Title

Low-salinity alters the bacterial community of sugar kelp, *Saccharina latissima* (*Phaeophyceae*)[1]

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Running title

Salinity alters *Saccharina*-associated bacteria

Abstract

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* and potentially *Saccharina*-associated bacteria. Bacteria influence host health and can facilitate or hinder host survival and acclimation to stressful abiotic conditions. Therefore, understanding how bacterial communities change under abiotic stress is critical to understand how host physiology will be affected by abiotic stress. We investigated the effect of low-salinity stress on *Saccharina*-associated bacteria and the host by surveying the bacterial community associated with *Saccharina* and the surrounding environment across naturally occurring salinity gradients during the spring freshet across two years at four field sites with contrasting salinity profiles around Vancouver, Canada (519 samples), coupled with salinity manipulation experiments repeated eight times (269 samples). Overall, *Saccharina* harbors a stable core bacterial community, which decreases in relative abundance under abiotic stress. In the field, both salinity and temperature shape the bacterial community, with temperature having higher explanatory power most of the time. In the lab, we confirm that the patterns observed in the field can be replicated by manipulating salinity alone. Decreased relative abundance of core bacteria and increased community dissimilarity in low-salinity in both the lab and field, suggest that host filtering is significantly impaired in low salinity. In the context of a stable host-associated core bacterial community during non-stressful conditions, the change in the community composition observed during conditions of abiotic stress indicates a host stress response.

Key index words

Saccharina latissima, Kelp, Bacteria, Core Microbiome, Salinity, Temperature, Abiotic Stress, Climate Change, Macroalgae, Phaeophyceae

Abbreviations

UBC: University of British Columbia

IndVal: indicator species analysis

ENA: European Nucleotide Database

PERMANOVA: Permutational multivariate analysis of variance

AKP: Anna Karenina Principle

AIC: Akaike information criterion

Introduction

Kelps (*Laminariales*, brown algae) are marine foundation species that form dense underwater forests across the globe, providing food and habitat for other organisms (Steneck et al., 2002). Through ecosystem services and commercial harvest, kelps contribute an estimated $674 billion CAD per year to the global economy (Eger et al., 2023). However, kelps are threatened by global change factors and an increased frequency and intensity of severe weather events; higher ocean temperatures, lower pH (Bindoff et al., 2019), increased coastal water turbidity (Filbee-Dexter et al., 2019), and lower ocean salinity (Davis et al., 2022; Filbee-Dexter et al., 2019; Andersen et al., 2011) are associated with reductions in kelp recruitment, reproduction, and survival. Thus, kelps and the ecosystem services they provide are vulnerable to climate change (Bindoff et al., 2019; Filbee-Dexter et al., 2019; Goldsmit et al., 2021).

There is a large body of evidence showing that low-salinity is stressful to kelp. Field observations from Australia (Davis et al., 2022) and Norway (Andersen et al., 2011) find up to 100% mortality of kelp sporophytes after freshwater floods, followed by a re-growth of kelp in the following months. Lab studies also show that low-salinity is stressful for kelps. Research of the microscopic gametophyte stage finds lower spore settlement and gemination rates in low-salinity (Lind & Konar, 2017). For the macroscopic sporophyte stage, lower photosynthetic efficiency (Bollen et al., 2016; Karsten, 2007), nitrogen uptake rates (Kumar et al., 2018), and growth rates (Mansilla et al., 2014) 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 (Bindoff et al., 2019; Filbee-Dexter et al., 2019; Goldsmit et al., 2021) and least predictable abiotic stressors that kelps will experience (Bindoff et al., 2019).

The bacterial community of macroalgae can influence growth and morphology (Marshall et al., 2006; Provasoli & Pintner, 1980) and tolerance of abiotic conditions (Dittami et al., 2016). One unknown is how changes in abiotic stressors, including low-salinity, will influence the kelp-associated bacterial community, and whether these changes exacerbate or ameliorate kelp stress tolerance.

Salinity is a strong determinant of bacterial community composition. In fact, a meta-analysis shows that host-associated bacterial community composition is primarily shaped by host-association and salinity, even when other variables, including pH and temperature are taken into account (Lozupone & Knight, 2007). Field studies (van der Loos et al., 2022) and manipulative lab experiments (Saha et al., 2020; Stratil et al., 2014) show that salinity is one of the most important factors in shaping the bacterial communities on non-kelp algal hosts. Studies of kelp (Lemay et al., 2018; Weigel & Pfister, 2019; Davis et al., 2023) and *Fucus* (Davis, 2022) have shown that seasonal changes and site differences are also important in shaping the bacterial community of brown algae. Time series in the field across multiple sites are needed to disentangle these factors, particularly when paired with lab experiments to isolate the relative importance of various abiotic factors in altering the bacterial community (Trevathan-Tackett et al., 2019). The need for paired lab and field studies is apparent in the kelp literature; field studies across sites at one timepoint show that salinity, but not temperature significantly alter the bacterial community associated with *Nereocystis* (Weigel & Pfister, 2019), but in the lab, high temperatures alter the bacterial community of *Ecklonia* (Qiu et al., 2019). We are not aware of lab-based studies that have examined how salinity alters the bacterial community of kelps.

Bacterial community composition typically changes in response to change in abiotic gradients across host-microbe systems, though the nature of these changes and whether they are

correlated with positive, negative, or neutral host outcomes varies across systems. In studies examining the bacterial community of hosts under non-stressful salinity gradients, including in green algae (*Ulva sp.*) from localities with different salinity (van der Loos et al., 2022) and transplanted seagrasses (Adamczyk et al., 2022), changes in the bacterial community composition are not associated with changes in host condition. However, under stressful salinity gradients, changes in bacterial community composition may not be benign to host functioning. For a freshwater strain of the brown alga *Ectocarpus sp.*, the bacterial community associated with the strain is essential to alga's ability to grow in fresh water (Dittami et al., 2016), showing that the bacterial community can improve brown algae's ability to tolerate low-salinity.

An important step in assessing the relationship between abiotic conditions and the bacterial community is to establish if the bacterial community is generally stable under the normal range of conditions, and only exhibits major changes under stressful conditions. A stable host-associated bacterial community is a requirement for the Anna Karenina principle (AKP) to potentially apply. AKP predicts that in stressful conditions, the bacterial community alpha and beta-diversity increase, along with a decrease in the core bacterial community (Zaneveld et al., 2017). Presumably, this destabilization is a result of the disruption of host filtering—host traits or mechanisms that selectively influence microbiome assembly—that typically maintains a stable, low-diversity core bacterial community. On sponges (Pita et al., 2018) and corals (McDevitt-Irwin et al., 2017), patterns that follow AKP predictions under abiotic stress are sometimes, but not always observed. A meta-analysis looking at how temperature affects the bacterial community on a very wide range of taxa finds that alpha-diversity decreases are more common and that beta-diversity shows no significant change over temperature treatments (Li et al., 2023), suggesting that AKP may not be the predominant stress response in the host-associated bacterial

community. Alternatively, there may be a directional change in the bacterial community in stressful conditions. Establishing causality in the link between host condition and change in host-associated bacterial communities in response to stress is an open challenge that requires experimental manipulation (Egan et al., 2013; McDevitt-Irwin et al., 2017; Pita et al., 2018). Field transplant studies of corals (Ziegler et al., 2017) and lab studies that manipulated the bacterial community of anemones (Baldassarre et al., 2022) show that warm-adapted host-associated bacterial communities are associated with improved host-condition in warmer waters, suggesting a directional change in the bacterial community rather than destabilization to a dysbiotic state as predicted by AKP.

We conducted a two-year field survey from April to July in 2021 and 2022 and a concurrent lab study in 2022 to isolate the effect of salinity on the bacterial community the subcanopy kelp *Saccharina latissima* ((L.) C.E. Lane, C. Mayes, Druehl, and G. W. Saunders). Specifically, we tested if the observed shifts in the bacterial community are consistent with 1) a shift to a distinct low salinity community (turnover) or 2) AKP predictions. Existing data suggests that *Saccharina* has a relatively stable core bacterial community (King et al., 2022). If there is a shift to a distinct low-salinity community, we predict that the core bacterial community of *Saccharina* would be replaced by a new core bacterial community in stressful abiotic conditions. If the changes in the bacterial community are consistent with AKP principles, we predict a loss of core bacterial community paired with increased alpha-diversity and community dissimilarity (beta-diversity) in stressful abiotic conditions.

Our extensive dataset shows that the *Saccharina* bacterial community is largely stable with a consistent suite of core bacteria that are maintained across time, space, and salinity gradients. Layered on this broad pattern of stability, we find that salinity has a small but

significant effect on the overall bacterial community composition in the lab and field and that lower salinity significantly decreases the relative abundance of core bacteria while increasing mean community dissimilarity. In the field, temperature generally has a stronger influence on the bacterial community than salinity. Together, these results suggest that host filtering by *Saccharina* is impaired in low salinity, and that high temperature and low-salinity likely function as additive stressors.

Methods

Field Site Description

Five field sites near Vancouver, Canada, were visited across two successive years (Figure 1A). Both years, sites were sampled every two weeks (Figure 1B) at low tide, from mid-April until mid-July (2021) or early-August (2022) during the time of the annual freshwater influx caused by snow melt in late spring, hereafter called the spring freshet. We selected sites based on the presence of *Saccharina* and their salinity profiles through the freshet (Ryan et al., 2019), aiming for two sites that maintained relatively high salinity (above 20 psu) and two that dropped to salinities stressful for kelp (10 psu -15 psu); sites are numbered 1-5 from lowest to highest salinity. In 2021, we collected data at Site 1 (Lighthouse Park; 49.329, -123.264), Site 2 (Third Beach, Stanley Park; 49.302, -123.158); Site 3 (Sandy Cove Park; 49.333, -123.222), and Site 5 (Girl in Wetsuit, Stanley Park; 49.304, -123.126; Figure 1A). Salinity dropped below 15 psu at three of the four sites (Site 1, Site 2, and Site 3; Figure 1C), so in 2022 we replaced Site 1 with Site 4 (Cates park; 49.300, -122.958; Figure 1A), which maintained higher salinity through the freshet and led to a more balanced sampling design. Within a sampling event, West Vancouver

sites (Site 3 and Site 1 or Site 4) were always sampled on the same day and the Vancouver sites (Site 2 and Site 5) were sampled the subsequent day due to the tide height required and travel time.

Site-Conditions

In 2021 and 2022 we recorded water salinity (Figure 1C) and water temperature (Figure 1D) at the start of each site visit. 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.

Temperature loggers and fake-kelp installation

In 2022, two temperature loggers (ElectricBlue, EnvLogger with 27 mm, hardware v2.4) and two brown silicone *Saccharina* analogs (fake-kelp hereafter, 30 cm long by 15 cm wide) per site were installed using an impact drill and underwater epoxy (GoToMarine, Z-Spar Splash Zone A-788 Kit). Temperature loggers were set at 0.5°C of precision and collected temperature data every 30 minutes. One temperature logger was placed above and one below 1 m chart datum to capture the temperatures experienced by *Saccharina* across tide heights (Figure S2); the chart datum height is in the logger metadata. Both fake-kelps at Site 4 and the higher intertidal fake-kelp at Site 5 were lost partway through the freshet, likely removed by members of the public.

Field bacterial data collection

In both years, two water samples, two rock swabs, and at least six *Saccharina* swabs were collected each site visit to capture changes in the bacterial community throughout the spring freshet.

We swabbed the bottom 10 cm of the *Saccharina* thallus (the meristem region) because it is the newest and most selective tissue (Lemay et al., 2021), presumably hosting the bacterial community most indicative of *Saccharina.* Samples were taken wearing gloves sprayed with 70% ethanol between samples. Rocks were sampled to capture the background biofilm communities, for which we selected regions roughly 5 x 5 cm that were free of visible organisms. We gently rinsed the *Saccharina* and rocks with 0.22 µm filter-sterilized seawater before swabbing the surface for 10 s with a cotton tipped swab (VWR, CA10805-154). The swab was then broken off into a cryovial (VWR, CA66021-993). After taking bacterial samples, we recorded the presence of any blisters on the kelp thallus. Blisters were observed only once on June 11th, 2021 at Site 2 on three *Saccharina* individuals, and the blistered tissue was not sampled.

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 stored in a cooler with ice packs at -20°C until they could be brought to the lab (within three hours of sample collection), where they were then stored at -70°C until extraction.

