## Title

Low salinity significantly alters the bacterial community of the marine foundation species *Saccharina latissima* (sugar kelp)

## Short title

Salinity alters *Saccharina* bacteria

## Author information

Siobhan Schenk[1], sschenk@mail.ubc.ca

Connor Glen Wardrop[1], connor.wardrop@brentwood.ca

Laura Wegener Parfrey[1,2,3], lwparfrey@botany.ubc.ca

[1]Biodiversity Research Center, Department of Botany, University of British Columbia, 6270 University Blvd, Vancouver, British Columbia V6T 1Z4, Canada

[2]Biodiversity Research Center, Department of Zoology, University of British Columbia, 6270 University Blvd, Vancouver, British Columbia V6T 1Z4, Canada

[3] Hakai Institute, PO Box 25039, Campbell River, British Columbia V9W 0B7, Canada

## Correspondence

Siobhan Schenk

2212 Main Mall, Room 170, Vancouver, BC, Canada, V6T 1Z4
sschenk@mail.ubc.ca

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As climate change progresses, the intensity and variability of freshwater outflow into the ocean is predicted to increase. The resulting increase in low salinity events will be a source of stress for *Saccharina latissima* (sugar kelp) and potentially *Saccharina's* associated bacterial community. Bacteria are important to the health of their host because host-associated bacteria can facilitate or hinder host survival in stressful abiotic conditions. Therefore, understanding how bacteria change under abiotic stress is critical to our understanding of how host physiology will be affected by abiotic stress. In this study, sampled the bacterial community associated with *Saccharina* and the surrounding environment across two years of field sampling at four sites with contrasting salinity profiles around Vancouver, Canada during the 2021 and 2022 spring freshet (562 samples) coupled with salinity manipulation experiments in the lab repeated eight times in 2022, concurrently with field sampling (269 samples). Our illumina data show that salinity is a significant factor in shaping the overall bacterial community of *Saccharina*. We find significant increases in bacterial community diversity and paired with a significant reduction in the relative abundance of core bacteria in low salinity across lab and field data. Our findings suggest that host-filtering is significantly impaired in low salinity, raising questions surrounding how resilient some existing

*Saccharina* populations may be to more intense and more variable freshwater influx.

Kelps (*Laminariales*, brown algae) are marine foundation species that form dense underwater forests across the globe, providing food and habitat for other organisms [1]. Through ecosystem services and commercial harvest, kelps contribute an estimated $674 billion CAD per year to the global economy [2]. However, increased frequency and intensity of severe weather events, higher ocean temperatures, lower pH [3], increased coastal water turbidity [4], and lower ocean salinity [4–6] are associated with reductions in kelp recruitment, reproduction, and survival. Thus, kelps and the ecosystem services they provide are vulnerable to climate change [3, 4, 7].

There is a large body of evidence showing that low salinity is harmful to kelp. Field observations from Australia [5] and Norway [6] find up to 100% mortality of kelp sporophytes after freshwater floods. Lab studies on the effects of low salinity on kelps agree with field data. Research of the microscopic gametophyte stage finds lower spore settlement and gemination rates in low salinity [8]. For the macroscopic sporophyte stage, lower photosynthetic efficiency [9, 10], nitrogen uptake rates [11], and growth rates [12] are reported in low salinity treatments. As anthropogenic climate change progresses, lower ocean salinity is predicted by modeling studies to be one of the most intense [3, 4, 7] and least predictable abiotic stressors that kelps will experience [3].

One unknown is how changes in abiotic stressors, including low salinity, will influence the kelp bacterial community, and whether these changes exacerbate or ameliorate kelp stress tolerance. The bacterial community of macroalgae can influence growth and morphology [13, 14] and tolerance of abiotic conditions [15].

Both free living and host-associated bacterial communities change with abiotic conditions. Seasonal cycling can drive predictable change in environmental [16, 17] and host-associated [18] bacterial communities. Additionally, local abiotic conditions, including salinity, can significantly shape the bacterial community in the water column [19, 20] and on non-kelp algal hosts [21–23]. Temperature and temperature significantly alter the bacterial community of the kelp *Ecklonia radiata* in the lab [24], but it is not known how salinity influences the bacterial community of kelps.

Across host-microbe systems, changes in bacterial community composition in response to abiotic stress are correlated with negative, positive, or no effects on host condition. In some marine hosts, including anemones [25], corals [26], and sponges [27], a change in the bacterial community is associated with increased host tolerance to abiotic stress. In other cases, including in green algae (*Ulva sp.*) from localities with different salinity [23] and transplanted seagrasses [28], changes in the bacterial

community composition are not associated with changes in host-health. Finally, declines in host condition in response to stress have been associated with two patterns: directional shifts in community composition and a destabilization of a typically stable bacterial community. In kelp, a change in the bacterial community composition is correlated with decreased host health [24, 29]. For the brown algae *Ectocarpus sp.* a single bacterial strain is responsible for the algae's ability to grow in freshwater [15], showing how destabilization of a small part of the bacterial community can severely impact the host's ability to tolerate particular abiotic conditions. Destabilisation has also been observed systems such as corals and sponges, and is characterized by declines in the core microbiome, increased alpha diversity, and increased dispersion in community composition (Anna Karenina principle) [30]. Presumably, destabilization is a result of the disruption of the host filtering that typically maintains a stable, low diversity, core community. However, in most cases, associations between host condition and bacterial community changes are correlative. Assigning causality in the link between host condition and change in host-associated bacterial communities in response to stress is an open challenge [27, 31, 32].

In this study, we asked how low salinity stress affects the subcanopy kelp *Saccharina* ((L.) C.E. Lane, C. Mayes, Druehl, and

G. W. Saunders) and its bacterial community. We define stress as a condition that adversely affects growth via damage and/or resource allocation associated with damage prevention and cellular repair [33, 34]. For our two-year field survey from April to July in 2021 and 2022, and the concurrent lab study in 2022, we predicted that (i) salinity would shape the bacterial community of *Saccharina* as observed in other algal systems [22, 23] and that (ii) alpha and beta-diversity would increase in lower salinity, accompanied by a decrease in relative abundance core bacteria, as observed in other marine host systems experiencing abiotic stress [27, 30, 32].

Our extensive dataset shows that salinity has a small but significant effect on the overall bacterial community composition and that lower salinity significantly decreases the relative abundance of the core bacterial community while increasing mean community dissimilarity, suggesting that host-filtering by *Saccharina* is impaired in low salinity.

## Methods

### *Field Site Description*

Five field sites near Vancouver, Canada were visited across two successive years. Both years, sites were sampled every two weeks at low tide, from mid-April until mid-July (2021) or early August (2022) during the time of the annual freshwater influx, due to snow melt in late spring, hereafter called the spring freshet.

In 2021, we collected data at Lighthouse Park (lhp; 49.329, -123.264), Starboat Cove Park (scp; 49.333; -123.261), Third Beach (tb; 49.302, -123.158); and Girl in a Wetsuit (gw; 49.304, -123.126; Fig. 1). In 2022, lhp was replaced by Cates Park (cp; 49.300, -122.958; Fig. 1). The West-Vancouver sites (lhp, scp, and cp) were always sampled on the same day and the Vancouver sites (tb and gw) were sampled the subsequent day. It was not possible to sample at all four sites on the same day due to the tide height required and travel time.

