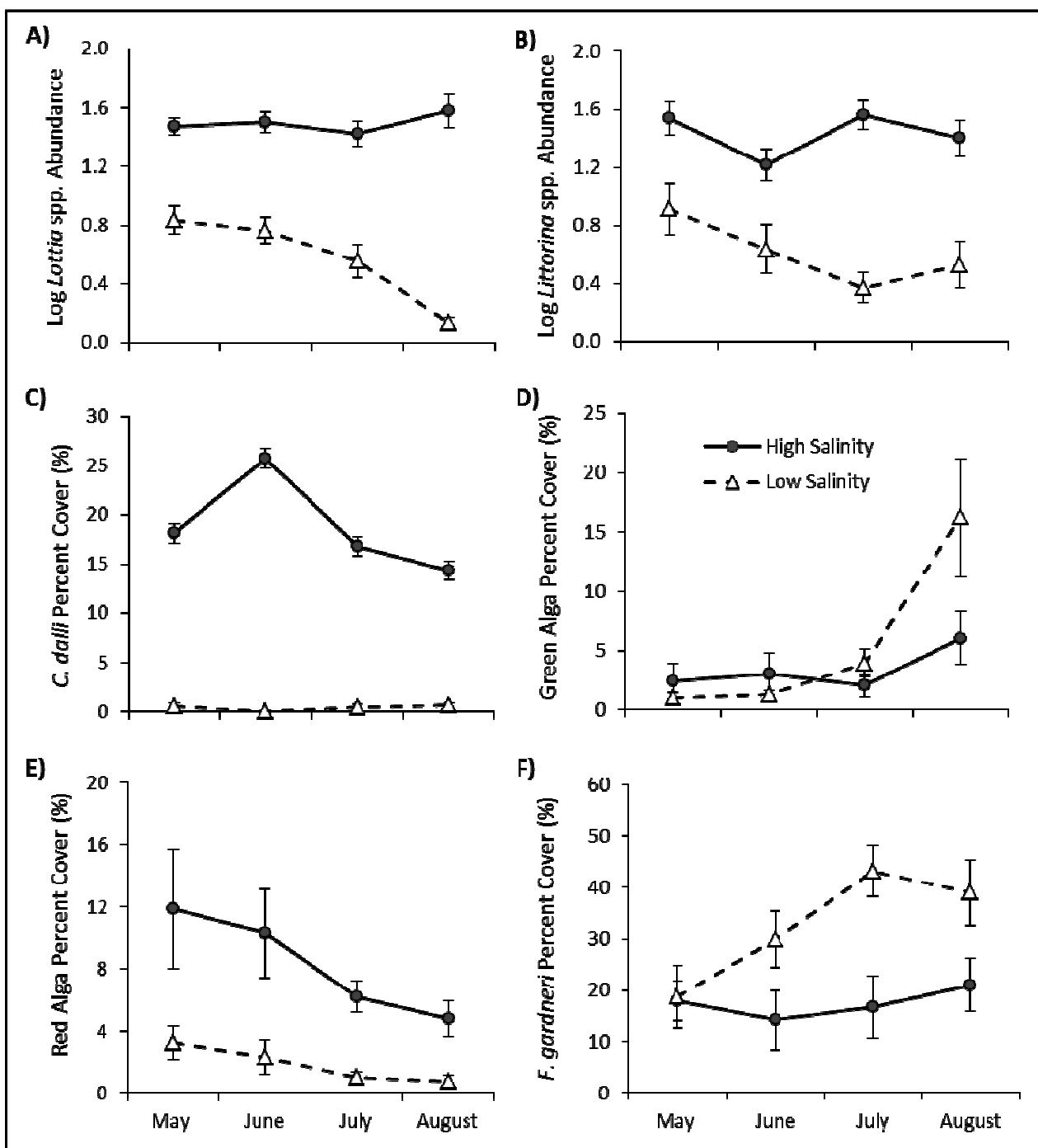


Figure 4. Abundance of invertebrates (A,B,C) and percent cover of algae (D,E,F) from transect surveys in high salinity sites (Gulf Islands) and low salinity sites (West Vancouver). Error bars indicate standard errors.

	Salinity			Month			Salinity*Month		
	df	F	p	df	F	p	df	F	p
<i>Lottia</i> spp.	1,42	180.55	<0.001	3,40	5.19	0.004	3,40	10.81	<0.001
<i>Littorina</i> spp.	1,42	81.93	<0.001	3,40	2.56	0.069	3,40	6.09	<0.001
<i>B. glandula</i>	1,42	5.50	0.024	3,40	13.28	<0.001	3,40	0.72	0.545
<i>C. dalli</i>	1,42	85.36	<0.001	3,40	4.49	0.008	3,40	5.49	0.003
<i>M. trossulus</i>	1,42	235.29	<0.001	3,40	5.71	0.013	3,40	5.02	0.024
Green Algae	1,42	2.49	0.122	3,40	7.74	<0.001	3,40	4.81	0.006
Red Algae	1,42	12.12	0.001	3,40	2.59	0.066	3,40	0.75	0.530
<i>F. gardneri</i>	1,42	9.84	0.003	3,40	5.61	0.003	3,40	4.35	0.010

Table 2. Statistical analyses of the effect of Salinity (high vs low) and Month (May through August) on the abundance of organisms (Repeated Measures MANOVA). Significant effects ($p<0.05$) are highlighted in bold.

Percent cover of *Chthamalus dalli* was greater in the high salinity region and remained consistently near zero in the low salinity region (Figure 4C). The greatest difference in percent cover occurred in June, with peak abundance in the high salinity region, and the smallest in August, as abundance in the high salinity region decreased. Both salinity and month also had significant effects on the abundance of *Balanus glandula* and *Mytilus trossulus* (Appendix A, Figures i and ii).

Green alga cover was dominated by *Ulva* spp. in both regions. Percent cover was low from May to July and increased in August in both regions, though much more substantially in the low salinity region (Figure 4D). *Urospora* spp. was present but rare. Percent cover of red algae was greater in the high salinity region, and showed a weak and non-significant decrease over the sampling period in both regions (Figure 4E). Red algae in the high salinity region were consistently dominated by *Mastocarpus* spp. and also included *Endocladia muricata*, *Microcladia* spp. and *Hildenbrandia* spp. In the low salinity region red algae included *Mastocarpus* spp., *Polysiphonia* spp. and *Melanosiphon intestinalis*. *Porphyra perforata* was present in high quantities in the high salinity region in May and June, but decreased significantly

in July and August (Appendix A, Figure iii). It was not present in the low salinity region during our sampling period. *Fucus gardneri* showed an increasing trend in both regions over time, but increased more rapidly and was more abundant in the low salinity region (Figure 4F). The greatest difference in abundance occurred in July.