Saccharina field collection

The microbial communities of six *Saccharina* individuals were sampled by swabbing at each site visit. In 2021, individuals were selected haphazardly, with their position (out of the water, in the wave splash zone, fully submerged in the ocean, or in tidepools) recorded. These positions differ in conditions experienced, including desiccation, exposure, and in the case of tidepools, low water flow and a wider range of temperatures and oxygenation. All of these variables may influence the bacterial communities associated with *Saccharina*. We systematically tested the influence of being in a tidepool versus ocean on the bacterial community of *Saccharina* in 2022 by sampling an additional six *Saccharina* from tidepools at Site 2 and comparing to individuals submerged in the ocean at this site. We used Site 2 because Site 2 consistently has accessible *Saccharina* in tidepools and the ocean whereas the other sites do not. We used marginal PERMANOVAs including water salinity, water temperature, and position to test the influence of position in the intertidal on *Saccharina* bacterial communities; neither position in the intertidal in general (2021 position: pseudo-$F_{2,113}$=1.071, R^2=0.018 p=0.348) nor position in or out of tidepools (Site 2 in 2022 in or out of tidepool: QIAGEN pseudo-$F_{1,19}$=1.5199, R^2=0.059, p=0.160, Zymo pseudo-$F_{1,15}$=0.944, R^2=0.051, p=0.509) explained any additional variation in the bacterial community of *Saccharina*. Therefore, we include *Saccharina* from all positions in our analyses. We only sampled *Saccharina* submerged in the ocean in 2022 at all other sites to increase consistency across samples.

Lab experiment protocol

In 2022 we performed a manipulative lab experiment to isolate the effect of low-salinity stress (Figure 1E). We define stress as a condition that adversely affects growth via damage and/or resource allocation associated with damage prevention and cellular repair (Davison & Pearson, 1996; Harley et al., 2012). We performed eight experimental rounds through the 2022 field season, corresponding to each field sampling event (Figure 1B), to assess the influence of salinity over time. After collecting the bacterial samples as described above, 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 Site 5 (approximately 1 h transit). *Saccharina* from Site 5 were used in the experiment because Site 5 has by far the largest population of *Saccharina*, and salinity stays relatively high.

Saccharina were incubated in seawater at 10°C on a 12h light:12h dark photoperiod with bubblers to induce water motion. Each experimental round included six 8 L aquaria (two per treatment), with three *Saccharina* meristems per aquaria (Figure 1E), in one of three salinity treatments: 10 psu, 20 psu, and unaltered sea water (full-strength hereafter). The low salinity treatment (10 psu) represents the lowest salinity observed at our sites (Figure 1C) and is reported to be stressful as measured by significantly lower effective quantum yield in sporophytes (Karsten, 2007). Significantly increased death and damage were observed between 6 psu and 11 psu for *Saccharina* germlings (Peteiro & Sánchez, 2012). A salinity of 20 psu is commonly experienced at our sites (Figure 1C) and was previously associated with differential gene expression (Monteiro et al., 2019). The full-strength seawater is pumped from 30 m depth in Burrard Inlet and brought to UBC by truck. The salinity fluctuated between 31 psu and 32 psu depending on the culturing round (Table S1). Salinity was lowered by adding deionized water to

the full-strength seawater as described previously (Gerard et al., 1987; Peteiro & Sánchez, 2012) and salinity was checked with a refractometer.

Most experimental rounds lasted four days with samples taken on Day 0 (in the field), Day 1 (24 h after collection), and Day 4. Our first experimental round lasted six days (Figure 2A). On Day 6, the *Saccharina* in 10 psu salinity died (turned green and fell apart to the touch; Figure 2A), so we shortened the incubation time to three days for culturing round two. We did not observe significant *Saccharina* damage on Day 3, so we extended the culturing duration to four days for the remaining experimental rounds (Figure 2A). For culturing round five, there are no dry weight measurements on Day 4 because we extended the culturing time to fourteen days, to see if the kelp in the 10 psu salinity would fall apart in as they did in round one. They did not.

Lab bacterial collection protocol

All lab bacterial sampling (Figure 1E) 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 with a sieve before taking bacterial samples. Note, the Day 0 *Saccharina* swabs are the Site 5 field samples. Since only six kelps are swabbed in the field, this means most lab experiment kelps are not swabbed on Day 0. Day 0 water and substrate samples were taken prior to adding the kelp to the aquaria. Lab *Saccharina* samples (Day 1 and Day 4) are swabs of all three kelps in the same aquaria to avoid pseudoreplication because we assumed the bacterial community was homogenized within the same aquaria (Chen & Parfrey, 2018). In total, we collected one of each sample type per aquaria, per lab sampling day (2 replicates for each treatment) and repeated the experiment eight times (Figure 1E).

Lab Saccharina condition

After taking bacterial samples, the condition of each *Saccharina* 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 (Figure 1E). These measurements serve as a phenotypic read-out to test if the biologically relevant salinity treatments chosen for this experiment are stressful according to the definition presented above (Davison & Pearson, 1996; Harley et al., 2012) by testing the effect of salinity on growth in particular.

Blistering occurrence was recorded as in other kelp studies (Davis et al., 2022; Qiu et al., 2019). 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 *Saccharina* for five minutes on a damp cloth. Then, we placed the optical fiber over the thallus base (just above the stipe) and took the measurement. Wet weight measurements (holdfast, stipe, blade section) were taken after blotting dry the *Saccharina*, after which the kelp was promptly returned to its aquaria.

Dry weights were obtained as follows. After taking wet weight measurements on the last experimental day, stipes and holdfast were cut off from the blade section (Bollen et al., 2016). We weighed the blade sections before drying them in at 60°C for at least 24 h, and re-weighed them (Bollen et al., 2016; van Ginneken, 2018).

DNA extraction and sequencing

In 2021, all DNA was extracted with QIAGEN DNeasy PowerSoil Pro Kit 96-well plate; 47017 or single tube; 47014 following the manufacturer's protocol. In 2022, 71% of field swabs (*Saccharina*, water, and rock) samples were extracted with QIAGEN 96-well kits. The remaining 29% of field samples were extracted with ZymoBIOMICS 96 MagBead DNA/RNA Kit (Zymo, R2136). All lab samples in 2022 were extracted with the Zymo kit. 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. The extraction method for each sample is indicated in the microbial metadata. The change was due to global supply chain issues. Each extraction plate included a blank swab or Sterivex™ membrane (extraction blank, n=12).

Primers for PCR were 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' (Parada et al., 2016; Quince et al., 2011). 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 (indicated in the microbial 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. 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; (Posit team, 2022; R Core Team, 2022)). Reads were processed following the dada2 pipeline (v1.24.0; Callahan et al., 2016). 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 (McLaren, 2020) formatted for the dada2 pipeline (McLaren & Callahan, 2021).

The merged sequence table, the taxonomy table, and metadata were grouped into a phyloseq object for filtering (v1.40.0; McMurdie & Holmes, 2013). 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; *Pseudomonas* is very likely a Zymo 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, all samples from one 10 psu 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 (Eppley & Bovell, 1958) and pH alters the bacterial community of kelps (Qiu et al., 2019). We also removed the last field sampling event in 2022 (July 26th and 27th) to make the time covered in 2021 and 2022 consistent as our sampling ended earlier in 2021. In total, filtering retained 17,438,672 of 24,862,382 total paired reads, 3,040 of an initial 29,105 ASVs, and 796 of the initial 1,095 samples, with a mean of 21,432 reads per sample. We converted the data to an iNEXT (v3.0.0; Hsieh et al., 2022) compatible format with the metagMisc package (v0.0.4; Mikryukov, 2022) to perform coverage-based rarefaction. The sample coverage was set to 0.8 and iterated 1000 times.

We tested the influence of DNA extraction kit on diversity. By Kruskal-Wallis test comparing field *Saccharina*, rock, and water samples followed by a Benjamini-Hochberg correction for multiple comparisons, *Saccharina* (χ_1^2 = 120.07, p<0.001) and rock samples (χ_1^2 =

25.465, $p<0.001$) extracted with the Zymo kit had higher richness than those extracted with the Qiagen kit, but not water samples ($\chi_1^2 = 2.64$, $p=0.10$). We tested for differences in beta-diversity using PERMANOVA comparing samples extracted with Zymo versus QIAGEN for they same sample type and at a similar time of year (Julian Day 160 to 192; 3 sampling rounds). We found significant differences between extraction kits for *Saccharina*, rock, and water samples (Figure S3), indicating that the different extraction kits capture different bacterial communities. Therefore, we accounted for extraction kit in all analyses that include samples extracted with different kits (2022 field samples).

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; Kassambara, 2022b).

For all field bacterial data analysis, we include all 2021 sampling rounds (Julian day 105 to 188) and sampling rounds one to seven (Julian day 107 to 192). We exclude the sampling round starting on Julian day 206 from the field data analysis because it is not matched in the 2021 data, For all lab bacterial analysis, we include all 2022 rounds.

We ran permutational analyses of variance (PERMANOVA) on rarefied data with the adonis2 function in the package vegan (v2.6-4; Oksanen et al., 2022) 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 betadisper function (equal unless stated otherwise) and ran post-hoc Pairwise Adonis tests in the pairwise.adonis package (v0.4.1; Arbizu, 2017).

We calculated the Shannon-Weiner diversity index with the diversity function in package vegan (Oksanen et al., 2022). The Bray-Curtis dissimilarity index between samples was calculated with the divergence function in the package microbiome (v1.22.0; Lahti & Shetty, 2012) as in Lesser et al. (2016).

To compare means between 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; Fox & Weisberg, 2019) and validated the assumption of normality with qq-plots. When the assumption of normality was violated, we ran a Kruskal-Wallis test followed by a post-hoc Wilcoxon test (R Core Team, 2022).

To identify the core *Saccharina* ASVs, we selected all field samples collected at salinity 20 psu or greater and ran an indicator species analysis (IndVal) on non-rarefied data with the function multipatt from the package indicspecies (v1.7.12; Caceres & Legendre, 2009) with 999 permutations, comparing substrate types (*Saccharina*, water, rock, and fake-kelp). We used a threshold of 0.7 IndVal statistic, which required ASVs to be enriched and highly prevalent on *Saccharina* compared to other sample types present on a majority of *Saccharina* samples. To calculate the relative abundance of the core accross temperature and salinity gradeints, we ran linear regression models. We selected the best model by backwards Akaike information criterion (AIC) and tested the linear regression assumptions with pots.

Taxa summary plots were generated by calculating the 10 taxa with the greatest relative abundance across all *Saccharina* samples in the field at the order and genus level. We ran linear regression models for each taxa identified as part of the top 10 most abundant taxa in the taxa plot to check for trends in relative abundance along the temperature and/or salinity gradients. We corrected for multiple-comparisons with a Benjamini-Hochberg correction (R Core Team, 2022).

All plots were made with the ggplot2 (Wickham, 2016) and ggh4x (v0.2.3; Brand, 2022) packages and were saved as pdf files with ggpubr (v0.5.0; Kassambara, 2022a). Text modifications for plot labels were made with the package stringi (v1.7.12; Gagolewski, 2022).

Reanalysis of published data

Raw data from King et al. (2023) were downloaded from ENA (PRJEB50679) and https://doi.org/10.6084/m9.fgshare.19453889.v1, processed with the dada2 pipeline, and filtered as described above and in their original publication. Core *Saccharina* ASVs were identified as those with a prevalence ≥ 0.8, replicating methods in King et al. (2023) because there are no comparison environmental samples available.

Results

Factors shaping the bacterial community of Saccharina in the field.

We examined how salinity shapes bacterial community diversity and composition in the field in comparison to temperature on *Saccharina* and in the surrounding environment. We surveyed *Saccharina*-associated bacterial communities bi-weekly at four sites (Figure 1A) with contrasting salinity (Figure 1C) and temperature (Figure 1D) profiles for two consecutive years during the annual low salinity event that occurs because of the spring freshet of the Fraser River near Vancouver, Canada. Salinity varied over time and across sites. Salinity varied from 31 psu (typical ocean salinity in the Salish Sea) to a minimum of 10 psu (Figure 1C). Variation in salinity is negatively correlated with temperature, and both salinity and temperature are correlated with seasonality, as measured by Julian day (Figure S1), so we do not include Julian day as a variable in the model to avoid overfitting.

Across the 519 samples in the field dataset, bacterial community composition on *Saccharina*, water, rock, and latex kelp analogs (fake-kelp) we installed at our sites in 2022 to assess the biofilm community on substrate with similar morphology are all significantly different from each other by PERMANOVA (Figure S4).