### *Site-Conditions*

In 2021 and 2022 we recorded water temperature and salinity at the start of each site visit (Fig. 1). In 2021, all measurements were conducted with a YSI ProQuatro Multiparameter Metre. On two sequential samplings in 2022, salinity only measurements were taken with a refractometer because

the YSI was not available. In all cases, abiotic measurements were repeated three times per site visit and the measurement instrument was calibrated per manufacture instructions prior to each sampling.

*Saccharina field collection*

In 2021, six *Saccharina* individuals were collected haphazardly at each site visit, including individuals that were out of the water, in the wave splash zone, or fully submerged in the ocean or tidepools. Given the strong influence of *Saccharina* position seen in the 2021 microbial data (supplementary results; Fig. S3), in 2022, only individuals that were submerged in the ocean were sampled (six per site). At tb in 2022, six additional individuals were collected from tidepools starting in May 2022 in case only tidepool *Saccharina* were accessible at tb later during the summer and to better understand the differences between tidepool and ocean *Saccharina* bacterial communities (supplementary results; Fig. S4).

*Field bacterial data collection*

In both years two water samples, two rock swabs, and at least six *Saccharina* meristem swabs (bottom 10 cm of the thallus) were collected each site visit to capture changes in the bacterial community throughout the spring freshet. Samples were taken wearing gloves sprayed with 70% ethanol between samples.

We swabbed the meristem because it is the newest and most selective tissue [35], presumably hosting the bacterial community most indicative of *Saccharina*. We gently rinsed *Saccharina* and rocks with 0.22 μm filter-sterilized seawater; then swabbed the surface for 10 s with cotton tipped swab (VWR, CA10805-154). The swab was then broken off into a cryovial (VWR, CA66021-993).

Water column bacterial samples were collected by pre-filtering seawater through a 150 μm mesh before filtering the water through a 0.22 μm membrane (MilliporeSigma, Sterivex™ Filter Unit) until the filter clogged or 500 mL of water had been sampled. The Sterivex™ was stored in a Whirl-Pak (VWR, 13500-390).

All samples were placed in a cooler with ice packs at -20°C until returning to the lab (within three hours of sample collection), where samples were stored at -70°C until extraction.

*Lab experiment protocol*

We performed eight experimental rounds manipulating salinity through the 2022 field season, corresponding to each field sampling event at gw. After collecting the field bacterial samples, 18 *Saccharina individuals* were collected (the holdfast, stipe, and bottom 15 cm of the blade), numbered with flagging tape wrapped around the stipe, and transported in a cooler filled with seawater from gw (approximately 1 h transit).

*Saccharina* were cultured in seawater at 10°C on a 12h light:12h dark photoperiod with bubblers to induce water motion. Each culturing round included six 8 L aquaria (two per treatment), with three *Saccharina* individuals per aquaria (Fig. 1), in one of three salinity treatments: 10, 20, and unaltered sea water (full strength hereafter). The full strength seawater is pumped from 30 m depth in Burrard Inlet and brought to UBC by truck. The salinity fluctuated between 31 and 32 depending on the culturing round (Table S1).

Most experimental rounds lasted four days with samples taken on day zero (field collection day), day one (24 h after collection), and day four. Our first culturing round lasted six days; on day six the *Saccharinas* in 10 salinity were green and fell apart to the touch, so we shortened the culturing time to three days for culturing round two. We did not observe significant *Saccharina* damage on day three, so we extended the culturing duration to four days for the remaining experimental rounds. For culturing round five, there are no dry weight measurements on day four (Fig. 2) because we extended the culturing time to fourteen days, to see if the *Saccharinas* in the 10 salinity would fall apart in as they did in round one. They did not.

*Lab bacterial collection protocol*

All lab bacterial sampling (Fig. 1) followed the protocol in the field section with the following modifications. The substrate samples were taken by swabbing the airline tubing of the bubbler rather than rocks and the aquaria water was not pre-filtered before taking bacterial samples. Note, the day zero *Saccharina* swabs are the gw field samples. Day zero water and substrate samples were taken prior to adding the *Saccharinas* to the aquaria. Lab *Saccharina* samples (day one and day four) are swabs of all three *Saccharinas* in the same aquaria, because we assumed the bacterial community was homogenized. In total, we collected one of each sample type per aquaria, per lab sampling day (Fig. 1).

*Lab Saccharina condition*

After taking bacterial samples, *Saccharina* condition was assessed on an individual basis (n=6 per salinity treatment/timepoint/experimental round) by quantifying blistering occurrence, wet weight, estimated quantum yield, and ratio of dry weight to wet weight on the last day of each experimental round.

Blistering occurrence was recorded as in other kelp studies [5, 24]. Estimated quantum yield, measurements were taken with a Junior-PAM Chlorophyll Fluorometer as described in the user manual. Briefly, we calibrated the PAM and dark acclimated the

*Saccharinas* for five minutes on a damp cloth. Then, we placed the optical fiber over the base of *Saccharina* (just above the stipe) and took the measurement. Wet weight measurements were taken after blotting dry the *Saccharina* before promptly returning the individual to its respective aquaria.

Dry weights were obtained as follows. After taking wet weight measurements on the last experimental day, stipes were cut off from the *Saccharinas* [9]. Then, we weighed the *Saccharinas* again, dried them in at 60°C for at least 24 h, and re-weighed them [9, 36].

*DNA extraction and sequencing*

In 2021, all DNA was extracted with QUIAGEN DNeasy PowerSoil Pro Kit 96-well plate; 47017 or single tube; 47014 following the manufacturer's protocol. In 2022, 36% of field swabs (rock and *Saccharina*) samples were extracted with QIAGEN 96-well kits and 64% with ZymoBIOMICS 96 MagBead DNA/RNA Kit (Zymo, R2136). All water samples from the 2022 field season and all lab samples in 2022 were extracted with the Zymo kit (see metadata). The combination was a result of global supply chain issues. For the extractions with the Zymo kit, the QIAGEN PowerSoil Max beads (QIAGEN, 12988-10) were used for the initial bead-beating step, and then the Zymo kit was used following

the manufacturers protocol. Each extraction plate included a blank swab or Sterivex™ membrane (extraction blank, n=12).

Primers for PCR were the 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3'), which target the V4 region of the 16S rRNA gene. Both primers have an Illumina adaptor and golay barcode on the 5' [37, 38]. PCR reactions were as follows: 1 µL extracted DNA (for extracted samples) or water (for PCR blanks, 1 per PCR plate, n=12), 15 µL Phusion™ High-Fidelity DNA Polymerase (Thermo Fisher, F530S), 2.4 µL of forward primer, 2.4 µL reverse primer (primers are from a 2.5 µM working stock solution), to a final reaction volume of 30 µL with molecular water (Thermo Fisher, SH30538FS). PCR reactions consisted of 30 s initial denaturation at 98°C, 25 cycles of amplification (30 s at 98°C, 30 s at 55°C, and 20 s at 72°C), and a final elongation step at 72°C for 10 min, which are the conditions recommended for the Phusion™ polymerase. Samples where a band was not visible on a DNA gel after two attempts with 25 cycles were amplified with 35 cycles (see metadata).

PCR reactions were cleaned with the QIAGEN QIAquick PCR Purification Kit (28104 or 28181) and quantification was performed with the Quan-IT PicoGreen assay kit (Thermo Fisher, P7589), following the manufacturers protocol. Samples were pooled

to equal concentrations and sent for Bioanalyzer at the UBC Sequencing Facility, Vancouver, British Columbia. In 2021, the library was sent for Illumina MiSeq at the Hakai Institute, Campbell River. In 2022, both libraries were sent for illumina MiSeq at the UBC Sequencing Facility at the same time. In all cases, libraries were constructed with the Illumina, MS-1023003 kit (MiSeq v3, 2x300).

