Climate change impacts on kelp:
Physiological responses across habitats, species, and populations

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Abstract

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Climate change is impacting organisms globally and increasing stressors like warming in both the atmosphere and ocean. Foundation species have disproportionate effects on their local environments through the provision of habitat therefore, declines can have major ecological impacts. Kelps are important foundation species in coastal marine ecosystems that support diverse understory algae, invertebrate, and fish assemblages and serve as a food source in natural systems and aquaculture. The environmental requirements of kelp, cold and nutrient rich waters, make them especially at risk from ocean warming. The Salish Sea is an extensive fjord estuary complex with a natural gradient of temperatures and nutrients with warm water temperatures in inlets like southern Puget Sound. This region is also a hotspot for intertidal thermal risk due to warm air temperatures in spring and summer coinciding with low tides. The combination of a gradient in water temperature and warm air temperatures during low tide makes this region ideal for studying multiple types of warming and stressors. In order to support conservation and management of ecologically important kelp species, we tested the effects of warming and multiple stressors across habitats (intertidal and subtidal), species (*Hedophyllum sessile*, *Nereocystsis luetkeana*, and *Saccharina latissima*), and populations in laboratory and field studies by measuring a comprehensive suite of physiological responses including blade growth, bleaching, photosynthesis, respiration, photosynthetic yield, nutrient uptake, and tissue C:N. We leveraged an extreme heatwave (2021 Western North American Heatwave) to understand mechanisms of survival through self-shading and protection of the meristem in *H. sessile* (Chapter 1). We built thermal performance curves for 8 *N. luetkeana* populations and found a trend of higher optimal temperatures in kelp from warmer sites but, surprisingly, the same critical thermal maximum of 22°C across all sites (Chapter 2). Finally, we tested the interactive effects of short-term exposure to warming and nitrogen limitation on *N. luetkeana* and *S. latissima* and found high temperatures but not low nitrogen availability was stressful for kelps (Chapter 3). Together, these studies indicate that inland seas can act as harbingers of climate change, some species are more resilient to warming more than others, management actions should protect sites that can serve as thermal refugia, and mitigation and restoration actions should be considered for sites nearing thermal tipping points.

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Morphology, bleaching, and growth shapes the resilience of an intertidal foundation species (*Hedophyllum sessile*) to aerial heatwaves

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Abstract

Climate change is increasing the frequency and severity of extreme events that have detrimental effects ranging from lethal to sublethal on many species, including foundation species that create habitat and ameliorate stress for other organisms. In order to understand how foundation species respond to extreme events, we investigated the physiological tolerance of an intertidal kelp, *Hedophyllum sessile*, to natural and simulated aerial heat stress during low tide in the Salish Sea (WA, USA), a location known for prolonged daytime aerial exposure in summer. Our field study that leveraged an extreme heatwave (2021 Western North American Heatwave); we measured survival as well as blade growth and bleaching of adult kelp sporophytes at the start and end of each spring tide sequence from May to August 2021. During spring and summer, we exposed entire sporophytes in the laboratory to simulated 3-hour low tide treatments at hot and ambient air temperatures, or no aerial exposure, for 4 consecutive days and measured physiological metrics and survival during the experiment and after a submerged recovery period. Together, these studies indicate *H. sessile* can survive extreme heat waves due to its morphology, but with extensive distal blade tissue bleaching and subsequent loss. Moreover, aerial heatwaves in spring may be worse than summer due to acclimation to warmer temperatures in summer. Since *H. sessile* has strong interactions with its understory community that rely on the provision of shade from the canopy, heatwave-induced blade shortening of *H. sessile* has the potential to incite a trait-mediated break down in community interactions.

Introduction

As extreme events (e.g. hurricanes, heatwaves, droughts, floods, wildfires) become more frequent due to climate change (Fischer et al., 2021; Swann, 2018; IPCC 2022), it is important to understand how foundation species respond to these stressors. Within ecological communities, foundation species such as trees, grasses, mangroves, corals, and kelp often ameliorate stressful conditions through the provision of more hospitable or benign microhabitats. Sometimes this amelioration is enough to reduce thermal risk for associated species utilizing the habitat even with climate warming (Jurgens et al., 2022). Since losses of foundation species can cause cascading impacts on the communities that they support (Harley et al., 2012; Munday et al., 2008; Reyer et al., 2013), we need to understand how these organisms survive and respond in stressful environments including extreme heat.

Plants, many of which are foundation species, have evolved multiple morphological mechanisms that increase their resilience to biotic and abiotic stress. For example, grasses have intercalary meristems that protect the growing region from being consumed by grazers (Branson, 1953). Meanwhile, trichomes are small leaf hairs that defend plants against heat stress as well as water loss (Gutschick, 1999; Kaur & Kariyat, 2020). Other plant morphologies that minimize water loss during transpiration have small compound leaves or stomatal crypts (Miller et al., 1995; Vico et al., 2013). Yet similar morphological mechanisms for thermal resilience are less well known in photosynthetic marine organisms.

It is also increasingly recognized that thermal extremes, more so than long term averages, have dramatic effects on population dynamics (Dowd et al., 2015; Gaines & Denny, 1993; Sanford et al., 2019). Heatwaves can have lasting long-term effects on populations that are pushed past their thermal limits. For example, a marine heatwave caused mass mortality of seagrass that triggered a community change and declines in the health of large herbivores (Thomson et al., 2015). Furthermore, thermal safety margins are expected to shrink in a warming world, causing temperatures to more frequently exceed thermal limits of primary producers, which puts many biomes at risk (O’Sullivan et al., 2017).

Both aerial (in the atmosphere) and marine (in the ocean) heatwaves act as major disturbances in intertidal ecosystems (Menge et al., 2022). Organisms inhabiting the intertidal zone experience periodic fluctuations in physical conditions due to the tidal cycle, making the intertidal zone an ideal environment to assess two types of warming. During spring tides, when the tidal amplitude is the greatest, intertidal organisms experience multiple days with long periods of exposure to terrestrial conditions so that they must tolerate hot or freezing temperatures, solar radiation, and desiccation to survive (Davison & Pearson, 1996). Spring tides occur twice monthly regardless of season. During neap tides, when the tidal amplitude is less extreme, organisms inhabiting the low shore remain submerged. While aerial heatwaves can warm surface waters, marine heatwaves are normally caused by a combination of mechanisms including atmospheric and oceanic contributions (Holbrook et al., 2019). The northeast Pacific Ocean experienced extremes of both types of heatwaves within the last decade. The Northeast Pacific Blob was a severe (category III; Hobday et al. 2018) marine heatwave with an extremely long duration from 2013-2016. The “Blob” caused mass bird and marine mammal mortality, homogenization of rocky intertidal community structure, and declines in habitat-forming brown seaweeds (Arafeh-Dalmau et al., 2019; Cavole et al., 2016; Weitzman et al., 2021). The 2021 Western North American Heatwave (2021 WNAH), also referred to as the Pacific Northwest heat dome, was an extreme aerial heatwave that brought record-breaking air temperatures to Oregon, Washington (USA) and southern British Columbia (CAN) (Bartusek et al., 2022; Overland, 2021) for 4 days; this was dangerous for human health (Silberner, 2021) as well as the environment. In the Salish Sea, the heatwave also coincided with afternoon spring low tides, which exposed intertidal organisms to the extreme aerial temperatures and caused mass die-offs of intertidal organisms, particularly the bay mussel *Mytilus trossulus* (Raymond et al., 2022). Impacts on other foundational species, such as intertidal seaweeds, showed large declines to localized extinctions of the rockweed, *Fucus*, in British Columbia (Harley et al. *in prep*).

Seaweeds are important primary producers and foundation species that respond to changes in environmental conditions including heatwaves (Filbee-Dexter et al., 2020; Harley et al., 2012; Smale et al., 2019; Straub et al., 2019). Generally, organismal metabolism increases with temperature and peaks at an optimum temperature after which function rapidly decreases (Huey & Stevenson, 1979). Therefore, the magnitude of warming impacts the direction of the effect on seaweed physiology (Díaz-Acosta et al., 2021). Seaweed photosynthesis and growth increase with temperature up to a tipping point (Bell, 1993; Colvard et al., 2014; Dudgeon et al., 1989; Maberly & Madsen, 1990; Supratya et al., 2020; Williams & Dethier, 2005). Román et al. (2020) found lethal effects on seaweeds during aerial heatwaves but sublethal effects on photosystem II and growth during marine heatwaves. Other sublethal effects of warming on seaweeds include bleaching, the loss of pigment from blades, which indicates physiological damage and can impair productivity (Hereward et al., 2020; Qiu et al., 2019).

Unlike other regions of the US West Coast, extreme low tides in the Salish Sea occur during daylight hours in summer, making this region a hot spot for aerial temperatures with a large number of low tide days with high thermal risk compared to other regions (Harley & Helmuth, 2003; Mislan et al., 2009). As extreme events like heatwaves increase in frequency and severity with climate change, the number of risky days in the Salish Sea will grow (Fischer et al., 2021; Meehl & Tebaldi, 2004; Oliver et al., 2018). The high thermal risk during low tide in the Salish Sea makes this region ideal for testing hypotheses surrounding organismal responses to climate change.

The low intertidal kelp, *Hedophyllum sessile* (formerly *Saccharina sessilis*), is an important foundation species that supports a diversity of organisms under the shade and protection of its canopy (Barner et al., 2016; Burnaford, 2004; Dayton, 1975). *Hedophyllum* *sessile* has strong interactions with its understory community including the large chiton, *Katharina tunicata* (Burnaford, 2004; Dayton, 1975; Duggins & Dethier, 1985; Paine, 2002). Additionally, *H. sessile* is an interesting case study in intertidal physiological ecology due its habitat, growth rate, and morphology. Most kelps reside in the subtidal but *H. sessile* inhabits a higher tidal elevation and niche, and therefore experiences long periods of terrestrial conditions, from 2.7 to 3.4 hours during low tides (Burnaford et al., 2014). Kelps (Laminariales), including *H. sessile*, undergo an alternation of generations and have a high growth rate, up to 2 cm/day per blade depending on the species, during their macroscopic sporophyte stage (hereafter, sporophyte), making it possible to assay changes in growth through elongation over short periods of time (Mann, 1973; Nicholson, 1970). Laminariales includes 25 genera of kelps (Starko et al., 2019) and only species in the genus *Hedophyllum* (e.g. *H. sessile, H. druehlii*) have a morphology that lack a stipe during their adult sporophyte life history phase (Starko et al., 2018; Widdowson, 1965). Without a stipe, the blade arises directly from the holdfast (see Fig. 1A), creating a canopy positioned very close to the substrate. The intercalary meristem of the blade (Fig. 1A) is at the base near the holdfast. We hypothesize that the unique morphology of *H. sessile* facilitates resilience to thermal stress by self-shading and protection of the meristem.

In this study, we evaluated how a foundational species that has strong community interactions (*Hedophyllum sessile*) grows, survives, photosynthesizes, and recovers during and after aerial heatwaves that coincide with low tides in the Salish Sea using field and laboratory studies. Using the 2021 Western North American Heatwave as a natural experiment, we examined temporal patterns in blade growth and bleaching across spring and neap tides to assess the role of emersion in growth and tissue loss. Additionally, we examined seasonal responses in growth and photo-physiology to low tide stressors in a laboratory study to assess the role of seasonal thermal acclimatation. Together, these studies allow us to understand how *H. sessile* responds to low tide stressors and aerial heatwaves.

Methods

Field observations

At Deadman Bay, San Juan Island, Washington, USA (48°30’47 N, 123°08’47 W), which has a robust bed of *Hedophyllum sessile* in the intertidal zone, we surveyed the kelp bed, measured *in situ* temperatures, and collected sporophytes for the simulated atmospheric heatwave experiment. Temperatures adjacent to the *H. sessile* bed at Deadman Bay were recorded by a Hobo Pendant Temperature/Light Data Logger (Onset) anchored to the substrate but not shaded by the *H. sessile* canopy. We cleared seaweeds around the logger during bi-spring tide sampling (described below). Temperatures recorded by the logger represent both air and water temperatures since the logger was submerged and exposed with the tides. Note that the logger temperatures are influenced by solar radiation during emersion; the *in situ* temperatures therefore do not approximate *H. sessile* sporophyte temperature but do capture extreme changes in thermal experience over time.

From April 27 to August 10, 2021, we monitored tagged adult sporophytes (n = 40-43) along a 15 m permanent transect at 0.25 m tidal height in the *H. sessile* bed. We tagged each sporophyte with labeled flagging tape tied around the holdfast. We performed sampling on the first and final tide below -0.15 m mean lower low water (on average 7 days apart) during spring tide sequences, the multi-day period when tides are the lowest, to capture differences in maximum length and bleaching during spring and neap tides.

We assessed survival of sporophytes during every sampling period. We considered the sporophytes to be dead if there were no blades remaining even if the holdfast was still attached because holdfasts were often present for weeks after mortality. We measured the maximum length and proportion of unhealthy or bleached (necrotic) tissue of the two longest blades of each sporophyte. Blade tissue was categorized as unhealthy or bleached if it was green or white in color and healthy if it was brown in color. We averaged the two measurements of maximum blade length or bleaching as a total sporophyte measurement for analyses (Burnaford et al., 2020). We calculated the daily change in maximum length as the percent change in healthy (unbleached) tissues since the last sampling period. We calculated the extent of bleached tissue as the length of bleached blade divided by total length times 100%.

Simulated atmospheric heatwave experiment

We collected *H. sessile* sporophytes of a similar size and tidal height from the same site as the field surveys for spring and summer experimental trials (on April 27 and June 21, 2021, respectively). In the laboratory, we punched 0.5 cm holes through the holdfast with a cork borer and threaded flagging tape through the holes to attach the sporophytes to nylon ropes. Prior to the start of the experiment, we removed herbivores, including *Lacuna* snails and *Pentidotea* isopods, and rinsed off diatoms with seawater from all sporophytes.

The experiment was designed to mimic the local air temperatures and emersion times during spring tides and neap tides (Friday Harbor Laboratories Weather Station, Carrington et al. 2022; NOAA Tides and Currents, Station 9449880), which we simulated by moving the sporophytes between water and air. We randomly assigned sporophytes to treatments (described below) and then hung the nylon ropes horizontally from the floating dock at Friday Harbor Laboratories (FHL), 0.5 m below the surface, where they received natural ambient light but were not shaded by the dock. We allowed sporophytes to acclimate to FHL dock conditions and heal from wounds for 24 hours before experimental treatments began. We simulated low tides by removing sporophytes from the water and exposing them to aerial conditions in the laboratory for 3 hours daily for 4 consecutive days. April low tide exposures began earlier in the day than June to correspond to the local timing of tides that change seasonally. Outside of the aerial exposures, the sporophytes remained underwater on the FHL floating dock.

We had 3 experimental treatments (n = 8 sporophytes per treatment): ambient aerial temperature (hereafter: “ambient”), elevated aerial temperature with heat lamps (“heatwave”), and no aerial exposure (“submerged”). We randomly placed the sporophytes on the benchtop with their holdfasts down to mimic a *H. sessile* bed during aerial exposure. We elevated aerial temperatures in the heatwave treatment above ambient laboratory temperature using ceramic heat lamps (#LF-15, Zoo Med) placed 40 cm above the sporophytes. Temperature controllers (True Temp #JB1235, JBJ Lighting) modulated power to the lamps to maintain an air temperature of 28°C, the maximum recorded by the FHL weather station in 2020 (Carrington et al. 2022). The ambient and heatwave treatments received ambient light from windows during exposure and outside of low tide exposure all treatments received ambient natural light while submerged. After 4 days with 3-hour aerial exposures, we allowed the treatments to remain submerged for 5 days as a recovery period that simulated a neap tide.

We monitored light and temperature conditions for each treatment with a HOBO Pendant Temperature/Light Data Logger (Onset) that moved with the sporophytes between the laboratory and water (Fig. 3). Meanwhile, we measured air temperature and relative humidity in the laboratory with a HOBO MX1101 Data Logger (Onset).

During the 4-day aerial exposure period, we monitored growth, desiccation, and light harvesting efficiency daily. For the two treatments with aerial exposures (ambient and heatwave) we measured blotted wet biomass at the beginning and end of each emersion period and calculated desiccation as the percent change in wet biomass. We measured growth as wet biomass standardized by initial wet biomass at the start of emersion. At the beginning and end of the emersion period, we measured light harvesting efficiency of photosystem II (Fv/Fm), which is a measure of chloroplast function that is sensitive to heat stress, with a Pocket PEA fluorimeter (Hansatech Instruments) after 15 minutes of dark adaptation under leaf clips. We collected Fv/Fm measurements on two regions of the blade, the base of the blade and 5 cm away from the base, and then averaged to create a single sporophyte measurement for analyses. The base of the blade had same Fv/Fm as the region 5 cm above the base (linear model: P = 0.9; Fig. S3). For the submerged treatment, we only measured wet biomass and Fv/Fm once per trial day to limit exposure time to air.

Statistical analysis

We performed all analyses in R version 4.0.3 (R Core Team, 2021). We assessed changes in field measurements of *H. sessile* size specific change in maximum length, percent of length with bleached tissue, and maximum length with linear models (LM). Specifically, we performed LMs on each response metric with a continuous date and tide (spring or neap) and their interaction as fixed effects. For the laboratory experiment, we analyzed differences in temperatures and relative humidity between seasons with t-tests. Meanwhile, we analyzed percent change in wet biomass, Fv/Fm recovery and desiccation in the laboratory experiment in separate linear mixed models (LMMs) with treatment, season, trial day, and the interaction between treatment and season as fixed effects and individual as a random effect. To assess the differences in Fv/Fm during emersion, we used a linear model with treatment, trial day, season, and the interactions between treatment and season as fixed effects. Finally, we used a binomial GLM to assess laboratory survivorship following the recovery period with treatment and season as fixed effects. LMMs and GLMs were performed in package *lme4* (Bates et al., 2015).

Results

Field observations

Emersed logger temperatures during low tides over the course of the *Hedophyllum sessile* surveys ranged normally from 9 to 41°C and reached maximum of 46°C during the historic 2021 Western North American Heatwave (2021 WNAH, Fig. 1D). Water temperatures ranged from 8 to 12°C and reached 14°C maximum following the 2021 WNAH (Fig. 1D).

We found no natural mortality (0%) of tagged adult sporophytes before the 2021 WNAH and low mortality immediately following the heatwave (3/43 sporophytes, 7%). The effect of tide depended on date for blade growth and bleaching ($P < 0.01$, Table 1, Fig 2). For example, growth during spring tides was 8–16 % per day in May and June but there was no growth in summer (July and August; Fig. 2A). Bleaching ranged 15–50% and followed spring tides only, with the greatest amount of bleaching (8 cm on average) during the 2021 WNAH (Table 1, Fig. 2B). Maximum length was greater following spring than neap tides in June, but after the heatwave (end of June), was similar between tides. Overall, blades were shorter by 4.5 cm at the end of summer compared to the start of measurements in April (Table 1, Fig. 2C).

