SALINITY VARIATION, PLANT-HERBIVORE INTERACTIONS,

AND COMMUNITY STRUCTURE ON ROCKY SHORES

by

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**Abstract**

Species respond to variation in physical environmental conditions both directly, based on physiological tolerances, and 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 than at sites near the mouth of the Fraser River where salinity was lower and seasonally variable. Conversely, green and brown algae were more abundant 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 exceed the tolerance of limpets, *Lottia pelta*, despite a capacity for local acclimatization or adaptation, but not that of the green alga, *Ulva sp*. Field based herbivore exclusion experiments demonstrated that the effects of salinity on the abundance of *Ulva spp*. and the grazer-associated barnacle *Chthamalus dalli* are indirect and driven by limpet abundance. Our results emphasize the importance of community level interactions in determining spatial and temporal patterns of distribution and abundance across environmental gradients.

**Introduction**

Intertidal communities vary in space and time 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 the indirect effects on the interactions between species (Underwood 1999; Dahlhoff et al. 2002; Longtin et al. 2009). Interspecific interactions may alter, inhibit or exacerbate species responses to environmental stress (Harley et al. 2006; Hawkins et al. 2009). Such effects may influence the recruitment, settlement or survival of intertidal species, affecting patterns of succession and community dynamics (Farrell 1991; Connolly et al. 2001). Additionally, variation in abiotic conditions, which can occur daily, seasonally and in the long term in response to large scale geographical and climatological processes, further influences the dynamic nature of intertidal communities (Helmuth et al. 2002; Hsieh et al. 2005; Menge et al. 2011).

In estuarine ecosystems, salinity is one of the most important drivers of the distribution, abundance, and performance of organisms (Ritter et al. 2005; Schoch et al. 2006). Exposure to fresh water has been shown to induce physiological stress responses in marine gastropods, including decreased heart rate, reduced haemolymph osmolality and mortality (De Pirro et al. 1999; Chelazzi et al. 2001; Firth and Williams 2009), as well as disrupt ecological processes such as feeding, activity, reproduction and larval development rate (Cheung 1997; Zimmerman and Pechenik 1991). Similarly, low salinity levels have been found to reduce the survival, development and settlement of barnacle larvae, and subsequently influence adult distribution (Qiu and Qian 1999; Dineen and Hines 1994; Starczak et al. 2011). Hyposaline conditions also inhibit the growth and photosynthetic rate of many algal species (Luo and Liu 2011; Connan and Stengel 2011; Karsten 2007), although several species have demonstrated a wide salinity tolerance range (Chang et al. 1999; Rath and Adhikary 2005), as well as a capacity for local adaptation to low salinities (Nygard and Ekelund 1999, 2006; Nygard and Dring 2008). Salinity variation can therefore have broader implications for species distribution and abundance by mediating trophic interactions (Witman and Grange 1998; Nielsen and Gosselin 2011) and can influence larger scale diversity patterns (Zacharias and Roff 2001; Hampel et al. 2009; Rubal et al. 2012).

Estuarine salinity varies spatially, with salinity increasing with distance from the source of freshwater runoff, as well as temporally, in response to variation in precipitation and river outflow (Zacharias and Roff 2001; Ysebaert and Herman 2002). The Strait of Georgia, British Columbia, presents a unique and ideal environment for studying the effects of salinity on coastal communities. The 220 km 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 (Fig. 1). Seasonal variation in freshwater influx via the Fraser River, regularly exceeding a mean outflow rate of more than 7000 m³/s in summer months (Environment Canada 2012), causes a corresponding variation in sea surface salinity near the Fraser plume, with an annual drop from approximately 25 psu to less than 15 psu during peak discharge (Held and Harley 2009; Halverson and Pawlowicz 2011). This effect, however, declines with increasing distance from the estuary, with waters southwest of the Southern Gulf Islands maintaining salinities of 23 psu to 30 psu year round (Tully and Dodimead 1957; Halverson and Pawlowicz 2011).

