Title: Salinity controls rocky intertidal community structure via suppression of herbivory

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Abstract

Climate change impacts ecosystems directly through differences in species specific responses as well as indirectly through changes to the strength of species interactions. To predict how species will be impacted by ongoing environmental change, we need to better understand the relative roles of these direct and indirect effects. Salinity is a strong driver of ecological patterns and processes, and salinity regimes in coastal regions are expected to be altered by climate change through intensification of the hydrological cycle and via climate-driven shifts in the timing and strength of the spring freshet. We hypothesized that hyposalinity can indirectly affect the intertidal community by excluding a dominant herbivore. To test this hypothesis, we 1) conducted intertidal diversity surveys in regions of high vs. seasonally low salinity in the Strait of Georgia, British Columbia, 2) conducted laboratory salinity tolerance trials for two important grazers (*Lottia pelta* and *Lottia digitalis*) and one primary producer (*Ulva* sp.), and 3) experimentally manipulated the abundance of grazers in these two regions. We show that rocky intertidal shores from two regions of disparate salinity regimes are distinct in their intertidal communities: low salinity sites were composed primarily of *Mytilus trossulus*, *Fucus distichus* and *Ulva* sp., whereas high salinity sites were dominated by *Chthamalus dalli*, *Lottia* spp., and *Mastocarpus* sp. Our laboratory trials confirmed that freshwater inputs experienced in the low salinity region resulted in hyposaline levels which exceeded the tolerance of *Lottia* spp., but not that of *Ulva* sp. Further, we show that by excluding grazers in high salinity sites, these communities more closely resemble that of the low salinity sites than they do of other high salinity sites with grazers present. Together, these results demonstrate that the pattern of distinct estuarine intertidal communities in low vs. high salinity regions in the Strait of Georgia may be

largely driven by the indirect effects of freshwater inputs, mediated by salinity-driven differences in herbivore population size and thus grazing pressure.

Introduction

Understanding organisms' direct responses to abiotic stress is an important first step in explaining the distribution of species across environmental gradients in time and space. The tolerance of individual species to environmental stressors is frequently used to predict the likelihood that they will persist in the face of climate change (Louthan et al. 2021). However, predictions made solely on the basis of tolerance limits to stress and without reference to the influence of interacting species can lead to misleading results and fail to explain the observed distribution and abundance of species (Brooker et al. 2007; Davis et al. 1998; Hein et al. 2012; Van der Putten et al. 2010; Wallingford & Sorte 2019; but see Thierry et al. 2021). A growing body of evidence suggests that environmental stressors impact community development and structure not only via direct effects on the physiology and survival of organisms, but also through indirect effects mediated by the interactions among species (Barton and Ives 2014; Diamond et al. 2017; Kordas, Harley, and O’Connor 2011; Underwood 1999). To better understand patterns of community composition and diversity and forecast how these patterns will shift due to ongoing environmental change, we need to integrate the direct effects of the environment on any given species with the indirect forcing that arises through environmentally driven shifts in trophic interactions, competition, and facilitation.

Environmental forcing is often mediated, or amplified, by changes in the strength of interspecific interactions (Kroeker and Sanford 2022). Interactions, such as predation, herbivory, facilitation, and competition, are altered by both changes in the abundance of one or more interacting species, as well as changes in per capita interaction strength (Agüera et al. 2015; Kordas, Harley, and O'Connor 2011). Stressors that alter key ecological rates such as feeding, through any of these pathways, will therefore also have an indirect impact on prey species, which can have further cascading impacts on tertiary species (Paine 1974). For example, tropical herbivorous fish have migrated into new habitats while tracking shifts in thermal isoclines, triggering a phase shift from a kelp-dominated system to a rocky barren, with a coinciding loss of kelp-associated diversity (Vergés et al. 2014). Further, environmental drivers can disproportionately affect some species over others; when 'leverage species' (*sensu* Power et al. 1996) are particularly sensitive to changes in the environment, small alterations in the abiotic world can drive considerable change to community structure (Harley et al. 2006).

In addition to interspecific variation in environmental tolerance, differing abiotic conditions across a species range can resulting in divergent selection among populations, contributing to intraspecific variation in environmental tolerance (Sanford and Kelly 2011). For example, despite gene flow among populations, an alpine grass species showed clear differentiation in fitness-related traits along an elevation gradient (Gonzalo-Turpin and Hazard 2009). Likewise, juveniles of *Nucella lamellosa* originating from an area of high salinity experienced higher mortality under hyposaline stress, compared to juveniles originating from a region of low salinity (Covernton and Harley 2020). Ultimately, the interplay between direct and indirect effects of abiotic factors at both a species and a population level will determine aggregate community level properties such as diversity and community structure.

We investigated the importance of direct and indirect environmental controls on estuarine rocky shores. Rocky intertidal shores are a unique system to study such ecological processes as they are highly dynamic in their physical environment, being at the interface of land and sea and exposed to the conditions of both with the rise and fall of the tides (Kunze, Wölfelschneider, and Rölfer 2021). Fluctuations in abiotic conditions occur daily, seasonally and in the long term in response to large scale geographical and climatological processes, further influencing the dynamic nature of intertidal communities (Hsieh et al. 2005). Along with the natural variability in abiotic conditions, rocky shores are highly tractable, making these systems ideal for testing questions related to how species interactions mediate the impacts of abiotic stress on species composition.

In estuarine rocky intertidal ecosystems, salinity is one of the most important drivers of performance of organisms at multiple scales of biological organization, and thus has cascading impacts on population and community structure (Ritter, Montagna, and Applebaum 2005). Exposure to fresh water can induce physiological stress responses in marine animals, including decreased heart rate, reduced haemolymph osmolality, and mortality (Chelazzi, De Pirro, and Williams 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, decreased salinity levels have been found to reduce the survival, development, and settlement of marine larvae, and subsequently influence adult distribution (Dineen and Hines 1994; Starczak et al. 2011). Further, hyposaline conditions inhibit the growth and photosynthetic rate of many marine algal species (Connan and Stengel 2011; Karsten 2007), although several algal species have demonstrated a wide salinity tolerance range (Rath and Adhikary 2005), as well as a capacity for local adaptation to low salinities (Nygård and Dring 2008). As species and populations can have individualistic responses to salinity across a food web, salinity variation can result in changes in community structure and ecological processes that influence biodiversity (Hampel, Elliott, and Cattrijsse 2009; Witman and Grange 1998; Zacharias and Roff 2001).

Here, we sought to understand the direct and indirect effects of spatial variation in salinity in the Strait of Georgia for intertidal algal-herbivore interactions and the resulting community structure. We hypothesized that limpets are disproportionately vulnerable to hyposalinity stress relative to other species, including resource species such as the green macroalga *Ulva* sp. We further hypothesized that differences in community structure between high and low salinity areas would be directly related to differences in limpet grazing pressure due to hyposalinity limits to limpet abundance, and therefore only indirectly driven by salinity. To test these hypotheses, we combined laboratory salinity tolerance trials on limpets, specifically *Lottia pelta*, and the green macroalgae *Ulva* sp., with observational surveys and a manipulative herbivore exclusion experiment at six sites, three each within an area of consistently high salinity, the Southern Gulf Islands, Canada, and in an area of seasonally low salinity, West Vancouver, Canada. First, we predicted a lower abundance of limpets in the periodically low salinity environments than in the consistently high salinity areas. Second, we predicted that any local adaptation to salinity in limpet populations, if present, would not be enough to overcome the minimum salinity level in West Vancouver to maintain a population level comparable to the rocky shores of the Southern Gulf Islands. Third, we predicted that a reduction in grazing pressure from limpets would result in an increase in algal cover and concomitant decrease in barnacle cover in low salinity sites.

Materials and Methods

Study system

This research took place in the Strait of Georgia, British Columbia. 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). Freshwater inputs are dominated by the Fraser River, which regularly exceeds a mean outflow rate of more than 7000 m^3/s in summer months during the freshet (ECCC 2012). The late spring peak in river discharge causes a corresponding reduction in sea surface salinity in the southern Strait of Georgia, with an annual drop from approximately 25 psu to less than 10 psu at coastal sites near the river mouth during peak discharge (Jarníková et al. 2022). This effect, however, declines with increasing distance from the Fraser River, with waters southwest of the Southern Gulf Islands maintaining salinities of 23 psu to 32 psu year-round (MacCready et al. 2021). The contrasting salinity regimes on either side of the Strait make this system a unique and ideal environment for disentangling the direct and indirect effects of this environmental stressor on coastal marine communities.

The field studies described here took place on the traditional, ancestral, and unceded territory of the Sḵwx̱wú7mesh (Squamish), xʷməθkʷəy̓əm (Musqueam), səl̓ilw̓ətaʔɬ (Tsleil-Waututh), Stz’uminus, Quw’utsun (Cowichan), Semiahmoo, scəw̓aθən məsteyəxʷ (Tsawwassen), SȾÁUTW̱ (Tsawout), Penelakut and Hwlitsum nations. We conducted field studies at three sites within each of two regions with contrasting salinity regimes: West Vancouver (LS1, LS2, LS3) and the Southern Gulf Islands (HS1, HS2, and HS3; Fig. 1). The Southern Gulf Island sites are located on the southwest side of the island chain and are not directly exposed to the Fraser River plume. Because of this, the HS sites remain at consistently high salinities year-round, while the LS sites experience reduced salinities during the summer

(Fig. 2). Water samples for salinity determination were collected from shore at a depth of ~15 cm and analyzed with a handheld refractometer, approximately once a month throughout the summers of 2010 and 2011, as well as once for each site in the following winters. Sea surface temperatures in the two regions are comparable, ranging from 5.0 to 18.5°C in West Vancouver and 6.0 to 18.5°C in the Southern 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 in the Southern Gulf Islands. All sites used in this study were composed of granitic rock except for HS1, which was sandstone. Areas selected for surveys and experiments were gently sloping (<40°) bedrock, with varying aspect (see Table S1).