Efficacy of temperature treatments in simulated aerial heatwaves & experimental conditions

As expected, water temperatures on the FHL dock and air temperatures differed between spring and summer trials (Fig. 3). Dock water temperatures (mean ± SE, spring: 9.4 ± 0.01°C, summer: 11.8 ± 0 .02°C) were warmer by 2.4 degrees on average during the summer (One-tailed t-test: t = -130.8, df =5846, P < 0.001). Ambient air temperatures (spring: 19.7 ± 0.1°C, summer: 21.8 ± 0.2°C) were also warmer by 2.1 degrees on average during the summer (One-tailed t-test: t = -9.2, df = 387, P < 0.001). But the heatwave treatment air temperatures (spring: 30.4 ± 0.5°C, summer: 28.6 ± 0.4°C) were on average 1.6 degrees warmer during the spring (t-test: t = 2.8, df = 384, P = 0.005). Meanwhile, relative humidity (spring: 49.0 ± 0.4%, summer: 64.5 ± 0.2%) in the laboratory during the experimental trials was 15% higher during summer (t-test: t = -37.3, df = 688, P < 0.001).

Laboratory experiments

During the emersion period in experimental trials (days 1 – 4), there was 100% survival in both seasons. Following the submerged recovery period (day 10), all sporophytes in the submerged and ambient treatments survived (100%) but in the heatwave treatment there were differences in survival between seasons. At the end of the recovery period, there was a significant effect of treatment (P < 0.001) and season (P = 0.002) on survival (binomial GLM) but we did not test the interaction due to limited degrees of freedom. Specifically in the heatwave treatment, there was no sporophyte survival (0%, no blades remained following bleaching) in spring but there was moderate sporophyte (63%) survival during summer, indicating a likely interactive effect of season and treatment.

Growth, measured as daily percent change in wet biomass, significantly increased by 36-40% in the submerged and ambient treatments but decreased by in the heatwave treatment (P < 0.001, Table 2, Fig. 4). Trial day had a positive effect on growth (P < 0.001) but season and the interaction between season and treatment were not significant ($P > 0.05$, Table 2). In post hoc Tukey tests, there were significant differences in growth between the heatwave and submerged treatments (estimate = -8.3, $P = 0.01$) and the heatwave and ambient treatments (estimate = -7.3, $P = 0.03$), but not between the ambient and submerged treatments (estimate = -1.0, $P = 0.9$). Meanwhile, desiccation following the aerial exposure significantly differed across treatments (heatwave: 20-37%, ambient: 8-14%) and season and their interaction, with the highest desiccation in spring in the heatwave treatment (Fig. S1, Table S1).

We found differences in PSII recovery among treatments and seasons ($P < 0.001$, Table 2, Fig. 5). Post hoc Tukey tests indicated Fv/Fm in the heatwave treatment was lower than in the ambient and submerged treatments (estimate = -0.12 and -0.1, respectively, $P < 0.001$), which were in turn statistically indistinguishable (estimate = -0.01, $P = 0.9$). There was less recovery of PSII in the heatwave treatment during spring than summer ($P < 0.001$, Table 2, Fig. 5). Meanwhile, the change in Fv/Fm from the beginning to end of the emersion period was significantly different between treatments and seasons ($P < 0.01$, Table 2, Fig. S2) with positive change in Fv/Fm in the ambient treatment but not the heatwave treatment.

Discussion

We found surprisingly high (93%) survival of a foundational intertidal kelp during an extreme natural aerial heatwave, despite declines in growth and photosynthetic function with increased bleaching of blades. Low tide exposure in mid spring had positive effects on *Hedophyllum sessile* growth in the field, but exposure became stressful in summer and following the 2021 Western North American Heatwave (2021 WNAH) and resulted in bleaching, tissue loss, and decreased growth. Our simulated heatwaves laboratory experiment revealed even higher survival and recovery during summer compared to spring experiments after simulated aerial heatwave of the same temperature. Sporophytes experiencing aerial exposure to ambient temperatures, when compared those continuously submerged, exhibited equivalent growth and slightly improved light harvesting efficiency, indicating that aerial exposure is not always stressful and can have benefits.

Other studies on seaweed physiological responses to warming and heatwaves have found effects varying from positive, to sublethal negative, to lethal. Menge et al. 2021 found increases in *H. sessile* growth rate during warm phases of climate patterns (ENSO, PDO), consistent with the higher growth we found during spring low tides before the 2021 WNAH. Net photosynthesis of intertidal seaweeds during low tide increases with warming while hydrated up to a tipping point (Bell, 1993; Williams & Dethier, 2005); the heatwave that reached over 35°C acted as a tipping point for *H. sessile* growth. Burnaford et al. (2020) also found declines in maximum quantum yield in *H. sessile* during low tide exposure in summer.

Seasonality can also play a role in stress tolerance and thermal acclimation. The lower survival of sporophytes in our laboratory study in the spring may be due to differences between ambient aerial temperatures in spring (20°C) than the summer (22°C) that created a larger difference between the heatwave temperatures during spring (+8° vs. +6°C). The sporophytes in the summer lab experiment and the field surveys during the 2021 WNAH may have acclimated to warmer temperatures (Dowd & Denny, 2020). In this manner, heatwaves of the same maximum temperature in spring could have greater negative impacts than summer. Similarly, Hereward et al. (2020) found greater negative effects on photosystem II in *Laminaria digitata* from simulated aerial heatwaves during a cooler season.

The unique morphology of *H. sessile* may play a role in its ability to live in an intertidal habitat and survive heatwaves. The blade morphology of *H. sessile* is variable across environments (Widdowson, 1965), with smooth blades under high flow or wave action and bullate blades in calmer flow (Milligan & DeWreede, 2004). San Juan Island within the Salish Sea, where our study was performed, is a regional hot spot for intertidal thermal risk (Mislan et al., 2009) with seasonally low wave action. Here, *H. sessile* only has bullate morphologies during spring and summer (Armstrong, 1988; Widdowson, 1965). The bullations hold water during low tide (Burnaford et al., 2020) and may increase humidity, which in turn can lower mortality risk as seen in other biogenic habitats (Jurgens et al., 2022). Additionally, *H. sessile* adult sporophytes do not have a stipe (Abbott & Hollenberg, 1976; Widdowson, 1965), which positions their blade close to the holdfast. We propose that the combination of no stipe and a bullate blade, which creates a cabbage-like morphology for which *H. sessile* is known (common name is sea cabbage) is ideal for protecting the meristem at the base of the blade during low tide through self-shading so only older distal tissue is exposed to the extreme damage, bleaching, and subsequent loss (Fig. 1B).

While we found low mortality of *H. sessile* after the 2021 WNAH, we would expect the response to be different if there were compounded perturbations via multiple aerial heatwaves or a heatwave with a duration encompassing multiple spring tides. Since compounded perturbations likely will have a more serious effects on long-term alteration of communities (Paine et al., 1998), repeated heatwaves would not allow enough regrowth (0.4 cm per day) to sustain self-shading and meristem protection. Considering the bleaching and growth rates measured in this study, *H. sessile* would need approximately 20 days to regrow the 8 cm of blade tissue that was lost due the 2021 WNAH. If perturbations were closer together than 20 days, detrimental impacts could be greater. Additionally, with repeated heatwaves and decreased survival, the buffering provided by high density beds could be lost, which would further compound negative effects (Hoos & Harley, 2021).

The 2021 WNAH occurred during an afternoon spring tide sequence and in a year with increased emersion times due to a high lunar declination (25°, NASA Jet Propulsion Laboratory), the angle at which the moon orbits the earth relative to the equator. Declination ranges from 18-28° on a 18.6 year cycle, modulating tidal fluctuations and emersion times for intertidal organisms (Denny & Paine, 1998). *H. sessile* is known to decline in cover with increases in emersion time due to high lunar declination angles (Burnaford et al., 2014), therefore moderately low cover of *H. sessile* was expected at the time of the 2021 WNAH. The cycle will continue to create longer emersion times until it peaks in 2025, so *H. sessile* cover will continue to decline until emersion times are less extreme. As heatwave events become more frequent with climate change (Fischer et al., 2021; Meehl & Tebaldi, 2004; Oliver et al., 2018), years coinciding with high lunar declination will become especially risky for organisms inhabiting the intertidal zone.

Disturbances and sub-lethal impacts on foundation species can have important community level consequences. For example, decreased maximum length and canopy area will likely decrease shade and amelioration of desiccation for the algae and invertebrate community that lives beneath *H. sessile* blades. This kelp is an ecological dominant (Dayton, 1975) with complex and variable interactions with its understory community (Dethier & Duggins, 1984, 1988; Duggins & Dethier, 1985). The chiton, *Katharina tunicata*, relies on the shaded microhabitat provided by the *H. sessile* canopy (Burnaford, 2004) and either dies or emigrates when the brown algal canopy is removed (Dayton, 1975; Duggins & Dethier, 1985). *Katharina tunicata* also helps *H. sessile* maintain dominance over the annual kelp, *Alaria marginata* (Paine, 2002). Yet, *K. tunicata* weakens the holdfasts of juvenile sporophytes (Markel & DeWreede, 1998). *Hedophyllum sessile* juvenile sporophytes are often found beneath the adult canopy on articulated corallines (Barner et al., 2016) where they have the greatest attachment strengths (Milligan & DeWreede, 2000). Removal of the coralline understory causes a strong negative effect on *H. sessile* cover and recruitment (Barner et al., 2016). Therefore, the strong community interactions in this system may compound negative effects of canopy loss via blade shortening and create a trait mediated breakdown of community interactions associated with blade length (Fig. 6). Following high temperatures (Fig. 6B), shorter sporophytes may have less self-shading capacity, making them more susceptible to further heat damage. Additionally, low density beds and smaller sporophytes are less likely to survive intense warming and heatwaves during low tide (Hoos and Harley 2021). With out the *H. sessile* canopy the understory community diversity would be lower (Fig. 6C).

Morphological mechanisms for survival, such bleaching distal blade area and self-shading of the meristem in *H. sessile*, are necessary since climate change is increasing stressful conditions. Yet the non-lethal effects of the *H. sessile* canopy shortening will likely impact the understory community following the 2021 WNAH via a trait mediated breakdown. Heatwaves like the 2021 WNAH, which had unprecedented severity for the region, are projected to become more frequent in the future (Dong et al., 2023). Impacts of extreme events like heatwaves can have lasting impacts on ecosystems, especially when foundation species are damaged (Nepper-Davidsen et al., 2019; Smale et al., 2019; Thomsen et al., 2021). Therefore, conservation efforts should target foundation species like *Hedophyllum sessile*, which have a mechanism for surviving under extremely stressful conditions and ameliorate abiotic conditions for its understory community.

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References

Abbott, I. A., & Hollenberg, G. J. (1976). *Marine algae of California*. Stanford University Press.

Arafeh-Dalmau, N., Montaño-Moctezuma, G., Martínez, J. A., Beas-Luna, R., Schoeman, D. S., & Torres-Moye, G. (2019). Extreme marine heatwaves alter kelp forest community near its equatorward distribution limit. *Frontiers in Marine Science*, *6*, 1–18. https://doi.org/10.3389/fmars.2019.00499

Armstrong, S. L. (1988). Mechanical properties of the tissues of the brown alga *Hedophyllum sessile* (C. Ag.) Setchell: Variability with habitat. *Journal of Experimental Marine Biology and Ecology*, *114*(2–3), 143–151. https://doi.org/10.1016/0022-0981(88)90134-7

Barner, A. K., Hacker, S. D., Menge, B. A., & Nielsen, K. J. (2016). The complex net effect of reciprocal interactions and recruitment facilitation maintains an intertidal kelp community. *Journal of Ecology*, *104*(1), 33–43. https://doi.org/10.1111/1365-2745.12495

Bartusek, S., Kornhuber, K., & Ting, M. (2022). 2021 North American heatwave amplified by climate change-driven nonlinear interactions. *Nature Climate Change*, 1–8. https://doi.org/10.1038/s41558-022-01520-4

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Bell, E. C. (1993). Photosynthetic response to temperature and desiccation of the intertidal alga *Mastocarpus papillatus*. *Marine Biology*, *117*(2), 337–346. https://doi.org/10.1007/BF00345679

Branson, F. A. (1953). Two new factors affecting resistance of grasses to grazing. *Rangeland Ecology & Management*, *6*(3), 165–171.

Burnaford, J. L. (2004). Habitat modification and refuge from sublethal stress drive a marine plant–herbivore association. *Ecology*, *85*(10), 2837–2849. https://doi.org/10.1890/03-0113

Burnaford, J. L., Nguyen, T.-V. T., & Henderson, S. Y. (2020). Linking physiology to ecological function: Environmental conditions affect performance and size of the intertidal kelp *Hedophyllum sessile* (Laminariales, Phaeophyceae). *Journal of Phycology*, 47.

Burnaford, J. L., Nielsen, K. J., & Williams, S. L. (2014). Celestial mechanics affects emersion time and cover patterns of an ecosystem engineer, the intertidal kelp *Saccharina sessilis*. *Marine Ecology Progress Series*, *509*, 127–136. https://doi.org/10.3354/meps10876

Cavole, L. M., Demko, A. M., Diner, R. E., Giddings, A., Koester, I., Pagniello, C. M. L. S., Paulsen, M.-L., Ramirez-Valdez, A., Schwenck, S. M., Yen, N. K., Zill, M. E., & Franks, P. J. S. (2016). Biological impacts of the 2013–2015 warm-water anomaly in the Northeast Pacific: Winners, losers, and the future. *Oceanography*, *29*(2), 273–285. JSTOR.

Colvard, N. B., Carrington, E., & Helmuth, B. (2014). Temperature-dependent photosynthesis in the intertidal alga *Fucus gardneri* and sensitivity to ongoing climate change. *Journal of Experimental Marine Biology and Ecology*, *458*, 6–12. https://doi.org/10.1016/j.jembe.2014.05.001

Davison, I. R., & Pearson, G. A. (1996). Stress tolerance in intertidal seaweeds. *Journal of Phycology*, *32*(2), 197–211. https://doi.org/10.1111/j.0022-3646.1996.00197.x

Dayton, P. K. (1975). Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological Monographs*, *45*(2), 137–159. https://doi.org/10.2307/1942404

Denny, M. W., & Paine, R. T. (1998). Celestial mechanics, sea-level changes, and intertidal ecology. *The Biological Bulletin*, *194*(2), 108–115.

Dethier, M. N., & Duggins, D. O. (1984). An "indirect commensalism" between marine herbivores and the importance of competitive hierarchies. *The American Naturalist*, *124*(2), 205–219. JSTOR.

Dethier, M. N., & Duggins, D. O. (1988). Variation in strong interactions in the intertidal zone along a geographical gradient: A Washington-Alaska comparison. *Marine Ecology Progress Series*, *50*(1/2), 97–105.

Díaz-Acosta, L., Barreiro, R., Provera, I., & Piñeiro-Corbeira, C. (2021). Physiological response to warming in intertidal macroalgae with different thermal affinity. *Marine Environmental Research*, *169*, 105350. https://doi.org/10.1016/j.marenvres.2021.105350

Dong, Z., Wang, L., Xu, P., Cao, J., & Yang, R. (2023). Heatwaves similar to the unprecedented one in summer 2021 over western North America are projected to become more frequent in a warmer world. *Earth's Future*, *11*(2), e2022EF003437. https://doi.org/10.1029/2022EF003437

Dowd, W. W., & Denny, M. W. (2020). A series of unfortunate events: Characterizing the contingent nature of physiological extremes using long-term environmental records. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192333. https://doi.org/10.1098/rspb.2019.2333

Dowd, W. W., King, F. A., & Denny, M. W. (2015). Thermal variation, thermal extremes and the physiological performance of individuals. *Journal of Experimental Biology*, *218*(12), 1956–1967. https://doi.org/10.1242/jeb.114926

Dudgeon, S. R., Davison, I. R., & Vadas, R. L. (1989). Effect of freezing on photosynthesis of intertidal macroalgae: Relative tolerance of *Chondrus crispus* and *Mastocarpus stellatus* (Rhodophyta). *Marine Biology*, *101*(1), 107–114. https://doi.org/10.1007/BF00393483

Duggins, D. O., & Dethier, M. N. (1985). Experimental studies of herbivory and algal competition in a low intertidal habitat. *Oecologia*, *67*(2), 183–191. JSTOR.

Filbee-Dexter, K., Wernberg, T., Grace, S. P., Thormar, J., Fredriksen, S., Narvaez, C. N., Feehan, C. J., & Norderhaug, K. M. (2020). Marine heatwaves and the collapse of marginal North Atlantic kelp forests. *Scientific Reports*, *10*(1), 13388. https://doi.org/10.1038/s41598-020-70273-x

Fischer, E. M., Sippel, S., & Knutti, R. (2021). Increasing probability of record-shattering climate extremes. *Nature Climate Change*, *11*(8), 689–695. https://doi.org/10.1038/s41558-021-01092-9

Gaines, S. D., & Denny, M. W. (1993). The largest, smallest, highest, lowest, longest, and shortest: Extremes in ecology. *Ecology*, *74*(6), 1677–1692. https://doi.org/10.2307/1939926

Gutschick, V. P. (1999). Biotic and abiotic consequences of differences in leaf structure. *New Phytologist*, *143*(1), 3–18. https://doi.org/10.1046/j.1469-8137.1999.00423.x

Harley, C. D. G., Anderson, K. M., Demes, K. W., Jorve, J. P., Kordas, R. L., Coyle, T. A., & Graham, M. H. (2012). Effects of climate change on global seaweed communities. *Journal of Phycology*, *48*(5), 1064–1078. https://doi.org/10.1111/j.1529-8817.2012.01224.x

Harley, C. D. G., & Helmuth, B. S. T. (2003). Local- and regional-scale effects of wave exposure, thermal stress, and absolute versus effective shore level on patterns of intertidal zonation. *Limnology and Oceanography*, *48*(4), 1498–1508. https://doi.org/10.4319/lo.2003.48.4.1498

Hereward, H. F. R., King, N. G., & Smale, D. A. (2020). Intra-Annual variability in responses of a canopy forming kelp to cumulative low tide heat stress: Implications for populations at the trailing range edge. *Journal of Phycology*, *56*(1), 146–158. https://doi.org/10.1111/jpy.12927

Hobday, A., Oliver, E., Sen Gupta, A., Benthuysen, J., Burrows, M., Donat, M., Holbrook, N., Moore, P., Thomsen, M., Wernberg, T., & Smale, D. (2018). Categorizing and naming marine heatwaves. *Oceanography*, *31*(2). https://doi.org/10.5670/oceanog.2018.205

Holbrook, N. J., Scannell, H. A., Sen Gupta, A., Benthuysen, J. A., Feng, M., Oliver, E. C. J., Alexander, L. V., Burrows, M. T., Donat, M. G., Hobday, A. J., Moore, P. J., Perkins-Kirkpatrick, S. E., Smale, D. A., Straub, S. C., & Wernberg, T. (2019). A global assessment of marine heatwaves and their drivers. *Nature Communications*, *10*(1), 2624. https://doi.org/10.1038/s41467-019-10206-z

Hoos, J. J., & Harley, C. D. G. (2021). The sign and magnitude of the effects of thermal extremes on an intertidal kelp depend on environmental and biological context. *Climate Change Ecology*, *2*, 100015. https://doi.org/10.1016/j.ecochg.2021.100015

Huey, R. B., & Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *American Zoologist*, *19*(1), 357–366. https://doi.org/10.1093/icb/19.1.357

Jurgens, L. J., Ashlock, L. W., & Gaylord, B. (2022). Facilitation alters climate change risk on rocky shores. *Ecology*, *103*(2). https://doi.org/10.1002/ecy.3596

Kaur, J., & Kariyat, R. (2020). Role of trichomes in plant stress biology. In *Evolutionary Ecology of Plant-Herbivore Interaction* (pp. 15–35). Springer International Publishing. https://doi.org/10.1007/978-3-030-46012-9_2

Maberly, S. C., & Madsen, T. V. (1990). Contribution of air and water to the carbon balance of *Fucus spiralis*. *Marine Ecology Progress Series*, *62*(1/2), 175–183.