*Sequence data processing*

Raw, demultiplexed reads were downloaded from the Illumina hub and imported into RStudio (R version 4.2.2, RStudio v2022.10.31; [39, 40]). Reads were processed following the dada2 pipeline (v1.24.0) [41]. Briefly, primers were removed, reads were truncated to maintain high quality for downstream analysis with the filterAndTrim function (275 bp for forward and 200 bp for reverse in 2021 and 230 bp for forward and 175 bp for reverse in 2022), error rates were calculated, paired forwards and reverse reads were merged, a sequence table was constructed, and chimeric reads were removed. The 2021 and 2022 sequence tables were merged (mergeSequenceTables) and amplicon sequence variants (ASVs) that differed by only the end base pair were collapsed (collapseNoMismatch), prior to assigning taxonomy with SILVA v138 [42] formatted for the dada2 pipeline [43].

The merged sequence table, the taxonomy table, and metadata were grouped into a phyloseq object for filtering (v1.40.0) [44]. Unassigned taxa and taxa assigned to chloroplasts, mitochondria, or eukaryotes were removed in addition to sequences assigned as *Pseudomonas*. *Pseudomonas* were present at high relative abundance in multiple extraction blanks and was abundant in nearly all samples extracted with the Zymo kit, but present in only 1 sample extracted with the Qiagen kits across both years; hence, *Pseudomonas* is very likely a kit contaminant. Next, samples with less than 1000 reads were removed; then, ASVs representing less than 0.001% of total reads in the dataset were removed; counts in the ASV table that were five or less were converted to zero (per sample filtering, to minimize the effect of barcode switching), and ASVs found in less than two samples were removed. Finally, samples with more than 200,000 reads were removed (four samples) and all samples from one 10 salinity aquaria in experimental round five were removed (four samples total) because we noticed that there was *Desmerestia viridis* lodged in the holdfast. *Desmerestia sp.* produce sulfuric acid [45] and pH alters the bacterial community of kelps [24]. In total, filtering retained 16,643,912 of 24,862,382 total paired reads, 3,016 of an initial 29,105 unique ASVs, and 838 samples from the initial 1,095 samples (Table S2 and S3), with a mean of 19,861 reads per sample.

We converted the data to an iNEXT (v3.0.0) [46] compatible format with the metagMisc package (v0.0.4) [47] to perform coverage-based rarefaction. The sample coverage was set to 0.8 and iterated 1000 times.

Following filtering and coverage-based rarefaction, we noticed a large difference in richness between samples extracted with the Zymo kit compared to the Qiagen kit. We investigated to see if this was due to the kits detecting a different suite of taxa. This is not the case (see supplementary methods and results).

*Statistical analysis*

To compare blistering incidence between salinity treatments, we used a Fisher's exact test with a Benjamini-Hochberg correction with the package rstatix (v0.7.1) [48]

To compare means between treatment groups, we ran an analysis of variance (ANOVA) followed by a Tukey post-hoc test. In all cases, we tested the assumption of equal variance between groups with Levene's test in the package car (v3.1-1) [49] and validated the assumption of normality with qq-plots. When the assumptions of equal variance was violated, we performed a Welsh ANOVA with the function oneway.test with "var.equal = FALSE" followed by a Games-Howell post hoc test in the package biostat (v0.0.20) [50]. When the assumption of normality was violated, we

ran a Kruskal-Wallis test followed by a post-hoc Wilcoxon test with a Benjamini-Hochberg correction [40].

We ran permutational analyses of variance (PERMANOVA) on rarefied data with the adonis2 function in the package vegan (v2.6-4) [51] with the distance metric set to Bray-Curtis. Marginal PERMANOVAs were ran the same way, adding the by = “margin” argument. In all cases, we tested for equal dispersion with the betadispers function (equal unless stated otherwise) and ran a post-hoc Pairwise Adonis test in the pairwise.adonis package(v0.4.1) [52]

To calculate the Bray-Curtis dissimilarity index between samples, we used the divergence function in the package microbiome (v1.22.0) [53] as in [54]. Pearson’s correlation tests were performed using the cor.test() function and linear regression models were performed with the lm() function and assumptions were assessed graphically.

To identify the core *Saccharina* ASVs, we selected all field samples collected when the ocean salinity was 20 or greater and ran an indicator species analysis (IndVal) on non-rarefied data with the function multipatt from the package indicspecies (v1.7.12) [55] with 999 permutations, comparing substrate types (*Saccharina*, water, rock). The core ASVs are those assigned to *Saccharina* with an IndVal statistic $\geq 0.7$ and a p-value $\leq 0.05$.

*Reanalysis of published data*

Raw data from King et al. (2023) were downloaded, processed with the dada2 pipeline, and filtered as described above and in their original publication. Core *Saccharina* ASVs were identified with IndVal analysis and used a prevalence ≥ 0.8, replicating methods in King et al. (2022)

## Results

*Lab salinity treatments differentially induced stress in Saccharina.*

We verified that lab salinity treatments differentially induced stress in *Saccharina* by measuring published stress-associated kelp phenotypes. We assessed blistering incidence on day four by Fisher's Exact Test followed by a pairwise Fisher post-hoc test, which shows that our lowest salinity treatment had significantly greater blistering incidence on day four than the other salinity treatments (Fig. 2A). Experimental round one lasted six days and experimental round two lasted three days. We did not observe any blistering on the last day of these rounds; however, on day six of round one, the *Saccharina* in the10 salinity were green and disintegrated when touched, indicating death.

Next, we compared the dry weight to wet weight ratio on day four by ANOVA (Fig. 2B) followed by a Tukey Post-Hoc test. The 10 salinity has a significantly lower dry weight to wet weight ratio compared to the other salinity treatments. Then, we compared the ratio of wet weight on day four compared to day zero (field collection day) by ANOVA. There are no significant differences between salinity treatments (Fig. 2C). These results paired with our dry weight data suggest that *Saccharina* is accumulating moisture but not additional biomass in low salinity. Finally, we analyzed photosynthetic efficiency found no significant difference between

salinities on day four by ANOVA (Fig. 2D). Hence, *Saccharina* remains alive in our lab cultures for at least four days.

*Salinity treatments in the lab shapes the bacterial community.*

We assessed changes in bacterial community diversity and composition in response to salinity treatments. We ran a PERMANOVA to test if salinity alters the bacterial community for each lab substrate type on day four (Table S4) and made NMDS plots (Fig. 3, Fig. S5). The bacterial community composition is significantly different between the full-strength and 10 salinity treatments on day four for *Saccharina* and water (Fig. 3, Table S4), but not for substrates (Fig. S5, Table S4) samples.

To compare variance in the bacterial community dissimilarity between salinity treatments, we calculated the Bray-Curtis dissimilarity index between samples from the same salinity treatment and sample type on day 4 (Fig. 4, Fig. S6). Then, we compared the mean dissimilarity between treatments and found significant differences for *Saccharina* (20 more dissimilar that full-strength) and water (full strength more dissimilar than 10 and 20) samples (Fig. 4); substrate samples did not differ (Fig. S6). We also compared the mean Shannon-Weiner diversity index between salinity treatments found that by ANOVA, salinity does not

significantly alter alpha diversity in the lab for any sample type (Fig. 4, Fig. S6). These data show that *Saccharina* bacteria are more dissimilar between individuals, but not more diverse, in lower salinity treatments.