Transect surveys

We conducted monthly surveys during low tide at each of the six study sites from May to August 2011. Because the tidal range differs between the two areas, we carried out surveys at the vertical height corresponding to approximately 30% immersion 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 rock face and eight randomly selected points were surveyed using a 25x25 cm quadrat. We counted all motile invertebrates and quantified sessile invertebrate and algal percent cover.

Salinity tolerance experiments

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

To determine the salinity tolerance of a common grazer, the limpet *Lottia pelta*, and whether the salinity tolerance was contingent on source population, we conducted an experiment with two populations: one from a high salinity site and one from a low salinity site. We collected *L. pelta*, 20±5 mm in length, from HS1 (salinity of 27 psu) on June 2, 2011, and from LS3 (salinity of 10 psu) on June 6, 2011. Limpets from HS1 were randomly divided into eighteen 1 L Ziploc® containers with mesh walls, for a total of six limpets in each. We placed each container 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 acclimate to this salinity for ten days. We also randomly divided limpets from LS3 into an additional eighteen containers (six limpets per container) and placed all containers into aquaria containing seawater at 10 psu, increasing the salinity at increments of 2.5 psu per day to 20 psu. These limpets were allowed to acclimate to the final salinity of 20 psu for six days. After the acclimation period was complete, we randomly arranged containers 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, with three aquaria for each salinity. Aquaria were covered, bubbled with compressed air, and placed in a recirculating sea water system to maintain a water temperature of 12°C. We lowered salinities at a rate of 3 psu every 30 minutes until the desired salinity was reached, and limpets remained submerged for twenty-eight days. Each day, we examined limpets for signs of mortality, including tissue damage, discolouration, and rigidity; any dead limpets were removed. The experiment continued for twenty-eight days, and limpets were not fed during this time.

ii). Salinity tolerance of *Lottia spp*. with tidal emersion

To determine whether the salinity tolerance of limpets is influenced by the periodic emersion from hyposaline conditions experienced during low tides, we conducted a salinity tolerance experiment with *L. pelta* and *L. digitalis,* which incorporated a mimic of tidal exposure. See additional methods for this experiment in the supplementary material.

iii). Salinity tolerance of *Ulva* sp.

We collected *Ulva* sp. from LS2 in West Vancouver, from a salinity of 28 psu on December 7, 2011. Approximately 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 between 0 and 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 0 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 m^{-2} s^{-1} of continuous light. After three weeks, we blot dried and weighed all samples, and 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. We dark-acclimated samples for one prior to measuring rapid light curves, fitted as:

$$ETR = ETR_{max} \times tanh\,(\frac{\alpha \times PFD}{ETR_{max}})$$

where ETRmax is the maximum ETR, alpha is the apparent photosynthetic efficiency, and PFD is the photon flux density in µmol photons m^{-2} s^{-1} (Jassby & Platt 1976).

Herbivore exclusion experiment

We manually cleared all organisms at seven subsites within each of the six study sites. Similar to the transect surveys, these plots were placed at a tide height corresponding to approximately 30% immersion time. Each subsite included a limpet inclusion, exclusion, and control plot. 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 are effective barriers to limpets (Harley 2002) and partial barriers to periwinkles (Harley 2006). We marked one circular plot within each area, also 28.5 cm in diameter, with steel bolts to serve as a control. The position of control and treatments was randomized within each subsite. We did not include copper controls in this study, as previous work has shown that partial copper treatments lead to partial effects which are difficult to interpret (Johnson 1992). Ultimately, local salinity levels exceeded the lower tolerance limit of limpets in the low salinity sites, and the inclusion plots were not effective at retaining *L. pelta*. Because of this, we only analyzed exclusion and control plots at each subsite. We maintained the exclusion treatments by removing limpets, along with any other grazers found inside the rings, every two weeks. We censused plots once per month during low tide, from May to August. We used a 10 x 10 cm quadrat to count motile 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

All analyses were completed using R version 4.1.2 (R Core Team 2020). To test whether salinity regimes between our study areas were different, we performed a student’s two sample t-test on the 10th percentile of salinities measured across two years at each of the six sites.

We analyzed community data from the transect surveys using the *vegan* package (v2.5-7; Oksanen et al. 2017). Species abundances were first relativized with a double Wisconsin transformation. This standardized species to equal maxima, then sites to equal totals, putting equal emphasis among sample units and among species. We ordinated the data with non-metric multidimensional scaling (nMDS). We then performed a permutational multivariate analysis of variance (PERMANOVA) to test the null hypothesis that the centroids of the groups are equivalent. To respect the dependence of sampling blocks within a site, we restricted the permutation scheme such that all quadrats along a transect were always permuted together. In doing so, 999 permutations were run on a matrix of Bray-Curtis dissimilarities. Because PERMANOVA cannot distinguish between differences in centroid location or levels of dispersion, we also used a PERMDISP test with 999 permutations, to test if the variances of the groups are different. We then conducted a Similarity of Percentages (SIMPER) analysis to investigate which species contributed most to the observed differences in salinity regions.

To test whether survival of *L. pelta* populations originating from a low and high salinity region differ along a gradient of salinity levels, we conducted a survival analysis with the *survival* package (v3.3-1; Therneau et al. 2023). I modeled the probability of survival with the Kaplan-Meier method, which is a non-parametric method to estimate survival probability from observed survival events. We then used these model fits to calculate the restricted mean survival time (RMST), for each site by population combination. Using the RMST is an alternative to proportional hazards modelling, which requires a constant relative hazard over time that is not met with our data. To determine differences in net productivity of *Ulva* sp., we used a least-squares regression to analyze the change in biomass before and after the treatments, as well as ETR_{max} at the end of the experiment.

Due to a lack of recruitment in spring months and a late summer heat wave in August 2011 that resulted in the die-off of many species during our herbivore exclusion experiment, we analyzed community data in the penultimate sampling point of July only. We used the same methods as described above in the transect surveys subsection. We restricted permutations similarly, keeping treatment plots within a subsite together. We then conducted a SIMPER analysis to investigate which species contribute the most to observed differences among salinity regions, grazer treatments and the interaction between the two. Prior to running the SIMPER analysis, we removed grazers from the site-species matrix, to ensure that our analysis does not identify a species that differed among treatments due to experimental manipulation. Finally, we fit generalized linear models, using the *glmmTMB* package (v1.1.2.3: Brooks et al. 2017), to the abundance of two species which were consistently identified as the most influential taxa in the SIMPER analysis, as well as had abundance patterns that were markedly altered by our experimental design: *Ulva* sp., and the barnacle *Chthamalus dalli*. Both models included an interaction between the two fixed effects, region and treatment, and site as a random effect nested within salinity region. To model percent cover of *Ulva* sp., we first attempted to fit a model with a beta error distribution, but this model failed to converge, therefore we used a tweedie error distribution with variance among regions modeled independently. We modelled abundance of *C. dalli* with a negative binomial error distribution, with a zero-inflation parameter. Both models were fitted with a type III sums of squares Wald chi squared test. We checked model diagnostics with the *DHARMa* package (v 0.4.5; Hartig 2022), by running K-S tests, Levene's test and plotting scaled residuals against each predictor variable.

Results

Abiotic conditions

Salinity varied in the Strait of Georgia over both space and time. Salinity decreased as a result of increasing Fraser River discharge in the spring and summer in both regions but these seasonal differences were much more pronounced in the low salinity region compared to the high salinity region (Fig. 2). The tenth percentile of salinity measured from 2010 to 2012 in the LS sites was 9.6 psu (± 0.9 SE), over 15 psu lower than the tenth percentile of 26 psu (± 0.9 SE) measured in the HS sites ($t_4 = -13.35$, $P < 0.001$).

Transect surveys

Communities belonging to low or high salinity regions had substantial differences in community composition from each other (PERMANOVA, $F_{1,184} = 81.18$, $P = 0.001$; Fig. 3). Additionally, low salinity sites changed through time whereas the high salinity sites remained similar (region x month, PERMANOVA, $F_{3,184} = 1.70$, $P = 0.001$; Fig. 3). Different months of the summer also had significantly different communities across salinity regions (PERMANOVA, $F_{3,184} = 4.48$, $P = 0.001$; Fig. 3). As groups with different dispersions may result in misleadingly low p values, PERMANOVA results must be interpreted cautiously. Dispersion among salinity groups was equal (PERMDISP, $F_{1,190} = 0.23$, $P > 0.05$; Table S2), however May and June had less variance than September (PERMDISP, $F_{3,188} = 6.52$, $P = 0.002$; Table S2). The following species contributed the most to differences between salinity groups: the bay mussel *Mytilus trossulus*, the acorn barnacles *Balanus glandula* and *Chthamalus dalli*, the brown alga *Fucus distichus*, and the *Petrocelis* phase of the red algae *Mastocarpus* sp. LS sites were composed of more *M. trossulus* and *F. distichus*, while HS sites were composed of more *B. glandula*, *C. dalli*, *Mastocarpus* sp., and *Lottia paradigitalis* (SIMPER; Table S3). Species-specific responses are shown graphically in Fig. S1a-h.