Mann, K. H. (1973). Seaweeds: Their productivity and strategy for growth. *Science*, *182*(4116), 975–981.

Markel, R., & DeWreede, R. (1998). Mechanisms underlying the effect of the chiton *Katharina tunicata* on the kelp *Hedophyllum sessile*: Size escapes and indirect effects. *Marine Ecology Progress Series*, *166*, 151–161. https://doi.org/10.3354/meps166151

Meehl, G. A., & Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. *Science*, *305*(5686), 994–997. https://doi.org/10.1126/science.1098704

Menge, B. A., Close, S. L., Hacker, S. D., Nielsen, K. J., & Chan, F. (2021). Biogeography of macrophyte productivity: Effects of oceanic and climatic regimes across spatiotemporal scales. *Limnology and Oceanography*, *66*(3), 711–726. https://doi.org/10.1002/lno.11635

Menge, B. A., Gravem, S. A., Johnson, A., Robinson, J. W., & Poirson, B. N. (2022). Increasing instability of a rocky intertidal meta-ecosystem. *Proceedings of the National Academy of Sciences*, *119*(3), e2114257119. https://doi.org/10.1073/pnas.2114257119

Miller, M. W., Barlow, P., Bennett, J. P., Bourdeau, P. F., Bryan, J., Conger, A. D., Conger, B. V., D’Amato, F., Davidson, D., & Evans, L. S. (1995). Methods for reducing the adverse effects of temperature stress on plants: A review. *Environmental and Experimental Botany*, *35*(3), 251–258.

Milligan, K. L. D., & DeWreede, R. E. (2000). Variations in holdfast attachment mechanics with developmental stage, substratum-type, season, and wave-exposure for the intertidal kelp species *Hedophyllum sessile* (C. Agardh) Setchell. *Journal of Experimental Marine Biology and Ecology*, *254*(2), 189–209. https://doi.org/10.1016/S0022-0981(00)00279-3

Milligan, K. L. D., & DeWreede, R. E. (2004). Morphological variations do not effectively reduce drag forces at high wave-exposure for the macroalgal species, *Hedophyllum sessile* (Laminariales, Phaeophyta). *Phycologia*, *43*(3), 236–244.

Mislan, K. a. S., Wethey, D. S., & Helmuth, B. (2009). When to worry about the weather: Role of tidal cycle in determining patterns of risk in intertidal ecosystems. *Global Change Biology*, *15*(12), 3056–3065. https://doi.org/10.1111/j.1365-2486.2009.01936.x

Munday, P. L., Jones, G. P., Pratchett, M. S., & Williams, A. J. (2008). Climate change and the future for coral reef fishes. *Fish and Fisheries*, *9*(3), 261–285. https://doi.org/10.1111/j.1467-2979.2008.00281.x

Nepper-Davidsen, J., Andersen, D., & Pedersen, M. (2019). Exposure to simulated heatwave scenarios causes long-term reductions in performance in *Saccharina latissima*. *Marine Ecology Progress Series*, *630*, 25–39. https://doi.org/10.3354/meps13133

Nicholson, N. L. (1970). Field studies on the giant kelp *Nereocystis*. *Journal of Phycology*, *6*(2), 177–182. https://doi.org/10.1111/j.1529-8817.1970.tb02378.x

Oliver, E. C. J., Donat, M. G., Burrows, M. T., Moore, P. J., Smale, D. A., Alexander, L. V., Benthuysen, J. A., Feng, M., Sen Gupta, A., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Straub, S. C., & Wernberg, T. (2018). Longer and more frequent marine heatwaves over the past century. *Nature Communications*, *9*(1), 1324. https://doi.org/10.1038/s41467-018-03732-9

O'Sullivan, O. S., Heskel, M. A., Reich, P. B., Tjoelker, M. G., Weerasinghe, L. K., Penillard, A., Zhu, L., Egerton, J. J. G., Bloomfield, K. J., Creek, D., Bahar, N. H. A., Griffin, K. L., Hurry, V., Meir, P., Turnbull, M. H., & Atkin, O. K. (2017). Thermal limits of leaf metabolism across biomes. *Global Change Biology*, *23*(1), 209–223. https://doi.org/10.1111/gcb.13477

Overland, J. E. (2021). Causes of the record-breaking Pacific Northwest heatwave, late June 2021. *Atmosphere*, *12*(11), 1434. https://doi.org/10.3390/atmos12111434

Paine, R. T. (2002). Trophic Control of Production in a Rocky Intertidal Community. *Science*, *296*(5568), 736–739. https://doi.org/10.1126/science.1069811

Paine, R. T., Tegner, M. J., & Johnson, E. A. (1998). Compounded Perturbations Yield Ecological Surprises. *Ecosystems*, *1*(6), 535–545. https://doi.org/10.1007/s100219900049

Qiu, Z., Coleman, M. A., Provost, E., Campbell, A. H., Kelaher, B. P., Dalton, S. J., Thomas, T., Steinberg, P. D., & Marzinelli, E. M. (2019). Future climate change is predicted to affect the microbiome and condition of habitat-forming kelp. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1896), 20181887. https://doi.org/10.1098/rspb.2018.1887

R Core Team. (2021). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/*.

Raymond, W. W., Barber, J. S., Dethier, M. N., Hayford, H. A., Harley, C. D. G., King, T. L., Paul, B., Speck, C. A., Tobin, E. D., Raymond, A. E. T., & McDonald, P. S. (2022). Assessment of the impacts of an unprecedented heatwave on intertidal shellfish of the Salish Sea. *Ecology*, *103*(10), e3798. https://doi.org/10.1002/ecy.3798

Reyer, C. P. O., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R. P., Bonfante, A., de Lorenzi, F., Dury, M., Gloning, P., Abou Jaoudé, R., Klein, T., Kuster, T. M., Martins, M., Niedrist, G., Riccardi, M., Wohlfahrt, G., de Angelis, P., de Dato, G., François, L., … Pereira, M. (2013). A plant's perspective of extremes: Terrestrial plant responses to changing climatic variability. *Global Change Biology*, *19*(1), 75–89. https://doi.org/10.1111/gcb.12023

Román, M., Román, S., Vázquez, E., Troncoso, J., & Olabarria, C. (2020). Heatwaves during low tide are critical for the physiological performance of intertidal macroalgae under global warming scenarios. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-78526-5

Sanford, E., Sones, J. L., García-Reyes, M., Goddard, J. H. R., & Largier, J. L. (2019). Widespread shifts in the coastal biota of northern California during the 2014–2016 marine heatwaves. *Scientific Reports*, *9*(1), 4216. https://doi.org/10.1038/s41598-019-40784-3

Silberner, J. (2021). Heat wave causes hundreds of deaths and hospitalisations in Pacific north west. *BMJ*, n1696. https://doi.org/msr

Smale, D. A., Wernberg, T., Oliver, E. C. J., Thomsen, M., Harvey, B. P., Straub, S. C., Burrows, M. T., Alexander, L. V., Benthuysen, J. A., Donat, M. G., Feng, M., Hobday, A. J., Holbrook, N. J., Perkins-Kirkpatrick, S. E., Scannell, H. A., Sen Gupta, A., Payne, B. L., & Moore, P. J. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change*, *9*(4), 306–312. https://doi.org/10.1038/s41558-019-0412-1

Starko, S., Boo, G. H., Martone, P. T., Lindstrom, S. C., Starko, S., Boo, G. H., Martone, P. T., & Lindstrom, S. C. (2018). A molecular investigation of *Saccharina sessilis* from the Aleutian Islands reveals a species complex, necessitating the new combination *Saccharina subsessilis*. *ALGAE*, *33*(2), 157–166. https://doi.org/10.4490/algae.2018.33.4.2

Starko, S., Soto Gomez, M., Darby, H., Demes, K. W., Kawai, H., Yotsukura, N., Lindstrom, S. C., Keeling, P. J., Graham, S. W., & Martone, P. T. (2019). A comprehensive kelp phylogeny sheds light on the evolution of an ecosystem. *Molecular Phylogenetics and Evolution*, *136*, 138–150. https://doi.org/10.1016/j.ympev.2019.04.012

Straub, S. C., Wernberg, T., Thomsen, M. S., Moore, P. J., Burrows, M. T., Harvey, B. P., & Smale, D. A. (2019). Resistance, extinction, and everything in between – The diverse responses of seaweeds to marine heatwaves. *Frontiers in Marine Science, 6*. https://doi.org/10.3389/fmars.2019.00763

Supratya, V. P., Coleman, L. J. M., & Martone, P. T. (2020). Elevated temperature affects phenotypic plasticity in the bull kelp (*Nereocystis luetkeana*, Phaeophyceae). *Journal of Phycology*, *56*(6), 1534–1541. https://doi.org/10.1111/jpy.13049

Swann, A. L. S. (2018). Plants and drought in a changing climate. *Current Climate Change Reports*, *4*(2), 192–201. https://doi.org/10.1007/s40641-018-0097-y

Thomsen, M. S., Mondardini, L., Thoral, F., Gerber, D., Montie, S., South, P. M., Tait, L., Orchard, S., Alestra, T., & Schiel, D. R. (2021). Cascading impacts of earthquakes and extreme heatwaves have destroyed populations of an iconic marine foundation species. *Diversity and Distributions*, *00*, 1–15. https://doi.org/10.1111/ddi.13407

Thomson, J. A., Burkholder, D. A., Heithaus, M. R., Fourqurean, J. W., Fraser, M. W., Statton, J., & Kendrick, G. A. (2015). Extreme temperatures, foundation species, and abrupt ecosystem change: An example from an iconic seagrass ecosystem. *Global Change Biology*, *21*(4), 1463–1474. https://doi.org/10.1111/gcb.12694

Vico, G., Manzoni, S., Palmroth, S., Weih, M., & Katul, G. (2013). A perspective on optimal leaf stomatal conductance under CO2 and light co-limitations. *Agricultural and Forest Meteorology*, *182–183*, 191–199. https://doi.org/10.1016/j.agrformet.2013.07.005

Weitzman, B., Konar, B., Iken, K., Coletti, H., Monson, D., Suryan, R., Dean, T., Hondolero, D., & Lindeberg, M. (2021). Changes in rocky intertidal community structure during a marine heatwave in the northern Gulf of Alaska. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.556820

Widdowson, T. B. (1965). A taxonomic study of the genus *Hedophyllum* Setchell. *Canadian Journal of Botany*, *43*(11), 1409–1420. https://doi.org/10.1139/b65-150

Williams, S. L., & Dethier, M. N. (2005). High and dry: Variation in net photosynthesis of the intertidal seaweed *Fucus gardneri*. *Ecology*, *86*(9), 2373–2379. https://doi.org/10.1890/04-1569

Tables

Table 1. Summary linear models of field measurements of growth and bleaching over time.

Model Parameter	MS	F	P
Size specific change in maximum length			
Date	41.0	1.39	0.2
Tide (spring vs neap)	123.9	4.21	0.04*
Date × Tide	1568	53.3	< 0.001***
Percent length of bleached tissue			
Date	12.0	0.06	0.8
Tide (spring vs neap)	40183	189.3	< 0.001***
Date × Tide	1362	6.42	0.01*
Maximum length			
Date	3408	71.4	< 0.001***
Tide (spring vs neap)	1373	28.8	< 0.001***
Date × Tide	567.2	11.9	< 0.001***

Table 2. Summary of linear mixed models and linear models of wet biomass and Fv/Fm in the laboratory experiment.

Model Parameter	MS	F	P
Wet biomass percent change			
Treatment	1008	21.1	<0.001***
Season	0.2	0.005	0.5
Trial day	17625	369	<0.001***
Treatment × Season	2	0.04	0.9
Recovery of Fv/Fm			
Treatment	0.06	10.6	<0.001***
Season	0.08	13.6	<0.001***
Trial day	0.001	0.26	0.7
Treatment × Season	0.02	2.70	0.07
Change in Fv/Fm during emersion period			
Treatment	0.36	58.7	<0.001***
Season	0.06	10.0	0.002 **
Trial day	0.003	0.41	0.5
Treatment × Season	0.02	2.95	0.09

Figures

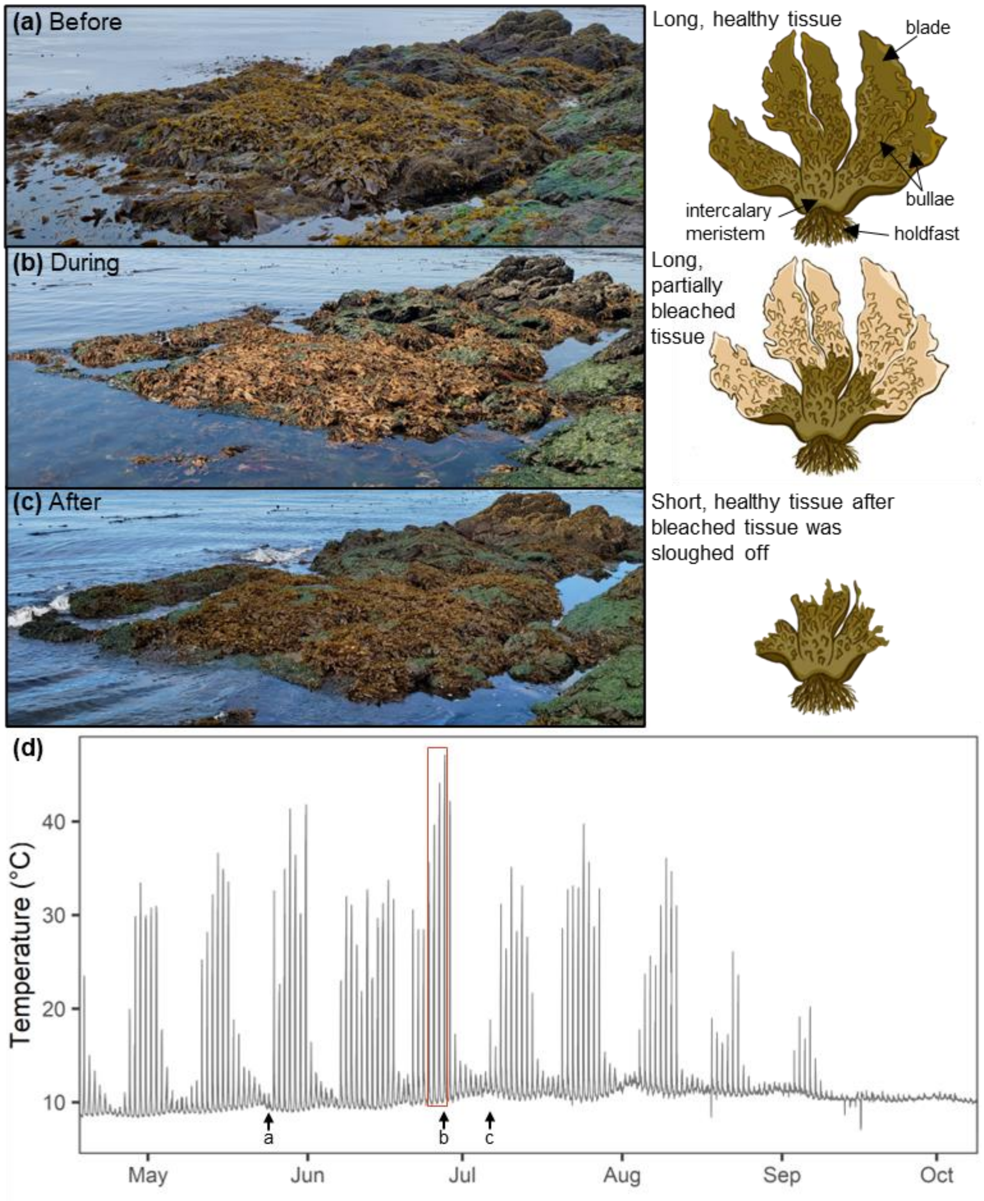

Figure 1. *Hedophyllum* bed and temperatures before, during, and after the 2021 Western North American Heatwave at Deadman Bay, San Juan Island, Washington. Photos were taken one month before the heatwave on (a) May 25th, (b) during the fourth day of the heatwave on June 28th, (c) and after on July 8th. Drawings show the change in one individual from (a) long and healthy before the heatwave to (b) long but partially bleached to (c) shorter but healthy after the bleached tissue was sloughed off. Labels in (a) show morphological features and location of the intercalary meristem. *In situ* intertidal (air and water) temperatures (d) in the *Hedophyllum* zone recorded at 5-minute intervals from April to October 2021 with a red box around the heatwave. Letters in (d) correspond to the dates that the photos (a-c) were taken. Photographs by R.J. Fales and artwork by K.A. Murie.