*Factors shaping the bacterial community of Saccharina in the field.*

We examined how salinity shapes bacterial community diversity and composition the field in comparison to temperature and Julian Day. Note that salinity is significantly negatively correlated with Julian Day and water temperature, while water temperature and Julian Day are positively correlated (Fig. S1). We ran marginal PERMANOVAs including water salinity, temperature, and Julian day for each field substrate type to assess the unique explanatory power provided by each variable (Table 1). Julian day explains the most variation in bacterial community composition, followed by temperature, then salinity for all substrate types. Consistent with observations in the lab, salinity does not significantly shape the bacterial community of rocks (Fig. S5, Table S5). Salinity is significant for *Saccharina* and water samples (Fig. 3, Table 1, S5), although the explanatory power of salinity is relatively low.

*Community dissimilarity increases in low ocean salinity, but not diversity.*

We asked whether community dissimilarity and diversity change as salinity changes. We calculated the Bray-Curtis dissimilarity index for each field sample, comparing the same sample type within the same site and sampling visit (salinity, temp, and Julian day were the same). We also calculated the Shannon's diversity index for each field sample. In both cases, linear regression models tests show that for *Saccharina* samples, Bray-Curtis dissimilarity increases as ocean salinity decreases. We find no significant relationship between salinity and Shannon's diversity index (Fig. 4, Table S6). We found no significant correlation between diversity metrics and salinity for water (Fig. 4, Table S6) or rock samples (Fig. S6, Table S6).

*The relative abundance of the Saccharina core bacteria negatively correlated with salinity.*

We then identified the core bacterial community of *Saccharina* by conducting an IndVal analysis comparing *Saccharina* samples to water and rock samples from all sampling events where ocean salinity was greater or equal to 20. We used an IndVal statistic threshold of 0.7, which required core ASVs to be a

>50% prevalence and enriched on *Saccharina* compared to water and rocks. We identified six core ASVs: *Persicirhabdus sp.* (ASV1), *Litorimonas sp.* (ASV3), *Maribacter sp.* (ASV4), *Cocleimonas sp.* (ASV5), *Robignitomaculum sp.* (ASV7), and and unidentified G*ammaproteobacteria* (ASV15; Fig. 5, Table S7). We ran Pearson's correlation tests on the relative abundance of all core ASVs combined and each core ASV alone. The total core relative abundance, *Cocleimonas sp.*, and *Maribacter sp.* are significantly correlated with salinity in the field (Fig. 5).

We then asked if these changes are observed in the more controlled environment of the lab. We again find that in lower salinity, the relative abundance of the core overall is significantly lower and find that the relative abundance of different ASVs (*Litorimonas sp.* and *Robiginitomaculum sp.*) is significantly lower in low salinity (Fig. 5). Hence, the relative abundance of the core overall consistently decreases as salinity decreases, but core ASVs driving this pattern are inconsistent between lab and field.

The decline in core relative abundance could be a result of a different core bacterial community colonizing or blooming in low salinity. We tested for a low salinity core as above using samples ocean salinity was less than 20. There were no new core ASVs specific to low salinity. In the lab, we observed an increase in the relative abundance of Alteromonadales at low salinity compared to

higher salinity in the lab (Fig. S10), but which ASVs were enriched differed across sampling rounds was not consistent.

*Core bacteria are conserved across very large spatial and temporal scales.*

To compare our *Saccharina* core to data in published in the literature, we downloaded reads from a study performed in the United Kingdom from September and August 2015 [56]. We then performed another IndVal using a prevalence of ≥0.8 on *Saccharina* as a threshold to identify the *Saccharina* indicator ASV from the King *et al.* (2022) study. Then, we compared these ASV sequences to our own and found that our *Maribacter* (ASV4) and unidentified *Gammaproteobacteria* (ASV15) are perfect sequence matches to a ASVs identified as *Saccharina* indicator in their data. Our other core ASVs are not perfect matches to theirs, but all genera that are represented in our core are present in the King *et al. Saccharina* indicator taxa. These findings suggest that depending on the filtering threshold, *Saccharina* may have a global core bacterial ASV community.

## Discussion

The predicted [3, 4] and ongoing die back of kelp forests [3, 5, 57] due to low salinity represents a global concern because of the environmental, economic, and cultural value that these foundation species provide [2, 3]. Our research shows that salinity significantly alters the bacterial community of *Saccharina*, both in the lab and in the field.

*Saccharina* hosts a core bacterial community, which is stable across large spatial and temporal scales (King *et al.*, 2023 and this study). We show that in low salinity, the relative abundance of core bacteria significantly decreases in conjunction with a significant increase in bacterial community dissimilarity. Together, these findings suggest that more opportunistic colonizers associate with *Saccharina* in lower salinity conditions in the field, pointing to decreased host-filtering by *Saccharina* in low salinity [58]. Overall, our results shows that although *Saccharina* is persisting in the field and remains photosynthetically active throughout the lab experimental period, *Saccharina* is experiencing stress in low salinity.

We show that salinity is a relatively weak but significant factor in shaping the bacterial community of *Saccharina*, which agrees with other studies investigating the effect of salinity on the bacterial community of other algae [21, 23]. Salinity explains more

variation in the bacterial community of *Saccharina* in the lab (9.8% total variation) compared to the field (0.66% unique variation, 1.7% total variation). In the field, Julian day (day of the year; 3.48% of unique variation, 7.16% of total explained) is much better than both salinity and temperature at explaining the variation in the bacterial community of *Saccharina*. This is likely due to combined effects of many unmeasured variables corelated with Julian day including: dissolved organic carbon, pH [16], irradiance, and nitrogen availability [59]. The large discrepancy in the explanatory power of salinity between lab and field highlights the importance of pairing field observations, which provide a more holistic view of the system, with lab experiments, which permit the manipulation of a single variable (salinity) without confounding effects of other co-variates present in the field.

The variation in the bacterial community observed herein is driven by a significant decrease in relative abundance of core ASVs, paired with a significant increase in community dissimilarity in low salinity, but no significant difference in diversity, although the trends we observe suggest minor increases in diversity as salinity decreases. This suggests that there are not more bacterial ASVs on *Saccharina* in low salinity (alpha-diversity), but that the variation between *Saccharina* individuals increases in low salinity (beta-diversity). Overall, our data fit the Anna Karenina Principle, which

posits increased alpha and beta-diversity in a "disrupted microbiota" [30], which has been observed in corals [32] and sponges [27]. However, stating that the beta-diversity change we observed indicates a disrupted microbiota would be a narrow view [60]. The word disruption itself has a negative connotation [61], which is not always biologically supported. A disrupted microbiota is often associated with unhealthy hosts [62], and is proposed to make the algal host more susceptible to disease [31]. By disruption, what is often meant, is a different microbiota under different conditions. In fact, a variable bacterial community can be beneficial, including increasing host-tolerance to various abiotic conditions [25–27, 63]. Our study is not designed to test if the changes we observed in the bacterial community of *Saccharina* are beneficial or not to *Saccharina*'s ability to tolerate low salinity.