Tolerance experiments

i). Salinity tolerance and local adaptation of *Lottia pelta*

The survival of *L. pelta* was strongly reduced by low salinity, although the impacts of salinity differed between high and low salinity populations (Fig. 4). Limpets could only survive for a few days at the two lowest salinities tested (5 and 8 psu), but the low salinity population had a higher restricted mean survival time (5.6 ± 0.4 at 5 psu and 6.4 ± 0.4 days at 8 psu) than the high salinity population (4.4 ± 0.2 at 5 psu and 4.9 ± 0.3 days at 8 psu). The difference in survival among populations was amplified at 11psu, where most low salinity limpets survived for the duration of the experiment with survival time estimated as 21.4 ± 2.5 days, as compared to 11.7 ± 1.5 days for the high salinity population. Limpet survival was uniformly high at salinities of 14, 17, and 20 psu, and populations did not differ in their restricted mean survival time at these salinities. Survival of *L. pelta* was not impacted by simulated tidal emersion (see Supplementary material for detailed results; Fig. S2).

ii). Salinity tolerance of *Ulva* sp.

The net productivity of *Ulva* sp. was significantly and unimodally related to salinity (Figure 5a; $R^2 = 0.48$, $P < 0.001$), with the greatest gain in mass at 15 psu and net losses at both 0 psu and 30 psu. ETR_{max}, a proxy for photosynthetic capacity, showed a similarly significant unimodal relationship (Fig. 5b; $R^2 = 0.71$, $P = 0.002$), with a maximum value at 20 psu and minimum at 0 psu.

Herbivore exclusion experiment

Herbivore exclusion had little influence on community structure in the low salinity region but had a large effect in the high salinity region (Fig. 6). Notably, the communities in the high salinity herbivore exclusion plots were more similar to low salinity communities (with or without herbivore exclusion fences) than to high salinity plots with herbivores; salinity region did not have a significant effect on community structure (PERMANOVA, $F_{1,80} = 8.20$, $P = 0.418$; Fig. 6), but treatment (PERMANOVA, $F_{1,80} = 3.41$, $P = 0.002$), as well as the interaction between salinity region and treatment (PERMANOVA, $F_{1,80} = 1.52$, $P = 0.044$) did have a significant effect. Dispersion was unequal among salinity groups (PERMDISP, $F_{1,82} = 17.3$, $P = 0.008$; Table S4), as well as among treatment groups (PERMDISP, $F_{1,82} = 7.5$, $P = 0.001$; Table S4). Among the high salinity sites, the effect of grazing on composition was primarily due to differences in *Ulva* sp. and the barnacles *B. glandula* and *C. dalli* (SIMPER, Table S5). The same three species also had the highest contribution to between group differences in the high salinity and low salinity plots with grazers. Excluding grazers differentially affected the abundance of *C. dalli* and *Ulva* sp. across salinity regions. There was a consistent pattern of more *Ulva* sp. in the low salinity sites than in the high salinity sites, regardless of grazer treatment ($\chi^2 = 20.06$, df = 1, $P < 0.001$). However, excluding grazers had a strong impact on

Ulva sp. cover in the high salinity sites, such that the grazer exclusion plots in the HS sites had similar *Ulva* sp. cover to all the plots in the LS sites (grazer x salinity, $\chi^2 = 14.99$, df = 1, $P < 0.001$; Fig. 7a). Conversely, excluding grazers had the opposite effect on *C. dalli* barnacle abundance. Grazer-excluded plots in the HS sites had an order of magnitude fewer *C. dalli* barnacles than the control plots in the same region (grazer x salinity, $\chi^2 = 17.1$, df = 1, $P < 0.001$; Fig. 7b), resulting in *C. dalli* densities in high salinity grazer exclusion plots being similar to those in both grazer treatments in the low salinity region. Results for the other species are shown graphically in Fig. S3a-f.

Discussion

As climate change continues to alter the biophysical world and places increasing pressure on ecosystems, understanding the mechanistic link between the abiotic environment and the resultant patterns on community composition is imperative. A multitude of environmental drivers, which include salinity amongst others, are shifting in space and time due to anthropogenic effects (IPCC 2022). The combination of gradients in abiotic drivers and resultant shifts in community interaction webs can create complex changes in community structure and ecosystem function (drought: Amundrud & Srivastava 2016; Chase & Knight 2003; precipitation: Barton & Ives 2014; warming: Robinson et al. 2017; snowpack: Brodie et al. 2012). Although the correlation between the abiotic environment and species abundance and distribution is well studied (Walther et al. 2002), the extent to which this pattern is driven directly by changes to performance and survival or indirectly by changes to species interactions is not fully known (Bertness et al. 1999; Blois et al. 2013; Brown et al. 2001). Empirical studies that disentangle the impacts of direct environmental stress and the indirect effects of modified species interactions in natural communities are rare.

There were distinct differences in both salinity regime and intertidal community structure in our two study regions. As freshwater discharge from the Fraser River increased in the spring, salinity decreased in both LS and HS sites, but this reduction was much more pronounced in the LS sites, as these sites were in closer proximity to the river mouth. In the LS region, the average tenth percentile of salinity was 9.5 psu, which is in stark contrast to the 10th percentile of 26 psu experienced in the HS sites. In addition, there was very little overlap in the composition of communities inhabiting LS and HS regions. Species driving the differences in the community composition among salinity regions consisted of a greater abundance of *M. trossulus* and *F. distichus*, and in some places *Ulva* sp. (e.g., the control plots in our experimental manipulations) in the LS sites, and barnacles, red algae, and grazers like the limpet species *L. paradigitalis* and *L. pelta* in the HS sites. Interestingly, community composition changed as the summer progressed in the low salinity region but not the high salinity region. This context-dependent shift in community structure may correspond with differences in the seasonal variation in salinity in the two regions (i.e., strong seasonal swings in salinity in the LS sites and only weak seasonal changes in the salinity of HS sites), which are driving shifts in species presence or abundance.

Our results align with other studies that found similar patterns of distinct communities associated with distance from freshwater sources such as riverine input or glacial melt (Giménez et al. 2010; Hossain, Marshall, and Hall-Spencer 2019; McCabe and Konar 2021). This pattern of disparate communities among habitats that lie along a gradient of abiotic stress is not unique to salinity alone; documented differences in species composition have also been shown for freshwater phytoplankton communities along a thermal gradient, plant communities along an elevation gradient, and butterfly species in areas of low and high rainfall, to name a few (Beirão et al. 2017; Hailemariam and Temam 2020; Ikram, Uniyal, and Kumar 2022). Despite the importance of identifying differences in species composition among regions of varying environmental conditions, teasing apart the relative importance of whether indirect or direct impacts of abiotic stress in driving these patterns is challenging for purely observational datasets.

The divergent pattern in limpet abundance between regions over the summer likely reflects the influence of the low salinity riverine output. Limpets are osmoconformers, and thus are unable to regulate their extracellular osmolality in response to changes in their environment, leading to deleterious effects on both physiological and behavioral responses, and ultimately survival (Chaparro et al. 2008; Firth and Williams 2009; Morritt et al. 2007). In the HS sites, limpets were able to survive a small seasonal decrease in salinity and maintain a constant abundance throughout the summer. In contrast, limpets in the LS sites nearly disappeared as surface salinity approached freshwater levels, likely as a result of mortality related to osmotic stress.

Additional support for our hypothesis that regional differences in the abundance of limpets are driven by salinity tolerance comes from our lab assays of hyposalinity tolerance limits. Here, we show that limpet survival is strongly compromised below 11 psu in populations originating from both regions, but to a greater degree for limpets from the HS site. While limpets are broadcast spawners with moderate dispersal ability and are thus likely to possess a certain degree of population connectivity, even species with dispersive planktonic larval stages can show signatures of local adaptation when faced with strong enough selective pressures (Sanford & Kelly 2011). Despite a propensity for local adaptation or acclimatization, limpets – regardless of population of origin - were not able to survive exposure to the full range of salinities potentially encountered in the LS sites. In general, this intolerance to low salinity is in accordance with previous research on intertidal gastropods (e.g., Covernton & Harley 2020; Wilson et al. 2009).

Based on our results, limpet populations are expected to experience substantial mortality after less than a week of exposure to salinities less than 11 psu. Interestingly, while limpet abundance was exceedingly low in the LS sites over the summer, some individuals were able to persist in this hyposaline environment, at levels as low as 7 psu. Our lab experiment shows that this likely is not a result of daily aerial emmersion during low tides, but there are other potential, non-mutually exclusive explanations as to why some limpets could survive in the LS sites. For example, there are likely periods of elevated salinity due to tidal dynamics or wind-driven mixing that were not captured by our salinity sampling. Alternatively, source-sink dynamics may be at play, where populations are being replaced annually through recruitment from high salinity source populations.

Salinity-driven differences in limpet abundance had cascading consequences for community structure across the salinity gradient. When grazers had been excluded from plots in the HS sites, communities resembled the LS sites to a remarkable degree, with more *Ulva* sp. and fewer barnacles, particularly *C. dalli*. Although the role of herbivory in structuring communities has been documented in several other systems besides rocky shores, such as the role of Canada geese in wetlands or Rocky Mountain elk in high elevation riparian areas (Jobe et al. 2022; Parsons, Maron, and Martin 2012), it seems to be especially strong in marine systems where herbivore gain or loss can result in striking community shifts (Bellwood et al. 2004; Hughes 1994; Ledlie et al. 2007; Poore et al. 2012; Shurin, Gruner, and Hillebrand 2006). Herbivores can thus impact successional trajectories, habitat complexity, and alter the strength of competition in communities. Unfortunately, we were unable to determine the effects of grazing in the LS sites, as the poor salinity tolerance of limpets meant that they were unable to survive in the inclusion plots. Had these inclusion plots been effective at retaining limpets, we would predict a decrease in *Ulva* sp. cover, which would likely facilitate an increase in *C. dalli* and red algae in the LS sites, as these species were already present and therefore have a salinity tolerance that allows at least some population persistence here. Ultimately, we need a better understanding of the salinity tolerance of these other species to understand the full effects of limpet grazing in this system.