Because grazers, particularly limpets, are known to control high intertidal community structure in the Northeast Pacific (Cubit 1984; Farrell 1991), and because limpet species have been shown to be highly susceptible to low salinity stress compared to algae and other invertebrates (see previous examples), 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 further 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. 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. Increased algal abundance has been shown to negatively impact barnacle species (Farrell 1991), and we therefore predicted lower barnacle abundance in low salinity sites. 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 at three sites within each of two regions with contrasting salinity regimes: West Vancouver, which experiences reduced salinities during the summer, and the Southern Gulf Islands, which experience consistently high salinities year-round. West Vancouver is located approximately 30km north of the Fraser River outflow, and the Southern Gulf Islands are located approximately 30 km southwest (Fig. 1). Gulf island sites are located on the southwest side of the island chain, and are not exposed to the Fraser River plume. The two areas are similar in terms of climate and topography. Sea surface temperature in the two regions is comparable, ranging from 5.0 to 18.5 in West Vancouver and 6.0 to 18.5 in the Gulf Islands (Fisheries and Oceans Canada 2009). The tidal range is greater in West Vancouver, with extreme high tides reaching 4.7 m above Canadian chart datum (approximated as the lowest astronomical tide), compared to 3.4 m above chart datum in the Gulf Islands. All sites were composed of granite rock except HS1, which was sandstone. The slope of the rock face ranged from 1 to 37º and aspect ranged from 40 to 320º east of magnetic north (Table S1).

*Transect Surveys*

Surveys were conducted once per month during low tide from May 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% submersion time. This occurs at 2.1 m in the Southern Gulf Islands and 3.0 m in West Vancouver. Ten meters of transect tape were laid across the selected area and eight randomly selected points were surveyed using a 25x25 cm quadrat. Mobile invertebrates were counted and sessile invertebrates and algae were measured by percent cover.

*Salinity Tolerance Experiments*

i) Salinity and tidal emersion tolerance of *Lottia* spp.

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 mimic of tidal exposure. Two experiments were performed, one with *L. pelta* and the other with *L. digitalis*, collected from HS1, Galiano Island, from a salinity of 32 psu. Four limpets were randomly assigned to one of twenty-four 1000 cm³ Ziploc® containers with mesh walls and two containers were randomly assigned to each of twelve 20 L aquaria containing seawater at 30 psu. Aquaria were covered, provided with compressed air and placed inside of a flow through sea water system to maintain a water temperature of 12°C. Salinity treatments of 30 psu, 20 psu and 10 psu were randomly assigned to each aquarium. Salinities were lowered by 2.5 psu per day with chilled, dechlorinated freshwater until the desired salinity was reached. To control for water changes, those treatments that had already reached target salinity were subjected to daily water replacements using filtered sea water of the target salinity in place of dechlorinated freshwater.

One randomly selected container within each aquarium was designated as the “intertidal” container, and the other as the “subtidal” container. At 10:00 every morning, the intertidal containers were removed from their aquaria to simulate exposure during low tide. At 18:00 every evening, the containers were placed back inside their aquaria. Each day, Limpets were examined for signs of mortality, including tissue damage, discolouration and rigidity, and dead limpets were removed. The experiment continued for twenty-eight days, and limpets were not fed during this time.

ii) Salinity tolerance and local adaptation of *L. pelta*

*L. pelta*, 20±5 mm in length, were collected from HS1, Galiano Island, from a salinity of 27 psu and four days later from LS3, West Vancouver, from a salinity of 10 psu. Limpets from the high salinity site were randomly divided into eighteen containers, for a total of six limpets in each. Each container was placed inside of an aquarium containing seawater at 30 psu. The salinity of the water within these aquaria was lowered by 2.5 psu per day until a salinity of 20 psu was reached. Limpets were allowed to acclimatize to this salinity for ten days. Limpets from the low salinity site were randomly divided into an additional eighteen containers, and all containers were placed into aquaria containing seawater at 10 psu, which was increased at increments of 2.5 psu per day to 20 psu. These limpets were allowed to acclimatize for six days. After the acclimatization period was complete, containers were randomly arranged into eighteen aquaria, all containing seawater at 20 psu, so that each aquarium contained one container of limpets from the high salinity site and one from the low salinity site. Aquaria were randomly assigned salinity treatments of 5, 8, 11, 14, 17 and 20 psu. Salinities were lowered at a rate of 3 psu every 30 minutes until the desired salinity was reached, and limpets remained submerged for twenty-eight days.

iii) Salinity Tolerance of *Ulva* sp.