Our two-year field study and manipulative lab experiment, along with existing research showing that increased water temperatures and decreased pH significantly alter the bacterial community of kelp in the lab [24], and climate models predicting that the intensity and frequency of abiotic stressors will increase with climate change [3], suggests that the bacterial community of kelps will be affected by climate change. We propose that in stressful abiotic conditions, kelps will exhibit decreased host-filtering of their bacterial community. If kelps do not filter their

bacteria as effectively in the future, it is unclear how kelp health and resilience to abiotic stress will be affected in natural kelp forests and in mariculture operations. In particular, the significant decrease in core bacteria, which often perform functions that are beneficial for the host [27], highlights the importance of considering the microbiota [64] when predicting host performance and overall biodiversity under climate change.

**Data availability**

Raw reads for this study are available on the European Nucleotide Database (ENA) under project PRJEB60884. Code and metadata are available on Borealis https://borealisdata.ca/privateurl.xhtml?token=96717e7c-3f4d-4c3b-8301-d91bf887606d.

The data King et al. (2023) were downloaded from ENA (PRJEB50679) and https://doi.org/10.6084/m9.fgshare.19453889.v1.

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## Contributions

Siobhan Schenk: conceptualization (equal), data collection (equal), writing (equal), editing (equal), data analysis (equal). Connor G. Wardrop: data collection (equal), editing (equal). Laura W. Parfrey: conceptualization (equal), data collection (equal), writing (equal), editing (equal), data analysis (equal).

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## Main text figure and table legends

**Figure 1**. A) Map of the sampling sites with black arrows representing the path of main freshwater sources in the area, B) conductivity, and C) water temperature profiles across sampling years. The error bars are +/- one standard deviation around the mean. D) Representative schematic of 2022 lab experiment. Note, in C, missing temperature data from 2022 are from days where a refractometer, rather than the YSI were used to measure water parameters. Also note, in D, the n = 1 for *Saccharina* because all individuals in the same aquaria are swabbed with the same cotton swab. Plots showing the correlation between salinity, temperature, and Julian Day are in figure S1. Full temperature profiles (Fig. S2) show the same patterns across sites and time.

**Figure 2.** *Saccharina* stress phenotypes by lab salinity treatment. A) The presence (orange) or absence (grey) of blistering by lab culture day, experimental round, and salinity treatment by individual. Fisher's Exact Test ($p < 0.001$) followed by a Pairwise Fisher Test of the day four data, show that the 10 salinity has significantly more blistering than the 20 and full-strength salinity on day four ($p \leq 0.0357$). B) The ratio of dry weight to wet weight on lab day four. C) The ratio of wet-weight on lab day four to wet-weight on day

zero. D) The estimated effective quantum yield on lab day four by salinity. For B, C, and D, ANOVA output comparing the different salinity treatments are indicated in the corresponding panel. Note, round one and two have no day four observations for all panels. In addition, in panel B only, there are no day four observations for culture round five. Day one comparisons for panels A, C, and D are in the supplementary results.

**Figure 3.** NMDS plot of A) *Saccharina* and B) water samples on day four colored by salinity treatment along with C) *Saccharina* and D) water samples from the field. Note, corresponding PERMANOVA outputs are in Table 1, S4, and S5.

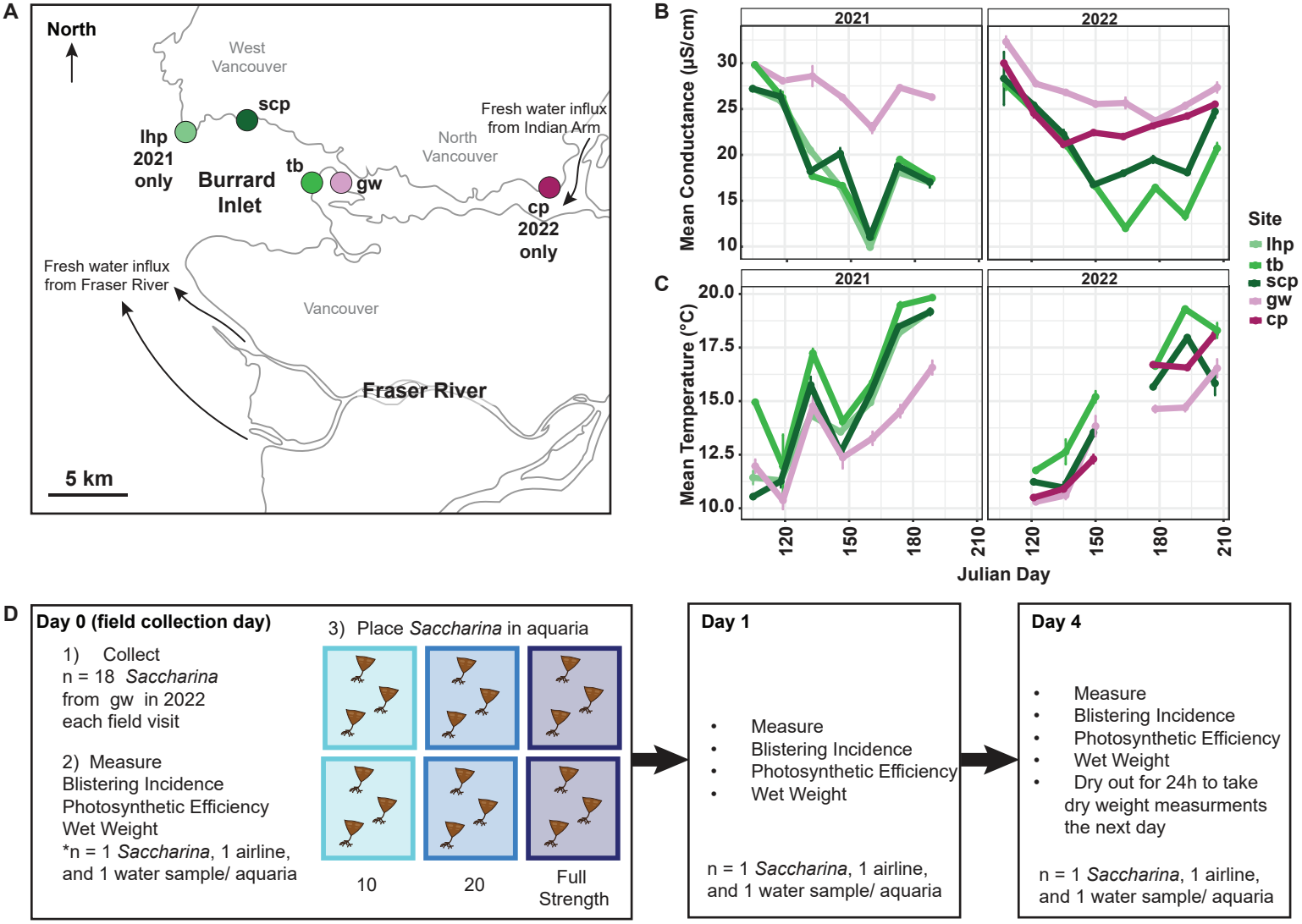
**Figure 4.** The per sample Bray-Curtis dissimilarity index (beta-diversity) of lab (A,B) and field (E,F), *Saccharina* and water samples. Beta-diversity is calculated within the same substrate type either by comparing samples within a salinity treatment (lab) or the same sampling site visit (field). Note, the full output of the linear regression models containing main effects and all possible interactions for salinity, water temperature, and Julian Day (E-F) are in Table S6. The t-values listed here are for the main effect of salinity in the linear regression model. The Shannon-Weiner