Ulva sp. often has a strong role in the successional trajectories of rocky shores as it is very effective at colonizing empty space made available by disturbance, has fast growth rates, and inhibits the settlement and growth of other algal species as well as invertebrates (Sousa 1979). While we did not capture strong differences in *Ulva* sp. among regions in our transect surveys, our experimental plots showed that in the absence of grazer manipulations (i.e., the control plots), *Ulva* sp. was approximately 10 times more abundant in the LS sites compared to the HS sites. Unlike limpets, several species belonging to the genus *Ulva* have demonstrated a wide tolerance for salinities as low as 5 psu (Ichihara, Miyaji, and Shimada 2013) and are therefore widespread in marine and brackish habitats (Rybak 2018). Our hypothesis that differences in grazing pressures, not salinity tolerance, was driving the asymmetry in regional abundance of *Ulva* sp. was supported by our results. Our lab experiment showed positive net gains in mass and minimal changes to ETR_{max} at a range of salinity levels from 5 to 25 psu, which encompasses the full range of salinities experienced at both the HS sites and LS sites during the summer. In the HS sites, the absence of limpets in the exclusion experiment allowed *Ulva* sp. to proliferate to abundances that matched that of the low salinity sites. Grazing by gastropods is known to have a direct negative impact on both the abundance and vertical zonation of intertidal foliose algae (Coleman et al. 2006; Hesketh, Schwindt, and Harley 2021), which is what was documented during our field manipulation. The effect of excluding limpets on algal cover is often strongest in the absence of barnacles, as barnacles increase the habitat structural complexity, impeding the movement of larger grazers (Geller 1991; Harley 2006). As our plots were completely cleared at the outset of the experiment, the lack of barnacles may have strengthened the ability of grazers to reduce *Ulva* sp. cover so effectively.

In contrast to *Ulva* sp., excluding grazers in the HS sites had the opposite effect on *C. dalli*, whose abundance decreased to match that of the abundance seen in the LS control plots. Limpets can have positive indirect effects on the abundance of *C. dalli* barnacles either by freeing up space for settlement of cyprid larvae that would otherwise be occupied by macroalgae, or by reducing interspecific competition by 'bulldozing' the barnacle *B. glandula* (Dayton 1971; Harley 2006). Because the presence of grazers did not have a negative effect on *B. glandula* abundance in the control plots in comparison to the exclusion plots, the facilitatory role limpets played in the recruitment of *C. dalli* was likely a result of grazing on algae, and not due to changes in interspecific competition. The former pathway is also supported by experimental limpet exclusions on the rocky shores of California and the Mediterranean, where algal cover increased and the congeneric species *Chthamalus montagui* and *Chthamalus stellatus* subsequently decreased (Arrontes et al. 2004; Benedetti-Cecchi 2000; Dungan 1986). In addition, herbivore presence may also serve as a settlement cue through chemical signals that indicate the presence of suitable habitat for *C. dalli*. Such a mechanism has been shown for *Chthamalus anispoma,* which has higher settlement in response to the chemical cues of species whose suitable habitat overlaps with its own (Raimondi 1988).

While our study did not set out to test the mechanisms controlling the abundance and distribution of each species, there are likely other indirect effects of salinity occurring in this system. For example, *M. trossulus* was present at a much higher abundance in the LS sites throughout the summer. As *M. trossulus* has a higher salinity tolerance than its main predators, sea stars and dogwhelks, its presence in the LS sites and absence in the HS sites is likely due to differences in predation pressure (Coverntton & Harley 2020; Held & Harley 2009; Qiu et al. 2002).

Taken together, our results show that indirect effects of abiotic drivers such as hyposalinity can propagate through levels of biological organization, having profound impacts on community structure. The estuarine rocky intertidal system of the Strait of Georgia is driven by osmotic-related suppression of key grazing species, with cascading effects through trophic interactions that ultimately restructure the entire community. Abiotic conditions that vary in both space and time can lead to dynamic patterns in species distribution and abundance along these spatiotemporal gradients. Climate change is expected to impact salinity regimes in coastal regions both by intensifying the hydrological cycle that impacts precipitation patterns and shifting the timing and strength of the spring freshet (Held and Soden 2006). Indeed, both short and long-term changes to the coastal salinity in the Strait of Georgia have already begun to take place. The 2021 North American heatwave, a climactic event that broke maximum daily temperature records in multiple locations by more than 5 °C, resulted in reduced soil water storage capacity, exacerbating autumnal flooding caused by an atmospheric river, and ultimately led to pronounced streamflow and freshwater inputs to coastal systems (White et al. 2023). Additionally, the mean annual salinity on the western boundary of the Strait of Georgia has increased by 3.9 psu since 1935 (Iwabuchi and Gosselin 2019). While intertidal populations have demonstrated a capacity for local adaptation, the continued expected changes to the global hydrological cycle are likely to alter the structure and composition of coastal communities. Understanding the effects of indirect effects on community dynamics may prove essential to predicting the direction of such change in coastal ecosystems.

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Figures

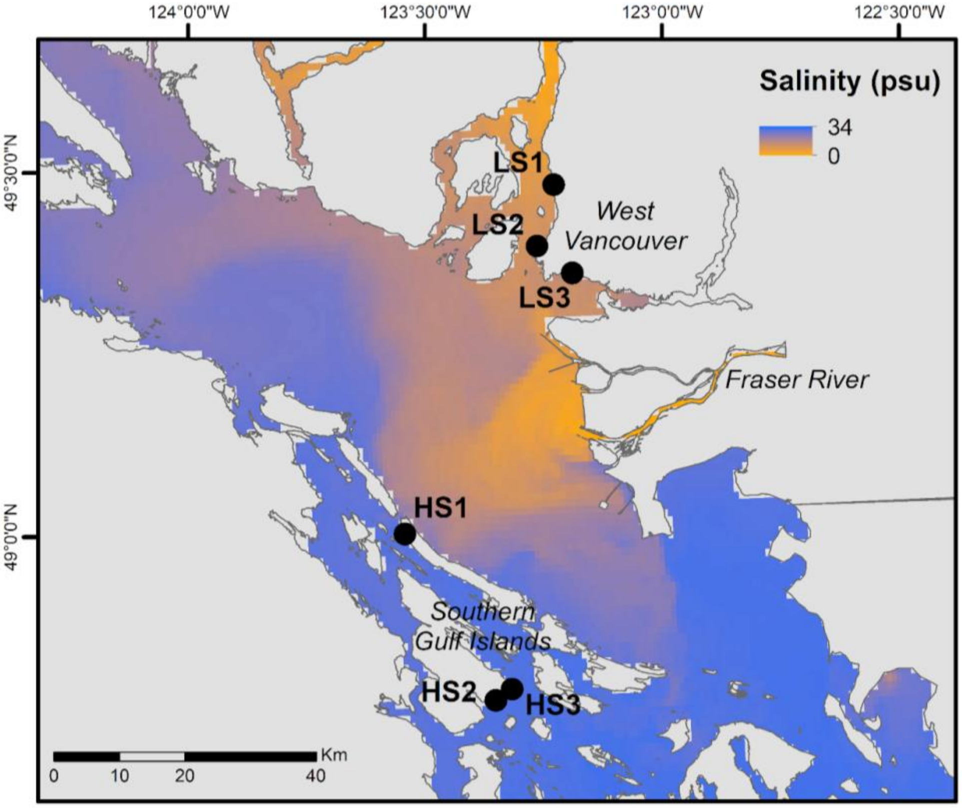

Figure 1 Map of the study region with LiveOcean modeled salinity for July 8, 2019 (MacCready et al. 2021). Low salinity sites are located in West Vancouver (LS1, LS2, and LS3). High Salinity sites are located in the Southern Gulf Islands (HS1, HS2, and HS3).