Tolerance Experiments

Survival of *L. pelta* from different salinity regions was significantly affected by salinity and the effect varied by population (Fixed Effects ANOVA, $p<0.0001$ for each factor, Figure 5). Survival of limpets from separate regions differed significantly only in the 11psu salinity treatment (ANOVA, $p=0.0232$, Figure 5C). Survival was high for both populations above 11psu and low for both populations below 11psu. There was no significant effect of tidal treatments on salinity tolerance (Appendix B, Figure i).

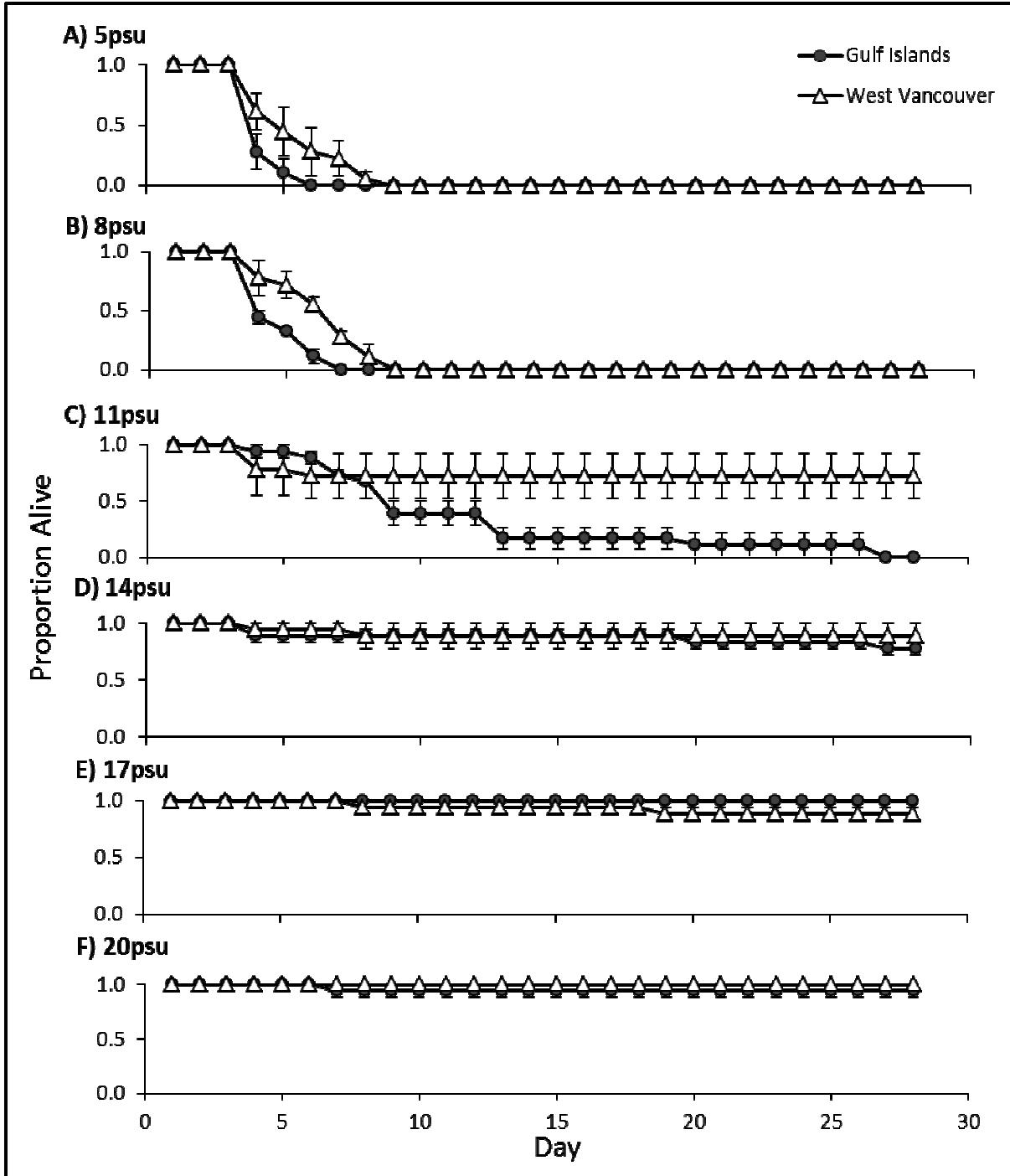


Figure 5. Mean proportion of limpets from the Gulf Islands and West Vancouver alive at each day for six salinity treatments (n=3). Error bars indicate standard error.

Net productivity of *Ulva* sp. was significantly affected by salinity treatment (Least-squares regression, $R^2=0.482$, $p<0.0001$) with the most positive change in mass at 15psu and the

lowest at both 0psu and 30psu (Figure 6A). ETRmax showed a similar relationship, with a maximum value at 20psu and minimum at 0psu ($R^2=0.712$, Figure 6B).

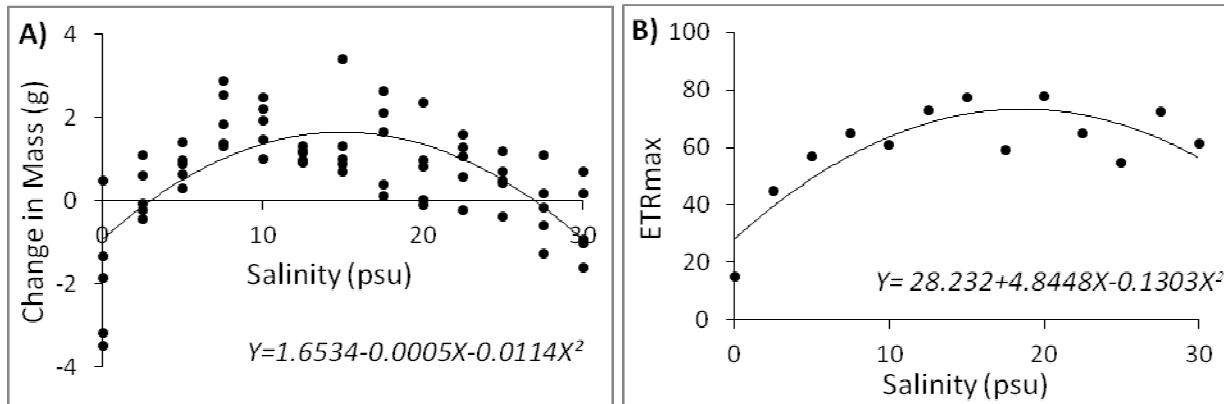


Figure 6. (A) Change in mass (g) and (B) ETRmax of *Ulva* sp. vs. salinity. Lines indicate least-squares regression.