Then, we analyzed the influence of salinity and temperature on *Saccharina*, water and rock separately using marginal PERMANOVAs, nesting within extraction kit. Fake-kelp samples were not analyzed further due to low sample size that resulted from loss. We employed a marginal PERMANOVA to assess the unique explanatory power of temperature and salinity, which are highly correlated so overall variation explained overlaps substantially. Salinity and temperature explain between 1 and 3% of unique variation in the bacterial community of *Saccharina* depending on the extraction kit, with temperature explaining more variation compared to salinity (Table 1; Figure S5A,D). The rock samples show no significant influence of temperature or salinity on the bacterial community (Table 1; Figure S5B,E). For water samples, water temperature and salinity generally explain much more unique variation compared to the *Saccharina* samples across both extraction kits (Table 1; Figure S5C,F). These results show that the observed trends are substrate-type specific and that the *Saccharina* bacterial community has less turnover than the water bacterial community and more than communities on rocks across the same environmental conditions.

Lab salinity treatments differentially induced stress in Saccharina.

We tested the influence of salinity on *Saccharina* and associated bacteria using replicated experiments throughout the spring freshet by incubating field-collected *Saccharina* in one of

three salinity treatments: 10 psu, 20 psu, and full-strength (31-32 psu; Figure 1E). These are biologically relevant salinity levels for *Saccharina* and are within the range of normal variation experienced at our sites (Figure 1C). We verified that these salinity treatments differentially induced stress in *Saccharina* by measuring four stress-associated kelp phenotypes. We assessed blistering incidence on Day 4 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 than the other salinity treatments (Figure 2A). The same pattern is observed on Day 1. 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 6 of round one, the *Saccharina* in the 10 psu salinity were green (Figure 2A) and disintegrated when touched, indicating death. This was not observed again throughout the experiment, even when we extended experimental round five to fourteen days, highlighting the importance of adequate biological replication.

We used ANOVA followed by Tukey Post-Hoc tests for the other comparisons. The 10 psu salinity treatment had a significantly lower dry weight to wet weight ratio compared to the other salinity treatments (Figure 2B). The ratio of wet weight on Day 4 compared to Day 0 did not differ by salinity treatment (Figure 2C), and the same results were observed on Day 1. We observed a no significant difference in estimated effective quantum yield on Day 4 (Figure 2D), or Day 1. These results suggest that *Saccharina* is accumulating moisture but not additional biomass in low-salinity and remains alive in our lab cultures for the duration of our four-day experiment.

After establishing that our biologically relevant salinity treatments were stressful for *Saccharina*, we assessed the bacterial community composition in the lab. We compared the

bacterial community of *Saccharina*, water, and the substrate samples with a PERMANOVA and found that on Day 4, all sample types are significantly different from each other (Figure S4).

Then, we assessed the impact of salinity and culturing round on the bacterial community for each sample type in the lab on Day 4 (Table 2; Figure S5). Our PERMANOVA results follow the same trend as the field data, with the water samples showing the most variation by salinity treatment (Table 2; Figure S5I), followed by *Saccharina* (Table 2; Figure S5G), with the substrate samples (Table 2; Figure S5H) showing no significant change in bacterial community composition by salinity. In our *Saccharina* and water samples, we also see a strong effect of culturing round, showing that the overall effect of salintiy in the lab is robust to different starting bacterial communities.

The Saccharina bacterial community is largely stable with a consistent core community

We assessed the stability of the *Saccharina* bacterial community by plotting the most abundant taxa (order and genera) over time and determining whether there is a stable core community on *Saccharina* at the ASV level.

Plotting the most abundant taxa observed on *Saccharina* through the freshet highlights the stability of the *Saccharina* microbiome over time as much of the community is consistently present (Figure S6A,B). To quantitatively assess the stability of the 10 most abundant taxa, we ran a linear regression model followed by Benjamini-Hochberg correction to identify taxa that significantly change in relative abundance over the salinity and temperature gradients (Table S3). At the order level (Figure S6A), there are no significant changes in relative abundance across the salinity or temperature gradient for either extraction kit. At the genus level (Figure S6B), the

QIAGEN-extracted samples detect a significant decrease of *Litorimonas* (*Caulobacterales*) with higher temperatures, whereas the Zymo-extracted samples show a significant increase in Pseudoalteromonas (*Alteromonadales*) relative abundance in lower salinity and higher temperature (Table S3).

We repeated these same analyses in the lab. At the Order level (Figure S6C), there is a significant decrease in Caulobacterales in the 10 and 20 psu treatments compared to full-strength salinity (Table S4). A similar pattern is observed at the genus level (Figure S6D), where there is a significant decrease in of *Litorimonas* and *Robiginitomaculum* (both *Caulobacterales*) in the lower salinity treatments (Table S4). These findings, paired with the field data, suggest that *Caulobacterales* decrease in relative abundance under abiotic stress conditions. We also observed a trend toward increasing Alteromondales and *Pseudoaltermonas* (Alteromonadales) at low salinity.

We identified the core bacterial community on *Saccharina* when the salinity was relatively high (20 psu or greater) and tested for changes in the relative abundance of the core in response to salinity and temperature. We excluded lower-salinity samples when defining the core because these conditions are likely to be stressful to the *Saccharina* populations studied here based on the results of the lab experiments (Figure 2). We used indicator species analysis with a threshold of 0.7 IndVal value to identify ASVs that were enriched on *Saccharina* compared to water and rock and at high frequency on *Saccharina* when ocean salinity was greater or equal to 20 psu. We identified six core ASVs (Table S5). We repeated the analysis with thresholds 0.5 to 0.8 IndVal to ensure the patterns observed were a robust choice of threshold for defining the core, which they were (Figure S7, Table S5).

We then ran a linear regression model with backward AIC model selection to test for a relationship between the total relative abundance of the *Saccharina* core ASVs identified with a IndVal threshold ≥0.7 with temperature and salinity (Figure 3A,B). We find that the best model ($F_{5,241}$=30.13, adjusted-R^2=0.372, p<0.001) includes both salinity and temperature. Within this model, for the QIAGEN samples both salinity (t=2.528, p=0.0121; Figure 3A) and temperature (t=-2.110, p=0.036; Figure 3B) and are significant, but for the Zymo samples, neither salinity (t=0.466, p=0.642; Figure 3A) or temperature (t=-1.250, p=0.213; Figure 3B) or are significant.

Then, we calculated the relative abundance of these core ASVs in the controlled lab environment. We find that the core persists, even at the lowest salinity, although by ANOVA ($F_{2,24}$=16.05, p<0.001; Figure 3C) the core decreases in relative abundance in the 10 and 20 psu treatments compared to the full-strength (p≤0.001; Figure 3C). The lab and field data show that core *Saccharina* taxa are maintained at all salinities in the field and in the controlled lab environment, showing a highly stable core bacterial community, further supporting the broad patterns of stability in the *Saccharina* bacterial community. The decreased relative abundance of the core observed both in the lab and field further support that abiotic stress—temperature (field only) and salinity (lab and field)—alters the bacterial community of *Saccharina*.

Then, we investigated the distribution of core bacteria ASV on *Saccharina* across much larger spatial scales by comparing our data to a study performed in the United Kingdom (UK) from September and August 2015 (King et al., 2023). The UK study did not include environmental comparison samples, so core bacteria were defined solely based on prevalence of 0.8 or higher; this resulted in 27 core ASVs. Comparing the core ASVs between the two datasets, 4/6 core ASVs detected in our BC population are also found in association with *Saccharina* in the UK, with ASV7 (*Robiginitomaculum* sp.) being a core ASV in both datasets (Table S5).

Nineteen of the twenty-seven core ASVs in the UK dataset are present in our *Saccharina* samples, including ASV7. However, many of these ASVs are present at low prevalence, highlighting the importance of including environmental samples in these types of analyses. When we compare the BC and UK datasets at the genus level, we find that all genera that include core *Saccharina* ASVs are present in the other dataset, indicating that functional redundancy within a genus, paired with other factors is likely in part responsible for differences in ASV-level overlap (Table S5). Overall, these comparisons show that to a large extent, the *Saccharina* bacterial community is somewhat consistent across global scales.

Is bacterial community change due to turnover or Anna Karenina patterns?

We asked whether changes in the bacterial community associated with *Saccharina* are consistent with a shift to a distinct low salinity community (turnover) or with Anna Karenina patterns (AKP) that are predicted to occur in stressful conditions for hosts that have an otherwise stable microbiome, with the assumption that stress reduces host filtering. AKP specifically predicts decreased relative abundance of the core taxa and increased diversity and community dissimilarity in samples from low-salinity in our dataset. We already identified that *Saccharina* has a generally stable bacterial community and shows a significant decrease in the relative abundance of the core in low-salinity in the field and in the lab data (Figure 3, Figure S6). Here, we test for the predicted increase in alpha and beta-diversity. The lab experiment allows us to isolate the influence of salinity, and two years of field survey data allow us to determine whether the patterns observed in the lab are consistent with observed dynamics on natural populations. Interpretation of patterns in the field are complicated by the fact that decreasing salinity in the

field is correlated with seasonality patterns generally, and increasing temperature in particular (Figure S1), which is likely an additive stressor for kelp in the Salish Sea.

The bacterial community of Saccharina does not turn-over by salinity.

We identified the core bacterial community of the field samples from below 20 to check if there is a new core bacterial community in low-salinity in the field that is distinct from higher salinity samples. We found that the core *Saccharina* bacterial community from low-salinity field samples is made up of 10 ASVs (Table S5). This includes 5 of the 6 ASVs that are part of the high-salinity field core; the 6th (ASV15) is also present with an IndVal stat of 0.62 in low-salinity, slightly below the 0.7 threshold, though still common on *Saccharina* in low-salinity conditions (Table S5). The five other ASVs that are core in low-salinity (salinity < 20) but not high-salinity (salinity ≥ 20) in the field have IndVal stats ranging from 0.67 to 0.45 in the high-salinity field samples, indicating again, that these ASVs are commonly associated with *Saccharina* in higher salinity conditions. These data show that although the ASVs that are part of the core are somewhat different between the high and low-salinity field samples, these ASVs are present throughout all field samples, showing that there is not a distinct bacterial community in low-salinity in the field. This is also supported by the lab, where all ASVs are found on *Saccharina* in all salinity treatments (Table S5).

Low salinity increases community dissimilarity but not diversity.

We tested the AKP predictions that alpha-diversity and dissimilarity will increase in low salinity and high temperature, which represent stressful conditions. For the field data, we ran linear regression models including temperature and salinity, nested within extraction kit and selected the best model by backwards stepwise model selection using AIC. First, for alpha-diversity of the field *Saccharina* samples, the best model includes temperature, but not salinity (Figure 4A, Table S6); there is a significant increase in alpha-diversity for the Zymo-extracted samples from warmer water, but no change in the QIAGEN-extracted samples. Since the Zymo samples include few field sampling rounds, this trend may not be robust. The best model for the rock samples includes only extraction kit, showing no pattern in alpha-diversity by salinity or temperature (Figure 4B, Table S6). Lastly, for the water samples, the best model includes salinity, but not temperature, with the QIAGEN samples showing increased diversity in lower-salinity (Figure 4C, Table S6).

We then assessed the effect of salinity on alpha-diversity in the lab on Day 4 by ANOVA and found that for all of the sample types (*Saccharina*, substrate, water) there are no significant differences in alpha-diversity by salinity treatment (Figure 4D,E,F, Table S6). The lack of change in alpha-diversity by salinity in the lab, paired with the field data suggests that salinity is likely not responsible for the observed increase in alpha-diversity in the Zymo-extracted samples.

We repeated the same process to assess changes in dissimilarity. In the field samples, the best model for field *Saccharina* includes only temperature, with the QIAGEN-extracted sample showing a significant increase in dissimilarity in higher temperatures (Figure 4G, Table S6). The best model for the rock samples includes kit only, showing no pattern by abiotic conditions

(Figure 4H, Table S6). The best model for the water samples includes temperature and salinity, with QIAGEN-extracted showing a significant decrease in beta-diversity in higher temperatures (Figure 4I, Table S6). Overall, the field results for *Saccharina*, but not water or rock, support the predicted AKP pattern of increased dissimilarity in stressful condition and show that temperature has a stronger effect than salinity on community dissimilarity.