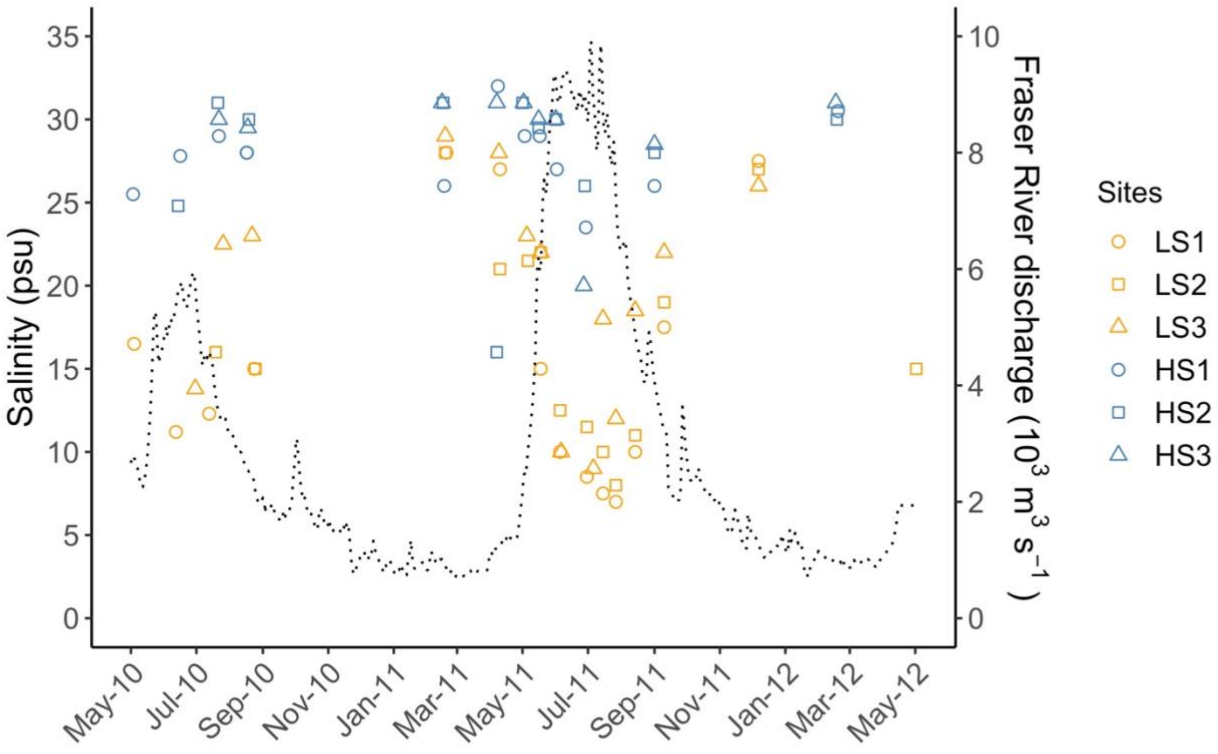

Figure 2 Measured surface salinity (psu) from sites in the Southern Gulf Islands and in West Vancouver, British Columbia. Dashed line indicates Fraser River discharge rate (10^3 m^3/s) measured at Hope, British Columbia (Environment Canada, 2012). Surface salinity for HS2, April 7, 2011, was influenced by heavy rainfall.

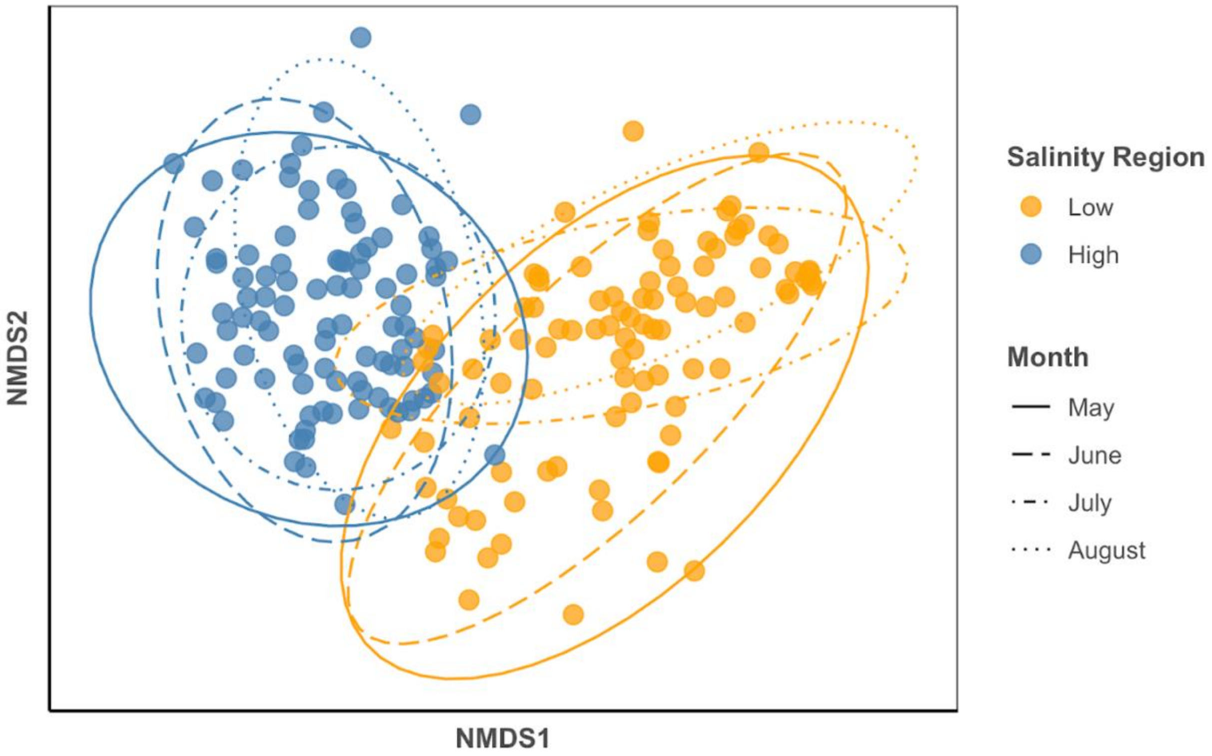

Figure 3 Non-metric multidimensional scaling (nMDS) of a Bray Curtis dissimilarity matrix based on species abundance data collected in May to August 2011, from transect surveys in regions of low and high salinity: West Vancouver (LS1, LS2, and LS3) and Southern Gulf Islands (HS1, HS2, and HS3). We used scree plots to identify the minimum number of dimensions that resulted in a stress lower than 0.15, resulting in an ordination with k = 3 (linear fit $r^2 = 0.864$, non-metric fit $r^2 = 0.978$). We analyzed the data with a PERMANOVA and show that salinity region (F = 81.18, $P = 0.001$), month (F = 4.48, $P = 0.001$), and the interaction between the two (F = 1.70, $P = 0.001$) all had a significant impact on community composition.

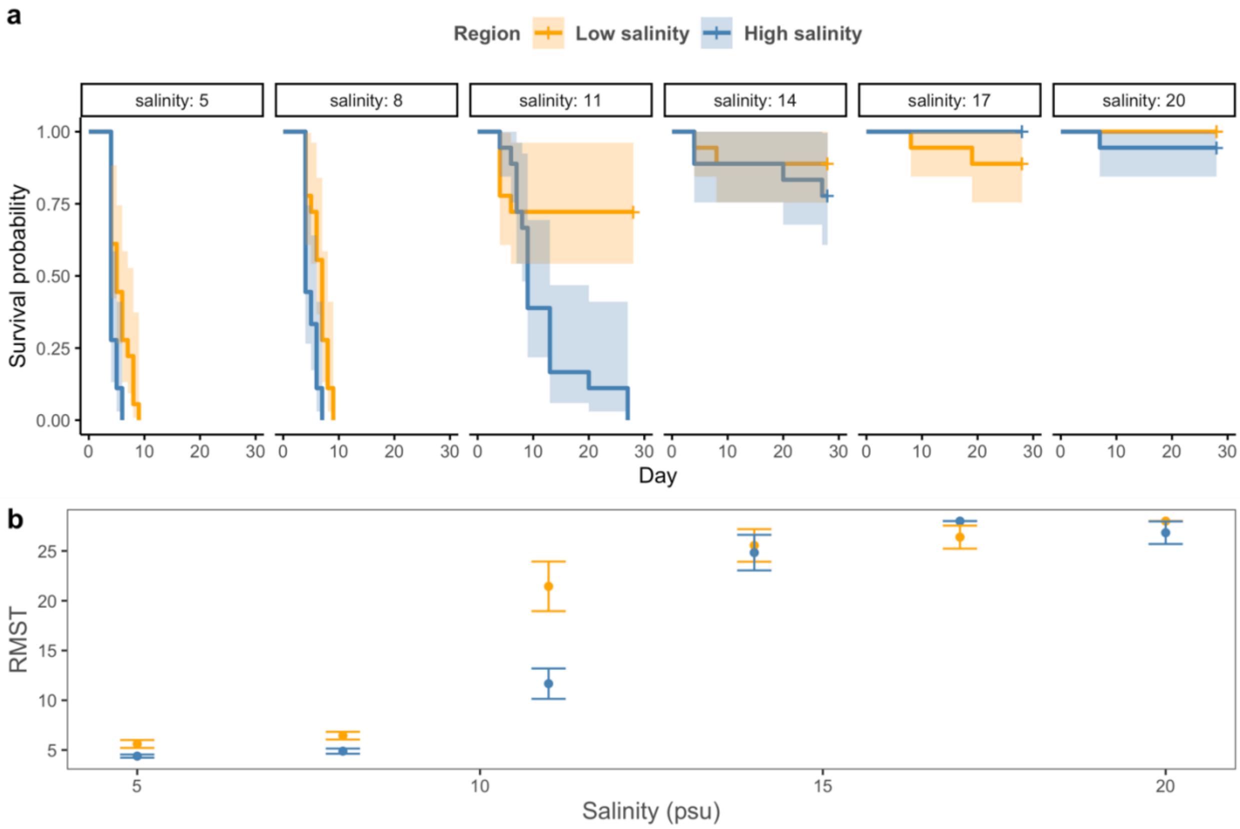

Figure 4 ***Lottia pelta*** **survival from HS1 and LS3 at a range of salinity levels**. (a) Kaplan-Meier survival curves for each of the population and salinity treatments over the 28-day experiment. Survival probability is indicated on the y-axis, where 1.0 is equivalent to 100% survival; shaded area indicates 95% confidence intervals. (b) Restricted Mean Survival Time (RMST) calculated for the duration of the 28-day experiment. Error bars represent standard error.