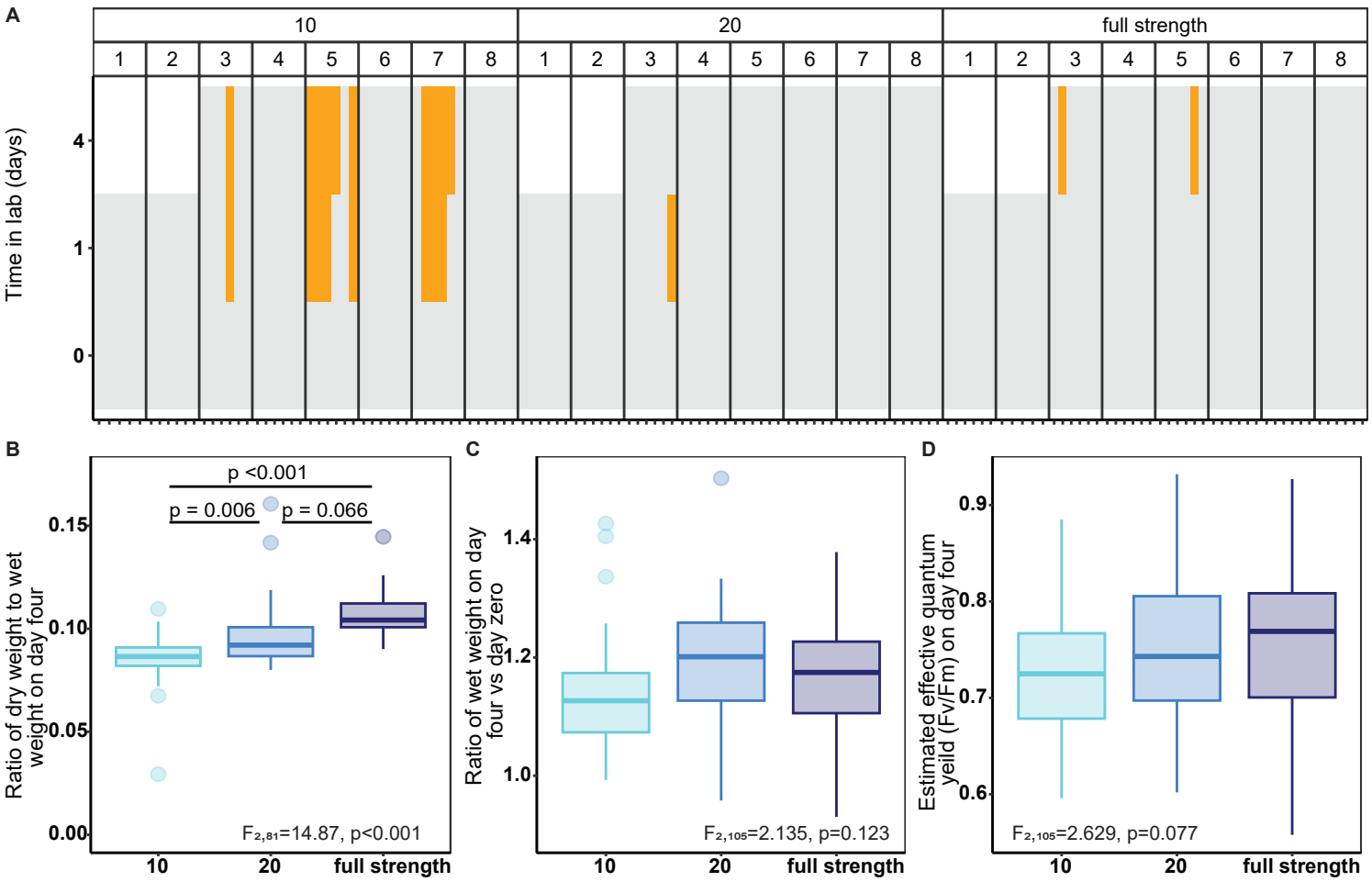
diversity index of lab (C,D) or field (G,H) *Saccharina* and water samples. For lab samples (A to D), p-values are indicated in if the salinity treatments are significantly different by ANOVA (Tukey post-hoc test) or Welsh ANOVA (Games-Howell post-hoc test).

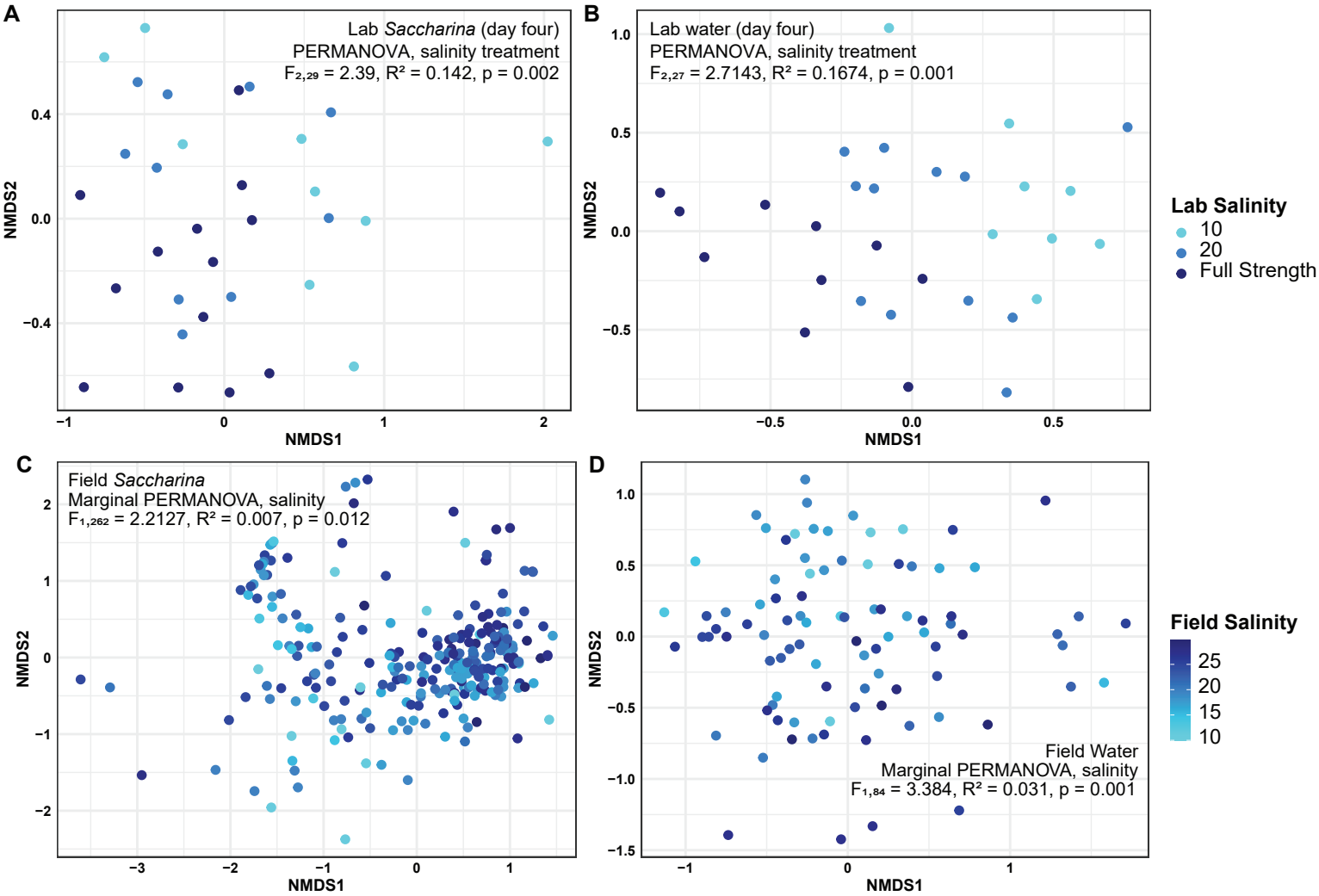
**Figure 5.** Relative abundance of core ASVs in the field (A, B) and in the lab on day four (C, D) on *Saccharina*. The output of the Pearson correlations test assessing the relative abundance of core ASVs as a percent of total reads by ocean salinity are indicated in the corresponding panels (A, B). For lab day four samples, the output comparing the mean relative abundance of core ASVs as a percent of total reads by salinity treatment is also indicated in the facets (C, D). We conducted either an ANOVA (F) or Kruskal-Wallis rank sum test (X) if the assumptions of the ANOVA were violated. When required, we conducted a Tukey post-hoc test for ANOVAs or post-hoc Wilcoxon rank sum test with a Benjamini-Hochberg correction for Kruskal-Wallis rank sum tests. Note, the different y-axes between the field and the lab. Also note, the mean relative abundance core ASVs on day zero (field collection day) is 0.5114 ± 0.2493 (sd), which is not significantly different from any salinity treatment on day four ($X_3^2 = 410.48$, $p = 0.5672$).