We then analyzed the lab samples, which allows us to isolate the role of salinity. The dissimilarity of the *Saccharina* samples in the 10 psu treatment is significantly higher compared to the 20 psu and full-strength salinities (Figure 4J, Table S6), but there is no significant change in the water and substrate samples (Figure 4K,L, Table S6). The lab data show that low salinity can increase the community dissimilarity of *Saccharina*, but the field data suggests that temperature may be more important. This contrast points to the fact that temperature and salinity are likely additive stressors in nature. In summary, our data support the predicted AKP patterns of increased beta-diversity, but not alpha-diversity, associated with abiotic stress.

Discussion

The predicted (Bindoff et al., 2019; Filbee-Dexter et al., 2019) and ongoing die back of kelp forests (Bindoff et al., 2019; Christie et al., 2019; Davis et al., 2022) due to climate change represents a global concern because of the environmental, economic, and cultural value that these foundation species provide (Bindoff et al., 2019; Eger et al., 2023). Our field survey and lab experiments at biologically relevant salinity levels show that low salinity induces stress in *Saccharina* and alters the *Saccharina*-associated bacterial community, resulting in reduced abundance of the core bacterial community. In the lab, bacterial community dissimilarity

increases in lower salinity, but in the field, we find that dissimilarity increases are better explained by water temperature than salinity.

Saccharina hosts a core bacterial community, which is somewhat stable across large spatial and temporal scales (King *et al.*, 2023 and this study). Some of the differences between the two studies could be explained by the differences in sampling. This study swabbed the meristematic region (up to 10 cm away from stipe) and King *et al.* (2023) scraped the tissue with a razor above the meristematic region (10 cm and more away from stipe). Blade sections of other kelps have differences in bacterial community composition (Lemay et al., 2021; Weigel & Pfister, 2019). In the field, we find that temperature is more important than salinity in shaping the bacterial community of *Saccharina*. However, it is difficult to fully disentangle the effects of temperature and salinity since they are so highly correlated in our data. In the lab, we find that salinity alone can lead to significant increases in beta-diversity in low-salinity, but do not see an effect of salinity on alpha-diversity. Therefore, our data do not fit the AKP, which posits a decreased relative abundance of an otherwise stable core bacterial community paired with increased alpha and beta-diversity in a “disrupted microbiota” (Zaneveld et al., 2017), which has been observed in corals (McDevitt-Irwin et al., 2017) and sponges (Pita et al., 2018). Additionally, the patterns predicted by AKP are not the most common patterns observed across host-associated bacterial communities. A meta-analysis studying the impact of temperature on environmental bacterial communities (Li et al., 2023) found that a decrease in alpha-diversity and no change in beta-diversity in stressful conditions is the most commonly observed pattern. This is also in contrast to our findings. Furthermore, we did not identify a turnover in the bacterial community in low salinity compared to higher salinity, showing that bacterial

community turnover is also insufficient to explain the observed trends in the *Saccharina* bacterial community.

A disrupted microbiota is often associated with unhealthy hosts (Shade & Handelsman, 2012), and is proposed to make algae more susceptible to disease (Egan et al., 2013). 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 (Baldassarre et al., 2022; Ghaderiardakani et al., 2020; Pita et al., 2018; Ziegler et al., 2017). Our study is not designed to test if the changes we observed in the bacterial community of *Saccharina* are beneficial or not to kelps' ability to tolerate low salinity. Regardless of the effect on *Saccharina* health, our data showing a decreased relative abundance of core taxa suggests that more opportunistic colonizers associate with *Saccharina* in stressful abiotic conditions, pointing to decreased host filtering by *Saccharina* in low-salinity. 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 and likely in higher temperatures.

We show that salinity is a relatively weak but significant factor in shaping the bacterial community of *Saccharina*. These findings generally agree with other studies investigating the effect of salinity on the bacterial community of other algae (Saha et al., 2020; van der Loos et al., 2022), but for kelps, the relative importance of temperature compared to salinity appears to be variable between datasets (Weigel & Pfister, 2019). Salinity explains more variation in the bacterial community of *Saccharina* in the lab compared to the field. This is due to much higher sample sizes in the field, two extraction kits, and the combined effects of many unmeasured variables corelated with temperature and salinity, which are themselves correlated. In these data,

temperature and salinity are correlated with time of the year (Julian Day), throughout which other variables including: dissolved organic carbon, pH (Madkaiker et al., 2023), irradiance, and nitrogen availability (Langdon & Atkinson, 2005) also change.

Our study 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. As we show with our field data, water temperature explains a greater proportion of variation in the bacterial community of *Saccharina* in the field. However, it is difficult to fully disentangle these variables as they are correlated with each other.

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 (Qiu et al., 2019), and climate models predicting that the intensity and frequency of abiotic stressors will increase with climate change (Bindoff et al., 2019), suggest that the bacterial community of kelps will be affected by climate change. We propose that in stressful abiotic conditions, which includes salinity as well as other non-focal variables discussed herein, 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 (Pita et al., 2018), highlights the importance of considering the microbiota (Weinbauer & Rassoulzadegan, 2007) 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. All code and metadata are available on Borealis https://borealisdata.ca/dataset.xhtml?persistentId=doi:10.5683/SP3/ILQ9UJ.

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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Conflict of interest

The named authors have no conflict of interest, financial or otherwise to report.

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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) Table showing if a site was assigned as high (black) or low-salinity (grey) for a field sampling round. The sampling rounds are numbered by the Julian Day of the first of the two sequential field days. White cells indicate that the site was not sampled. C) Conductivity (salinity) and D) water temperature profiles across sampling years with error bars showing +/- one standard deviation around the mean. E) Representative schematic of 2022 lab experiment. Note, in D, missing temperature data from 2022 are from days where a refractometer, rather than the YSI were used to measure water parameters. We include Julian day 206 in panels B, C, and D even though we exclude this sampling round in our field data analysis because experimental round 8 includes *Saccharina* collected during this sampling round. Also

note, in E, the n = 1 for *Saccharina* bacterial samples 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 from the temperature loggers show the same patterns across sites and time (Figure S2).

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 (in psu) for each *Saccharina* in lab culture. Experimental round 1 lasted six days and the *Saccharina* in the 10 psu condition became green and fell apart to the touch (indicated in green). Experimental round 2 lasted three days. Fisher's Exact Test ($p < 0.001$) followed by a Pairwise Fisher Test of the Day 4 data (experimental round 3 to 8), show that the 10 psu salinity has significantly more blistering than the 20 psu and full-strength salinity on Day 4 ($p \leq 0.036$). B) The ratio of dry weight to wet weight on lab Day 4. C) The ratio of wet-weight on lab Day 4 to wet-weight on Day 0. D) The estimated effective quantum yield on lab Day 4 by salinity (in psu). For B, C, and D, ANOVA output comparing the different salinity treatments are indicated in the corresponding panel. Note, round 1 and 2 have no Day 4 observations for all panels. In addition, in panel B only, there are no Day 4 observations for experimental round 5 because we extended the culturing time to see if the *Saccharina* would turn green. After 14 days, green *Saccharina* was not observed in any salinity treatment. Day 1 comparisons for panels A, C, and D are in the supplementary results.

Figure 3. Relative abundance of core ASVs in the field (A, B) and in the lab on Day 4 (C) on *Saccharina*. Plots A and B show the same samples, but arranged along the salinity (A) and

temperature (B) gradients observed in the field across both years. The color gradients in panels A and B show the abiotic gradient not plotted on the x-axis. Point and line shape show the extraction kit used to extract the samples. In C, the Tukey posy-hoc comparison p-values for the ANOVA are shown. Note, all three x-axes are arranged form least to most stressful abiotic condition.

Figure 4. The Shannon-Weiner diversity index for the field (A,B,C) and lab Day 4 (D,E,F) samples along with the Bray-Curtis dissimilarity index of the field (G,H,I) and lab Day 4 (J,K,L) samples. Beta-diversity is calculated within the same substrate type either by comparing samples within the same sampling site visit (field) or a salinity treatment (lab). The same sample types are arranged in columns and indicated in the panels. For the field samples, the point color represents salinity and the shape (point and line) indicates the extraction kit. For all panels, the full statistical output is in Table S6, but we indicate the significant factors in the nested linear regression model (field) or Tukey post-hoc test if the ANOVA showed significant differences between groups (lab). Note, all axes are ordered from least stressful to most stressful, with the field sampling plotted along the temperature gradient while the lab samples are plotted by salinity treatment.

Table 1. Output of field data marginal PERMANOVA showing the explanatory power marginal explanatory of salinity and water temperature by sample type within the same extraction kit. Statistically significant output is bolded.

Table 2. Output of lab Day 4 PERMANOVA showing the explanatory power salinity and culture round by sample type. We include the Tukey post-hoc comparisons for salinity treatments when the PERMANOVA showed significant differences between treatments. All three columns of the output for *Saccharina* and water are for the crossed PERMANOVA, while the substrate output is for a PERMANOVA with only salinity. We could not run the crossed PERMANOVA design for the substrate samples because the model is overfitted due to the smaller sample size of substrate samples (n=14) compared to *Saccharina* (n=26) and water samples (n=27). Statistically significant output is bolded.

Supplemental text figure and table legends

Figure S1. Dot plots with regression line showing the correlation between conductance (µS/cm; referred to as salinity in the text), temperature (°C), and Julian day for 2021 and 2022. Results of Pearson's Correlation Test indicated in the plot area.

Figure S2. The mean 5 (A) highest or (B) lowest daily recorded temperatures (°C) by each temperature logger. Error bars are +/- 1 standard deviation.

Figure S3. NMDS plots showing field A) *Saccharina*, B) rock, and C) water samples. Point color represents the Julian day sampling round (Figure 1B), with equivalent rounds being the same color. Darker shades are 2021 samples and lighter colors are samples from 2022. Point shape represents the extraction kit used to extract that sample. PERMANOVA results comparing extraction kits for each sample type are listed in the corresponding panel.

Figure S4. NMDS plots showing each sample type (color) and the extraction kit used (shape) for A) both years of field samples and B) lab Day 4 samples. PERMANOVA outputs are in the corresponding panels.

Figure S5. NMDS plots by sample type showing the distribution of samples across the salinity (A:C,G:H) and temperature (D:F) gradients in the study. Pannels A to F show field samples, while panels G to I show lab Day 4 samples. Sample types are in the same column, while the same abiotic gradient is shown across rows. Point shapes indicate the extraction kit used.

Figure S6. The relative abundance of the 10 most abundant orders (A, C) or genera (B, D) in the field samples. Field samples (A,B) are separated by extraction kit and salinity on the sampling day (facets) and the per-sample relative abundance is averaged across all samples from the same sampling day. Sample numbers are indicated in parentheses. (C,D) Each lab sample is shown (not averaged) and show the 10 most abundant taxa as identified in the field data. Colors are consistent between the field and lab Day 4 plots. Tables include the output of models comparing the relative abundance of taxa in the field (Table S3) and lab Day 4 (Table S4).

Figure S7. Scatter plot of the per-sample relative abundance of core ASVs on *Saccharina* in the field along the A) salinity or B) temperature gradient in the field arranged from least-stressful on the left to most-stressful on the right. Facets separate the samples by extraction kit and colors represent the different IndVal stat threshold values, meaning each sample is plotted for each IndVal stat value.

Table S1. Shipping dates and associated salinity of the full-strength seawater for each lab culturing round.

Table S2. Number of field bacterial samples that were sequenced per sample source, year, and sample type. Also, the sample number of the of lab 8 experimental rounds, showing the number of samples per lab experimental day. Note, all experimental rounds included three salinity levels (10 psu, 20 psu, and full-strength), with two replicate aquaria per salinity level. There were three *Saccharina* per aquaria in all instances except experimental round 1, where one of the 10 psu salinity aquaria only had two *Saccharina*.

Table S3. Output of linear regression model comparing the per-sample relative abundance of each of the 10 most abundant orders and genera in the field in field *Saccharina* samples. Models were run individually for each taxa and are nested within extraction kit, where trends in taxa relative abundance by salinity and temperature were assessed. P-values were adjusted for multiple comparisons with a Benjamini-Hochberg correction.

Table S4. Output of ANOVA model comparing the per-sample relative abundance of each of the 10 most abundant orders and genera in the field in the Day 4 lab *Saccharina* samples. ANOVAs were run individually for each taxa and p-values were adjusted for multiple comparisons with a Benjamini-Hochberg correction.