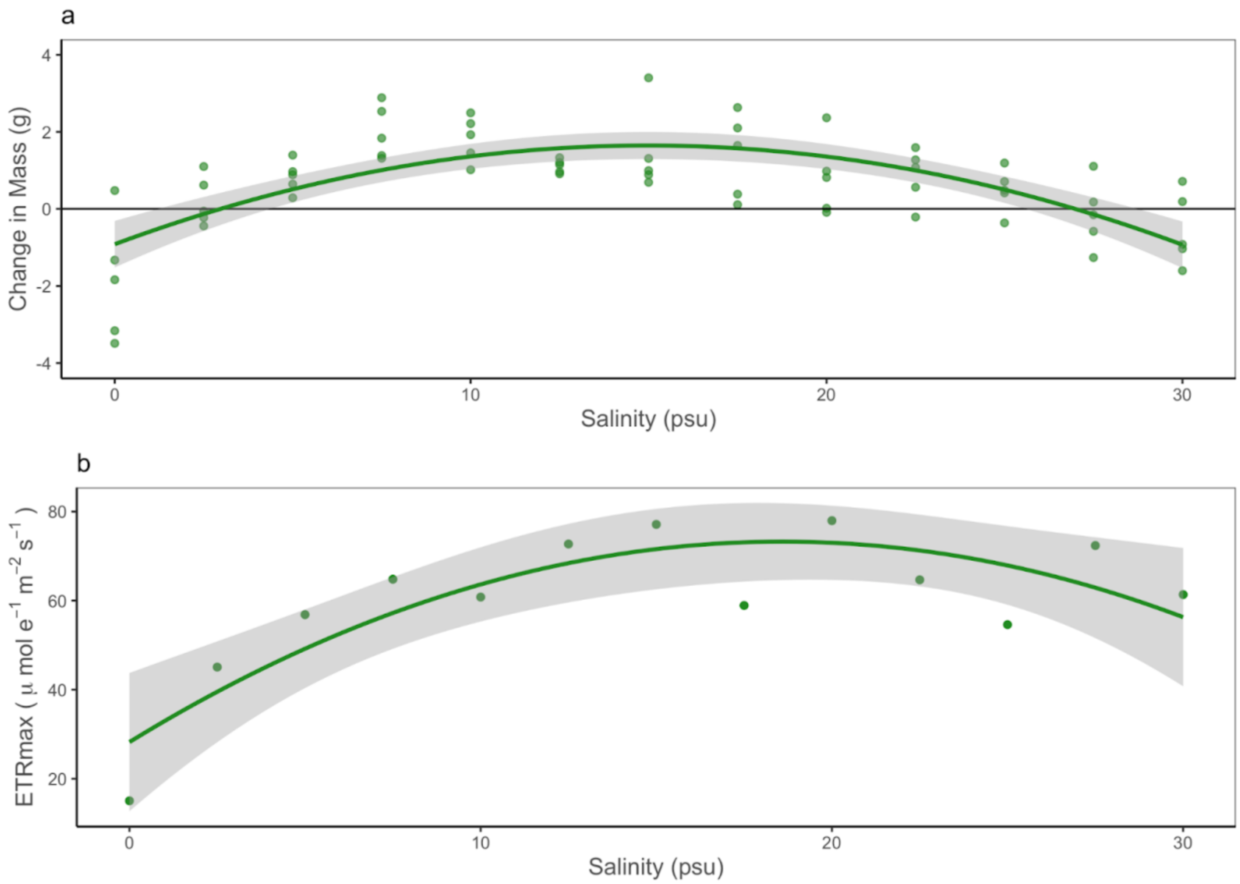

Figure 5 *Ulva* sp. salinity tolerance (A) Change in mass (g), (least squares regression, $y = -0.915 + .342x - 0.0114x^2$, $R^2 = 0.48$, $P < 0.001$) and (B) Maximum electron transport rate (ETR_{max}), (least squares regression, $y = 28.2 + 4.84x - 0.13x^2$, $R^2 = 0.71$, $P = 0.002$). The data were fitted to a second order polynomial, and the shaded area depicts 95% confidence intervals.

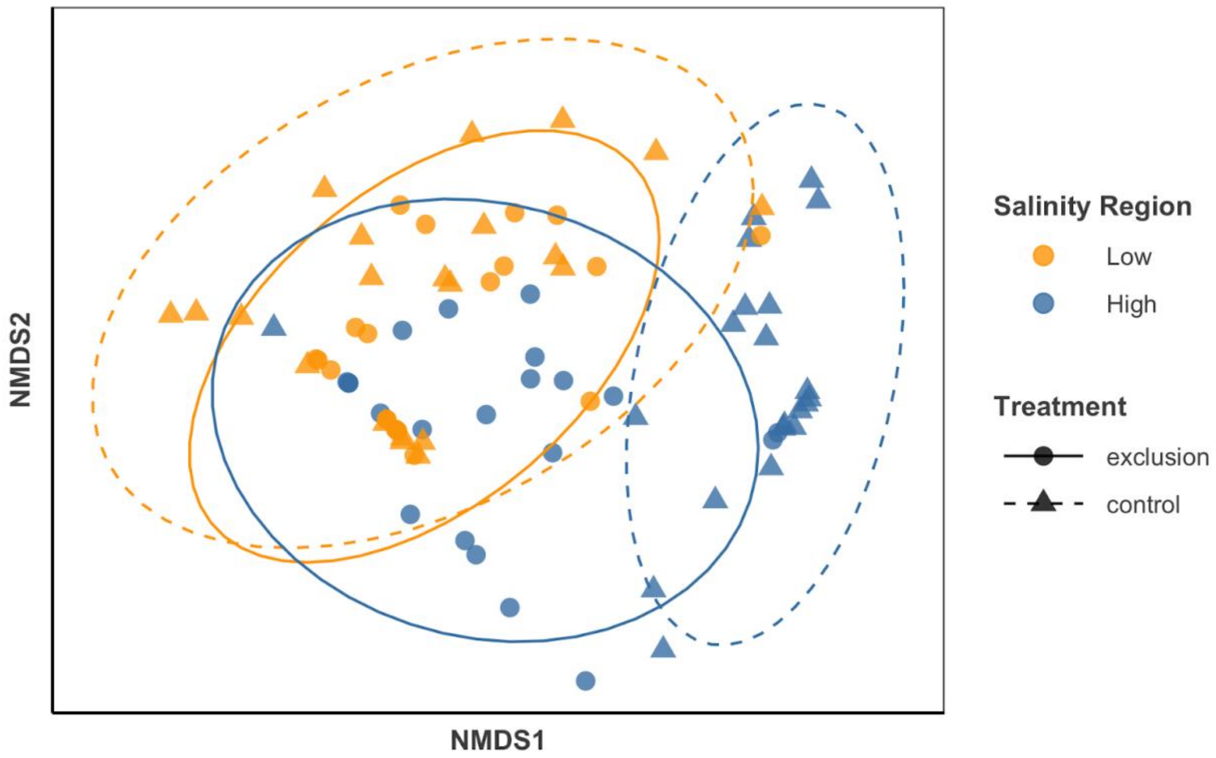

Figure 6 Non-metric multidimensional scaling (nMDS) based on a Bray-Curtis dissimilarity matrix of species abundance data collected in July 2011, from herbivore experimental plots (herbivore exclusions and control plots) at low (West Vancouver) and high salinity sites (Southern Gulf Islands). We used scree plots to identify the minimum number of dimensions that resulted in a stress lower than 0.15, resulting in an ordination with k = 3 (linear fit $r^2 = 0.932$, non-metric fit $r^2 = 0.989$). Both the grazer manipulation treatment (PERMANOVA, F = 3.41, *P* = 0.002), and the interaction between grazers and salinity region had a significant impact on community structure (PERMANOVA, F = 1.52, *P* = 0.044).

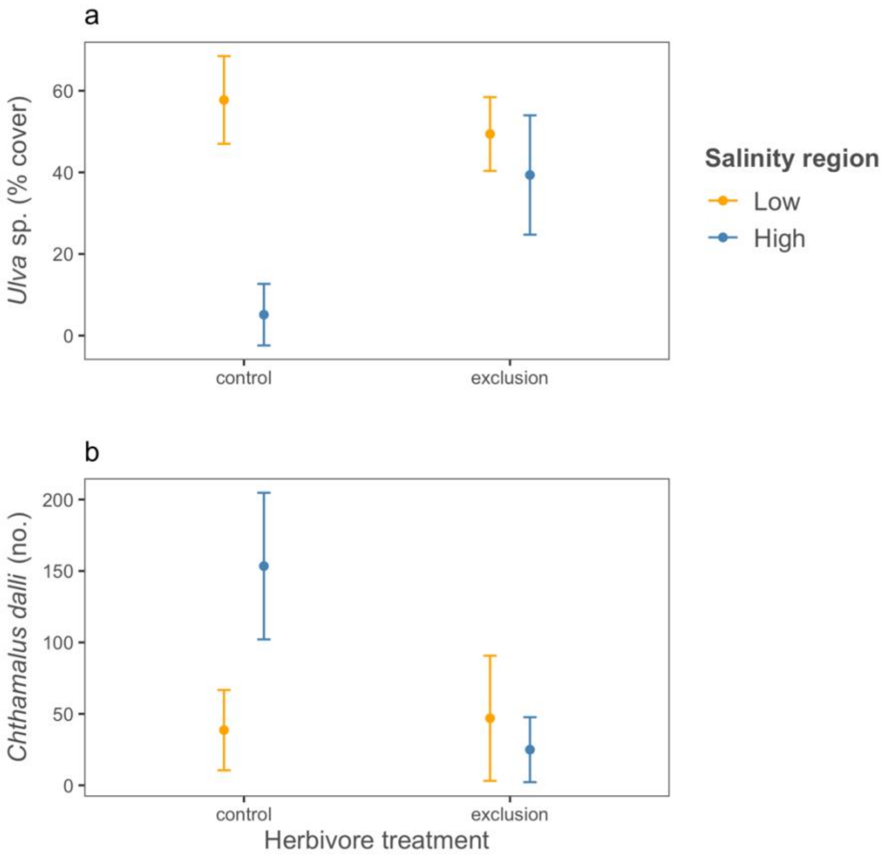

Figure 7 Mean abundance of (A) *Chthamalus dalli* and (B) *Ulva* sp. in the herbivore exclusion experiment in three low salinity (Southern Gulf Islands) and three high salinity sites (West Vancouver), censused in July 2011, two months after treatment establishment. Error bars represent standard error.