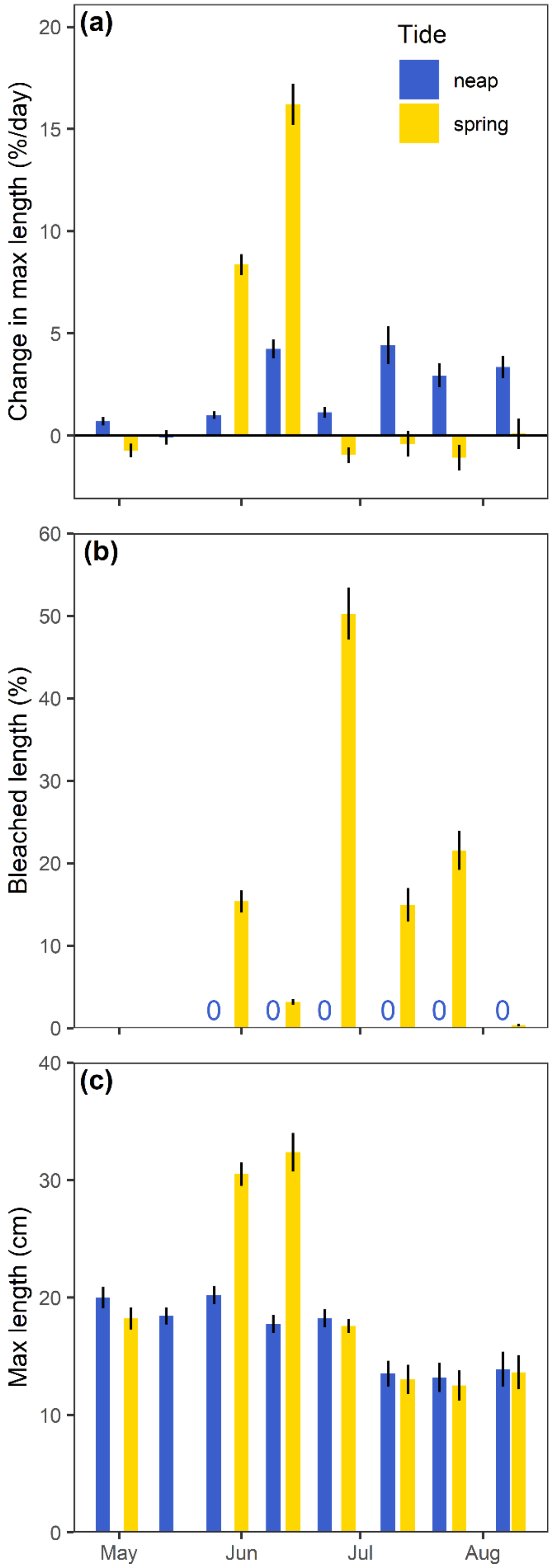

Figure 2. Changes in *Hedophyllum* growth and bleaching during spring (yellow) and neap (blue) tides from May to August 2021 including the Western North American Heatwave at the end of June, bars are mean ± SE (n = 29-42). (a) Change in the maximum length as a percentage per day, (b) percentage of blade tissue that bleached with blue zeros representing neap tides without bleaching, and (c) and maximum blade length.

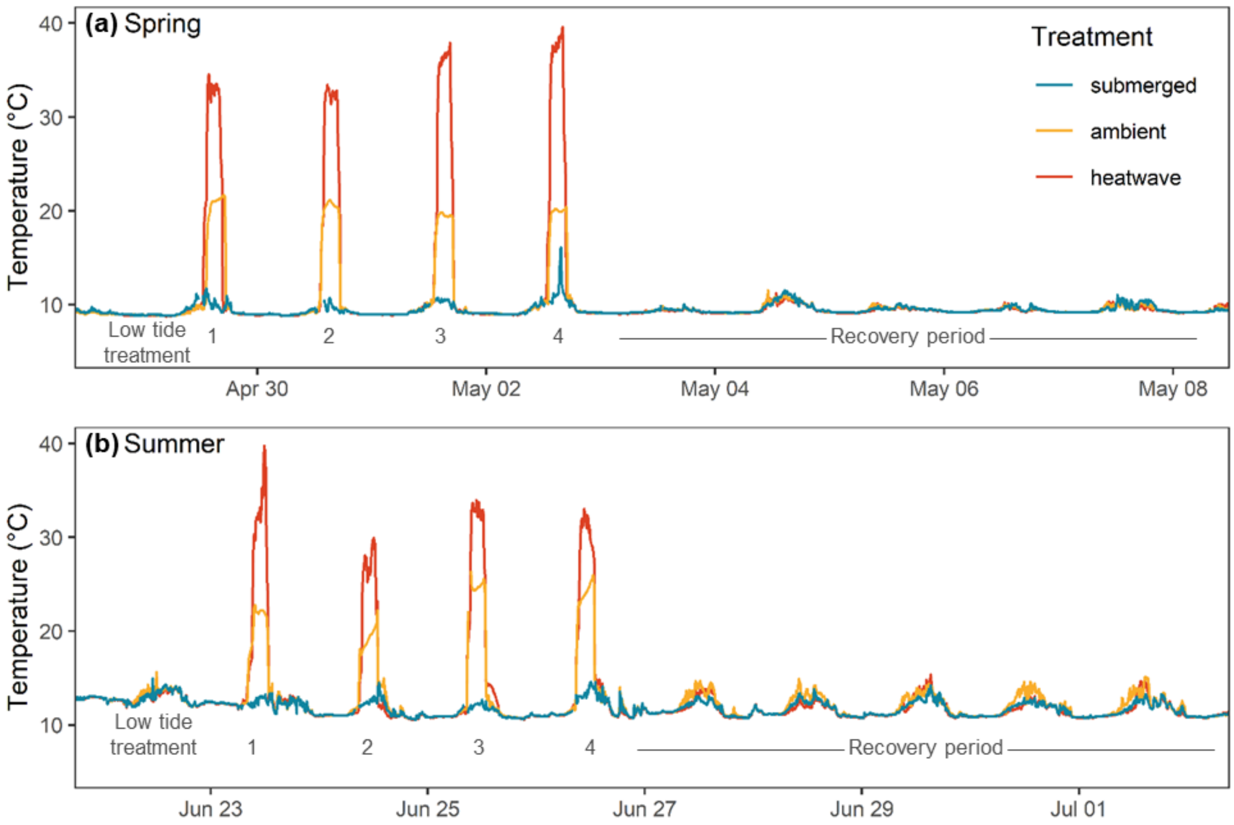

Figure 3. Environmental temperatures for each treatment in the laboratory experiments and recovery period in (a) spring and (b) summer. Loggers and treatments were either continuously submerged (blue), periodically exposed to ambient air (yellow), or heatwave (red) conditions.

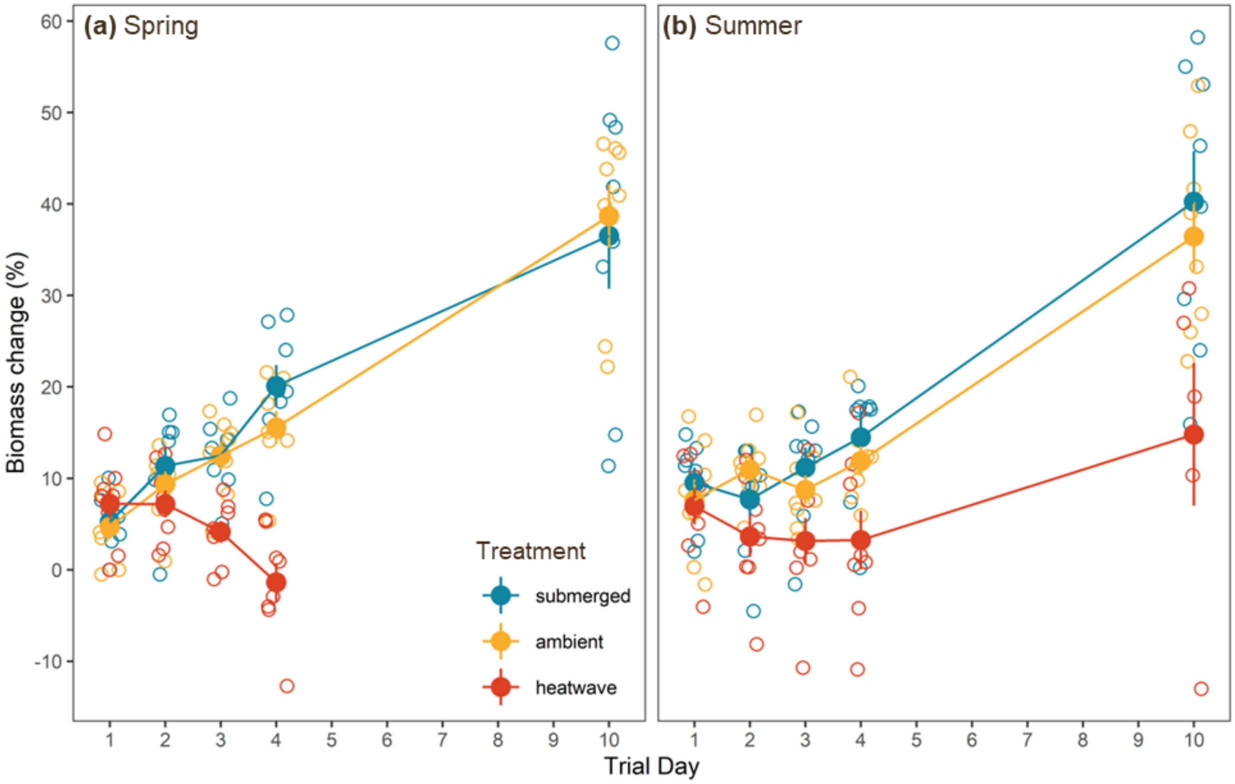

Figure 4. Daily change in wet biomass (%) during the emersion period (days 1-4) and after a 6-day recovery period in (a) spring and (b) summer. Open circles represent individual data points (n = 8 per treatment) and closed circles represent mean ± SE. Colors indicate treatments: submerged (blue), periodic exposure to ambient air (yellow), and heatwave (red) conditions.

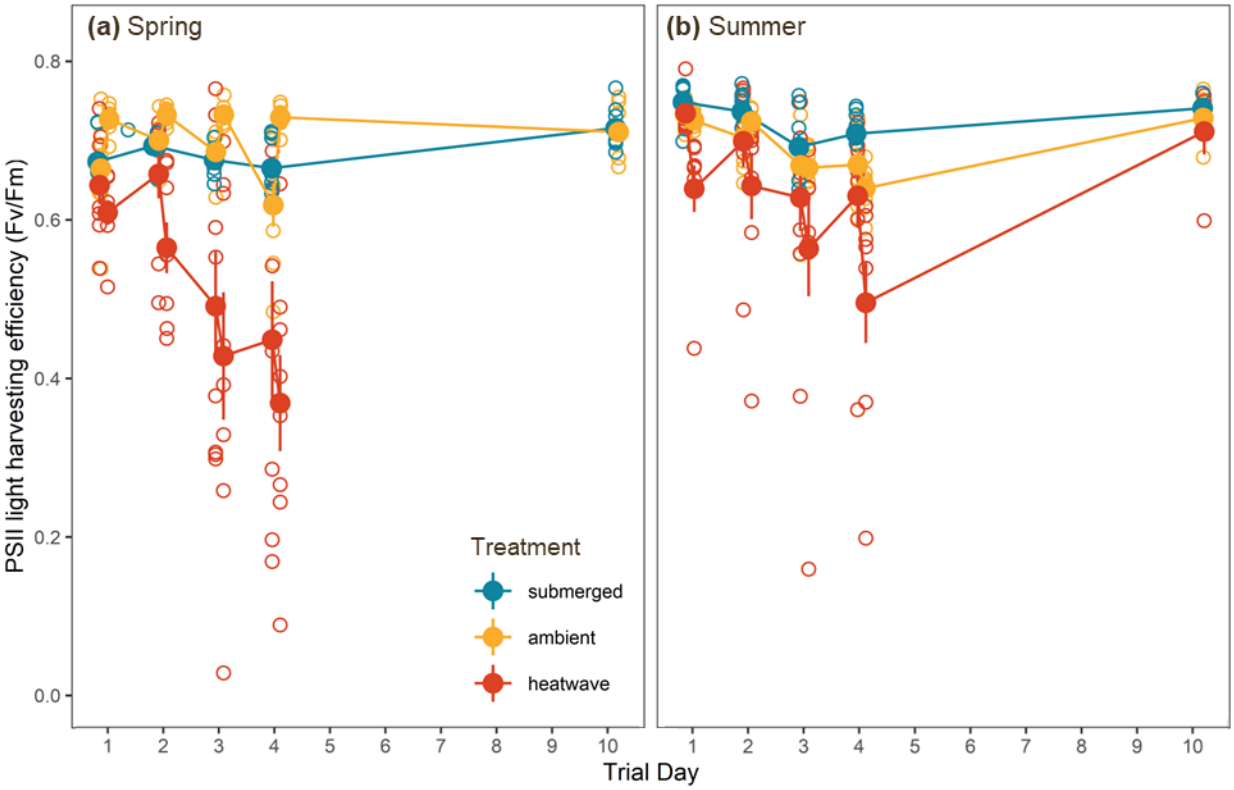

Figure 5. Photosystem II light harvesting efficiency (Fv/Fm) at the beginning and end of each emersion period (days 1-4) and after a 6-day recovery period during (a) spring and (b) summer. Open circles represent individual data points (n = 8 per treatment) and closed circles represent mean ± SE. Colors indicate treatments: submerged (blue), periodic exposure to ambient air (yellow), and heatwave (red) conditions.

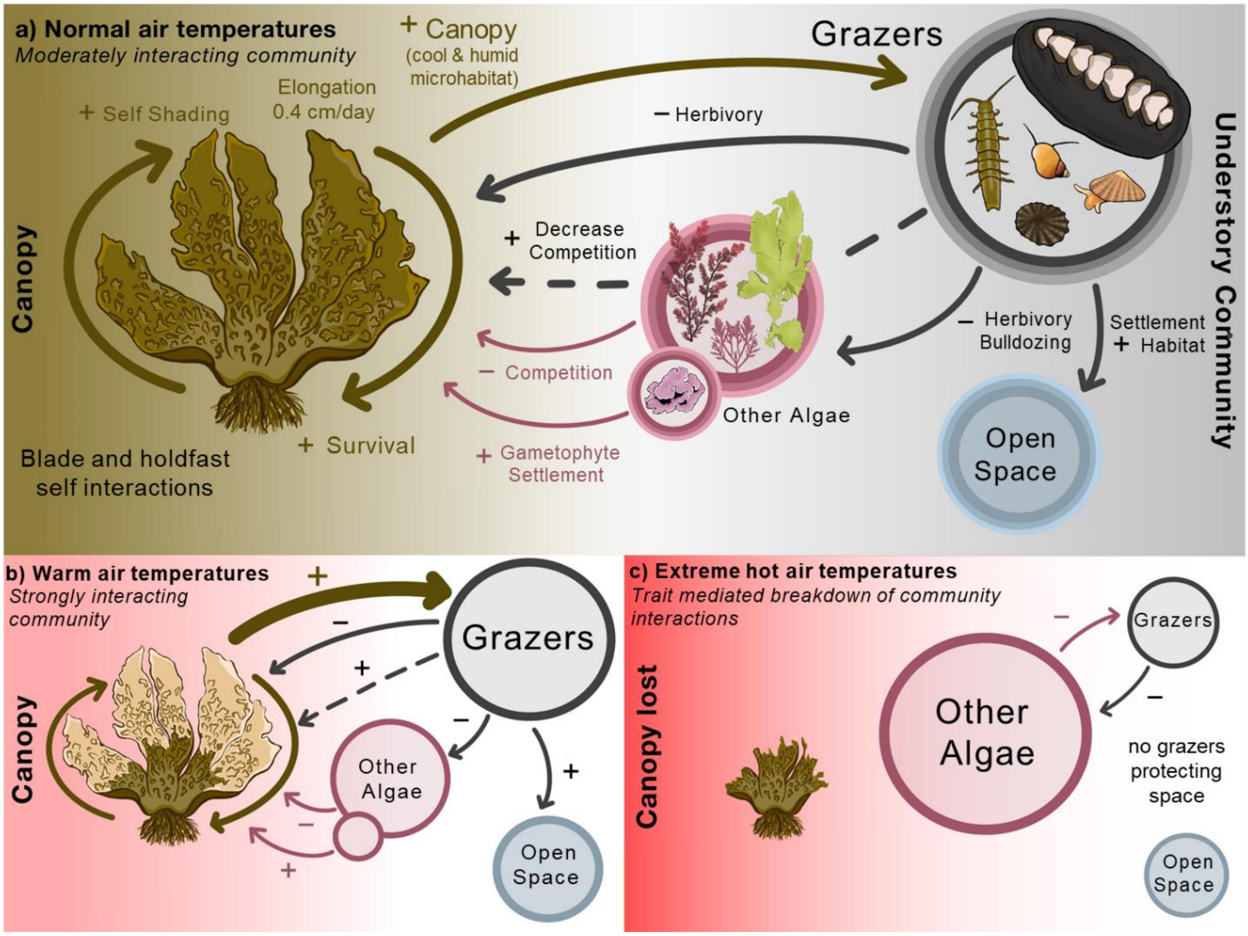

Figure 6. Conceptual diagram of interactions between (a) *Hedophyllum* blade and its own holdfast and (a-c) between *Hedophyllum* and the understory community. Panels show community interaction changes under different levels of thermal emersion stress: (a) normal air temperatures, (b) warm air temperatures, and (c) extremely hot air temperatures. Trait mediated interactions via blade elongation decline with increasing air temperature and shortening/loss of blade canopy area. Interactions with the understory community are based on published literature (Barner et al., 2016; Burnaford, 2004; Dayton, 1975; Dethier & Duggins, 1984, 1988; Duggins & Dethier, 1985; Markel & DeWreede, 1998; Paine, 2002). Artwork by K.A. Murie.

Chapter 1 – Supplementary Materials

Table S1. Summary of linear model for desiccation during low tide experiment.

Model Parameter	MS	F	P
Treatment	10536	837.7	<0.001***
Season	1098	87.3	<0.001***
Trial day	1.8	0.15	0.7
Treatment × Season	547.4	43.5	<0.001***

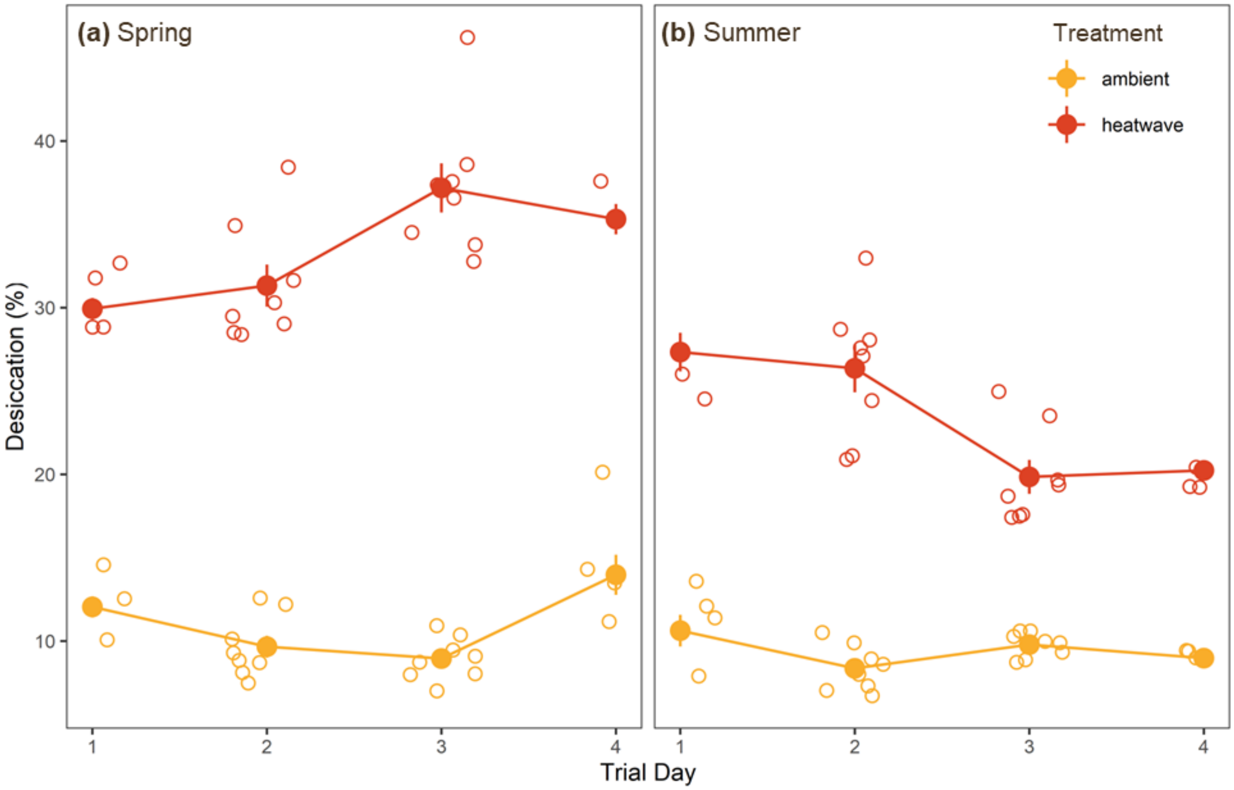

Figure S1. Desiccation (%) as the loss of wet biomass during emersion periods in the ambient (yellow) and heatwave (red) treatments during (A) spring and (B) summer. Closed circles represent mean ± SE and open circles represent individual data points (n = 8 per treatment).