Field Exclusion Experiments

Treatments in the high salinity region were successful in maintaining desired limpet densities between sampling events. Limpet abundance was significantly greater in the inclusions when measured prior to monthly grazer treatment adjustments (Tukey HSD, $p<0.001$, Table 3). Limpet abundance was consistently low in control plots and did not differ significantly from the exclusions at the time of sampling. Inclusion treatments in the low salinity region were not successful in retaining limpets. Limpet abundance in all low salinity treatments did not differ significantly from high salinity controls and exclusions (Appendix C, Figure i). Periwinkle abundance was greater in the high salinity region ($p<0.001$) but did not differ between treatments (Appendix C, Figure ii). These patterns were most apparent in June and July. May and August showed less differentiation across treatments and salinity regions.

	Salinity			Treatment			Salinity*Treatment		
	df	F	p	df	F	p	df	F	p
<i>Lottia</i> spp.	1	59.67	<0.001	2	16.19	<0.001	2	11.25	<0.001
<i>Littorina</i> spp.	1	66.55	<0.001	2	1.17	0.314	2	0.31	0.736
<i>B. glandula</i>	1	25.87	<0.001	2	5.41	0.006	2	1.61	0.204
<i>C. dalli</i>	1	24.94	<0.001	2	12.10	<0.001	2	15.78	<0.001
<i>M. trossulus</i>	1	2.35	0.128	2	0.93	0.397	2	0.85	0.431
Green Algae	1	49.62	<0.001	2	2.96	0.056	2	7.04	0.001
Red Algae	1	12.17	<0.001	2	1.09	0.340	2	0.85	0.431
<i>F. gardneri</i>	1	6.32	0.013	2	2.55	0.082	2	0.59	0.556

Table 3. Statistical analyses of the effects of salinity (high vs. low) and treatment (exclusion, control, inclusion) on the abundance of organisms (Fixed Effects ANOVA) for July. Significant effects ($p<0.05$) are highlighted in bold.

Abundance of *B.glandula* exceeded that of *C. dalli* across treatments. *B. glandula* was significantly lower only in the control plots in the low salinity region (Figure 7A). Abundance of *C. dalli* in the high salinity region was highest in control plots, followed by inclusions and lowest in the exclusions (Figure 7B). Abundance in all low salinity treatments did not differ significantly from high salinity inclusions or exclusions. There were no significant differences in *Mytilus trossulus* percent cover across treatments or salinity regions (Table 2).

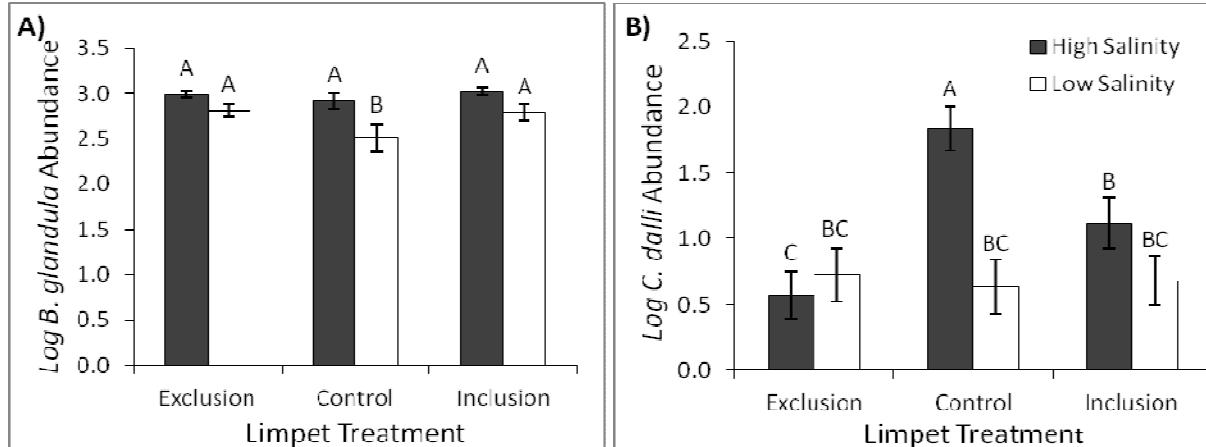


Figure 7. Mean abundance of A) *Balanus glandula* and B) *Chthamalus dalli* in each treatment at high and low salinity sites for July. Error bars indicate standard error. Letters indicate results of Tukey's HSD.

Percent cover of green algae consisted exclusively of *Urospora* sp. and *Ulothrix* sp. in both regions. Percent cover in the high salinity region was lowest in treatments exposed to grazing, the control and inclusion. Percent cover in exclusion treatments was high and did not differ significantly from all treatments in the low salinity region (Figure 8). Percent cover of red algae consisted almost exclusively of *Mastocarpus* spp. and was greater in the low salinity region, but with no effect of limpet treatment (Figure 9A). Similarly, percent cover of *Fucus gardneri* was significantly greater in the low salinity region (Figure 9B). There was a weak, non-significant trend toward higher abundance in the control plots.