*Ulva sp.* was collected from LS2 West Vancouver, from a salinity of 28 psu. 5-6 g of blot dried *Ulva sp*. was placed into each of sixty-four 1 L plastic bottles. Each bottle was randomly assigned a salinity treatment from 0-30 psu at intervals of 2.5 psu and provided with compressed air. The 0 psu treatment contained only distilled water, while all other treatments contained combinations of filtered seawater at 31 psu and dechlorinated freshwater at 1 psu. Bottles were placed inside of a flow through sea water system to maintain a water temperature of 12°C and provided 25±5 µmol 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*

Seven subsites within each of the six study sites (HS1, HS2, HS3, LS1, LS2 and LS3) were manually cleared of organisms. Within each subsite, a limpet inclusion, exclusion and control plot were set. Inclusions and exclusions were formed by securing two copper fences, 2.5 cm high and 28.5 cm in diameter, to the rock face using Quickcrete® quick drying cement. Copper enclosures/exclosures of this type have been shown to be effective barriers to limpets (Harley 2002) and partial barriers to periwinkles (Harley 2006). Four *L. pelta*, 20±5 mm in diameter, were collected from the nearby shoreline within each site and placed into rings designated as limpet inclusion treatments. This density of 0.63 limpets per 100 cm², approximately corresponds to the average density of limpets in low salinity sites as determined by previous survey data (0.55 per 100 cm² in low salinity sites and 3.65 per 100 cm² in high salinity sites). *L. pelta* were determined 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). Any other grazers found inside rings were removed. Limpet treatment densities were maintained every two weeks by adding or removing limpets as necessary. One circular plot within each area, also 28.5 cm in diameter, was marked with steel bolts and served as a control. The position of control and treatments was randomized within each subsite. Copper controls were not included in this study, as previous work has shown that partial copper treatments lead to partial effects which are difficult to interpret (Johnson 1992).

Sampling occurred once per month during low tide from May to August. A 10x10 cm quadrat was used to count mobile invertebrates and barnacles and estimate percent cover of algae and mussels within each treatment. Salinity samples were taken at each sampling event and measured using a refractometer (S/Mill-E, Atago Inc.).

*Statistical Analyses*

Survey data for each species were analyzed with repeated measures ANOVA to determine differences in abundance between salinity regions and over time. Site was nested within salinity region. *Littorina* spp., limpet and green and red alga species were lumped into respective groups for statistical analyses. Mobile invertebrate count data were log transformed.

Salinity tolerance of *L. pelta* from experimental manipulations was determined using fixed effects ANOVA to analyze 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 the month of July were analyzed for each species using fixed effects ANOVA to determine differences in abundance between treatments and salinity regions. Abundance of sessile organisms was low and similar across all treatments in May and June, and patterns were most pronounced for the month of July. Limpet, periwinkle, and green and red alga species were combined into respective groups. *Porphyra perforata* was considered a palatable species and was therefore analyzed separate from the unpalatable red alga group. Unidentifiable barnacle recruits (individuals <2mm basal diameter) were not included in the analyses of *Balanus glandula* and *C. dalli*. Count data for barnacles and mobile invertebrates were log transformed, and Post-hoc Tukey HSD analyses were performed to determine the degree of difference between group means.

**Results**

*Spatial and Temporal Variation in Salinity*

Surface salinities showed a seasonal pattern across all sites, with highest salinities occurring in the winter and early spring months (October to April) and lowest salinities occurring in the summer and early autumn (June to September) (Fig. 2). 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).

*Transect Surveys*

Limpets were significantly more abundant in the high salinity sites, and this difference increased throughout the summer (Fig. 3a; Repeated Measures MANOVA, P<0.001). *Lottia paradigitalis* was the numerically dominant limpet species in both regions, followed by unknown juveniles. Similarly, *Littorina* spp. abundance was significantly greater in high salinity sites, and this difference also increased over time (Fig. S1a; Table S2).

Percent cover of *Chthamalus dalli* was significantly greater in the high salinity region and remained consistently near zero in the low salinity region (Fig. 3b; Repeated Measures MANOVA, P<0.001). Abundance of both *B. glandula* and *Mytilus trossulus* increased significantly over time, but abundance of *B. glandula* was greater in the high salinity sites, whereas *M. trossulus* abundance was greater inlow salinity sites(Fig. S1b,c; Table S2).

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 (Fig. 3c). Filamentous green algae were present but rare. Percent cover of red algae was significantly greater in the high salinity region (Fig. 3d; Repeated Measures MANOVA, P<0.001). Red algae in the high salinity region were consistently dominated by *Mastocarpus* spp. in both the upright and crustose form 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 in July and August. 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 (Fig. S1d).