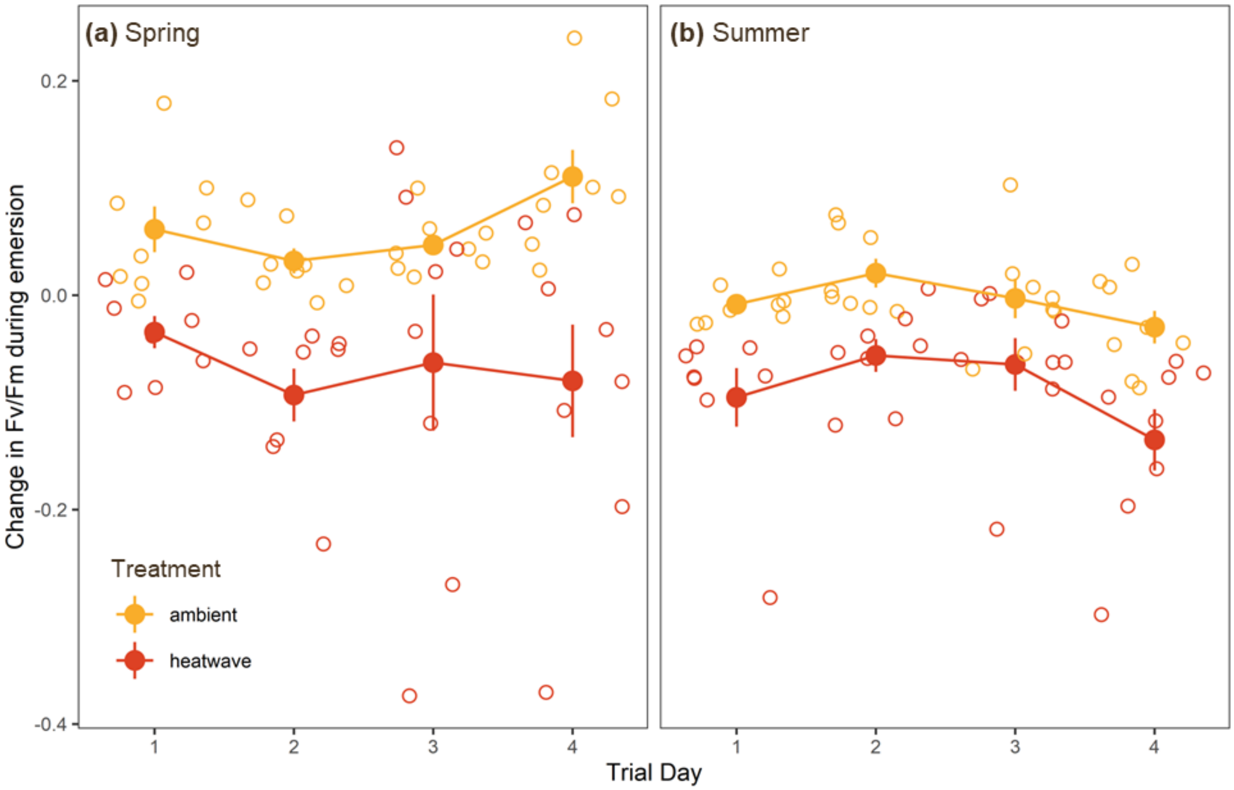

Figure S2. Change in dark adapted yield of photosystem II (Fv/Fm) in the ambient (yellow) and heatwave (red) treatments during low tide emersion in the laboratory experiment in (A) spring and (B) summer. Closed circles represent mean ± SE and open circles represent individual data points (n = 8 per treatment).

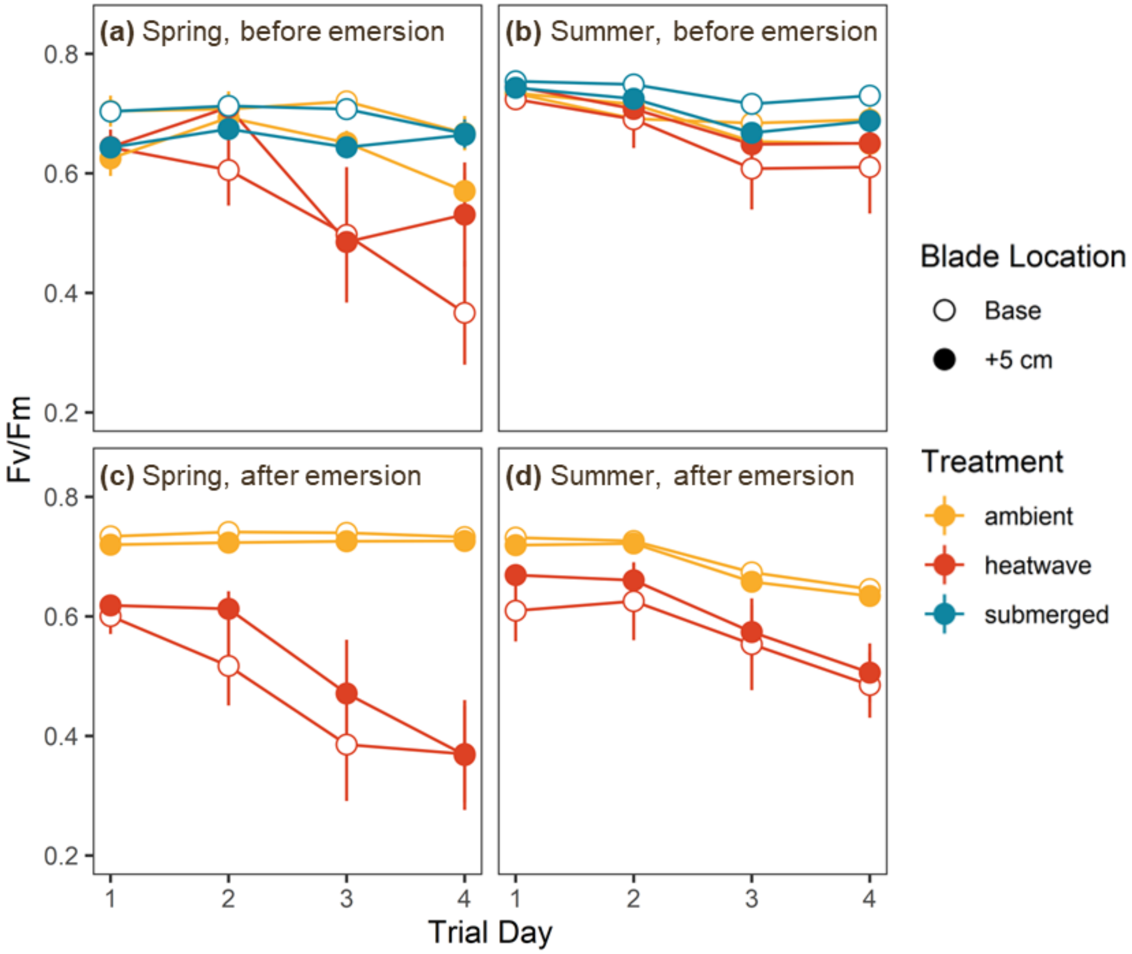

Figure S3. Dark adapted yield of photosystem II (Fv/Fm) as mean ±SE in each treatment: ambient (yellow), heatwave (red), and submerged (blue). Measurements were collected on two regions of the blade: the base (open circle) and 5 cm above the base (closed circle). Panels represent seasons and emersion for (A & C) spring and (B & D) summer and the time (A & B) before and (C &D) after emersion in the laboratory.

Thermotolerance of the canopy forming kelp, *Nereocystis luetkeana,* from a range of thermal environments in the Salish Sea

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Acronyms: marine heatwave (MHW), thermal performance curve (TPC), thermal optimum (T_{opt}), critical thermal maximum (CT_{max}), thermal safety margin (TSM), warming tolerance (WT), photosynthetically active radiation (PAR), mean lower low water (MLLW)

Abstract

Increased global temperatures have negatively impacted a variety of marine ecosystems. One strategy to mitigate these impacts is to focus conservation and restoration efforts on populations already capable of dealing with higher temperatures. A broad range of abiotic conditions exist in the Salish Sea of western USA & Canada, with colder waters near the open coast and warmer waters in more protected waters. Among the diverse cold-water kelp species in the Northeastern Pacific, *Nereocystis luetkeana* (bull kelp) is the only large canopy forming species within the Salish Sea; it is experiencing localized declines at sites associated with elevated temperatures. Therefore, we tested whether the thermotolerance of adult sporophyte blades varied across eight sites forming a natural gradient of temperatures. We measured kelp condition including pneumatocyst size, blade thickness index, C:N ratios, and performed a suite of physiology assays. We incubated N. *luetkeana* blade tissue plugs for 36 hours in constant temperatures ranging 9-27°C and quantified growth, net photosynthesis, and dark adapted yield of photosystem II (Fv/Fm) to estimate thermal performance curves. We found a trend of higher optimal temperatures (T_{opt}) in kelp from warmer sites but, surprisingly, the same critical thermal maximum (CT_{max}) of 20 ± 1°C across all sites. By contextualizing physiological performance with temperatures measured in natural kelp habitats, we found cool sites have a larger margin of safety and warm sites are dangerously close to exceeding their thermal limits. Our findings indicate that conservation efforts should be targeted towards sites that do not exceed 20°C and restorative actions are needed to increase resilience to warming for populations nearing the tipping point.

Introduction

With climate warming and increasing frequency and severity of marine heatwaves (MHWs) and other climatic extremes, understanding mechanistic responses to abiotic stressors across populations is increasingly important (Higham et al., 2021; Sulmon et al., 2015). During these extreme events, organisms can be pushed beyond their physiological limits, causing decreases in performance (metabolism, growth, reproduction) or even death. But not all populations will respond to ocean warming and heatwaves in the same manner; responses can differ due to thermal history, local adaptation or acclimation, or additional stressors in the habitat (Harley et al., 2006). The search for warm tolerant populations or phenotypes (Humanes et al., 2022; Kuo & Sanford, 2009; Sorte et al., 2011; Villeneuve et al., 2021) has become an increasingly prevalent goal as targets for conservation or donor populations for restoration efforts for climate adaptation and mitigation.

Habitat-forming species have disproportionate effects on their local environments; therefore, declines can have major ecological impacts. Kelps are important primary producers that form biogenic habitats in coastal marine ecosystems that support diverse understory algae, invertebrate, and fish assemblages and serve as a food source in natural systems and aquaculture (Duggins et al., 1989; Grebe et al., 2019; Teagle et al., 2017). The environmental requirements of kelp, cold and nutrient rich waters, make them especially at risk from ocean warming and MHWs (Smale, 2020). One third of kelp species are in decline globally (Krumhansl et al., 2016); there is an urgent need to understand how habitat forming species like kelps respond to heatwaves and climate change. By understanding kelp responses to warming, we can also draw conclusions about the health and stability of associated marine communities including economically important species like salmonids and crabs.

The Northeast Pacific Ocean has the highest diversity of kelps (Laminariales) in the world (Druehl, 1981; Starko et al., 2019), yet in the inland Salish Sea, the bull kelp *Nereocystis luetkeana* is the only large canopy forming species and no other species create a surface canopy (the giant kelp, *Macrocystis pyrifera* is not present, Druehl 1978, Mumford 2007). Puget Sound (southern Salish Sea) and Barkley Sound (outer coast, Vancouver Island) populations of *N. luetkeana* have declined, especially in areas with warmer waters (Berry et al., 2021; Starko et al., 2022). Northern California *N. luetkeana* forests have also declined, in association with a MHW and urchin overgrazing (Rogers-Bennett & Catton, 2019). Even though we have seen large declines with warming ocean temperatures, our knowledge about *N. luetkeana* thermotolerance is limited and largely site specific. Two genetic groups of *N. luetkeana* have been identified in the Salish Sea, with populations in Puget Sound differing from others in Washington state (Gierke 2019). In order to better support conservation of this important species, we need to determine whether there are warm adapted or acclimated populations and whether warm tolerance has a genetic basis.

Differences between populations of *N. luetkeana* are not well understood and can be influenced by light, flow, nutrients, salinity, depth, and biotic factors as well as genetics. For example, *N. luetkeana* blades are plastic in response to flow conditions and can change from a wavy to smooth blade morphology (Koehl et al., 2008; Koehl & Alberte, 1988). Within the Salish Sea, *N. luetkeana* populations have varying pneumatocyst sizes and number of blades. Meanwhile, Hurd et al. (1996) found *Macrocystis* growth was limited by nitrogen when C:N ratios were high (> 25). Nitrogen sufficiency is important for kelps and can increase thermotolerance (Fernández et al., 2020; Gao et al., 2016; Gerard, 1997). Other factors that can influence thermotolerance include local adaptation to warming as seen in *Laminaria* gametophytes (Martins et al., 2020; Schimpf et al., 2022; Strasser et al., 2022).

Generally, *N. luetkeana* has been classified as a cold-water species that thrives in high flow or wave environments, but this species can be found across a range of environments and temperatures. In the 1980s, Lüning and Freshwater (1988) determined the thermal survival range of *N. luetkeana* near San Juan Island was -1.5 to 18°C. Hamilton et al. (2020) suggested 16°C as the thermal limit due to persistence of kelp forests at a monthly maximum temperature of 14.5°C in Oregon, but local extinctions of forests experiencing 16°C in northern California during a MHW. Warm water discharge from a power plant increased temperatures in Diablo Cove, California by 3.5°C on average to a maximum nearing 19°C caused a 97% decline of *N. luetkeana* (Schiel et al. 2004). However, other studies report much higher temperature tolerances. In British Columbia, the growth rate of *N. luetkeana* sporophytes from one site was optimal at 12°C but was measurable, if low, up to 22°C (Supratya et al., 2020). Finally, during the early life-history gametophyte stage, Weigel et al. (in review) found temperatures above 18°C limited reproduction due to failure in the transition from gametophyte to sporophyte across 7 sites in the Salish Sea.

Despite the widespread research on kelp responses to warming, the use of a wide range of temperatures to build thermal performance curves (TPCs) is not common (but see notable exceptions: Borlongan et al. 2019, Supratya et al. 2020, Wernberg et al. 2016, Paine et al. 2021, Fernández et al. 2020). Performance of photosynthetic organisms can be measured via photosynthesis, respiration, dark adapted yield of photosystem II, and growth in order to understand which physiological responses are impacted by temperature. Responses to temperature in ectotherms are non-linear; thermal performance curves (TPCs) allow for the determination of critical thermal maximums and thermal optimums (Huey and Stevenson 1979). TPCs can be contextualized using habitat temperatures to estimate margins of safety including warming tolerance (difference between critical thermal maximum and habitat temperature) and thermal safety margin (difference between optimal temperature and habitat temperature) under various climate scenarios (Deutsch et al 2008). Therefore, a goal of this study is to use TPCs to calculate warming tolerance or thermal safety margins using temperatures measured in natural kelp habitats.

The Salish Sea is an extensive fjord estuary complex with a natural gradient of temperatures and nutrients; colder nutrient rich waters are closer to the open ocean in the Strait of Juan de Fuca while warmer nutrient poor water is found in inlets and seasonally associated with warmer freshwater inputs from rivers (Fig. 1). The southern Salish Sea is supplied with cold, nutrient rich oceanic water from the Pacific Ocean through the Strait of Juan de Fuca and therefore remains relatively cold. However, the southernmost region, south Puget Sound, has less exchange with oceanic water because it is farther away, and it experiences warm nutrient poor conditions during summer (Berry et al. 2021). To the north, the Strait of Georgia warms in the summer in part due a large volume of warm fresh water input from the Fraser River (Pawlowicz et al. 2007, Riche et al. 2014). The San Juan Islands, situated between the Strait of Georgia and the Strait of Juan de Fuca, experience a mixture of conditions via tidal exchange. Water motion in the Salish Sea is largely driven by strong tidal currents rather than wave action (Yang et al., 2021). Mean summer sea surface temperatures range from 10 -18°C (Table 1; Khangaonkar et al. 2018, Berry et al. 2021). Climate simulations predict an overall increase of 1.5°C across the Salish Sea (Khangaonkar et al., 2019) with some regions in Puget Sound already experiencing mean temperatures up to 18°C in summer (Berry et al., 2021).

We leveraged a natural temperature gradient in the Salish Sea (Harley 2011) to test whether there is variation in short-term *N. luetkeana* sporophyte physiology and thermal limits across populations. In this study, we aimed to 1) quantify variation in sporophyte morphology and elemental composition among populations, 2) develop thermal performance curves to estimate optimum and critical temperatures, 3) relate optimum and critical temperatures to natural kelp habitat, and 4) make useful predications for management, conservation, and restoration of *N. luetkeana* forests in the Salish Sea by identifying which forests are at risk due to warming and MHWs. Due to variability in abiotic conditions among sites, we expected to find differences in morphologies and elemental composition, with larger morphologies and higher nitrogen content in colder nutrient rich environments. We hypothesized that warmer sites will be more thermotolerant with higher optimal temperatures and critical maximum temperatures than cool sites due to local adaptation.