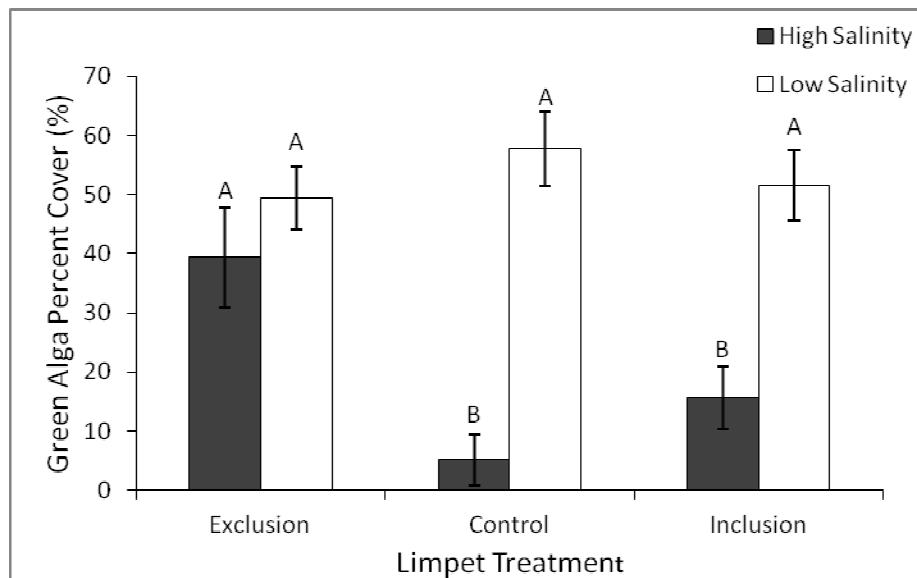


Figure 8. Mean percent cover of green algae in each treatment in high and low salinity regions for July. Error bars indicate standard error. Letters indicate results of Tukey's HSD.

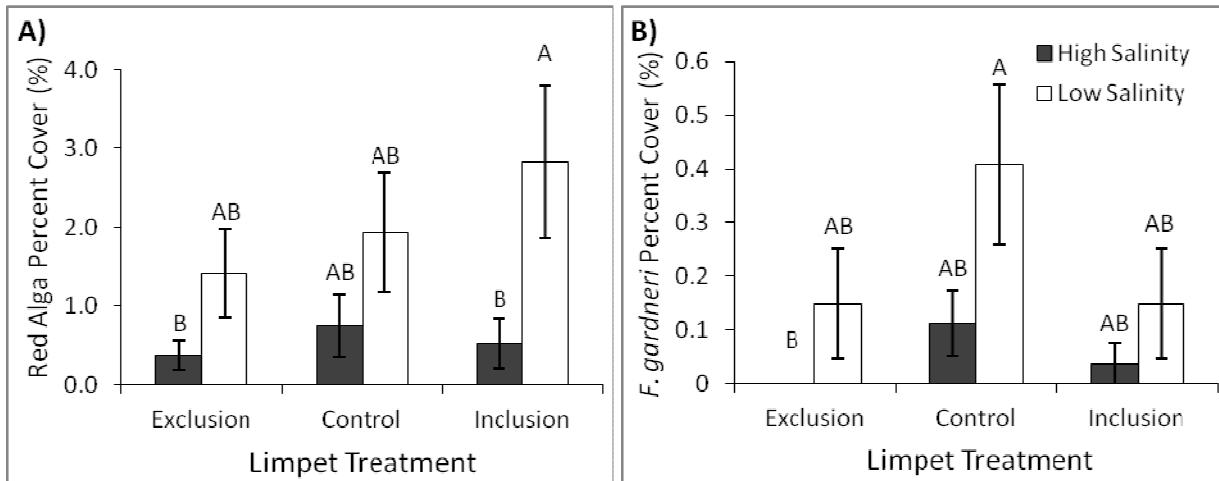


Figure 9. Mean percent cover of A) red algae and B) *Fucus gardneri* in each treatment in high and low salinity regions for July. Error bars indicate standard error. Letters indicate results of Tukey's HSD.

DISCUSSION

Rising atmospheric CO₂ concentrations are expected to heterogeneously alter oceanic salinity via changes in the global hydrological cycle (Held and Soden 2006). Over the past sixty years, oceanic surface salinity has decreased in the high latitudes due to a 7% increase in precipitation, and increased in the mid to low latitudes due to a 3% increase in evaporation, consistent with the enhanced hydrological cycle and sea surface warming predicted by climate change models (Helm et al. 2010; Durack and Wijffels 2010; Nurhati et al. 2011). In coastal areas, climatic models have shown that changing patterns of precipitation and snowmelt will alter runoff from major rivers, causing associated changes in the salinity of estuarine systems (Melack et al. 1997; Huang and Vikram 2010). Continued amplification of global freshwater flux may lead to changes in ocean circulation patterns and even reinforce salinity change by altering ocean thermal structure (Williams et al. 2006). Climate induced salinity changes may therefore prove influential in shaping the future of coastal communities.

Effects of Salinity on Species Abundance and Distribution

The patterns of surface salinity variation that emerged in our analysis are consistent with our expectations. Salinities show a seasonal pattern of decline following the period of peak runoff in mid-summer. Salinity in West Vancouver is lower than in the Gulf Islands, and this difference is more pronounced in the mid to late summer, with a minimum value of 7psu in late July in West Vancouver, compared to a minimum of 20psu in late June in the Gulf Islands. Surface salinity values for winter months are lacking for our sites during our study period, though the trends in our data and information available from nearby sites suggest a relatively uniform and high salinity across all sites in winter months (Fisheries and Oceans Canada 2012; Held and Harley 2009). Additionally, rainfall events can greatly affect surface salinity, and may account for some of the observed variability, particularly in early spring and late fall.

The physical environmental variables affecting intertidal organisms can vary widely across very small spatiotemporal scales, creating considerable heterogeneity across microhabitats within a single location (Lively *et al.* 1993; Menconi *et al.* 1999; Helmuth and Hofmann 2001). Despite this patch variability, several distinct patterns emerged from our transect data. The

divergent pattern in limpet abundance between regions over the summer reflects the influence of the low salinity riverine output. Limpets in the high salinity region were able to survive the high temperatures and increased desiccation stresses associated with afternoon low tides, and maintain a fairly constant abundance throughout the summer. Limpets in the low salinity region nearly disappeared as surface salinity approached freshwater levels, likely as a result of osmotic stress related mortality. Periwinkle abundance was much more variable in both regions, but showed a similar pattern of consistently high values in the high salinity region and decreasing values in the low salinity region. Abundance appeared to stabilize by the end of the summer, and this may be a result differences in behavioural response to salinity stress. Limpets respond to osmotic stress by tightly adhering to rock surfaces (Arnold 1957; Hoyaux *et al.* 1976), and this may be less effective than the strategy employed by *Littorina* spp., which involves tightly closing the operculum (Mcalister and Fisher 1968; Hoyaux *et al.* 1976). The large surface area of the limpet foot may also cause them to succumb more quickly to hypo-osmolality (Arnold 1957; Mcalister and Fisher 1968).