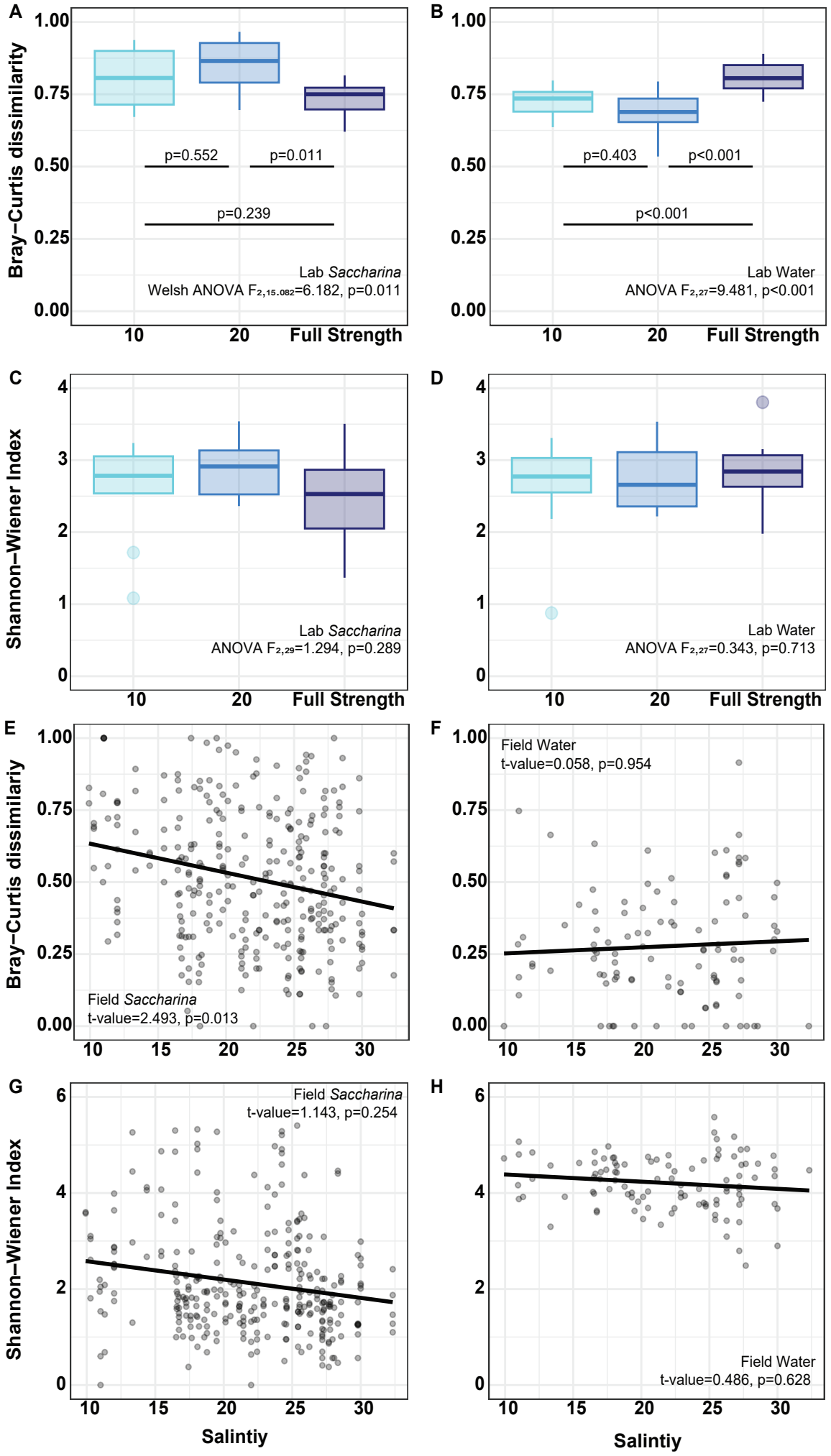
**Table 1.** Output of marginal PERMANOVA marginal showing the unique explanatory power marginal explanatory of salinity, water temperature, and Julian day for field samples. Output of PERMANOVA showing the total explanatory power of each variable are in Table S5.