Methods

Sites, conditions, and collections

We sampled populations of *Nereocystis luetkeana* from 8 sites, selected to span regions and temperature conditions across the U.S. portion of the Salish Sea (Fig. 1, Table 1). We visited sites shortly after adult sporophytes reached the surface of the water column (May 15 – June 24, 2022). At each site, we measured *N. luetkeana* pneumatocyst diameters, determined presence of other species of kelps, and collected tissue for laboratory assays and elemental analysis. Specifically, we measured adult pneumatocyst diameter in the field with calipers (±2 mm) to assess differences in sporophyte size across sites (n = 23-25 per site). We collected blade tissue samples for laboratory physiology assays at each site by boat by using a cork borer to excise 1.5 cm diameter tissue plugs (hereafter, plugs) from the blade 10 cm above the pneumatocyst. Each plug was stored in a 50 ml falcon tube filled with seawater collected from the site and kept cool (9 - 12°C) during transport to the laboratory (<5 hours). Additionally, we made note of which other species of kelp were present in the intertidal and shallow subtidal, the size of the kelp forest, and its relation to the shore (fringing or not) (Table 1).

We utilized available data sets from summer 2022 to estimate $T_{habitat}$ as the mean summer temperature. We also determined the maximum summer temperature for each site (Table 1). Turn Rock, Salmon Beach, Lincoln Park, Cherry Point, and Squaxin Island temperatures were from Weigel et al. (in review), which deployed temperature loggers within *N. luetkeana* forests at a depth of 3-5 m below mean lower low water (MLLW) with readings every 15 minutes. Puget Sound Restoration Fund provided temperature data from their Jefferson Head restoration site which was collected at 5 m below MLLW sampled every 15 minutes. Tongue Point and North Beach data were from nearby NOAA buoys, 9444090 and 9444900 stations respectively, at 3 m below MLLW sampled every 6 minutes.

Laboratory Assays

In the laboratory, we oven dried field collected blades and plugs from the assays to a constant mass. We calculated a thickness index for each plug as measured area (described below) divided by dry mass (n = 12-15 per site). Lastly, we pulverized field collected blades with a Retsch Mixer Mill 400 tissue grinder and sent the sampled to the Washington State University Stable Isotope Core Laboratory for elemental analysis (% N, % C, C:N, $\delta^{13}C$, and $\delta^{15}N$).

We incubated the plugs in 7 static temperature treatment tanks: 9, 12, 15, 18, 21, 24, and 27°C for 36 hours (n = 3 plugs per temperature). Each treatment was housed in clear plastic 18 x 18 cm containers filled with 2.5 L of artificial seawater (Instant Ocean, 30 PSU) and we added 20 µM nitrate as sodium nitrate ($NaNO_3$) and 1.25 µM phosphate as sodium phosphate (NaH_2PO_4). We ensured nutrients were not limiting by adding nitrate and phosphate which maintained the natural 16:1 ratio. To maintain temperature treatments, we used a water bath at 8°C to chill treatments below room temperature (9, 12, 15, 18, and 21°C) and an ambient temperature water bath for treatments that were above room temperature (24 and 27°C). We then warmed individual tanks to the treatment temperature with a heater controlled by a digital heat controller (True Temp #JB1235, JBJ Lighting). We placed tanks on a 14:10 light-dark cycle with 100 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) at the surface of the water, which is commonly used light level for *N. luetkeana* in the laboratory (Koehl & Alberte, 1988; Poulson et al., 2011; Supratya et al., 2020). Additionally, we maintained gentle flow in each tank with a micropump (Aquaneat BP, 300 liters per hour). At the end of the incubation, we determined survival of tissue plugs based on color and structural integrity; we considered bleached (tan to white in color) plugs that disintegrated when removed from the tank to be dead.

We measured oxygen mass-specific metabolic rates (net photosynthesis and dark respiration) in seawater using a Clark-type electrode system (Hansatech Chlorolab+ with DW3 chamber, Norfolk England) (Bell, 1993; Colvard et al., 2014; Maberly, 1992). The 10 mL reaction chamber was temperature-controlled by a water jacket connected to a recirculating chiller and, for net photosynthesis, the LED light source provided 400 µmol photons m^{-2} s^{-1} PAR, which is above saturating irradiance (I_k) but well below photoinhibition of *N. luetkeana* (Poulson et al., 2011). Chamber seawater was exchanged every 30 minutes to ensure measurements were collected in well-oxygenated seawater (~90-100% saturation). Gross photosynthesis was calculated as the difference between net photosynthesis and respiration. We also measured dark adapted yield of photosystem II (Fv/Fm) with a PAM (pulse-amplitude modulated) fluorometer (Hansatech Pocket Pea). We dark adapted the plugs for 15 minutes in leaf clips prior to collection of Fv/Fm measurements to ensure all photosystem II reaction centers were available. Following the photo-physiology assays, we took photographs of the plugs with a scale to measure change in area and condition. We quantified tissue growth (or loss) as change in area from initial size from photographs with a scale in ImageJ at 1 mm resolution (Schneider et al., 2012).

Thermal performance curves and statistics

We performed all analyses in R version 4.1.2 (R Core Team, 2021). We evaluated differences in pneumatocyst size, blade thickness index, $\delta^{13}C$, $\delta^{15}N$, percent nitrogen, percent carbon, and C:N ratios among sites with type III ANOVAs and post-hoc Tukey tests using the *car* and *emmeans* packages, respectively (Fox & Weisberg, 2019; Lenth et al., 2023). We fit thermal performance curves (TPCs) for growth, gross photosynthesis, and Fv/Fm with the *rTPC* package using non-linear least squares regressions to estimate the thermal optimum (T_{opt}) and critical thermal maximum (CT_{max}) (Padfield et al., 2021). Through AIC model selection, we selected the quadratic (Montagnes et al., 2008), Spain (Spain, 1983), and Johnson Lewin (Johnson & Lewin, 1946) models to build thermal performance curves. We assessed goodness of fit of the TPC models with residual standard errors (SE) for each site and assay. We used a residual resampling bootstrapping approach to estimate 95% confidence intervals for T_{opt} and CT_{max}. We considered sites to be different if the 95% confidence intervals were not overlapping. Using summer mean habitat temperature ($T_{habitat}$), we calculated warming tolerance ($WT = CT_{max}$

- $T_{habitat}$) and thermal safety margin (TSM = T_{opt} - $T_{habitat}$) following Deutsch et al. (2008). Lastly, we used separate regression analyses to test for a relationship between T_{opt} or CT_{max} with $T_{habitat}$ for each assay.

Results

Field condition and blade elemental composition

Across sites there were differences in the size, depth, and location (e.g. infringing (adjacent to shore) or not) of the kelp forests (Table 1). *Nereocystsis luetkeana* pneumatocyst sizes differed across sites ($F_7 = 97.9$, $P < 0.001$) with larger diameters at colder sites, smaller at warmer sites, and intermediate at Cherry Point (Tukey tests, Fig. 2A). The blade thickness index also differed across sites ($F_7 = 41.3$, $P < 0.001$) with thicker blades at colder sites and the thinnest blades at Lincoln Park (Tukey tests, Fig. 2B). In general, pneumatocyst diameter and blade thickness were approximately two-times greater at cold sites compared to warm sites.

$\delta^{13}C$ ratios in *N. luetkeana* blades were highly variable and different across sites ($F_7 = 16.4$, $P < 0.001$), highest at Tongue Point and Jefferson Head and lowest at Salmon Beach and Lincoln Park (Tukey tests, $P < 0.05$; Fig. 3A). $\delta^{15}N$ stable isotopes were also different across sites ($F_7 = 41.1$, $P < 0.001$); Salmon Beach had the highest $\delta^{15}N$ (mean: 12.4), Jefferson Head and Squaxin Island were intermediate (mean: 9.1), and the rest of the sites had the lowest $\delta^{15}N$, averaging 7.4 (Fig. 3B). Percent carbon in the blade significantly differed across sites ($F_7 = 4.2$, $P = 0.002$), but this trend was driven entirely by North Beach with 11% higher carbon relative to other sites (Fig. 3C). Percent blade nitrogen differed across sites ($F_7 = 7.9$, $P < 0.001$), with 1.2% higher nitrogen at the cool Strait of Juan de Fuca sites (Tongue Point, Turn Rock, North Beach) than Jefferson Head and Squaxin Island (Tukey tests, Fig. 3D). Lastly, differences in carbon to nitrogen ratios among sites ($F_7 = 12.1$, $P < 0.001$) were driven by a high C:N of 12.4 at Jefferson Head while the rest of the sites were similar in C:N with 9.0 - 9.7 (Tukey tests, Fig. 3E).

Laboratory Assays

Survival of *N. luetkeana* plugs after 36 hours in temperature incubations was consistent across all sites with 100% survival from 9 - 21°C and 0% survival at 24 or 27°C. Due to blade mortality, no physiology assays were measured above 21°C.

Thermal performance curves (TPC) goodness of fits varied among assays, with the best fits for Fv/Fm (residual standard error (SE) = 0.04 – 0.11), followed by growth (residual SE = 5.1 – 11.1, Table 2). Gross photosynthesis TPC model fits were poor (residual SE = 39.1 – 105.6) and should be interpreted with caution (Table 2).

Thermal optima (T_{opt}) for growth ranged from 12.0 to 14.4°C with an average of 13.6°C (Table 2, Fig. 4). Note that growth data were not available at the warmest site, Squaxin Island, due to weather constraints on sampling. The 95% confidence intervals of T_{opt} overlapped at Turn Rock, North Beach, Salmon Beach, Jefferson Head, and Lincoln Park but not for the coldest and warmest sites (Tongue Point and Cherry Point, respectively), where T_{opt} differed by 2.4°C (Fig. 7). The estimates of CT_{max} for growth ranged from 18.9 to 21.1°C with an average of 20.1°C (Table 2). As with T_{opt} for growth, all 95% confidence intervals for CT_{max} overlapped except between Cherry Point and Tongue Point, which differed by 1.6°C.

Gross photosynthesis TPCs were poor fits and we were not always able to estimate T_{opt}, CT_{max}, and 95% confidence intervals (Fig. 5). The confidence intervals, when present, were overlapping for both T_{opt} and CT_{max} indicating no difference across sites (Fig. 7). Overall, estimates of T_{opt} ranged from 9.0 to 19.2°C and the average was 13.7, but confidence intervals were very large (10°C range; Table 2). Meanwhile, confidence intervals were much narrower for CT_{max} and estimates ranged from 20.5 to 21.4°C across sites with an average of 21.1°C. Details of metabolism as net photosynthesis and dark respiration can be found in the supplement (Fig. S1).

T_{opt} estimates for Fv/Fm were higher and more variable than for growth, ranging from 15.6 to 20.0°C with an average of 18.2°C (Table 1). While curve fits were generally good for Fv/Fm, we were unable to calculate 95% confidence intervals for Salmon Beach due to a lack of a peak (see flat top curve, Fig. 6), which was also the site with the lowest optimum so this value should be interpreted with caution. All T_{opt} confidence intervals were overlapping, indicating no differences in optimum temperatures of Fv/Fm across sites (Fig. 7). CT_{max} of Fv/Fm ranged from 21.0 - 25.9°C with an average of 22.9°C across sites. As with Topt, all CT_{max} confidence intervals were overlapping indicating no significant differences across sites.

Warming tolerances (WT) and thermal safety margins (TSM) differed across sites associated with the differences in habitat temperatures ($T_{habitat}$). The range in $T_{habitat}$ was 11.1 – 14.7°C (Table 1), while WT ranged from 5.8 –14.4°C and TSM from 0 – 8.1°C between assays and sites (Table 2). Specifically, Jefferson Head had the lowest TSM for growth since $T_{habitat}$ overlapped with T_{opt} (Fig. 7A), while other sites were 1-2°C from T_{opt} (Fig. 7D). WT and TSM for Fv/Fm had more degrees of warming until reaching critical thresholds than growth (Table 2, Fig. 7D & E).

T_{opt} slightly increased with $T_{habitat}$ across all performance metrics but the relationship was not significant ($R^2 = 0.3 – 0.7$, Table 3, Fig. 8A). We found no relationship between CT_{max} and $T_{habitat}$ for any performance assays ($P > 0.05$; Table 3, Fig. 8B). Finally, we built a general TPC for growth for all sampled sites in the Salish Sea (Fig. 9). The overall T_{opt} of growth was 13.6°C (95% CI: 12.7 – 14.2) and CT_{max} of growth was 20.3°C (95% CI: 19.7 – 21.1).

Discussion

Nereocystsis luetkeana populations across the Salish Sea were surprisingly similar in their physiological responses to warming and we found limited evidence of warm adaptation or acclimation. The thermal optima for growth, photosynthesis, and photosystem II function were typically different for the coldest (Tongue Point) and warmest (Squaxin Island) site and there was a trend of higher T_{opt} at warmer sites indicating that there is some acclimation or adaptation to warmer temperatures. Yet the critical thermal maximum was the same across all sites, indicating that 20 ± 1°C will be a tipping point for *N. luetkeana* sporophyte declines in the future. Cool sites have a larger warming tolerance since the habitat temperature is much lower than the critical temperature. Meanwhile, warm sites are already dangerously close to exceeding their thermal safety margin. Given habitat temperatures are already warm, further warming temperatures will cause rapid declines when these sites surpass the thermal optimum.

In our study of eight populations of *N. luetkeana*, sites closer to the Strait of Juan de Fuca had larger pneumatocysts and thicker blades, which could indicate different ecotypes (Fowler-Walker et al., 2006; Graham et al., 2007; Innes, 1984) or healthier populations. Even if the Strait of Juan de Fuca populations are more robust, mortality occurred at the same temperature as the Puget Sound populations which means morphological traits were not an indicator of higher CT_{max}. The robust morphology and size of Strait of Juan de Fuca populations are potentially an indicator of good environmental conditions for kelp, especially since there were more kelp species present at these sites. Sites in the Strait of Juan de Fuca may also have more access to nitrogen, an important nutrient for kelps, since most nitrogen is marine in origin in the Salish Sea (Mohamedali et al., 2011; Newton et al., 2002). Lincoln Park and Cherry Point had similar $\delta^{15}N$ signatures to the Strait of Juan de Fuca sites indicating that their nitrogen is likely marine in origin as well. Salmon Beach had the highest $\delta^{15}N$ level and therefore likely has some anthropogenic source of nitrogen, but the C:N ratio and percent nitrogen was still similar to other sites so nitrogen stores were not enhanced by the different source. Nitrogen availability can increase thermotolerance of kelps (Fernández et al., 2020; Gao et al., 2016; Gerard, 1997), yet Jefferson Head and Cherry Point, which had the lowest blade nitrogen content, had similar performance and critical limits to other sites with higher percent nitrogen.

We found the thermal physiological limit, as CT_{max} , to be 21-22°C in the Salish Sea across all sites in this short-term study with tissue plugs. This value is higher than other studies from California and Oregon with mortality from 16-19°C (Hamilton et al., 2020; Schiel et al., 2004). Additionally, Lüning and Freshwater (1988) also report a CT_{max} of 18°C for the San Juan Islands, suggesting the upper end of the survival range may have increased by 3°C in the last 38 years. But similar to studies from British Columbia, Canada, we found *N. luetkeana* tissue had an optimum growth rate near 12°C (Supratya et al., 2020), Possibly, *N. luetkeana* populations in this study are better adapted or acclimated to warm temperature than in other parts of its range in California and Oregon but similar to British Columbia.

The *N. luetkeana* populations that we sampled allow us to draw important comparisons between kelp physiology and population genetics. The North Beach population was used as the source of sori for the Jefferson Head restoration of *N. luetkeana* (Puget Sound Restoration Fund non-profit, PSRF) and are therefore genetically similar to each other but experience different environmental conditions, with cooler water at North Beach than Jefferson Head. The Jefferson Head restoration kelp had the highest C:N ratio of all sites due to low blade nitrogen. The two sites had similar TPCs for growth but there was a trend of a higher T_{opt} for Fv/Fm at Jefferson Head. Yet the CT_{max} of Fv/Fm for all sites, including the donor and restoration populations, were similar (22°C) indicating the threshold for photosystem II failure is highly conserved between populations. At a broader geographic scale, a study that sampled *N. luetkeana* from Alaska, British Columbia, Salish Sea, Washington and California found 4 major genetic groups (Gierke, 2019). Our sites encompass two of those groups, with populations in Puget Sound differing from the WA coastal sites as well as Cherry Point (in the Strait of Georgia), which surprisingly grouped with the WA coastal sites. Additionally, Salmon Beach and Squaxin Island are known to be very genetically similar with low allelic richness (Gierke, 2019) and have more similar microbial communities compared to more oceanic sites (Weigel & Pfister, 2019). Salmon Beach and Squaxin Island photosynthesized similarly in the laboratory in our study. Yet in the field, the Salmon Beach population is abundant and healthy with thicker blades while Squaxin Island has been declining for many years and was nearing extinction in 2022 indicating that some factor other may be influencing differences between these populations (Berry et al. 2021, author pers. obs.).

The use of tissue plugs is common in seaweed physiological studies (e.g. Maberly 1992, Bell 1993, Colvard et al. 2014, Hereward et al. 2020, Liesner et al. 2020). This method typically assays a small piece of young healthy blade tissue, but may not reflect the physiological tolerance of a whole sporophyte. Additionally, our study was performed at 3-degree intervals so there is interpolation between temperatures that increases the variability around our estimates. Yet our results for T_{opt} of growth are similar to other studies of the same species which used whole blades (Supratya et al., 2020), as well as larger blade segments (40 cm) from Turn Rock and Cherry Point (Fales, Weigel, et al. in prep). Additionally, our physiological assays were measured on plugs in field collected condition, as opposed to a common garden experiment, therefore there is carry over from field conditions (Kawecki & Ebert, 2004; Kuo & Sanford, 2009). There were differences in starting conditions across sites, for example blades from southern Puget Sound were thinner than blades from Strait of Juan de Fuca sites (Tongue Point, Turn Rock, and North Beach). While this method of using field-condition blades has confounding factors, it does allow us to better predict the consequences of warming and MHWs for each population of *N. luetkeana*, which was a goal of this management focused study.

Many factors may contribute to the lack of local adaptation or acclimation of *N. luetkeana* to warming including population connectivity, complex life cycles, and seasonality. Maximum thermotolerance, as evidenced by CT_{max} and survival, was unexpectedly similar across sites. Similarly, *Laminaria digitata* had the same upper temperature limit across its wide range in the North Atlantic (Liesner et al., 2020). While it is common for marine macrophytes to have different thermal niches for geographically separated populations (King et al., 2018), *N. luetkeana* in the Salish Sea may not have enough isolation to allow for local adaptation. Alternatively, thermal selection could be acting on a different life-history stage than large sporophytes. For example, Weigel et al. (in review) found 18°C limited reproduction of *N. luetkeana* through failure of the transition from gametophyte to sporophyte across 8 sites in the Salish Sea. Lastly, thermotolerance of *N. luetkeana* could differ across the growing season as temperatures become warmer later in summer. Our experiment was performed in early summer (late May to June) before the hottest temperatures were reached; these early season measurements are therefore better suited for testing local adaptation than acclimation.