Table S5. Table showing the output of IndVal analysis for the field, lab, and King datasets. Taxonomy information for each ASV is provided in columns A to I. Columns J to M show the output of the IndVal for the field samples collected with salinity ≥ 20. The assigned_*Saccharina*_high column indicated if the ASV was assigned to *Saccharina* (1) or to another sample type (0) by the IndVal analysis. IndVal_stat_high shows the IndVal stat, Specificity_Saccharina_high indicates the specificity to *Saccharina* and Prevalence_Saccharina_high shows the prevalence on *Saccharina*. Columns N to Q are the same data and organization as columns J to M, but for field samples collected where salinity < 20. Columns R to T show the prevalence of each ASV in the lab data on Day 4 by salinity treatment. Column U (Prevalence_King) shows the prevalence of the ASV in the King dataset. Note, the BC core, with an IndVal stat ≥ 0.7 can be identified in column K (IndVal_stat_high). The UK core can be identified in column U (Prevalence_King), with a prevalence of ≥ 0.8, which corresponds to column M in the BC data.

Table S6. Output of the best nested linear regression model by backwards AIC for the field or ANOVA for the lab Day 4 samples. From left to right, the columns indicate if the samples analyzed are from the field or lab, if the model is analyzing alpha (Shannon-Weiner) or beta-diversity (Bray-Curtis Dissimilarity), the type of mode, the sample type analyzed, the output of

the overall model, the output for each factor in the model (field data only), and the output of the Tukey post-hoc test (lab only).

Tables

Table 1.

Surface	Kit	Salinity	Temperature
Saccharina	QIAGEN	**$F_{1,185}$=2.465, R^2=0.013, p=0.007**	**$F_{1,185}$=2.601, R^2=0.014, p=0.001**
	Zymo	$F_{1,56}$=1.178, R^2=0.012, p=0.260	**$F_{1,56}$=2.038, R^2=0.034, p=0.022**
Rock	QIAGEN	$F_{1,63}$=0.792, R^2=0.012, p=0.848	$F_{1,63}$=0.630, R^2=0.010, p=0.990

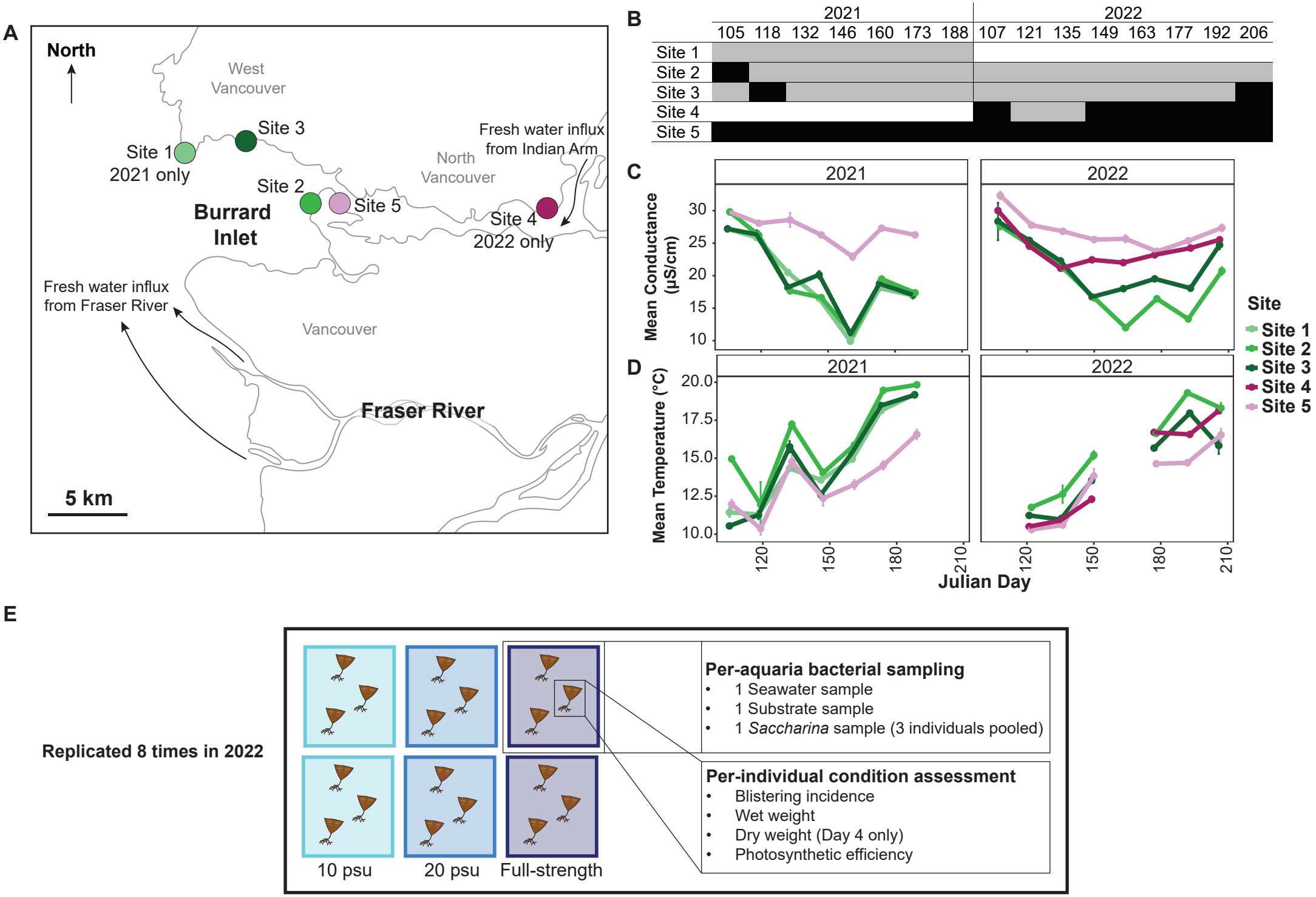
	Zymo	$F_{1,31}$=0.972, R^2=0.029, p=0.460	$F_{1,31}$=0.959, R^2=0.029, p=0.473
Water	QIAGEN	**$F_{1,48}$=2.261, R^2=0.041, p=0.011**	**$F_{1,48}$=3.651, R^2=0.066, p=0.001**
	Zymo	$F_{1,31}$=1.695, R^2=0.045, p=0.083	**$F_{1,48}$=4.240, R^2=0.112, p=0.001**

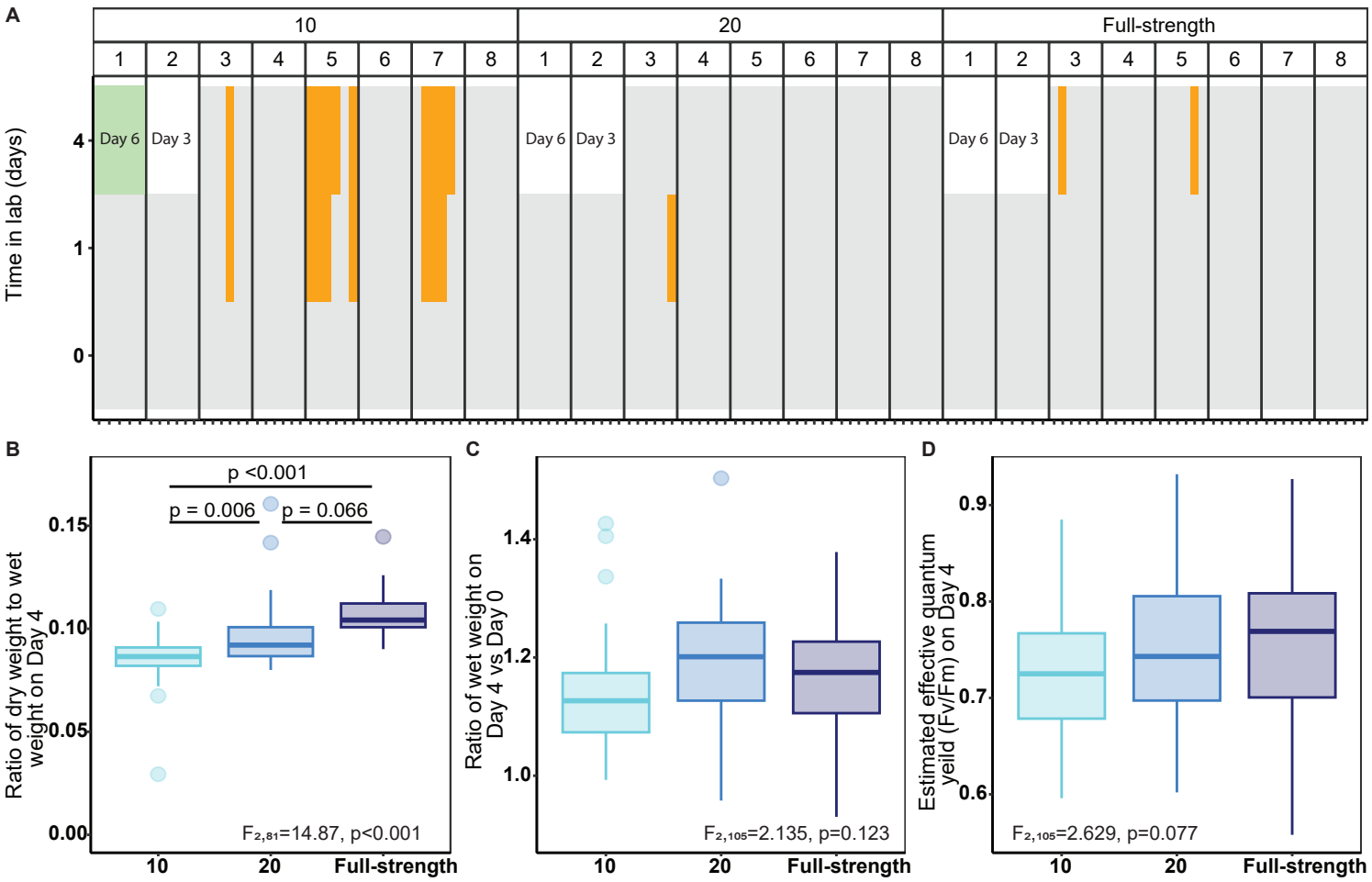
Table 2.

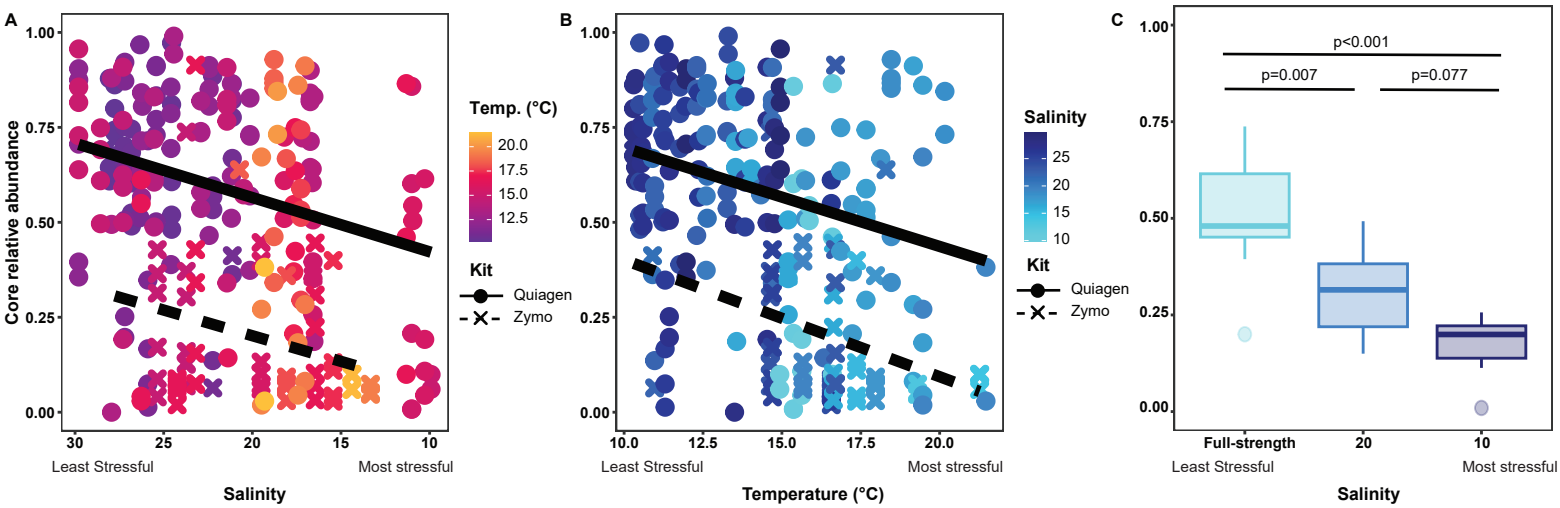
Surface	Kit	Salinity	Culture Round	Salinity*Culture Round
Saccharina	Zymo	**$F_{2,9}$=3.363, R^2=0.166, p=0.002** 10 vs 20 p=0.096 **10 vs full-strength p=0.003** 20 vs full-strength p=0.294	**$F_{5,9}$=2.692, R^2=0.331, p=0.001**	$F_{10,9}$=1.145, R^2=0.281, p=0.266
Substrate	Zymo	$F_{2,12}$=1.188, R^2=0.165, p=0.264	Not run	Not run
Water	Zymo	**$F_{2,11}$=4.626, R^2=0.186, p=0.001** 10 vs 20 p=0.189 **10 vs full-strength p=0.003** **20 vs full-strength p=0.039**	**$F_{6,11}$=3.273, R^2=0.394, p=0.001**	$F_{8,11}$=1.241, R^2=0.199, p=0.113