Percent cover of *Balanus glandula* was slightly higher in the high salinity region, though there was much variability across sites. Percent cover increased over the summer in both regions. Settlement of new recruits was observed only in May, and the increase in percent cover was likely a result of juvenile and adult growth. Studies of salinity effects on barnacles have shown that many species display a high tolerance to salinity stress, as well as a high capacity for adaptation, even within a single generation (Dineen and Hines 1994; Qiu and Qian 1999). Percent cover of *Chthamalus dalli* was consistently low in the low salinity region and variable and high in the high salinity region. Although salinity is likely to have some effect on this species, the lack of seasonal pattern resembling the change in salinity suggests other factors may be controlling *Chthamalus* sp. abundance. Presence of *Chthamalus* was observed, though not measured, at higher tidal heights in the low salinity region, which suggests that salinity may be directly or indirectly influencing zonation patterns. Percent cover of *Mytilus trossulus* was consistently low in the high salinity region, and increased over the summer in the low salinity region, showing an apparent positive response to low salinity conditions. Related work on this species has shown that low salinity conditions reduce predation by sea stars (Harley – unpublished data).

Percent cover of green algae remained stable and low throughout most of the summer, and then increased dramatically in both regions in August. This sudden increase may have been in response to improved abiotic conditions, such as nutrient availability, or a result of the timing of new recruitment of *Ulva* spp. The change was much more dramatic in the low salinity region, with percent cover increasing more than ten-fold compared to July levels. This suggests a positive effect of low salinity on green algae, which may be indirectly driven by limpet density. Percent cover of red algae in the high salinity region is greater, but decreases sharply in the late summer. This decline is likely a result of increased temperature and desiccation stress. Because the percent cover in the low salinity region never approaches that of the high salinity region, the exposure to hyposaline conditions in the summer may be enough to prevent red algae from ever reaching high abundance. Additionally, red algae may be influenced indirectly through community level interactions, such as competition with the highly abundant *F.gardneri*. Percent cover of *F.gardneri* increases over the summer in both regions, though much more rapidly in the low salinity region. The increase may be the result of improving abiotic conditions, and the difference between regions may be influenced by the abundance of limpets, which have been shown to affect adult abundance of *Fucus* spp. by grazing spores (Lodge 1948).

Salinity Tolerance of Limpets and Green Algae

Limpet survival is strongly compromised below 11psu. At higher salinities, limpets showed a dramatic increase in survival. The differences observed between the two populations at 11psu, and to a lesser extent at 8psu, suggest local adaptation of limpets from West Vancouver to hyposaline conditions. Despite this propensity for population adaptation, limpets were not able to survive exposure to the full range of salinities potentially encountered in West Vancouver. In a natural environment, limpets are released from salinity stress during low tides. However, periodic removal of limpets from the salinity treatments had no effect on survival, indicating that periodic release from salinity stress during low tides does not reduce salinity stress. Based on our results, limpet populations would be expected to experience substantial mortality after a week of exposure to salinity below 11psu. This strengthens our hypothesis that the observed decline in limpet abundance in the West Vancouver region was a result of low surface salinity, which dropped below 11psu in June.

Net productivity of *Ulva* sp. was significantly influenced by salinity. Change in both biomass and photosynthetic efficiency peaked between 15psu and 20psu, despite the fact that the surface salinity had risen to 28psu at the time of collection. *Ulva* sp. displayed a relatively high tolerance to low salinity conditions, with considerable tissue degradation occurring only at 0psu. The variability in the change of biomass may have also been influenced by the differential cellular water uptake occurring at different salinities, which would be expected to increase with decreasing salinity. This may explain deviation of peak performance from 20psu in photosynthetic efficiency to 15psu in biomass change. Photosynthesis occurred at all salinities, albeit very weakly in 0psu. In this experiment, it was necessary to use distilled water in order to achieve a salinity of 0psu. Therefore, a lack of dissolved nutrients may have further influenced productivity. While limpet survival was greatest at high salinity, *Ulva* sp. performance declined at salinities between 25psu and 30psu. This may indicate a greater sensitivity of *Ulva* sp. to hypersaline conditions, and adaptation to conditions experienced in the Fraser River estuary.

Effects of Limpet Presence on Community Structure

Limpet inclusions in the high salinity region were successful at maintaining high limpet density, though mortality was high and limpets were often replaced. Exclusions and controls showed similar limpet abundances when sampled during low tide, likely because limpets tend to seek shelter in rock crevices during low tide and actively graze during wash periods (Branch 1981). The exclusion treatment resulted in greater percent cover of green algae than the control, suggesting that control plots were frequented by limpets. Inclusions in the low salinity region did not differ in limpet abundance compared to controls or exclusions. All limpets had to be replaced at every sampling event, and frequently died within twenty-four hours. This suggests that the added handling stress coupled with salinity and desiccation stress may strongly affect limpet survival. Abundance of *Littorina* spp. was high in both the inclusion and exclusion plots of the high salinity region, despite removal of all individuals during sampling events. *Littorina* spp. are less susceptible to control by copper rings (Harley 2006) and all plots were therefore subject to periwinkle grazing. *Littorina* spp. abundance was low in all treatments in the low salinity region, consistent with the patterns observed from transects.

Abundance of sessile organisms in all plots consisted only of barnacle recruits during the month of May. Recruits became identifiable in June, and a small amount of algae began to

appear. Differences between regions and treatments became visible in July, but were greatly reduced in August, as abundances of all organisms in both regions declined. This pattern may have been a result of increased desiccation stress as summer temperatures increased, which would have been expected to affect all plots equally, and illustrates the sensitivity of undeveloped communities to physical stress.

Abundance of *B. glandula* was greater in the high salinity region, though not by a large extent. This is consistent with the observed patterns of abundance from transect surveys. In the high salinity region, abundance of *C. dalli* was highest in the control treatment, followed by limpet inclusions and then exclusions. Facilitative interactions have been proposed to exist between grazers and barnacles, in which barnacle shading provides protection from desiccation and grazing reduces competition for space between barnacles and algae (Benedetti-Cecchi 2000; Lohse and Raimondi 2007). Dungan (1986) found an increase in *Chthamalus* cover in the presence of *Lottia* sp, while Harley (2006) found that *B. glandula* was negatively influenced by *Littorina* spp. and *C. dalli* was positively influenced. Our results suggest that *C. dalli* is positively influenced by limpet presence, but this effect was not seen in *B. glandula*. Abundance of both *B. glandula* and *Littorina* spp. were greater in the high salinity region, however, no causal links can be made from these data. The mechanism by which limpets positively affect barnacle growth requires further investigation.