Global sea surface temperatures have already increased by 0.9°C in the last century (IPCC 2022) and climate projections for Salish Sea predict 1.5-3°C of warming by 2100 (Riche et al. 2014, Khangaonkar et al. 2019). Organisms inhabiting coastal oceans and inland seas are likely to reach their thermal tipping points sooner since temperatures are rising more rapidly. Therefore, inland seas like the Salish Sea can act as bellwethers for climate change. Our study indicates that temperatures above 22°C will cause severe declines in *N. luetkeana* physiological performance and high mortality. Daily maximum temperatures in two *N. luetkeana* forests already reached 18°C during the summer of 2022 showing some forests are only 4°C away from the critical threshold. This temperature threshold could be modulated by other stressors such as flow, light, salinity, and nutrient availability (Gunderson et al., 2016; Litchman & Thomas, 2023; Newcomb et al., 2019). Future-proofing and preparation for marine heatwaves is necessary since temperatures are likely to surpass *N. luetkeana* thresholds in the near future. Accordingly, efforts towards gametophyte banks which can be used for restoration should be continued and expanded.

Ultimately, we need to move towards contextualizing thermal performance curves for habitat forming species with local temperatures to make more relevant predictions of physiological responses to climate change. We can use thermotolerance to guide conservation of potentially heat tolerant populations or sites that can serve as thermal refugia. The search for thermotolerant populations of *N. luetkeana* may require greater geographic separation than encompassed by our study. Sites to the north, such as Barkley Sound (Starko et al., 2022) or the northern Strait of Georgia in the Salish Sea should be assessed. Management actions in the Salish Sea should prioritize conservation of locations that can serve as thermal refugia below the tipping point of 22°C and restorative actions that can increase resilience to warming for populations nearing the tipping point.

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References

Bell, E. C. (1993). Photosynthetic response to temperature and desiccation of the intertidal alga *Mastocarpus papillatus*. *Marine Biology*, *117*(2), 337–346. https://doi.org/10.1007/BF00345679

Berry, H. D., Mumford, T. F., Christiaen, B., Dowty, P., Calloway, M., Ferrier, L., Grossman, E. E., & VanArendonk, N. R. (2021). Long-term changes in kelp forests in an inner basin of the Salish Sea. *PLOS ONE*, *16*(2), e0229703. https://doi.org/10.1371/journal.pone.0229703

Colvard, N. B., Carrington, E., & Helmuth, B. (2014). Temperature-dependent photosynthesis in the intertidal alga *Fucus gardneri* and sensitivity to ongoing climate change. *Journal of Experimental Marine Biology and Ecology*, *458*, 6–12. https://doi.org/10.1016/j.jembe.2014.05.001

Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences*, *105*(18), 6668–6672. https://doi.org/10.1073/pnas.0709472105

Druehl, L. D. (1978). The distribution of *Macrocystis integrifolia* in British Columbia as related to environmental parameters. *Canadian Journal of Botany*, *56*(1), 69–79. https://doi.org/10.1139/b78-007

Druehl, L. D. (1981). Geographic Distribution. In C. S. Lobban & M. J. Wynne (Eds.), *The Biology of Seaweeds* (pp. 306–352). University of California Press.

Duggins, D. O., Simenstad, C. A., & Estes, J. A. (1989). Magnification of secondary production by kelp detritus in coastal marine ecosystems. *Science*, *245*(4914), 170–174.

Fernández, P. A., Gaitán-Espitia, J. D., Leal, P. P., Schmid, M., Revill, A. T., & Hurd, C. L. (2020). Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: Implications for acclimation under thermal stress. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-60104-4

Fowler-Walker, M. J., Wernberg, T., & Connell, S. D. (2006). Differences in kelp morphology between wave sheltered and exposed localities: Morphologically plastic or fixed traits? *Marine Biology*, *148*(4), 755–767. https://doi.org/10.1007/s00227-005-0125-z

Fox, J., & Weisberg, S. (2019). *An {R} Companion to Applied Regression, Third Edition.* https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Gao, X., Endo, H., Nagaki, M., & Agatsuma, Y. (2016). Growth and survival of juvenile sporophytes of the kelp *Ecklonia cava* in response to different nitrogen and temperature regimes. *Fisheries Science*, *82*(4), 623–629. https://doi.org/10.1007/s12562-016-0998-4

Gerard, V. A. (1997). The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). *Journal of Phycology*, *33*(5), 800–810. https://doi.org/10.1111/j.0022-3646.1997.00800.x

Gierke, L. G. (2019). *A seascape genetics approach to studying genetic differentiation in the bull kelp* Nereocystis luetkeana [M.S., The University of Wisconsin Milwaukee]. https://www.proquest.com/docview/2345864351/abstract/4EF0D3DD9AE43CBPQ/1

Graham, M. H., Vásquez, J., & Buschmann, A. H. (2007). Global ecology of the giant kelp *Macrocystis*: From ecotypes to ecosystems. In *Oceanography and Marine Biology: An Annual Review* (pp. 39–88). Taylor & Francis. https://doi.org/10.1201/9781420050943.ch2

Grebe, G. S., Byron, C. J., Gelais, A. St., Kotowicz, D. M., & Olson, T. K. (2019). An ecosystem approach to kelp aquaculture in the Americas and Europe. *Aquaculture Reports*, *15*, 100215. https://doi.org/10.1016/j.aqrep.2019.100215

Gunderson, A. R., Armstrong, E. J., & Stillman, J. H. (2016). Multiple stressors in a changing world: The need for an improved perspective on physiological responses to the dynamic marine environment. *Annual Review of Marine Science*, *8*(1), 357–378. https://doi.org/10.1146/annurev-marine-122414-033953

Hamilton, S. L., Bell, T. W., Watson, J. R., Grorud-Colvert, K. A., & Menge, B. A. (2020). Remote sensing: Generation of long-term kelp bed data sets for evaluation of impacts of climatic variation. *Ecology*, *101*(7), e03031. https://doi.org/10.1002/ecy.3031

Harley, C. D. G., Hughes, A. R., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S., Rodriguez, L. F., Tomanek, L., & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, *9*(2), 228–241. https://doi.org/10.1111/j.1461-0248.2005.00871.x

Hereward, H. F. R., King, N. G., & Smale, D. A. (2020). Intra-Annual variability in responses of a canopy forming kelp to cumulative low tide heat stress: Implications for populations at the trailing range edge. *Journal of Phycology*, *56*(1), 146–158. https://doi.org/10.1111/jpy.12927

Higham, T. E., Ferry, L. A., Schmitz, L., Irschick, D. J., Starko, S., Anderson, P. S. L., Bergmann, P. J., Jamniczky, H. A., Monteiro, L. R., Navon, D., Messier, J., Carrington, E., Farina, S. C., Feilich, K. L., Hernandez, L. P., Johnson, M. A., Kawano, S. M., Law, C. J., Longo, S. J., … Niklas, K. J. (2021). Linking ecomechanical models and functional traits to understand phenotypic diversity. *Trends in Ecology & Evolution*, *36*(9), 860–873. https://doi.org/10.1016/j.tree.2021.05.009

Humanes, A., Lachs, L., Beauchamp, E. A., Bythell, J. C., Edwards, A. J., Golbuu, Y., Martinez, H. M., Palmowski, P., Treumann, A., van der Steeg, E., van Hooidonk, R., & Guest, J. R. (2022). Within-population variability in coral heat tolerance indicates climate adaptation potential. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1981), 20220872. https://doi.org/10.1098/rspb.2022.0872

Hurd, C. L., Harrison, P. J., & Druehl, L. D. (1996). Effect of seawater velocity on inorganic nitrogen uptake by morphologically distinct forms of *Macrocystis integrifolia* from wave-sheltered and exposed sites. *Marine Biology*, *126*(2), 205–214. https://doi.org/10.1007/BF00347445

Innes, D. J. (1984). Genetic differentiation among populations of marine algae. *Helgoländer Meeresuntersuchungen*, *38*(3), 401–417. https://doi.org/10.1007/BF02027689

Johnson, F. H., & Lewin, I. (1946). The growth rate of *E. coli* in relation to temperature, quinine and coenzyme. *Journal of Cellular and Comparative Physiology*, *28*(1), 47–75. https://doi.org/10.1002/jcp.1030280104

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Khangaonkar, T., Nugraha, A., Xu, W., & Balaguru, K. (2019). Salish Sea response to global climate change, sea level rise, and future nutrient loads. *Journal of Geophysical Research: Oceans*, *124*(6), 3876–3904. https://doi.org/10.1029/2018JC014670

Khangaonkar, T., Nugraha, A., Xu, W., Long, W., Bianucci, L., Ahmed, A., Mohamedali, T., & Pelletier, G. (2018). Analysis of hypoxia and sensitivity to nutrient pollution in Salish

Sea. *Journal of Geophysical Research: Oceans*, *123*(7), 4735–4761. https://doi.org/10.1029/2017JC013650

King, N. G., McKeown, N. J., Smale, D. A., & Moore, P. J. (2018). The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography*, *41*(9), 1469–1484. https://doi.org/10.1111/ecog.03186

Koehl, M. A. R., & Alberte, R. S. (1988). Flow, flapping, and photosynthesis of *Nereocystis leutkeana*: A functional comparison of undulate and flat blade morphologies. *Marine Biology*, *99*(3), 435–444. https://doi.org/10.1007/BF02112137

Koehl, M. A. R., Silk, W. K., Liang, H., & Mahadevan, L. (2008). How kelp produce blade shapes suited to different flow regimes: A new wrinkle. *Integrative and Comparative Biology*, *48*(6), 834–851. JSTOR.

Krumhansl, K. A., Okamoto, D. K., Rassweiler, A., Novak, M., Bolton, J. J., Cavanaugh, K. C., Connell, S. D., Johnson, C. R., Konar, B., Ling, S. D., Micheli, F., Norderhaug, K. M., Pérez-Matus, A., Sousa-Pinto, I., Reed, D. C., Salomon, A. K., Shears, N. T., Wernberg, T., Anderson, R. J., … Byrnes, J. E. K. (2016). Global patterns of kelp forest change over the past half-century. *Proceedings of the National Academy of Sciences*, *113*(48), 13785–13790. https://doi.org/10.1073/pnas.1606102113

Kuo, E., & Sanford, E. (2009). Geographic variation in the upper thermal limits of an intertidal snail: Implications for climate envelope models. *Marine Ecology Progress Series*, *388*, 137–146. https://doi.org/10.3354/meps08102

Lenth, R. V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2023). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (1.8.6). https://cran.r-project.org/web/packages/emmeans/index.html

Liesner, D., Fouqueau, L., Valero, M., Roleda, M. Y., Pearson, G. A., Bischof, K., Valentin, K., & Bartsch, I. (2020). Heat stress responses and population genetics of the kelp *Laminaria digitata* (Phaeophyceae) across latitudes reveal differentiation among North Atlantic populations. *Ecology and Evolution*, *00*, 1–34. https://doi.org/10.1002/ece3.6569

Litchman, E., & Thomas, M. K. (2023). Are we underestimating the ecological and evolutionary effects of warming? Interactions with other environmental drivers may increase species vulnerability to high temperatures. *Oikos*, *2023*(2), e09155. https://doi.org/10.1111/oik.09155

Lüning, K., & Freshwater, W. (1988). Temperature tolerance of Northeast Pacific marine algae. *Journal of Phycology*, *24*(3), 310–315. https://doi.org/10.1111/j.1529-8817.1988.tb04471.x

Maberly, S. C. (1992). Carbonate ions appear to neither inhibit nor stimulate use of bicarbonate ions in photosynthesis by *Ulva lactuca*. *Plant, Cell & Environment*, *15*(2), 255–260. https://doi.org/10.1111/j.1365-3040.1992.tb01480.x

Martins, N., Pearson, G. A., Bernard, J., Serrão, E. A., & Bartsch, I. (2020). Thermal traits for reproduction and recruitment differ between Arctic and Atlantic kelp *Laminaria digitata*. *PLOS ONE*, *15*(6), e0235388. https://doi.org/10.1371/journal.pone.0235388

Mohamedali, T., Roberts, M., Sackmann, B., & Kolosseus, A. (2011). *Puget Sound dissolved oxygen model nutrient load summary for 1999-2008* (p. 172). Washington Department of Ecology. https://apps.ecology.wa.gov/publications/documents/1103057.pdf

Montagnes, D. J. S., Morgan, G., Bissinger, J. E., Atkinson, D., & Weisse, T. (2008). Short-term temperature change may impact freshwater carbon flux: A microbial perspective. *Global Change Biology*, *14*(12), 2823–2838. https://doi.org/10.1111/j.1365-2486.2008.01700.x

Mumford, T. F. (2007). *Kelp and eelgrass in Puget Sound*. Puget Sound Nearshore Partnership. https://doi.org/10.21236/ADA477318

Newcomb, L. A., George, M. N., O'Donnell, M. J., & Carrington, E. (2019). Only as strong as the weakest link: Structural analysis of the combined effects of elevated temperature and pCO2 on mussel attachment. *Conservation Physiology*, *7*(1), coz068. https://doi.org/10.1093/conphys/coz068

Newton, J. A., Albertson, S. L., Van Voorhis, K., Maloy, C., & Siegel, E. (2002). *Washington State Marine Water Quality, 1998 through 2000* (#02-03-056). Washington State Department of Ecology, Environmental Assessment Program.

Padfield, D., O'Sullivan, H., & Pawar, S. (2021). rTPC and nls.multstart: A new pipeline to fit thermal performance curves in r. *Methods in Ecology and Evolution*, *12*(6), 1138–1143. https://doi.org/10.1111/2041-210X.13585

Poulson, M. E., McNeil, A. J., & Donahue, R. A. (2011). Photosynthetic response of *Nereocystis luetkeana* (Phaeophyta) to high light. *Phycological Research*, *59*(3), 156–165. https://doi.org/10.1111/j.1440-1835.2011.00614.x

R Core Team. (2021). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.*

Rogers-Bennett, L., & Catton, C. A. (2019). Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports*, *9*(1), 15050. https://doi.org/10.1038/s41598-019-51114-y

Schiel, D. R., Steinbeck, J. R., & Foster, M. S. (2004). Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. *Ecology, 85*(7), 1833–1839. https://doi.org/10.1890/03-3107

Schimpf, N. M., Liesner, D., Franke, K., Roleda, M. Y., & Bartsch, I. (2022). Microscopic stages of North Atlantic *Laminaria digitata* (Phaeophyceae) exhibit trait-dependent thermal adaptation along latitudes. *Frontiers in Marine Science, 9*, 870792. https://doi.org/10.3389/fmars.2022.870792

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), Article 7. https://doi.org/10.1038/nmeth.2089

Smale, D. A. (2020). Impacts of ocean warming on kelp forest ecosystems. *New Phytologist*, *225*(4), 1447–1454. https://doi.org/10.1111/nph.16107

Sorte, C. J. B., Jones, S. J., & Miller, L. P. (2011). Geographic variation in temperature tolerance as an indicator of potential population responses to climate change. *Journal of Experimental Marine Biology and Ecology*, *400*(1–2), 209–217. https://doi.org/10.1016/j.jembe.2011.02.009

Spain, J. D. (1983). Basic Microcomputer Models in Biology. *FEBS Letters*, *162*(1), 202–202. https://doi.org/10.1016/0014-5793(83)81080-1

Starko, S., Neufeld, C. J., Gendall, L., Timmer, B., Campbell, L., Yakimishyn, J., Druehl, L., & Baum, J. K. (2022). Microclimate predicts kelp forest extinction in the face of direct and indirect marine heatwave effects. *Ecological Applications*, e2673. https://doi.org/10.1002/eap.2673

Starko, S., Soto Gomez, M., Darby, H., Demes, K. W., Kawai, H., Yotsukura, N., Lindstrom, S. C., Keeling, P. J., Graham, S. W., & Martone, P. T. (2019). A comprehensive kelp phylogeny sheds light on the evolution of an ecosystem. *Molecular Phylogenetics and Evolution*, *136*, 138–150. https://doi.org/10.1016/j.ympev.2019.04.012

Strasser, F.-E., Barreto, L. M., Kaidi, S., Sabour, B., Serrão, E. A., Pearson, G. A., & Martins, N. (2022). Population level variation in reproductive development and output in the golden kelp *Laminaria ochroleuca* under marine heat wave scenarios. *Frontiers in Marine Science*, *9*, 943511. https://doi.org/10.3389/fmars.2022.943511

Sulmon, C., van Baaren, J., Cabello-Hurtado, F., Gouesbet, G., Hennion, F., Mony, C., Renault, D., Bormans, M., El Amrani, A., Wiegand, C., & Gérard, C. (2015). Abiotic stressors and stress responses: What commonalities appear between species across biological organization levels? *Environmental Pollution*, *202*, 66–77. https://doi.org/10.1016/j.envpol.2015.03.013

Supratya, V. P., Coleman, L. J. M., & Martone, P. T. (2020). Elevated temperature affects phenotypic plasticity in the bull kelp (*Nereocystis luetkeana*, Phaeophyceae). *Journal of Phycology*, *56*(6), 1534–1541. https://doi.org/10.1111/jpy.13049

Teagle, H., Hawkins, S. J., Moore, P. J., & Smale, D. A. (2017). The role of kelp species as biogenic habitat formers in coastal marine ecosystems. *Journal of Experimental Marine Biology and Ecology*, *492*, 81–98. https://doi.org/10.1016/j.jembe.2017.01.017

Villeneuve, A. R., Komoroske, L. M., & Cheng, B. S. (2021). Environment and phenology shape local adaptation in thermal performance. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1955), 20210741. https://doi.org/10.1098/rspb.2021.0741

Weigel, B. L., & Pfister, C. A. (2019). Successional dynamics and seascape-level patterns of microbial communities on the canopy-forming kelps *Nereocystis luetkeana* and *Macrocystis pyrifera*. *Frontiers in Microbiology*, *10*. https://www.frontiersin.org/articles/10.3389/fmicb.2019.00346

Weigel, B. L., Small, S. L., Berry, H. D., & Dethier, M. N. (in review). Effects of temperature and nutrients on microscopic stages of the bull kelp (*Nereocystis luetkeana*, Phaeophyceae).