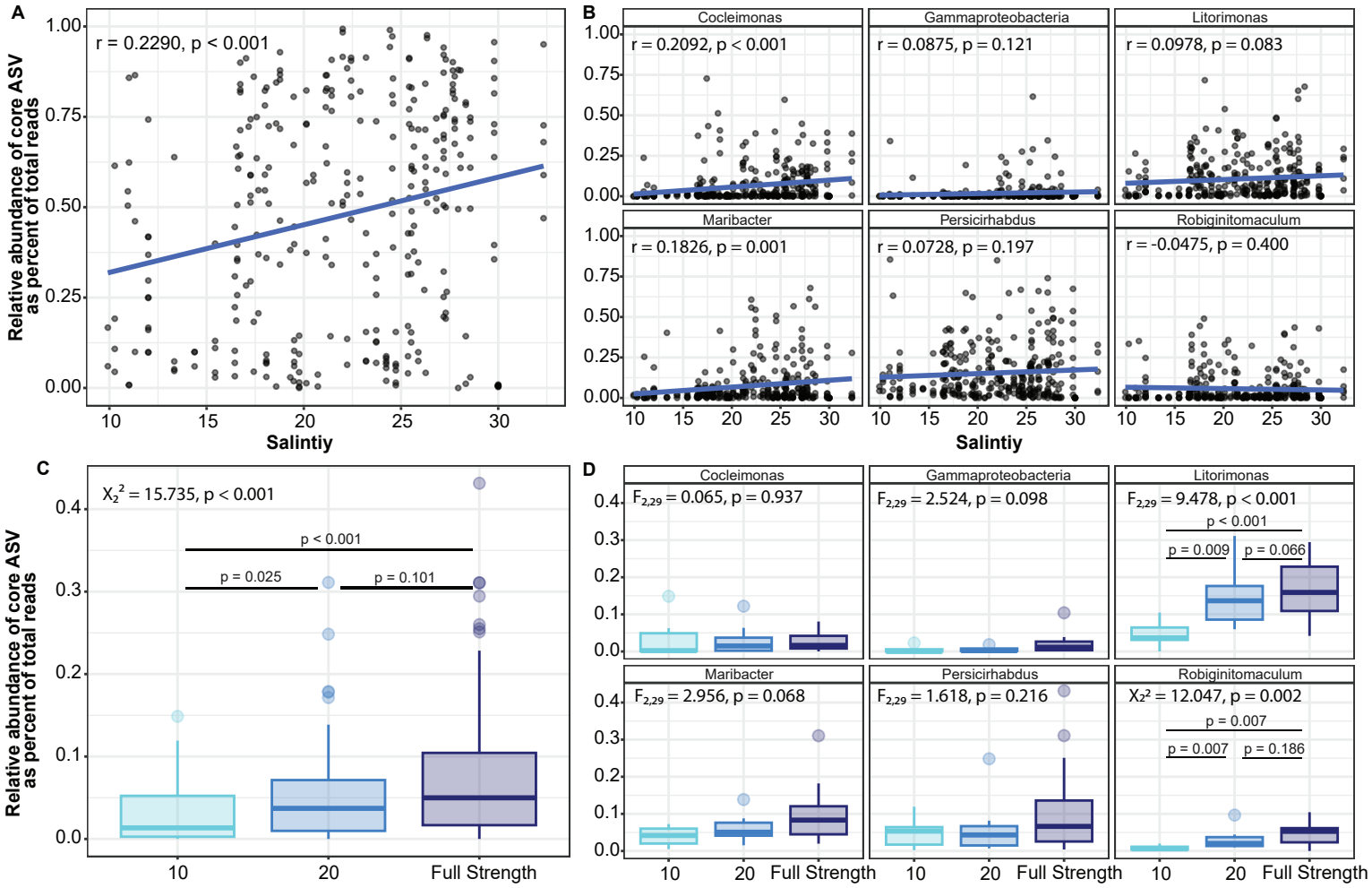
| Substrate | Variables in model | F | $R^2$ | p |
|---|---|---|---|---|
| ***Saccharina*** **(n=269)** | Salinity | 2.213 | 0.007 | 0.012 |
| | Temperature | 2.437 | 0.008 | 0.005 |
| | Julian Day | 10.289 | 0.035 | 0.001 |
| **Water (n=91)** | Salinity | 3.384 | 0.031 | 0.001 |
| | Temperature | 2.952 | 0.027 | 0.001 |
| | Julian Day | 4.094 | 0.037 | 0.001 |
| **Rock (n=101)** | Salinity | 0.833 | 0.008 | 0.787 |
| | Temperature | 1.505 | 0.014 | 0.020 |
| | Julian Day | 1.499 | 0.014 | 0.001 |


A
North
West Vancouver
scp
lhp
2021
only
Burrard
Inlet
tb
gw
North
Vancouver
Fresh water influx
from Indian Arm
cp
2022
only
Fresh water influx
from Fraser River
Vancouver
Fraser River
5 km
B
2021
2022
Mean Conductance (µS/cm)
30
25
20
15
10
C
Mean Temperature (°C)
20.0
17.5
15.0
12.5
10.0
120
150
180
210
Julian Day
Site
lhp
tb
scp
gw
cp
D
Day 0 (field collection day)
1) Collect
n = 18 *Saccharina*
from gw in 2022
each field visit
2) Measure
Blistering Incidence
Photosynthetic Efficiency
Wet Weight
*n = 1 *Saccharina*, 1 airline,
and 1 water sample/ aquaria
3) Place *Saccharina* in aquaria
10
20
Full
Strength
Day 1
• Measure
• Blistering Incidence
• Photosynthetic Efficiency
• Wet Weight
n = 1 *Saccharina*, 1 airline,
and 1 water sample/ aquaria
Day 4
• Measure
• Blistering Incidence
• Photosynthetic Efficiency
• Wet Weight
• Dry out for 24h to take
dry weight measurments
the next day
n = 1 *Saccharina*, 1 airline,
and 1 water sample/ aquaria


A
10
20
full strength
1 2 3 4 5 6 7 8
Time in lab (days)
4
1
0
B
p <0.001
p = 0.006
p = 0.066
Ratio of dry weight to wet weight on day four
0.15
0.10
0.05
0.00
F2,81=14.87, p<0.001
10
20
full strength
C
Ratio of wet weight on day four vs day zero
1.4
1.2
1.0
F2,105=2.135, p=0.123
10
20
full strength
D
Estimated effective quantum yeild (Fv/Fm) on day four
0.9
0.8
0.7
0.6
F2,105=2.629, p=0.077
10
20
full strength


A
Lab *Saccharina* (day four)
PERMANOVA, salinity treatment
$F_{2,29}$ = 2.39, $R^2$ = 0.142, p = 0.002
NMDS2
NMDS1
B
Lab water (day four)
PERMANOVA, salinity treatment
$F_{2,27}$ = 2.7143, $R^2$ = 0.1674, p = 0.001
NMDS2
NMDS1
Lab Salinity
10
20
Full Strength
C
Field *Saccharina*
Marginal PERMANOVA, salinity
$F_{1,262}$ = 2.2127, $R^2$ = 0.007, p = 0.012
NMDS2
NMDS1
D
Field Water
Marginal PERMANOVA, salinity
$F_{1,84}$ = 3.384, $R^2$ = 0.031, p = 0.001
NMDS2
NMDS1
Field Salinity
25
20
15
10


A
Bray–Curtis dissimilarity
1.00
0.75
0.50
0.25
0.00
p=0.552
p=0.011
p=0.239
Lab Saccharina
Welsh ANOVA F2,15.082=6.182, p=0.011
10
20
Full Strength
B
p=0.403
p<0.001
p<0.001
Lab Water
ANOVA F2,27=9.481, p<0.001
C
Shannon–Wiener Index
4
3
2
1
0
Lab Saccharina
ANOVA F2,29=1.294, p=0.289
D
Lab Water
ANOVA F2,27=0.343, p=0.713
E
Field Saccharina
t-value=2.493, p=0.013
15
25
30
F
Field Water
t-value=0.058, p=0.954
G
Field Saccharina
t-value=1.143, p=0.254
6
Salintiy
H
Field Water
t-value=0.486, p=0.628


A
r = 0.2290, p < 0.001
Relative abundance of core ASV as percent of total reads
Salintiy
B
Cocleimonas
r = 0.2092, p < 0.001
Gammaproteobacteria
r = 0.0875, p = 0.121
Litorimonas
r = 0.0978, p = 0.083
Maribacter
r = 0.1826, p = 0.001
Persicirhabdus
r = 0.0728, p = 0.197
Robiginitomaculum
r = -0.0475, p = 0.400
Salintiy
C
X2² = 15.735, p < 0.001
p < 0.001
p = 0.025
p = 0.101
Relative abundance of core ASV as percent of total reads
Full Strength
D
Cocleimonas
F2,29 = 0.065, p = 0.937
Gammaproteobacteria
F2,29 = 2.524, p = 0.098
Litorimonas
F2,29 = 9.478, p < 0.001
p < 0.001
p = 0.009
p = 0.066
Maribacter
F2,29 = 2.956, p = 0.068
Persicirhabdus
F2,29 = 1.618, p = 0.216
Robiginitomaculum
X2² = 12.047, p = 0.002
p = 0.007
p = 0.007
p = 0.186
Full Strength