Percent cover of green algae was significantly lower in treatments exposed to limpets than in those where limpets were absent. This pattern is consistent with our prediction that limpet grazing has a direct negative effect on the abundance of green algae. Percent cover of red algae was significantly greater in the low salinity region, in contrast to the pattern observed in the transect surveys. This cover consisted almost entirely of *Mastocarpus* spp. and, though different, was extremely small in both regions (0.5-2.5%). This pattern may reflect differences in the timing of settlement or species identity of *Mastocarpus* spp. between the two regions. The red alga community may also be influenced by more complex community interactions that we were not able to assess in this experiment. *F. gardneri* was more abundant in the low salinity region, similar to transect our results, though it did not appear to be affected by limpet grazing. The distributional patterns observed for these algae may therefore be predominately affected by other indirect mechanisms or abiotic factors not investigated in this study.

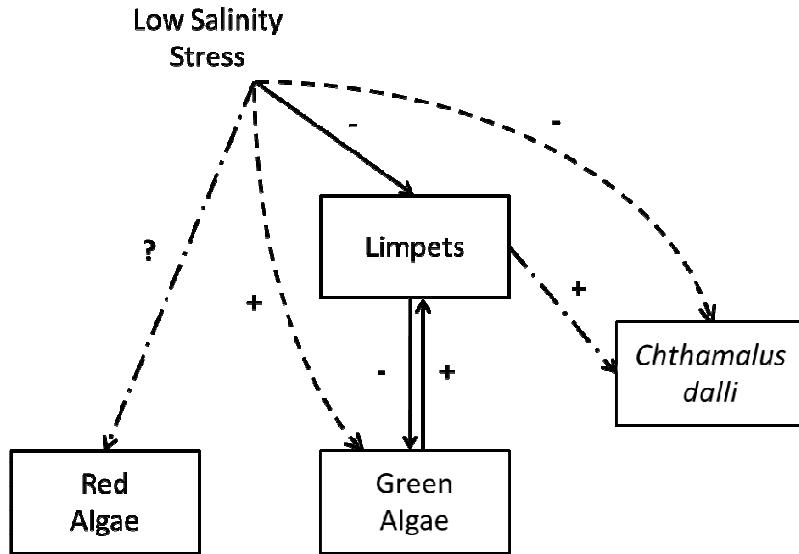


Figure 10. Conceptual diagram illustrating the effects of low salinity stress on intertidal communities. Low salinity has a direct, negative effect on limpet abundance, which has a direct negative effect on palatable green alga abundance due to grazing. Low salinity therefore has an indirect positive effect on green algae. Limpets have a positive effect on abundance of *Chthamalus dalli*, of which the mechanism is undetermined, thereby producing an indirect negative effect of low salinity on *C. dalli*. The effect of low salinity stress on red algae is unclear. Solid lines indicate direct effects, dashed lines indicate indirect effects, and dash-dot lines indicate unknown mechanisms. Symbols indicate the direction (positive, negative or unknown) of effects.

Conclusions

The effects of hypo-osmotic stress on intertidal organisms vary both within and among species. Large scale changes in oceanic salinity can therefore alter trophic dynamics, creating complex changes in community structure in coastal marine systems. Our surveys revealed dramatic differences in community composition between high and low salinity regions, in which the high salinity environments contained a high abundance of grazers, barnacles and red algae, whereas the low salinity environments were highly dominated by *F. gardneri*, *Mytilus trossulus* and green algae. The seasonal decline in salinity exceeded the tolerance of local limpets, suggesting that the physiological effects of low salinity directly drive the observed differences in limpet density between the two regions. Our experiments also confirmed that green algae have a wider tolerance for hyposaline conditions. When limpets are experimentally removed in high salinity regions, patterns of green algae and barnacle abundance resemble that of the low salinity region, suggesting that low salinity is indirectly driving these patterns through limpet mortality.

The results of our study illustrate how physiological stress can propagate through ecological systems, creating both positive and negative indirect effects on species abundance. Abiotic conditions that vary in both space and time can lead to dynamic patterns in species distribution and abundance along these spatiotemporal gradients. This study explored variation at a single depth over one season; future research may involve investigations of differences in vertical zonation patterns and across several seasons. Additionally, detailed studies on the mechanisms that drive these patterns would further our understanding of the ecology of this system.

The changes in oceanic salinity that are expected with climate change are patchy and highly dependent on the individual climatic trends in a given area. The effects of salinity changes in conjunction with increased temperature and acidification have not been largely considered. While intertidal populations have demonstrated a capacity for local adaptation, the expected changes to the global hydrological cycle are likely to alter the structure and composition of coastal communities. Understanding the effects of salinity on community level dynamics may therefore prove essential to predicting the direction of change in coastal ecosystems.

APPENDICES

Appendix A – Additional Transect Survey Results

Percent cover of *B. glandula* was greater in the high salinity region ($p=0.024$) and showed significant increase in both regions over the summer ($p<0.001$) (Figure 1). Percent cover of *M. trossulus* was greater in the low salinity region ($p<0.001$) (Figure 2). Percent cover increased over the summer in the low salinity region ($p=0.013$) while remaining consistently near zero in the high salinity region, creating an interactive effect salinity and time ($p=0.024$).

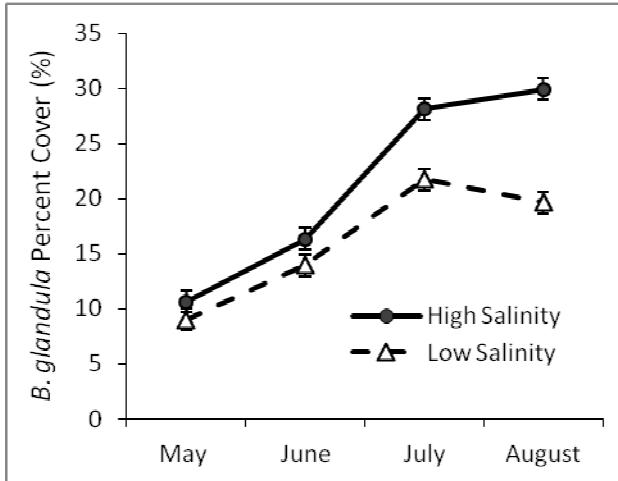


Figure i. Percent cover of *Balanus glandula* from transect surveys in high salinity sites (Gulf Islands) and low salinity sites (West Vancouver). Error bars indicate standard errors.