*Tolerance Experiments*

Survival of *L. pelta* from both regions was significantly greater above 11 psu than below (Fig. 4; ANOVA, P<0.0001). Survival of limpets from separate regions differed significantly only in the 11 psu salinity treatment, in which limpets from the low salinity region had greater survival (Fig. 4c; ANOVA, P=0.023). There was no significant effect of tidal treatments on salinity tolerance (Fig. S2, S3).

Net productivity of *Ulva* sp. was significantly affected by salinity treatment (Least-Squares Regression, R²=0.482, P<0.0001) with the greatest gain in mass at 15 psu and net losses at both 0 psu and 30 psu (Fig. 5a). ETRmax showed a similarly unimodal relationship, with a maximum value at 20 psu and minimum at 0 psu (Fig. 5b; R²=0.712).

*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 (Fig. S4a; Tukey HSD, P<0.001). Limpet abundance was consistently low in control plots and did not differ significantly from the exclusions at the time of sampling. Due to extensive mortality, inclusion treatments in the low salinity region were not successful in retaining limpets. Therefore, limpet abundance in all low salinity treatments did not differ significantly from high salinity controls and exclusions at the time of sampling.

Periwinkle abundance was greater in the high salinity region (P<0.001) but did not differ between treatments (Fig. S4b). *B. glandula* was more abundant than *C. dalli* across treatments (Fig. S4c). In the high salinity region *C. dalli* was most abundant in control plots, followed by inclusions and then exclusions (Fig. 6a).

Percent cover of green algae consisted exclusively of *Urospora* sp. and *Ulothrix* sp. in both regions. Green algae were less abundant in grazed treatments (control and inclusion) in the high salinity region, and were abundant and unaffected by treatment in the low salinity region (Fig. 6b). Percent cover of red algae consisted almost exclusively of crustose *Mastocarpus* sp., and both red algae and *F. gardneri* were greater in the low salinity region and unaffected by limpet treatments (Fig. 6c, S4d).

**Discussion**

The effects of hypo-osmotic stress on intertidal organisms vary both within and among species. Large scale changes in oceanic salinity can alter trophic dynamics, creating complex changes in the community structure of coastal marine systems. Our surveys revealed dramatic differences in community composition between high and low salinity regions; 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*, *M. 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 than limpets. 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 by reducing herbivore pressure (Fig. 7).

*Effects of Salinity on Species Abundance and Distribution*

Consistent with our expectations, sea surface salinity showed a seasonal pattern of decline during the period of peak Fraser River discharge in mid-summer. Salinity in West Vancouver was lower than in the Gulf Islands, and this difference was more pronounced in the mid to late summer. Surface salinity was sampled infrequently at our sites during the winter months, but the trends in our data and information available from nearby sites within the two regions suggest a relatively uniform and high salinity across all sites in winter months (Fisheries and Oceans Canada 2009; Held and Harley 2009). Rainfall events may account for some of the observed variability in surface salinity, particularly in early spring and late fall.

The difference 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 thereby maintain a fairly constant abundance throughout the summer. Limpets in the low salinity region nearly disappeared as surface salinity plummeted, 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. Periwinkles respond to osmotic stress by tightly closing the operculum (Mcalister and Fisher 1968; Hoyaux et al. 1976), which may be more effective than the strategy of tightly adhering to rock surfaces that is observed in limpets, which lack an operculum (Arnold 1957; 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).

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 acclimation (Dineen and Hines 1994; Qiu and Qian 1999). The barnacle, *B. glandula* was more abundant in the high salinity region, though with considerable variability among sites, and percent cover increased in both regions over the summer. Settlement of new recruits was observed only in May, and the increase in percent cover was likely a result of juvenile and adult growth. Percent cover of *C. dalli* was consistently low in the low salinity region and high, but variable, 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 *C. dalli* abundance. Presence of *C. dalli* was observed, though not measured, at higher tidal heights in both regions, which suggests that salinity may be directly or indirectly reducing the vertical distribution range of the species. *C. dalli* may be negatively influenced by the low abundance of *Littorina* spp. in the low salinity region, which have been shown to facilitate recruitment of *C. dalli* (Harley 2006).