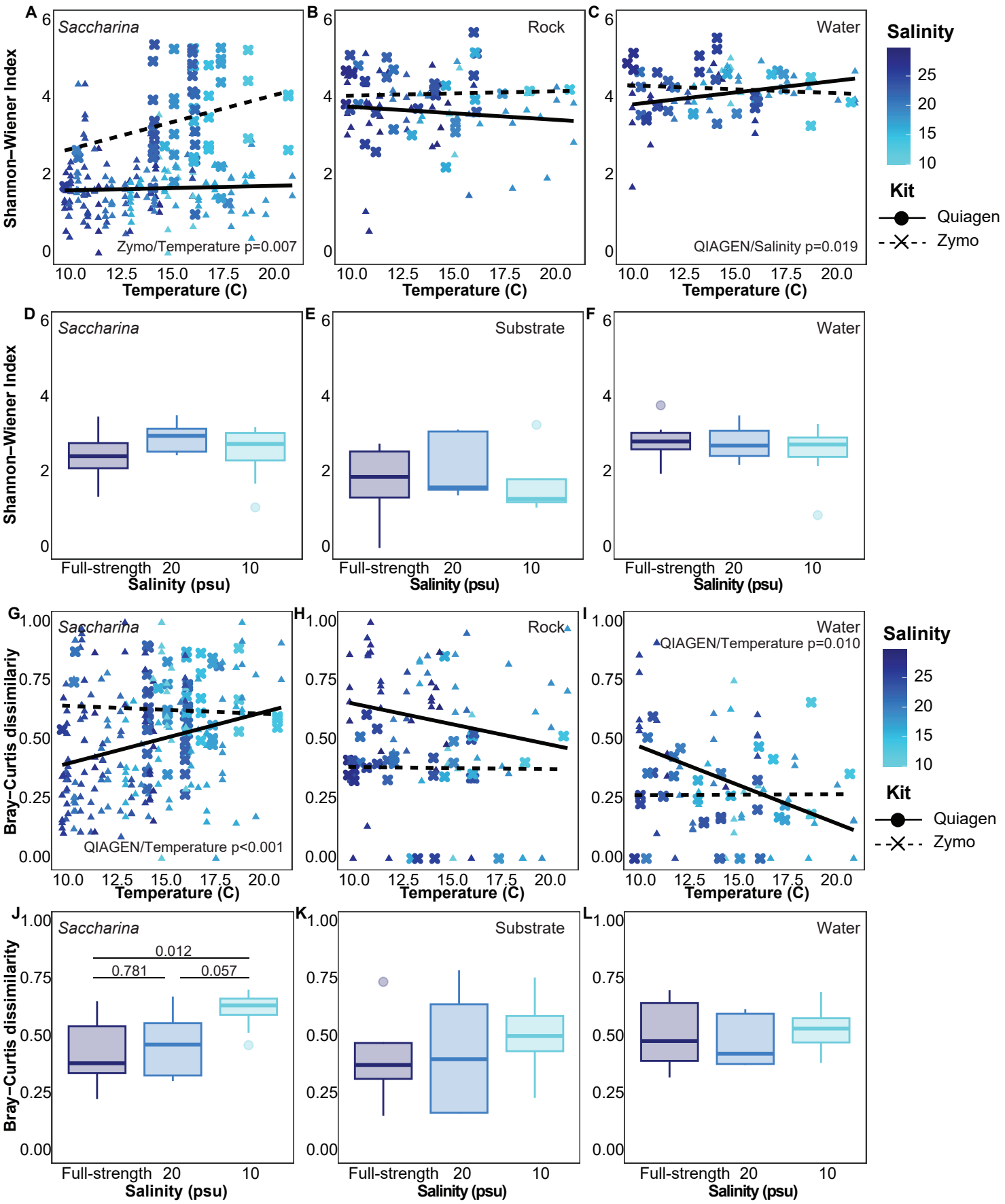
Table S1.

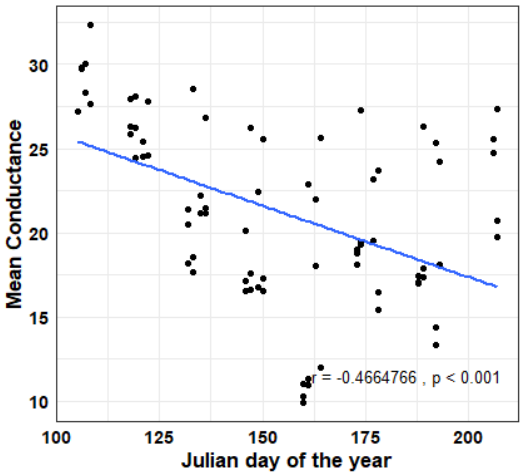
Culturing round number (start date)	New water shipment date	Full strength salinity
1 (April 19, 2022)	April 13, 2022	32
2 (May 3, 2022)	April 13, 2022	32
3 (May 17, 2022)	April 13, 2022	32
4 (May 31, 2022)	April 13, 2022	32
5 (June 14, 2022)	June 10, 2022	31
6 (June 28, 2022)	June 10, 2022	31
7 (July 12, 2022)	June 10, 2022	31
8 (July 207, 2022)	July 19, 2022	32

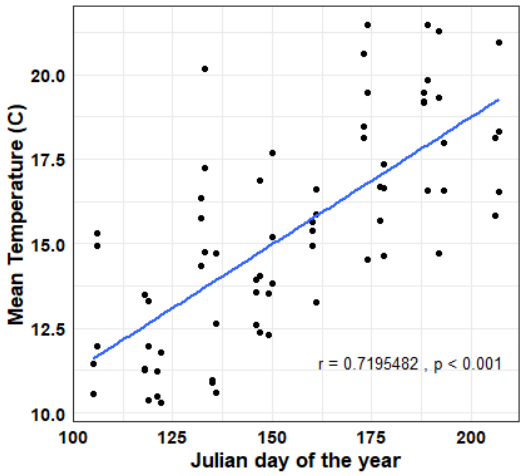

A
North
West Vancouver
Site 3
Site 1
2021 only
Site 2
Site 5
Burrard Inlet
North Vancouver
Fresh water influx from Indian Arm
Site 4
2022 only
Fresh water influx from Fraser River
Vancouver
Fraser River
5 km
B
2021
2022
105 118 132 146 160 173 188 107 121 135 149 163 177 192 206
Site 1
Site 2
Site 3
Site 4
Site 5
C
2021
2022
Mean Conductance (µS/cm)
30
25
20
15
10
D
Mean Temperature (°C)
20.0
17.5
15.0
12.5
10.0
120
150
180
210
Julian Day
Site
Site 1
Site 2
Site 3
Site 4
Site 5
E
Replicated 8 times in 2022
10 psu
20 psu
Full-strength
Per-aquaria bacterial sampling
• 1 Seawater sample
• 1 Substrate sample
• 1 Saccharina sample (3 individuals pooled)
Per-individual condition assessment
• Blistering incidence
• Wet weight
• Dry weight (Day 4 only)
• Photosynthetic efficiency

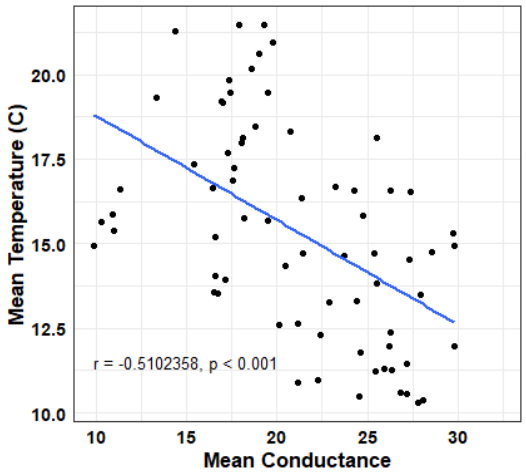

A
10
20
Full-strength
1 2 3 4 5 6 7 8
Time in lab (days)
4
1
0
Day 6
Day 3
B
p <0.001
p = 0.006
p = 0.066
Ratio of dry weight to wet weight on Day 4
0.15
0.10
0.05
0.00
F2,81=14.87, p<0.001
10
20
Full-strength
C
Ratio of wet weight on Day 4 vs Day 0
1.4
1.2
1.0
F2,105=2.135, p=0.123
D
Estimated effective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

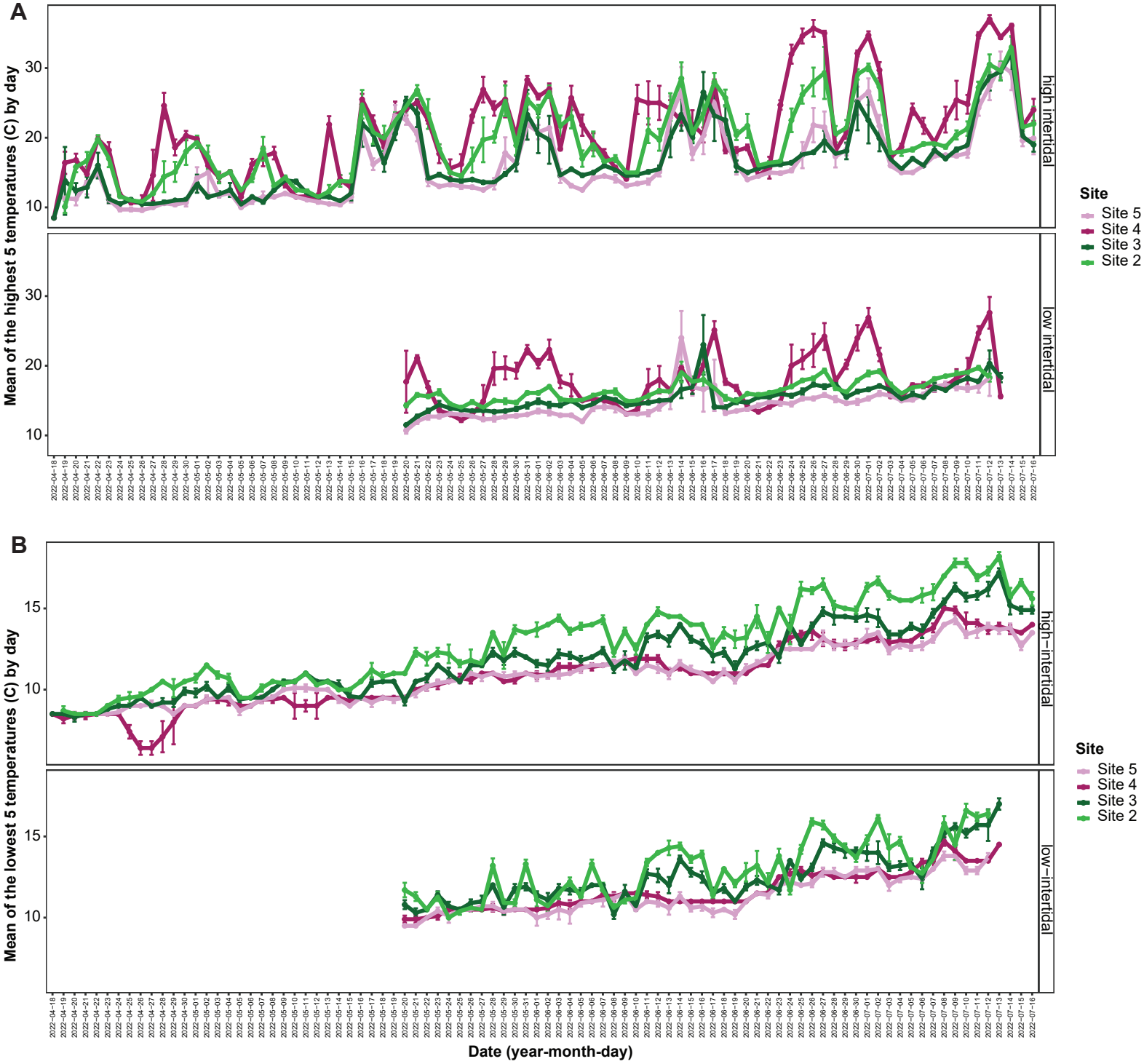

A
Core relative abundance
1.00
0.75
0.50
0.25
0.00
30
25
20
15
10
Least Stressful
Most stressful
Salinity
Temp. (°C)
20.0
17.5
15.0
12.5
Kit
Quiagen
Zymo
B
10.0
12.5
15.0
17.5
20.0
Temperature (°C)
Salinity
25
20
15
10
C
p<0.001
p=0.007
p=0.077
Full-strength