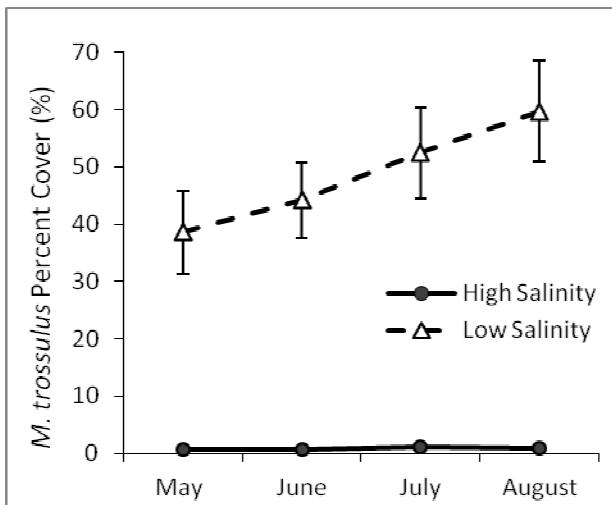


Figure ii. Percent cover of *Mytilus trossulus* from transect surveys in high salinity sites (Gulf Islands) and low salinity sites (West Vancouver). Error bars indicate standard errors.

Appendix B – Limpet Salinity Tolerance Experiments with Tidal Treatment

The proportion of *L. pelta* at the end of the experiment was significantly lower in the 10psu treatment than in the 20psu or 30psu treatment (Fixed Effects ANOVA, $p<0.0001$, Figure i). There was no significant effect of tidal treatment on survival ($p=0.8603$). An identical experiment performed with *L. digitalis* yielded a similar effect of salinity ($p<0.001$) and no effect of tidal treatment ($p=1.000$, Figure ii).

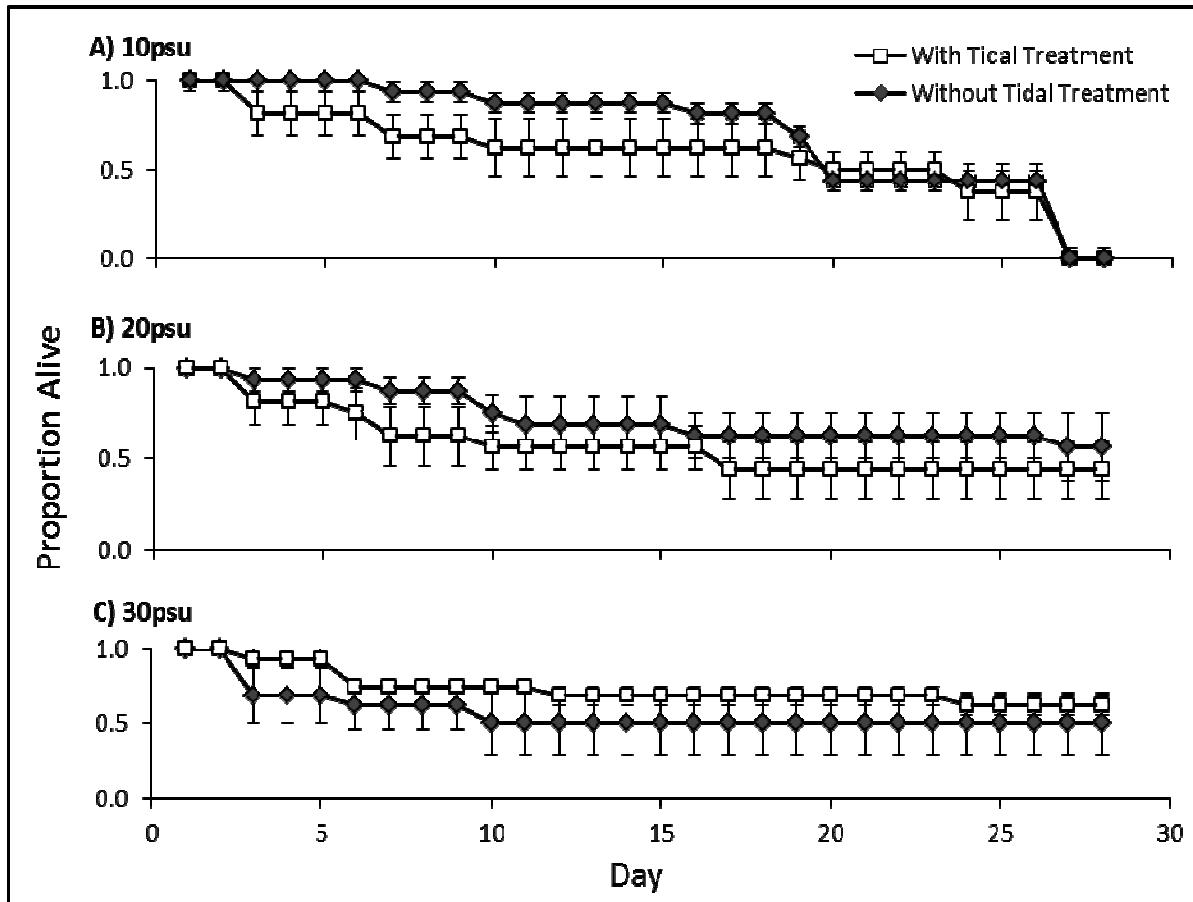


Figure i. Mean proportion of limpets (*Lottia pelta*) with and without tidal treatment alive at each day for six salinity treatments ($n=4$). Error bars indicate standard error.