Green algae remained stable and low throughout much of the summer and increased sharply in August. This 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. The brown alga, *F. gardneri*, similarly increased more rapidly in the low salinity region, and may also be influenced by the presence of limpets, which have been shown to affect adult abundance by grazing spores (Lodge 1948). Red algae was more abundant in the high salinity region than in the low, but decreased sharply in the late summer. This decline may have been the result of increased temperature and desiccation stress, suggesting reds may be less stress-tolerant than greens. Because the 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*.

*Salinity Tolerance of Limpets and Green Algae*

Based on our salinity tolerance experiments, we have shown that limpet survival is strongly compromised below 11 psu. The differences observed between the two populations at 11 psu, and to a lesser extent at 8 and 5 psu, suggest local acclimation or 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 salinities less than 11 psu. 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 11 psu in June.

Photosynthetic performance and net productivity of *Ulva* sp. was significantly influenced by salinity. Change in both biomass and photosynthetic efficiency peaked between 15 psu and 20 psu, despite the fact that the surface salinity had risen to 28 psu at the time of collection. *Ulva* sp. displayed a relatively high tolerance to low salinity conditions, with considerable tissue degradation occurring only at 0 psu. The variability in the change of biomass may have been influenced by the differential cellular water uptake occurring at different salinities, which would be expected to increase with decreasing salinity. This could explain deviation of peak performance from 20 psu in photosynthetic efficiency to 15 psu in biomass change. Photosynthesis occurred at all salinities, albeit very weakly in 0 psu. In this experiment, it was necessary to use distilled water in order to achieve a salinity of 0 psu, therefore, a lack of dissolved nutrients may have further influenced productivity. While limpet survival was greatest at 30psu, *Ulva* sp. performance declined at salinities between 25 psu and 30 psu, which indicates 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 had to be replaced often. Exclusions and controls showed similarly low 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 still being grazed by limpets. Inclusions in the low salinity region were not successful in maintaining limpets; the animals had to be replaced at every sampling event, frequently dying within twenty-four hours. This may be due to the inability of limpets to seek adequate refuge from low salinity waters, or to the added handling stress coupled with salinity and desiccation stress. Green algae were significantly lower in treatments successfully 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.

*Littorina* spp. was abundant in both the inclusion and exclusion plots of the high salinity region, despite repeated 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 surveys.

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. *B. glandula* was greater in the high salinity region, though not by a large extent. 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 structure provides protection from desiccation and grazing reduces competition for settlement space between barnacles and algae (Benedetti-Cecchi 2000; Lohse and Raimondi 2007). Dungan (1986) found an increase in *Chthamalus* spp. cover in the presence of *Lottia* spp., 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*. *B. glandula* and *Littorina* spp. Were both more abundant in the high salinity region, though no causal links can be made from these data. The mechanism by which limpets positively affect barnacle growth requires further investigation.

Percent cover of red algae was significantly greater in the low salinity region, in contrast to the pattern observed in the surveys. This cover consisted almost entirely of *Mastocarpus* spp. and, though significantly 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 survey 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.

*Implications*

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. 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. Rising atmospheric CO² concentrations are expected to heterogeneously alter oceanic salinity via changes in the global hydrological cycle (Held and Soden 2006). 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 Mehta 2010). The effects of salinity changes in conjunction with increased temperature and acidification are poorly understood at best. 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 such change in coastal ecosystems.

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**Figure Legends**

**Fig. 1** Map of the study region. Low salinity sites are located in West Vancouver and high salinity sites are located in the Gulf Islands.

**Fig. 2** 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 (10³m³/s) measured at Hope, British Columbia (Environment Canada, 2012). Surface salinity for Eagle Cove, April 7, 2011, was influenced by heavy rainfall.

**Fig. 3** Mean abundance of invertebrates (a,b) and mean percent cover of algae (c,d) from transect surveys in high salinity sites (Gulf Islands) and low salinity sites (West Vancouver). Error bars indicate standard errors.

**Fig. 4** Mean proportion of *L. pelta* from the Gulf Islands and West Vancouver alive at each day for four of six salinity treatments (n=3). Patterns observed at 17 and 20 psu were similar to that of 14 psu. Error bars indicate standard error.