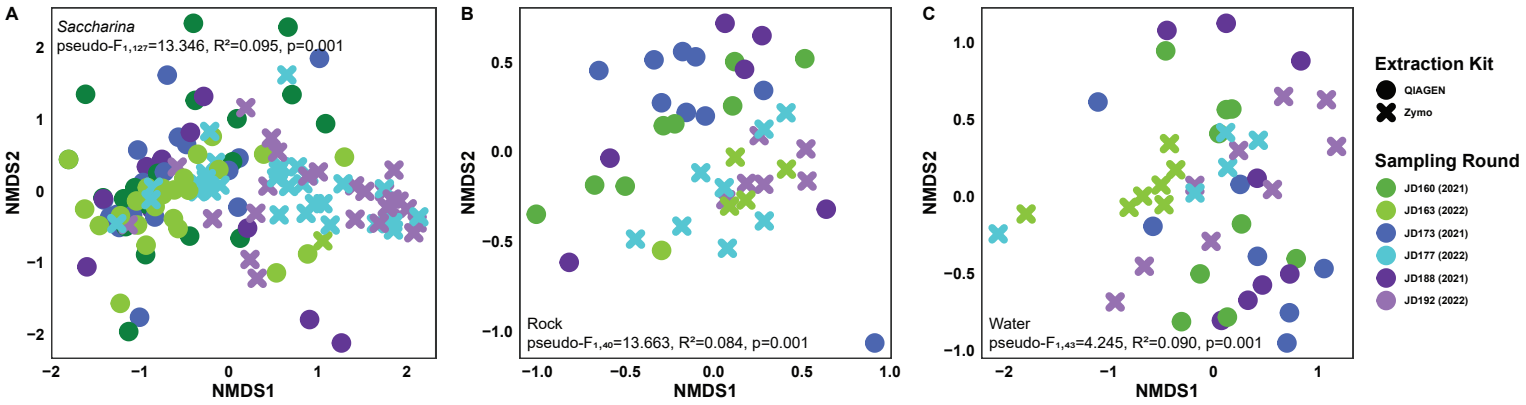

A
Saccharina
Zymo/Temperature p=0.007
B
Rock
C
Water
QIAGEN/Salinity p=0.019
Shannon–Wiener Index
Temperature (C)
Salinity
25
20
15
10
Kit
Quiagen
Zymo
D
Saccharina
E
Substrate
F
Water
Full-strength
20
10
Salinity (psu)
G
Saccharina
QIAGEN/Temperature p<0.001
H
Rock
I
Water
QIAGEN/Temperature p=0.010
Bray–Curtis dissimilarity
J
Saccharina
0.012
0.781
0.057
K
Substrate
L
Water

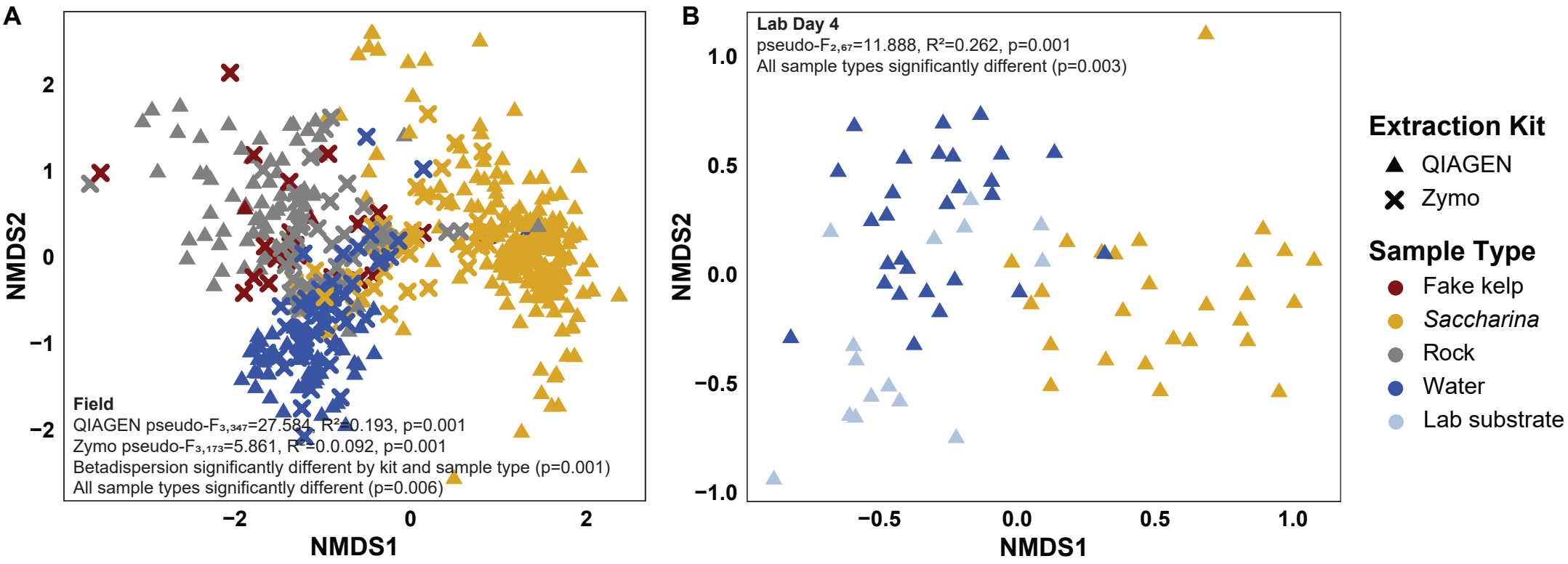

Mean Conductance
Julian day of the year
r = -0.4664766 , p < 0.001
30
25
20
15
10
100
125
150
175
200

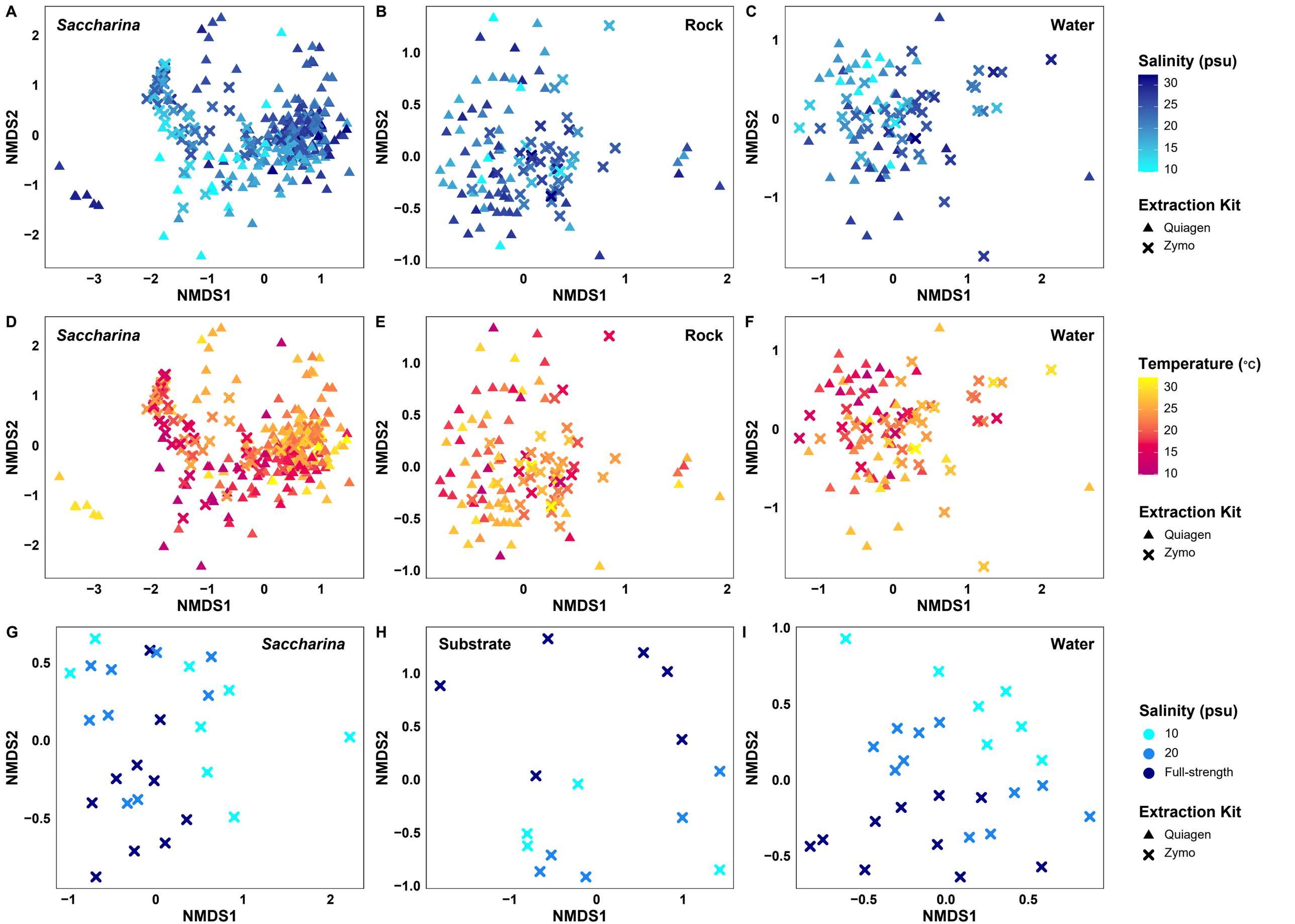

Mean Temperature (C)
Julian day of the year
r = 0.7195482 , p < 0.001
20.0
17.5
15.0
12.5
10.0
100
125
150
175
200