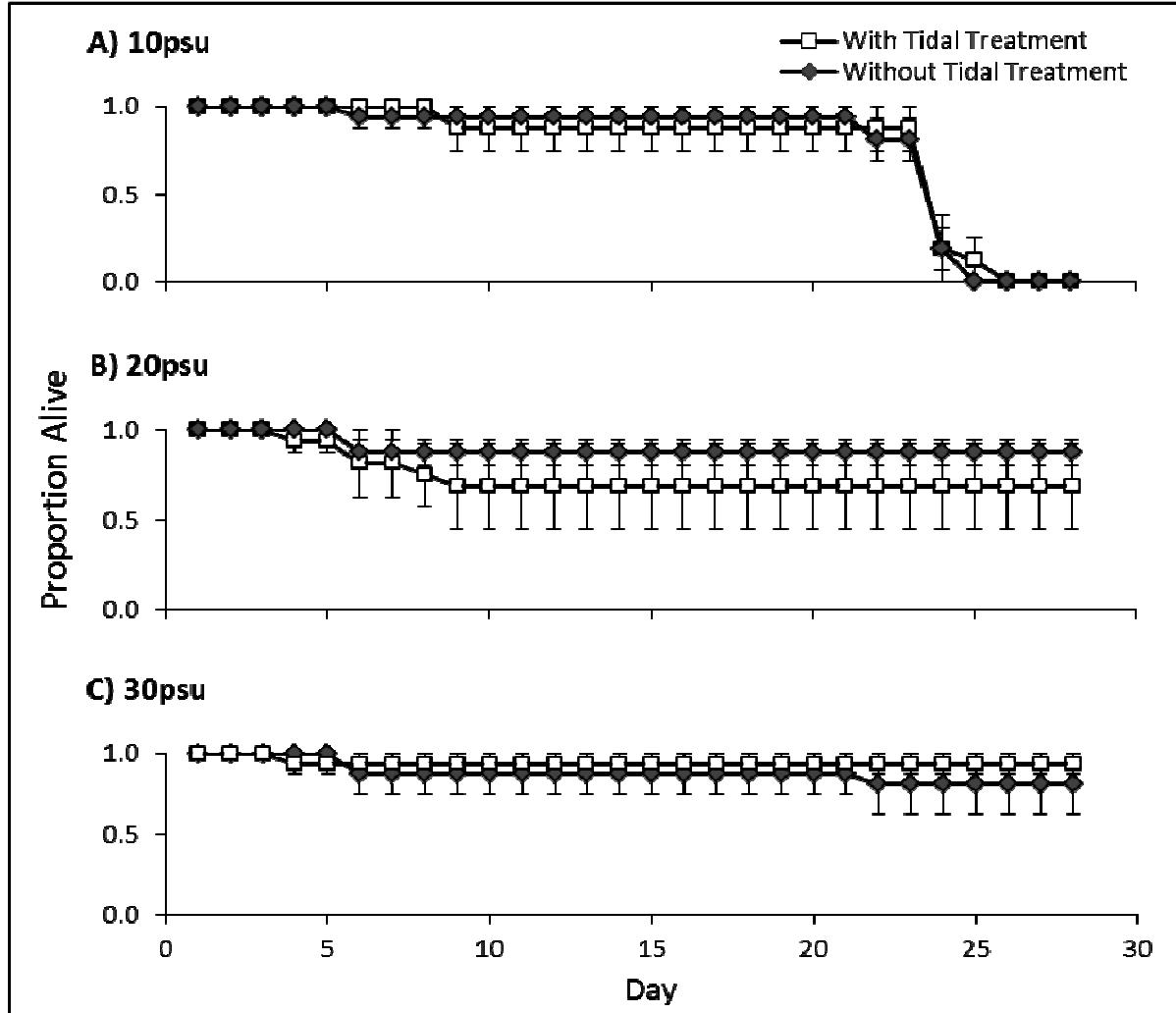


Figure ii. Mean proportion of limpets (*Lottia digitalis*) with and without tidal treatment alive at each day for six salinity treatments (n=4). Error bars indicate standard error.

Appendix C – Grazer Exclusion in Experimental Plots

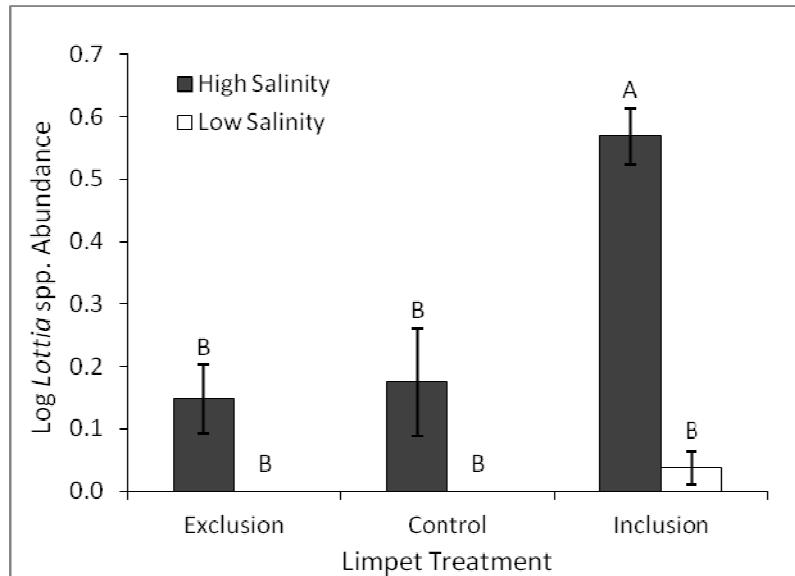


Figure i. Mean abundance of *Lottia* spp. in each treatment at high and low salinity for the month of July. Error bars indicate standard error. Letters indicate results of Tukey's HSD.

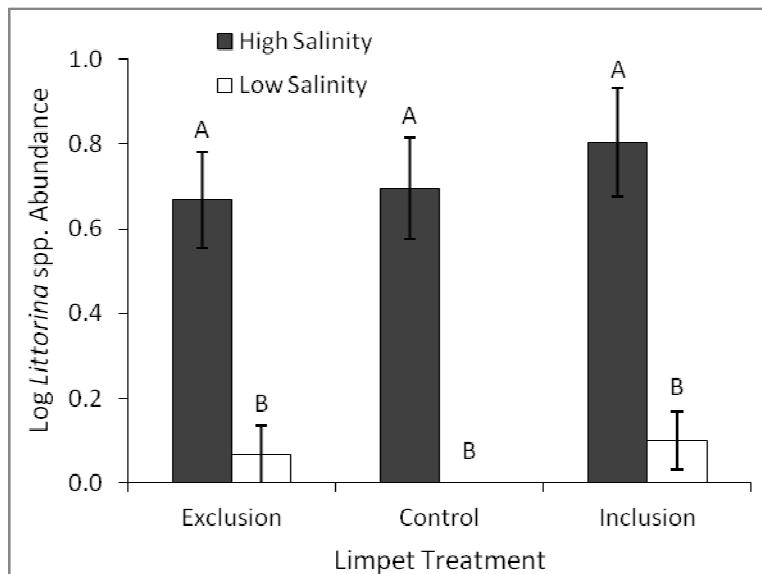


Figure ii. Mean abundance of *Littorina* spp. in each treatment at high and low salinity for the month of July. Error bars indicate standard error. Letters indicate results of Tukey's HSD.