**Fig. 5** (a) Change in mass (g) and (b) ETRmax of *Ulva* sp. vs. salinity. Lines indicate least-squares regression.

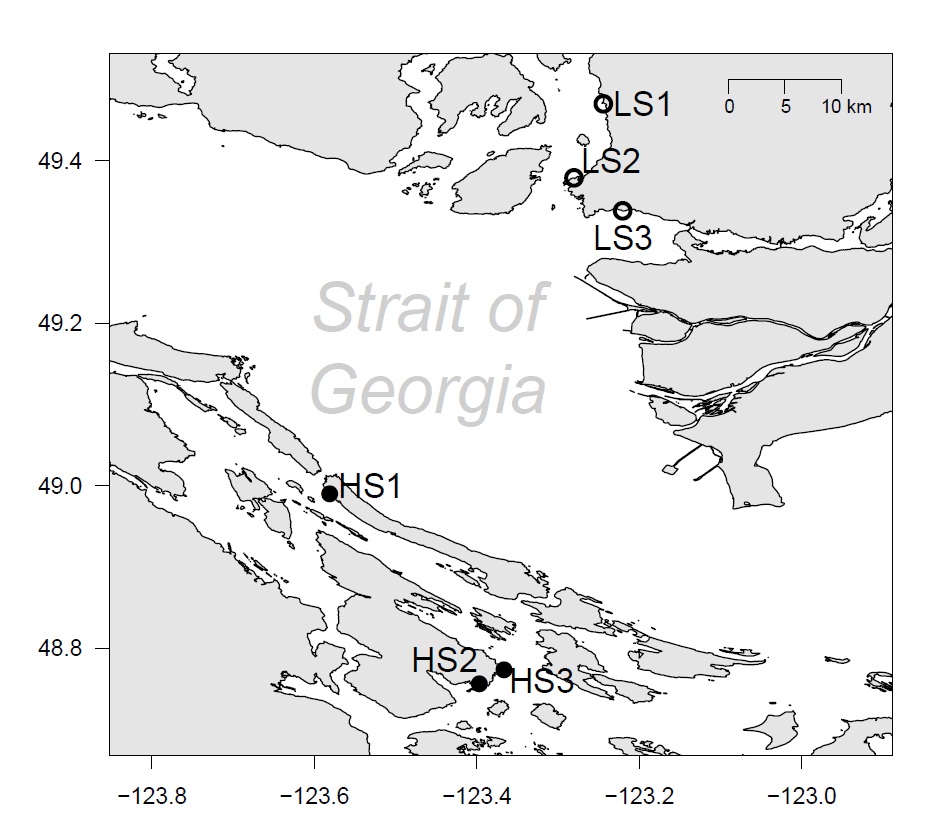
**Fig. 6** Mean abundance of a) *C. dalli* and percent cover of b) green algae and c) red algae in each treatment in high and low salinity regions for July. Error bars indicate standard error. Letters indicate results of Tukey’s HSD.

**Fig. 7** 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 *C. dalli*, though 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.

**Fig 1**

\*Unfinished – need to convert to .eps file

**Fig. 2**



\*Almost Finished – This one is an .eps file, but for some reason it looks strange in Word…still trying to figure it out…



**Fig. 3**

\*Almost Finished - .eps file, but looks weird in Word…also need to make error bars hide behind white boxes, and maybe connect axes?



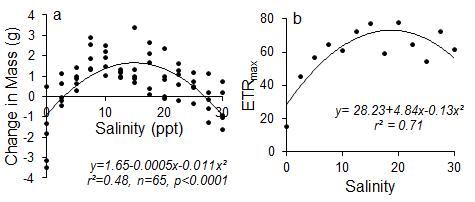
**Fig. 4**

**\***Not finished – this is an excel file of what the graph should look like



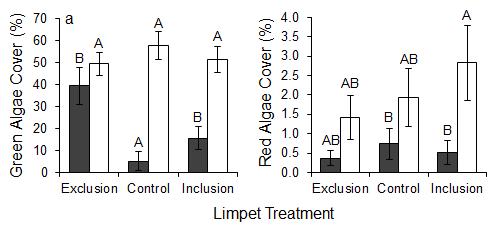
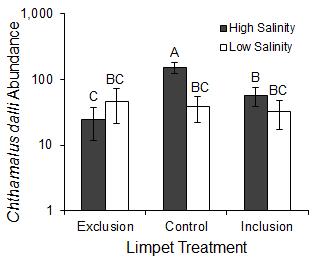
**Fig. 5**

\*Not Finished – This is an excel file



**Fig. 6**

\*Not Finished – this is an excel file (will be 3 graphs stacked vertically)



**Fig. 7**

\*Chris is working on this one ☺