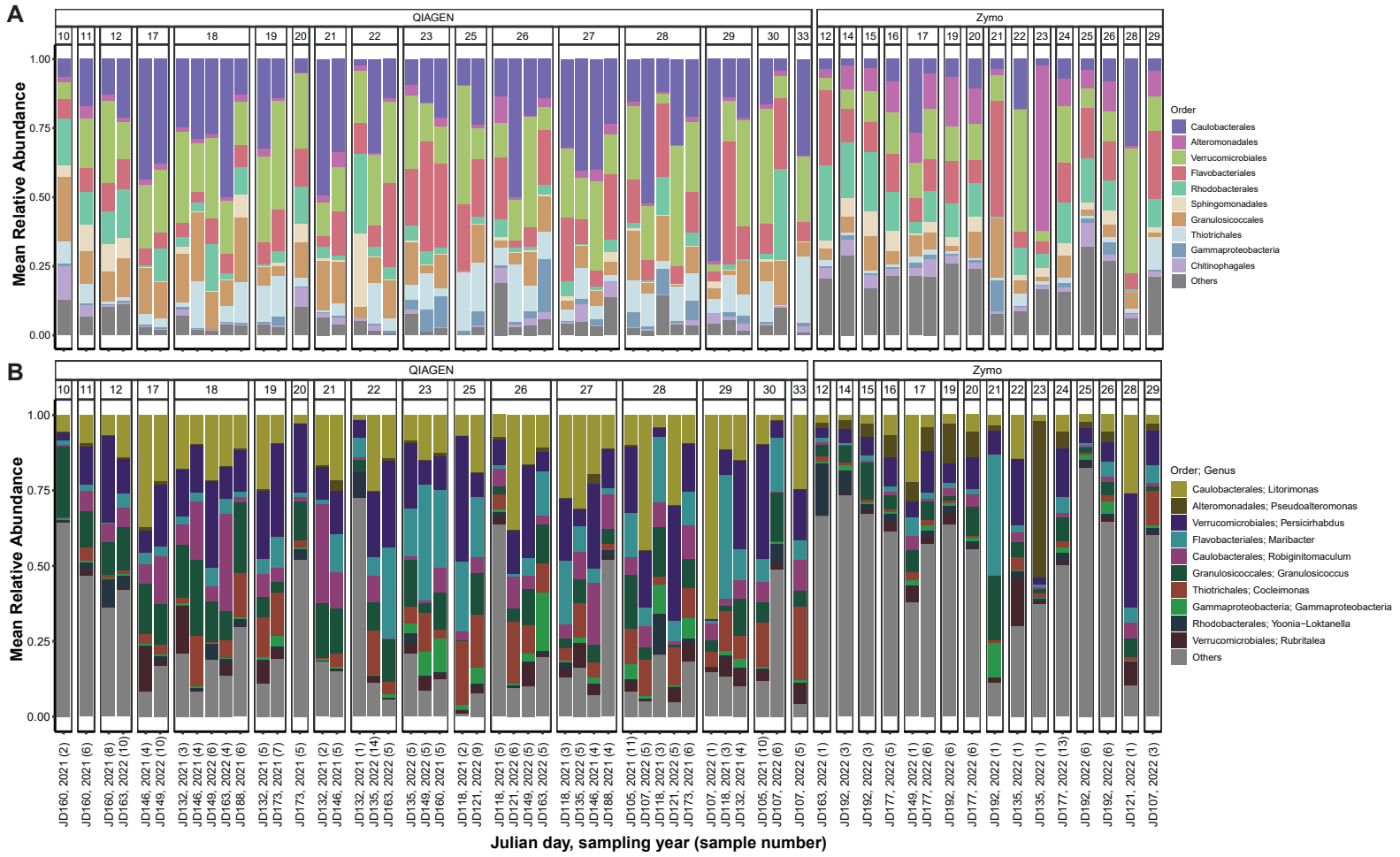

Mean Temperature (C)
Mean Conductance
r = -0.5102358, p < 0.001
20.0
17.5
15.0
12.5
10.0
10
15
20
25
30

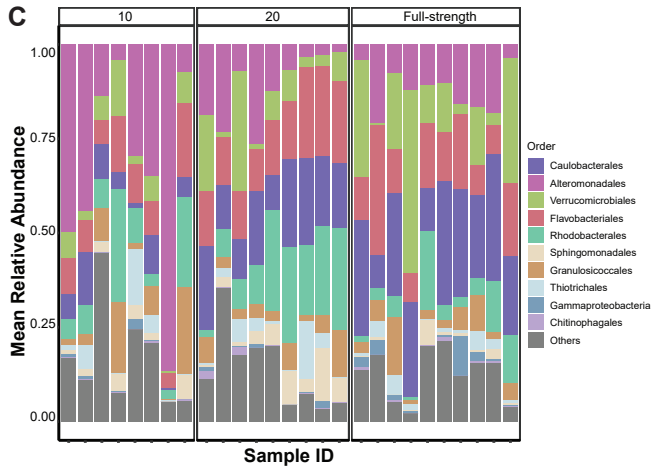

A
Mean of the highest 5 temperatures (C) by day
high intertidal
low intertidal
Site
Site 5
Site 4
Site 3
Site 2
B
Mean of the lowest 5 temperatures (C) by day
high−intertidal
low−intertidal
Site
Site 5
Site 4
Site 3
Site 2
Date (year-month-day)

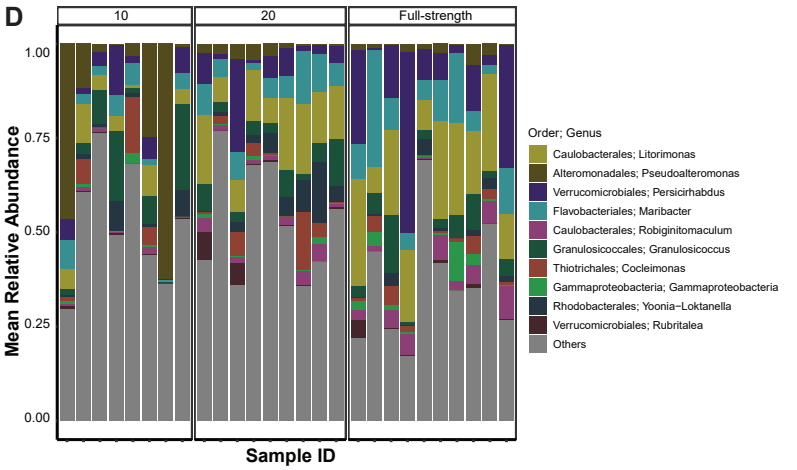

A
Saccharina
pseudo-F1,127=13.346, R²=0.095, p=0.001
NMDS2
NMDS1
B
Rock
pseudo-F1,40=13.663, R²=0.084, p=0.001
NMDS2
NMDS1
C
Water
pseudo-F1,43=4.245, R²=0.090, p=0.001
NMDS2
NMDS1
Extraction Kit
QIAGEN
Zymo
Sampling Round
JD160 (2021)
JD163 (2022)
JD173 (2021)
JD177 (2022)
JD188 (2021)
JD192 (2022)

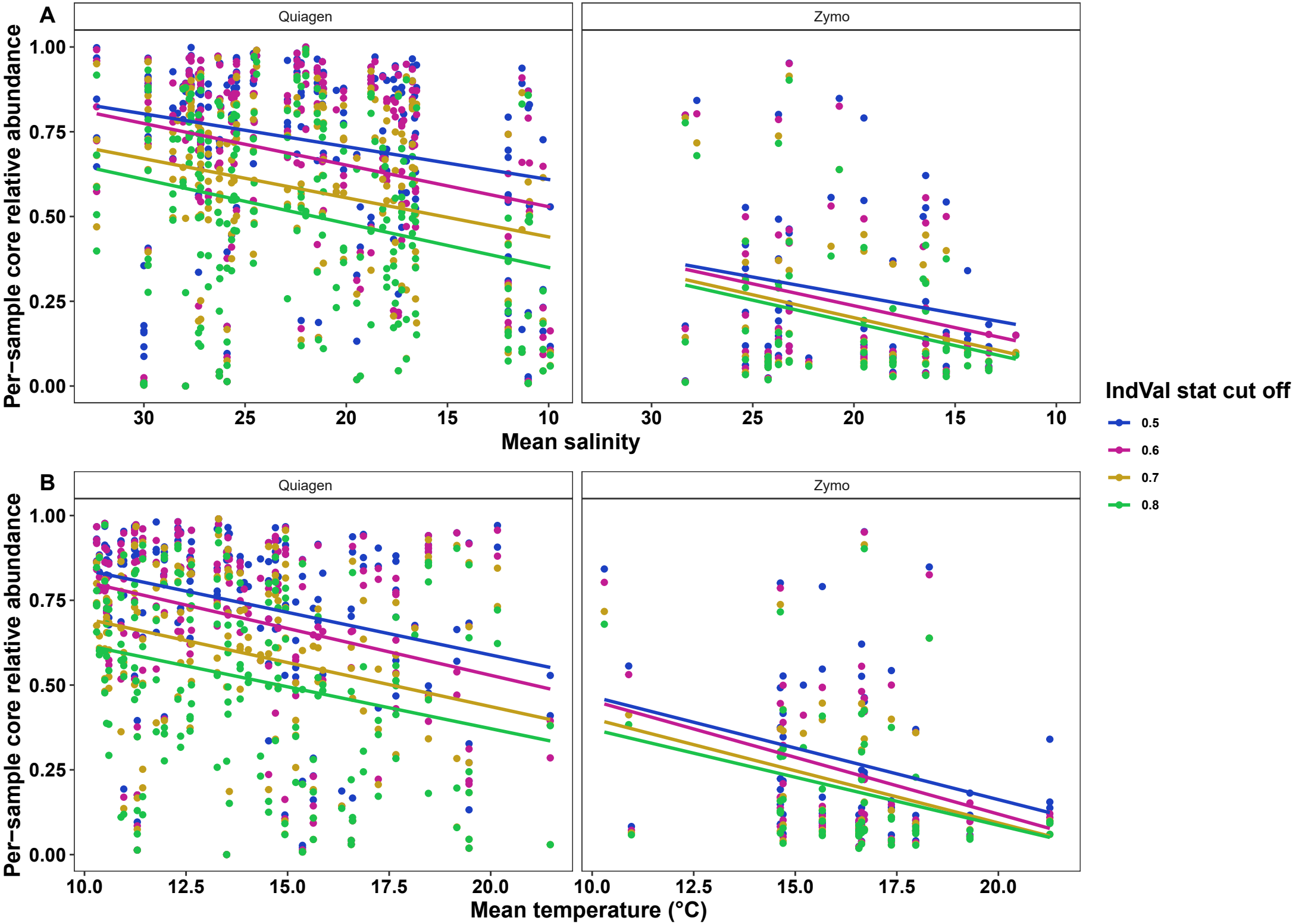

A
Field
QIAGEN pseudo-F3,347=27.584, R²=0.193, p=0.001
Zymo pseudo-F3,173=5.861, R²=0.0.092, p=0.001
Betadispersion significantly different by kit and sample type (p=0.001)
All sample types significantly different (p=0.006)
NMDS1
NMDS2
B
Lab Day 4
pseudo-F2,67=11.888, R²=0.262, p=0.001
All sample types significantly different (p=0.003)
NMDS1
NMDS2
Extraction Kit
QIAGEN
Zymo
Sample Type
Fake kelp
Saccharina
Rock
Water
Lab substrate


A
Saccharina
B
Rock
C
Water
D
Saccharina
E
Rock
F
Water
G
Saccharina
H
Substrate
I
Water
NMDS1
NMDS2
Salinity (psu)
30
25
20
15
10
Extraction Kit
Quiagen
Zymo
Temperature (°c)
30
25
20
15
10
Extraction Kit
Quiagen
Zymo
Salinity (psu)
10
20
Full-strength
Extraction Kit
Quiagen
Zymo


A
QIAGEN
Zymo
Mean Relative Abundance
Order
Caulobacterales
Alteromonadales
Verrucomicrobiales
Flavobacteriales
Rhodobacterales
Sphingomonadales
Granulosicoccales
Thiotrichales
Gammaproteobacteria
Chitinophagales
Others
B
Order; Genus
Caulobacterales; Litorimonas
Alteromonadales; Pseudoalteromonas
Verrucomicrobiales; Persicirhabdus
Flavobacteriales; Maribacter
Caulobacterales; Robiginitomaculum
Granulosicoccales; Granulosicoccus
Thiotrichales; Cocleimonas
Gammaproteobacteria; Gammaproteobacteria
Rhodobacterales; Yoonia−Loktanella
Verrucomicrobiales; Rubritalea
Others
Julian day, sampling year (sample number)


C
10
20
Full-strength
Mean Relative Abundance
Order
Caulobacterales
Alteromonadales
Verrucomicrobiales
Flavobacteriales
Rhodobacterales
Sphingomonadales
Granulosicoccales
Thiotrichales
Gammaproteobacteria
Chitinophagales
Others
Sample ID


D
10
20
Full-strength
Mean Relative Abundance
Order; Genus
Caulobacterales; Litorimonas
Alteromonadales; Pseudoalteromonas
Verrucomicrobiales; Persicirhabdus
Flavobacteriales; Maribacter
Caulobacterales; Robiginitomaculum
Granulosicoccales; Granulosicoccus
Thiotrichales; Cocleimonas
Gammaproteobacteria; Gammaproteobacteria
Rhodobacterales; Yoonia−Loktanella
Verrucomicrobiales; Rubritalea
Others
Sample ID


A
Quiagen
Zymo
Per−sample core relative abundance
1.00
0.75
0.50
0.25
0.00
30
25
20
15
10
Mean salinity
B
Quiagen
Zymo
10.0
12.5
15.0
17.5
20.0
Mean temperature (°C)
IndVal stat cut off
0.5
0.6
0.7
0.8