Supplementary material

Salinity and tidal emersion tolerance of *Lottia* spp.

Methods

To determine whether 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 simulated tidal exposure. Two experiments were performed, one with *Lottia pelta* and the other with *Lottia digitalis*, collected from HS1, Galiano Island, from a salinity of 32 psu. We randomly assigned four limpets to one of twenty-four 1 L Ziploc® containers with mesh walls and two containers to each of twelve 20 L aquaria containing seawater at 30 psu. We randomly assigned four aquaria to each salinity treatment - 30 psu, 20 psu and 10 psu. We then covered each aquaria, provided each with compressed air and placed them inside of a flow through sea water system to maintain a water temperature of 12°C. We lowered salinities by 2.5 psu per day with chilled, dechlorinated freshwater until the desired salinity was reached. To control for water changes, we also performed daily water replacements in treatments that had already reached target salinity, using filtered sea water 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, we removed the intertidal containers from their aquaria to simulate exposure during low tide. At 18:00 every evening, the containers were placed back inside their aquaria. While out of the water, containers housing the "intertidal" limpets were held at room temperature. Each day, we examined limpets for signs of mortality, including tissue damage, discolouration, and rigidity, and removed dead limpets. The experiment continued for 28 days, and we did not feed limpets during this time.

To test whether emersion during a simulated low tide had an impact on salinity tolerance of either *L. pelta* or *L. digitalis*, we conducted a survival analysis with the *survival* package, version 3.3-1 in R. We modeled the probability of survival with the Kaplan-Meier method, which is a non-parametric method to estimate survival probability from observed survival events

Results

The confidence intervals of the Kaplan-Meier survival curves overlap for the intertidal and subtidal treatments at 10, 20 and 30 psu, thus daily emersion from salinity stress had no effect on survival probability for *L. pelta* or *L. digitalis* (Fig. S2).

Tables

Table S1: **Physical characteristics of study sites for intertidal surveys and herbivore manipulation experiment.** 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 the compass direction faced by the substratum.

Site	Region	Latitude	Longitude	Vertical height (m)	Slope (°)	Aspect (°)
LS1 Lions Bay	WV	49.78472	123.4044	3.3	10-22	225-295
LS2 Copper Cove	WV	49.64056	123.4742	3.8	19-37	265-335
LS3 Sharon Cove	WV	49.57306	123.3819	3.3	9-16	95-225
HS1 Hailstorm	SGI	49.65056	123.9697	2.3	10-33	55-75
HS2 Ruckle Park	SGI	49.29250	123.6106	2	4-16	100-135
HS3 Eagle Cove	SGI	49.26750	123.6761	2.4	1-26	165-245

Table S2: Results from a PERMDISP test on species composition data from transect surveys conducted in West Vancouver (LS sites) and the Southern Gulf Islands (HS sites), in the summer of 2011. Dispersion among the two salinity regions were equal, but dispersion among months was not.

Df	SS	MS	F	N.Perm	*P*-value
Dispersion by salinity region					
1	0.004	0.004	0.23	999	0.642
190	3.420	0.018			
Dispersion by month					
3	0.141	0.047	6.515	999	0.002
188	1.355	0.007			

Table S3: **Contribution of species to the overall Bray-Curtis dissimilarity matrix of invertebrate and algal community composition in low vs. high salinity regions during the summer of 2011, estimated with a similarity of percentages (SIMPER) analysis.** Species which make up 70% of the cumulative contribution are shown, as well as their mean abundance in low and high salinity regions.

Taxon	Avg contribution (%)	Cumulative contribution (%)	Mean abundance low salinity	Mean abundance high salinity
Mytilus trossulus (%)	15.9	19.1	48.7	0.8
Balanus glandula (%)	8.6	29.4	16.1	21.3
Fucus distichus (%)	8.5	39.6	32.7	17.4
Chthamalus dalli (%)	8.1	49.3	0.4	18.8
Petrocelis (%)	4.3	54.5	0.7	2.3
Lottia paradigitalis (no.)	4.1	59.5	2.0	37.6
Barnacle recruits (%)	4.0	64.2	5.5	0.3
Lottia pelta (no.)	3.8	68.8	0.2	0.5

Table S4: **Results from a PERMDISP test on species composition data from grazer manipulation experiments conducted in West Vancouver (LS sites) and the Southern Gulf Islands (HS sites), in the summer of 2011**. Dispersion among the two salinity regions was unequal, as was dispersion among control and exclusion plots.

Df	SS	MS	F	N.Perm	*P*-value
Dispersion by salinity region					
1	0.155	0.155	7.463	999	0.008
82	1.707	0.021			
Dispersion by treatment					
1	0.451	0.451	17.318	999	0.001
82	2.136	0.026			

Table S5: **Contribution of species to the overall Bray-Curtis dissimilarity matrix of invertebrate and algal community composition of a grazer exclusion field experiment in low and high salinity regions**. The specific comparison is shown on the left side of the table, with the species' contribution to average between-group dissimilarity, as well as the mean abundance in each group of the comparison. Mean abundance group a and group b corresponds to the first and second group respectively, reported in the comparison on the left-hand side of the table. Grazer treatments are indicated with a '-' for grazer exclusions, and '+' for grazer controls. Species which make up 70% of the cumulative contribution are shown.

Taxon	Avg contribution (%)	Cumulative contribution (%)	Mean abundance group a	Mean abundance group b
high salinity – vs. high salinity +				
Ulva sp. (%)	18.4	28.9	39.4	5.1
Chthamalus dalli (no.)	15.6	53.4	24.9	153.4
Balanus glandula (no.)	10.8	70.3	1058.4	1138.4
high salinity – vs. high salinity +				
Ulva sp. (%)	18.2	30.6	39.4	57.7
Balanus glandula (no.)	13.8	54.0	1058.4	647.4
Fucus distichus (%)	7.3	66.3	0.0	0.4
Diatoms (%)	6.3	76.9	12.6	0.0
high salinity – vs. low salinity –				
Ulva sp. (%)	17.4	34.0	39.4	49.4
Balanus glandula (no.)	11.7	56.8	1058.4	803.8
Diatoms (%)	6.3	69.1	12.6	0.0
high salinity + vs. low salinity +				
Ulva sp. (%)	24.5	32.5	5.1	57.7
Chthamalus dalli (no.)	15.5	53.2	153.4	38.6
Balanus glandula (no.)	14.3	72.1	1138.4	647.4
high salinity + vs. low salinity -				
Ulva sp. (%)	25.1	36.5	5.1	49.4
Chthamalus dalli (no.)	15.7	59.3	153.4	46.9
Balanus glandula (no.)	11.5	76.1	1138.4	803.8
low salinity + vs. low salinity -				
Ulva sp. (%)	15.8	31.9	57.7	49.4
Balanus glandula (no.)	9.8	51.8	647.4	803.8
Fucus distichus (%)	8.9	69.7	0.4	0.1

Figures:

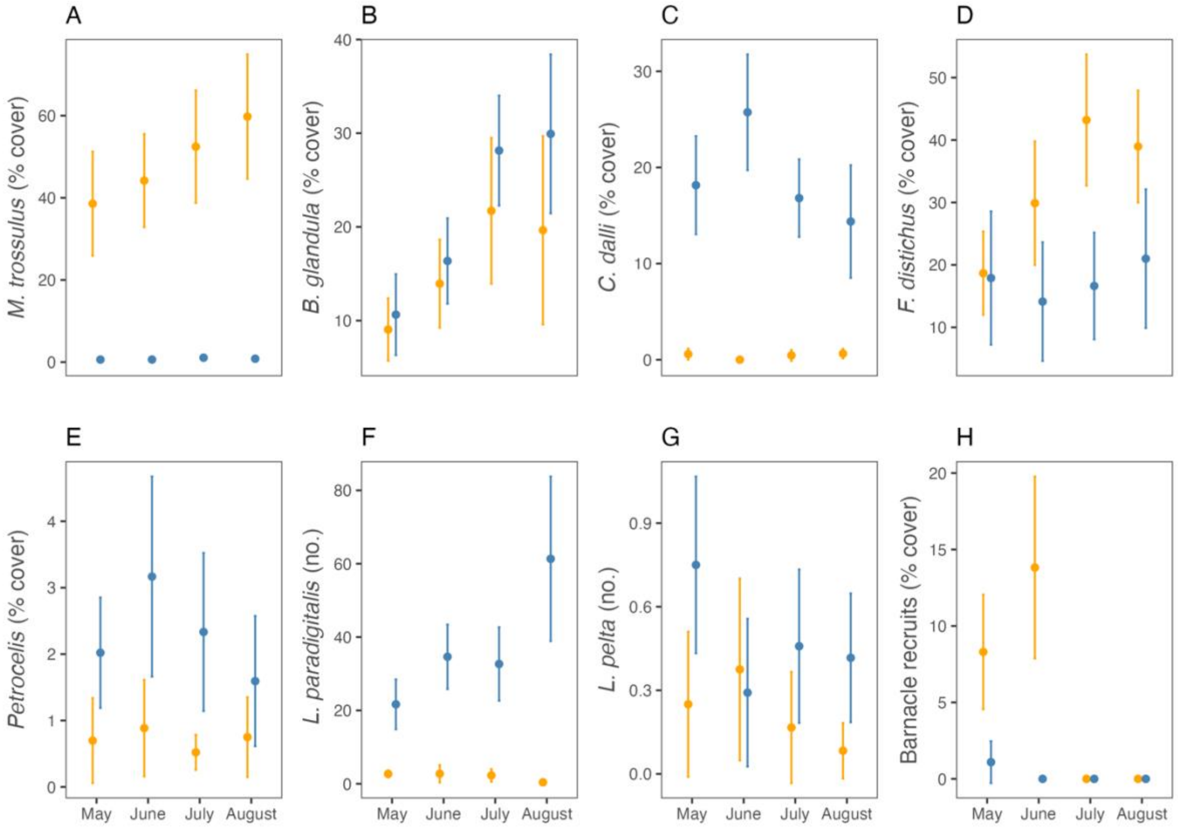

Figure S1: **Mean abundance of species which were key contributors**, as identified by a SIMPER analysis, to differences among intertidal communities in a low salinity region, West Vancouver, and a high salinity region, the Southern Gulf Islands during the summer of 2011. Error bars represent standard error.

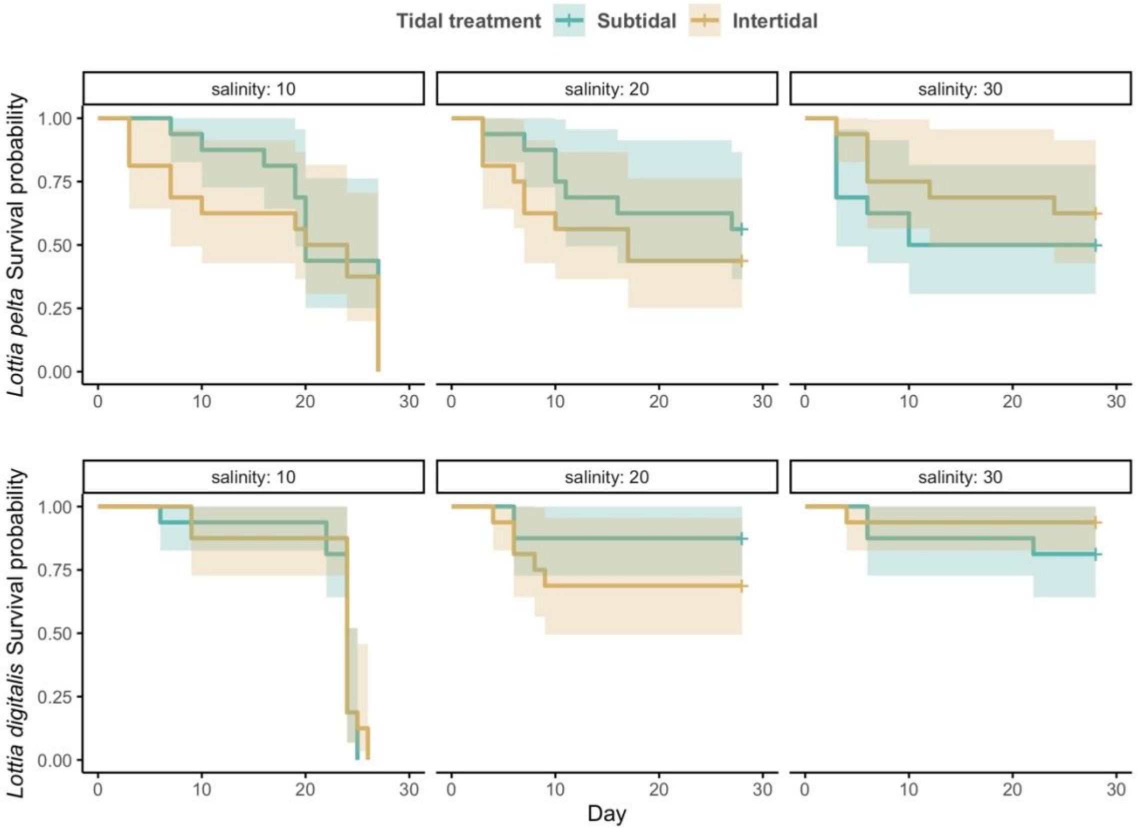

Figure S2: Kaplan-Meier survival curves for *Lottia pelta* and *Lottia digitalis*, both collected from HS1 in the Southern Gulf Islands, from a salinity of 32 psu. Limpets were exposed to a salinity of 10, 20 or 30 psu, in either a ‘subtidal’ treatment (immersed 24 hours/day), or an ‘intertidal’ treatment (8 hours/day of air exposure). Survival probability is indicated on the y-axis, where 1.0 is equivalent to 100% survival; shaded area indicates 95% confidence intervals

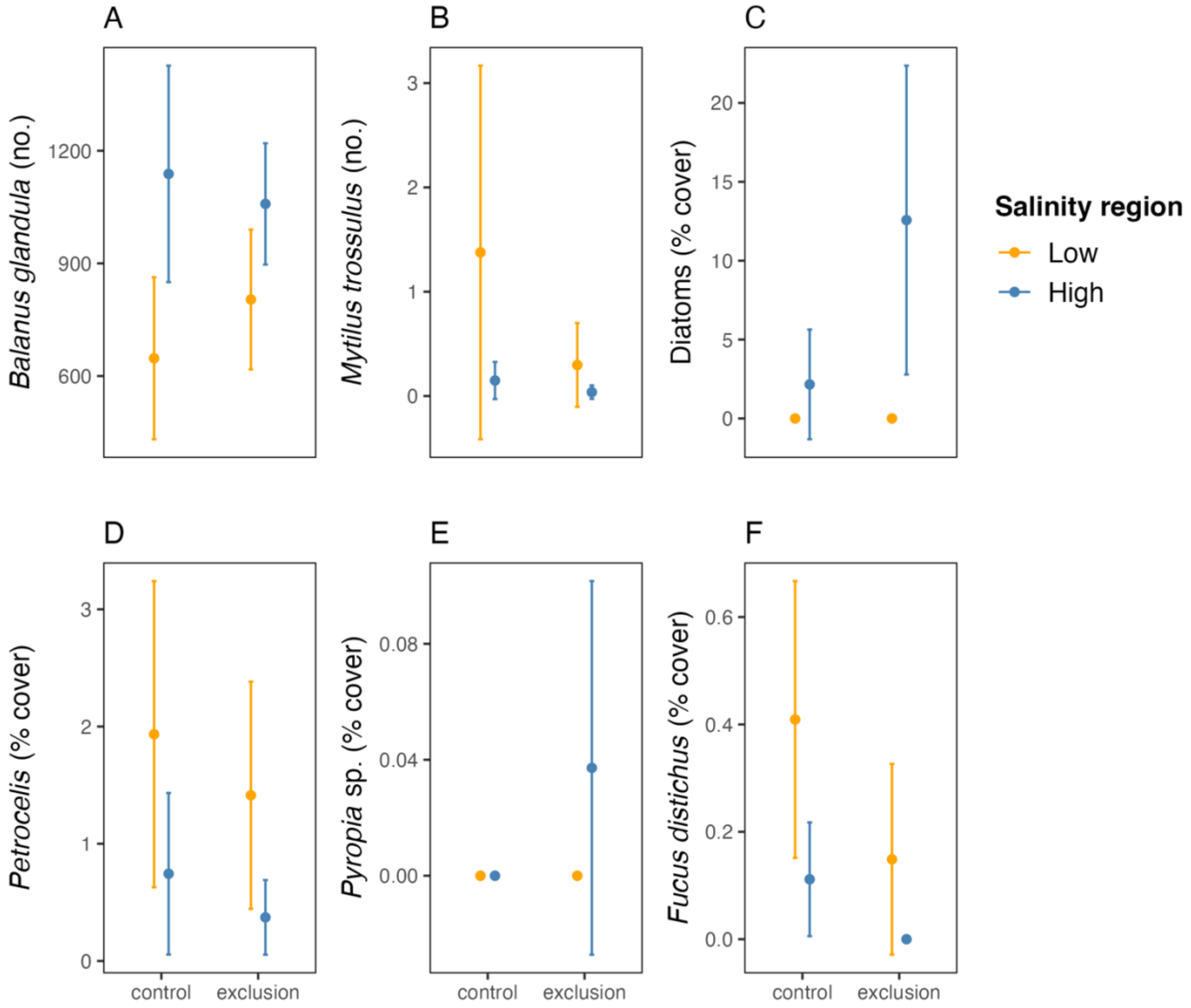

Figure S3: Mean abundance of species which were key contributors, as identified by a SIMPER analysis, to differences among intertidal communities of a grazer exclusion experiment in a low salinity region, West Vancouver, and a high salinity region, the Southern Gulf Islands. Error bars represent standard errors.