Yang, Z., Wang, T., Branch, R., Xiao, Z., & Deb, M. (2021). Tidal stream energy resource characterization in the Salish Sea. *Renewable Energy*, *172*, 188–208. https://doi.org/10.1016/j.renene.2021.03.028

Tables

Table 1. Sampling locations of *Nereocystis* beds ordered from cool to warm detailing: site name, region, population genetics, coordinates, location, general site characteristics, the number of other kelp (Laminariales) species present in the intertidal and subtidal, thermal regime (cold, moderate, or warm), and mean and maximum summer water temperatures. Population genetics from Gierke 2019 and Puget Sound Restoration Fund (PSRF). Summer water temperatures from 2022 sampled in kelp beds from Weigel et al. in review, NOAA buoys, and PSRF. Mean summer temperature is used as $T_{habitat}$. ND = no data

Site	Region	Population genetics	Latitude	Longitude	Characteristics	Other kelps	Thermal regime	Summer temp. (°C)	
								Mean	Max
Tongue Point	Strait of Juan de Fuca	WA coastal	48°09'58" N	123°41'53" W	Oceanic, nearshore, large forest	7	Cold	11.1	13.7
Turn Rock	San Juan Islands	WA coastal	48°32'07" N	122°57'52" W	Deep water, fast currents, large forest	9	Cold	11.1	15.2
North Beach	Strait of Juan de Fuca	WA coastal, Donnor for restoration	48°08'37"N	122°46'48"W	Nearshore, large forest	6	Cold	11.5	14.3
Salmon Beach	Puget Sound	Puget Sound	47°17'50" N	122°31'56" W	Nearshore, fast currents, moderate forest	ND	Moderate	12.3	14.4
Jefferson Head (PSRF restoration)	Puget Sound	Same as North Beach	47°44'37" N	122°29'01"W	Restored small kelp bed on seeded lines attached to benthos	3	Moderate	13.6	17.7
Lincoln Park	Puget Sound	Puget Sound	47°32'01" N	122°23'54" W	Nearshore, shallow kelp forest, small forest, nutrient poor	3	Moderate	12.7	15.3
Cherry Point	Strait of Georgia	WA coastal	48°51’24” N	122°44’40” W	Nearshore, close to Fraser River plume, large forest, low nutrients	3	Warm	13.4	20.5
Squaxin Island	Puget Sound	Puget Sound	47°10'04" N	122°53'38" W	Nearshore, shallow kelp forest, nutrient poor, in major decline	2	Warm	14.7	18.6

Table 2. Summary of model residual standard error (SE) and degrees of freedom of thermal performance curves for each assay and site as well as estimates of T_{opt} and CT_{max} with 95% confidence intervals (CI), warming tolerance (WT), thermal safety margin (TSM). ND = no data.

Site	Residual SE	DF	T_{opt}	CI T_{opt}	CT_{max}	CI CT_{max}	WT	TSM
Growth								
Tongue Point	9.32	11	12.0	9.0 - 13.4	18.9	17.9 - 19.7	7.8	0.9
Turn Rock	7.01	12	12.7	9.0 - 14.2	20.0	18.4 - 21.5	8.9	1.6
North Beach	7.49	12	14.1	13.3 - 14.7	21.2	20.4 - 22.0	9.7	2.6
Salmon Beach	5.45	12	13.6	12.8 - 14.3	20.3	19.5 - 21.0	8.0	1.3
Jefferson Head	9.04	12	13.6	10.7 - 14.5	21.2	20.2 - 22.8	7.6	0
Lincoln Park	11.06	9	14.5	12.0 - 16.5	18.5	15.1 - 20.9	5.8	1.8
Cherry Point	5.10	9	14.4	14.1 - 15.5	20.5	20.1 - 21.0	7.1	1.0
Gross photosynthesis								
Tongue Point	51.38	11	9.0	9.0 - 18.9	21.0	20.9 - 21.2	9.9	ND
Turn Rock	39.10	11	9.0	9.0 - 12.0	21.4	21.2 - 21.6	10.3	ND
North Beach	37.49	11	ND	ND	ND	ND	ND	ND
Salmon Beach	83.94	11	18.6	9.0 - 19.9	21.3	21.0 - 22.8	9.0	6.3
Jefferson Head	96.09	11	14.9	9.0 - 20.3	ND	ND	ND	1.3
Lincoln Park	102.40	10	16.1	13.8 - 17.2	20.5	20.2 - 21.1	7.8	3.4
Cherry Point	115.10	11	9.0	ND	ND	ND	ND	ND
Squaxin Island	105.60	11	19.2	9.0 - 20.2	21.4	21.1 - 22.6	6.7	4.5
Fv/Fm								
Tongue Point	0.11	11	19.2	18.0 - 19.9	21.5	21.1 - 21.6	10.4	8.1
Turn Rock	0.04	11	17.0	15.7 - 19.8	24.8	21.7 - 27.5	13.7	5.9
North Beach	0.03	11	16.8	15.3 - 19.7	25.9	21.9 - 29.3	14.4	5.3
Salmon Beach	0.07	11	15.6	ND	22.0	ND	9.7	3.3
Jefferson Head	0.03	11	20.0	19.0 - 20.1	21.8	21.8 - 21.9	8.2	6.4
Lincoln Park	0.11	10	19.5	17.3 - 19.7	21.0	18.7 - 21.2	8.3	6.8
Cherry Point	0.04	11	17.8	15.9 - 20.0	24.4	21.8 - 29.3	11.0	4.4
Squaxin Island	0.04	11	19.9	18.5 - 20.1	21.6	21.5 - 21.8	6.9	5.2

Table 3. Summary of linear regressions examining the relationship between T_{opt} or CT_{max} with $T_{habitat}$ for all performance assays.

Assay	DF	F	P	R^2	Slope
T_{opt}					
Growth	1, 5	3.73	0.1	0.43	0.56
Photosynthesis	1, 4	7.30	**0.05**	0.65	2.57
Fv/Fm	1, 6	2.21	0.2	0.27	0.66
CT_{max}					
Growth	1, 5	0.47	0.5	0.09	0.29
Photosynthesis	1, 3	0.03	0.9	0.01	0.03
Fv/Fm	1, 6	0.10	0.4	0.14	-0.54

Figures

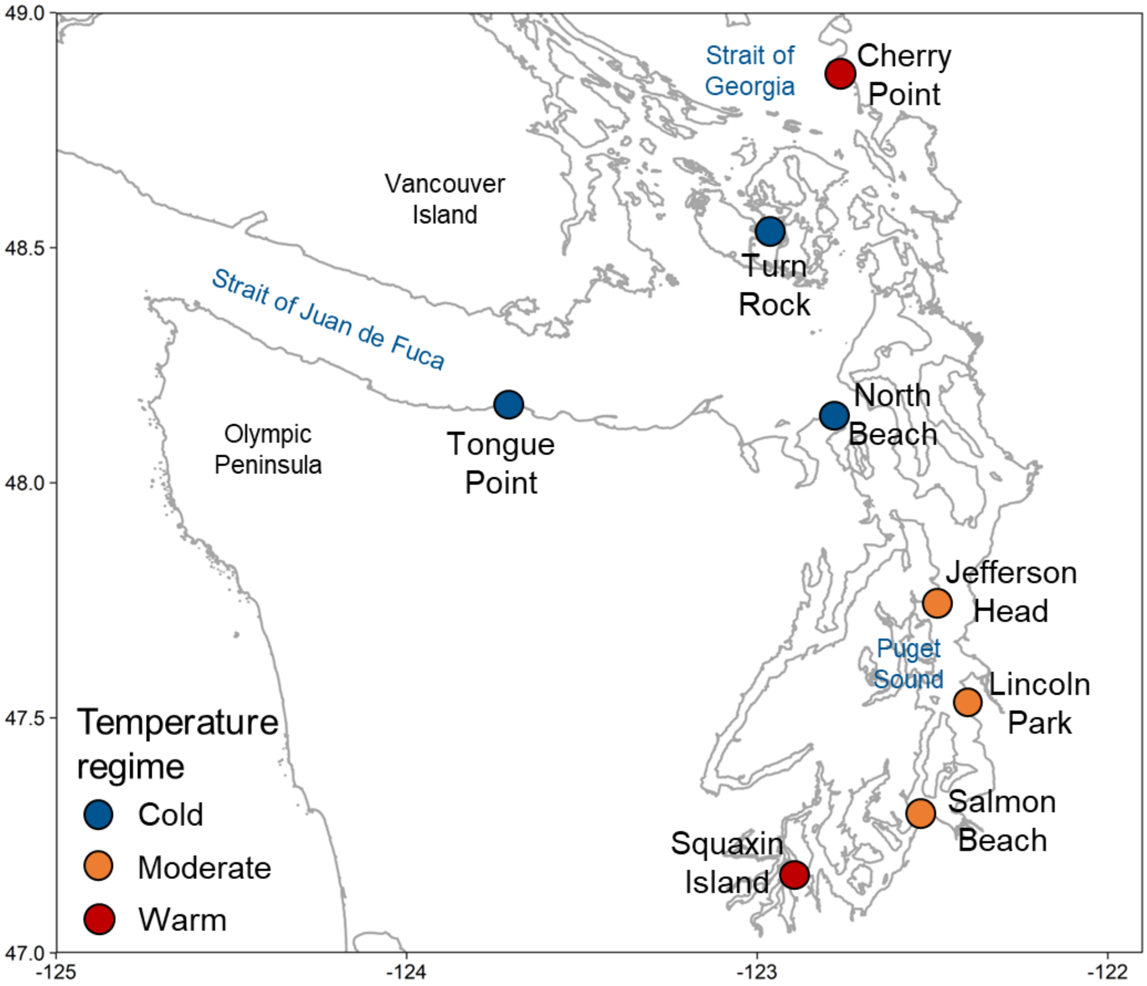

Figure 1. *Nereocystis luetkana* forests across the Salish Sea that we sampled, color coded by temperature (cold, moderate, or warm). Salish Sea regions are labeled with blue text and sites with black text.

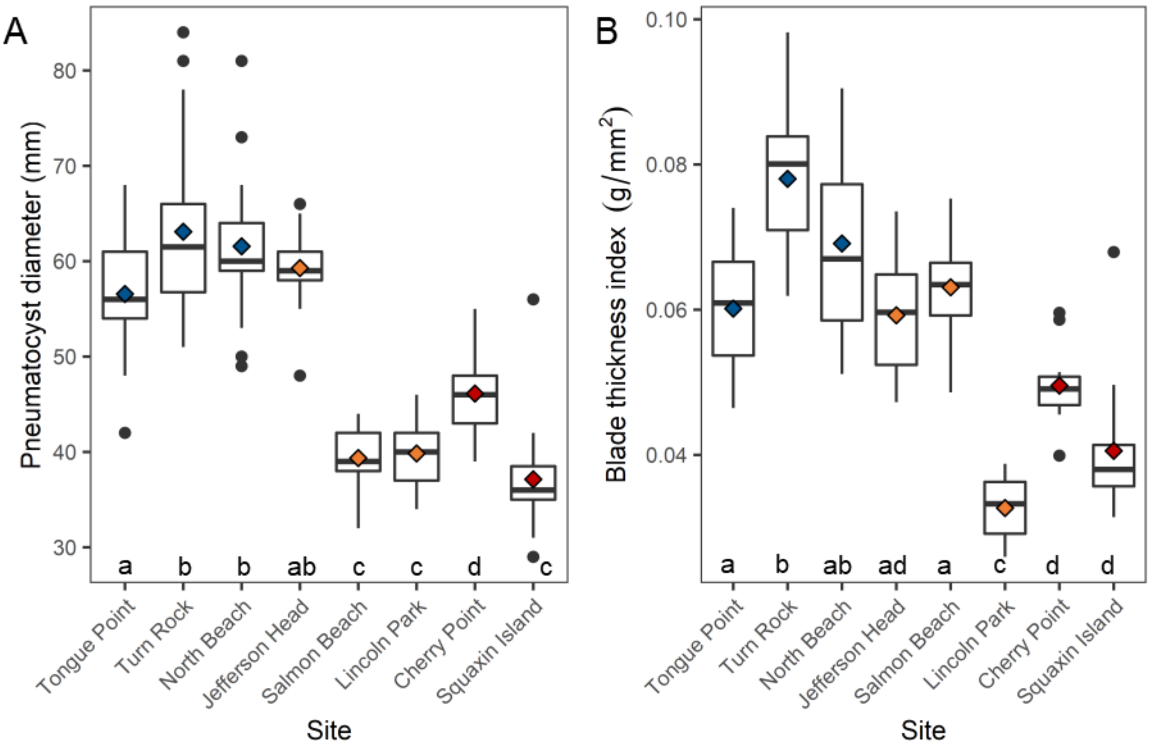

Figure 2. Morphological characteristics including (A) pneumatocyst diameter and (B) blade thickness index as boxplots and colored diamonds represent site means. Colors correspond to Fig. 1 with cold (blue), moderate (orange), and warm (red) temperature regimes. Sites are listed from cool to warm. Pneumatocysts were larger with thicker blades at cooler sites. Lowercase letters represent significant differences between sites from Tukey tests.

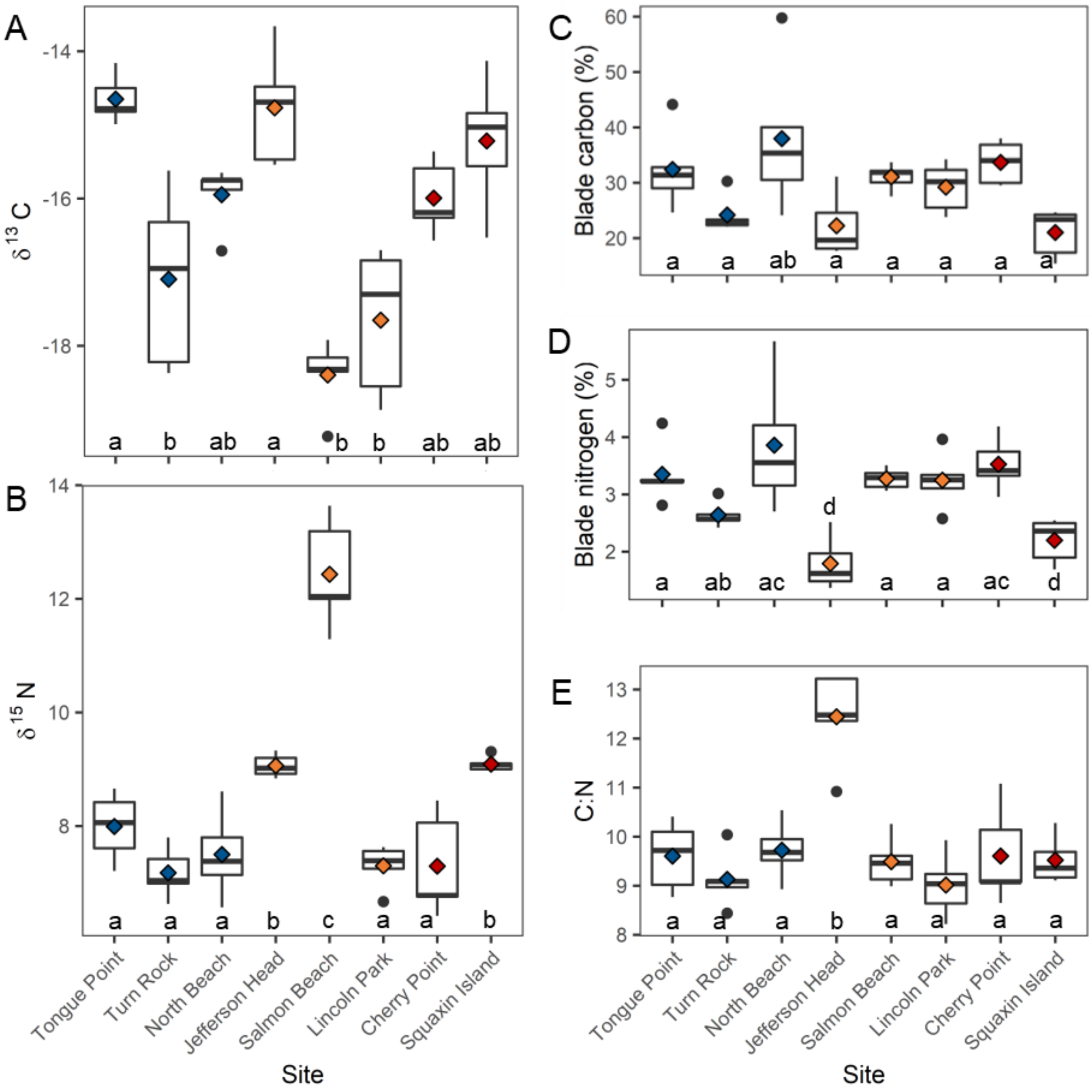

Figure 3. Nutrient characteristics of blades represented as boxplots with colored diamonds as site means for (A) δ^{13}C, (B) δ^{15}N, (C) percent blade carbon, (D) percent blade nitrogen, (E) and carbon to nitrogen ratio. Colors correspond to Fig. 1 with cold (blue), moderate (orange), and warm (red) temperature regimes. Sites are listed from cool to warm. Lowercase letters represent significant differences between sites from Tukey tests.

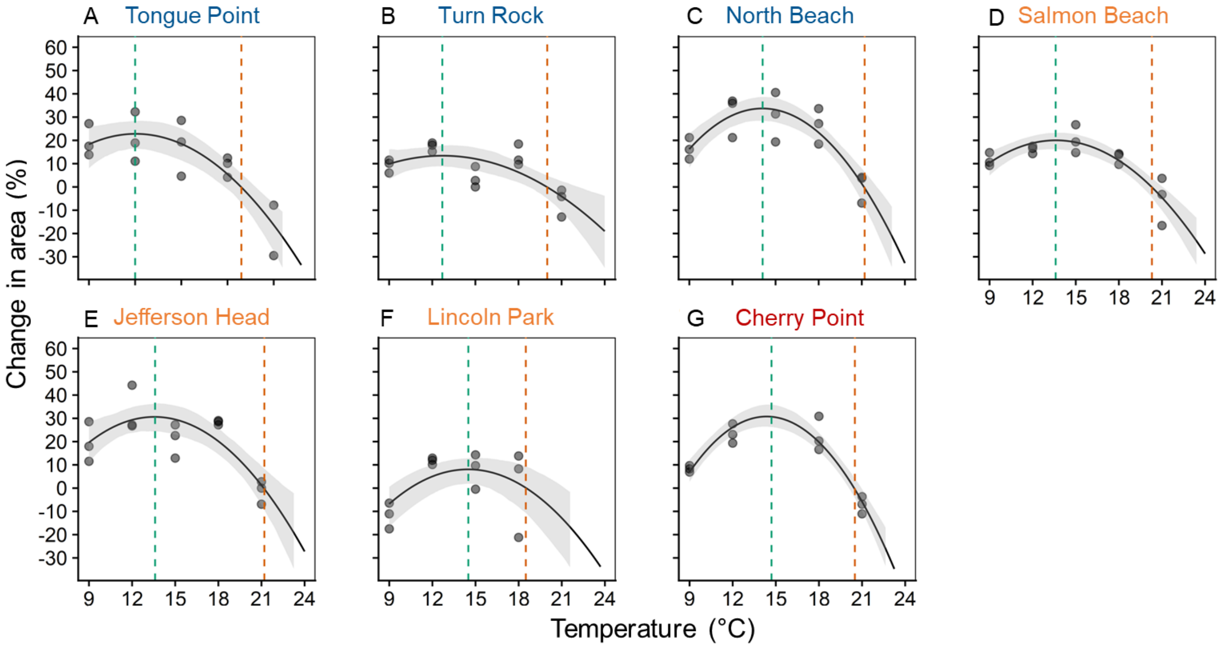

Figure 4. Thermal performance curves fit with a quadratic model for growth as percent change in area. Points represent biological replicates and gray shading indicates 95% confidence interval. T_{opt} is represented by green dashed lines and CT_{max} by orange dashed lines. Colors correspond to Fig. 1 with cold (blue), moderate (orange), and warm (red) temperature regimes. There was no growth data from Squaxin Island.

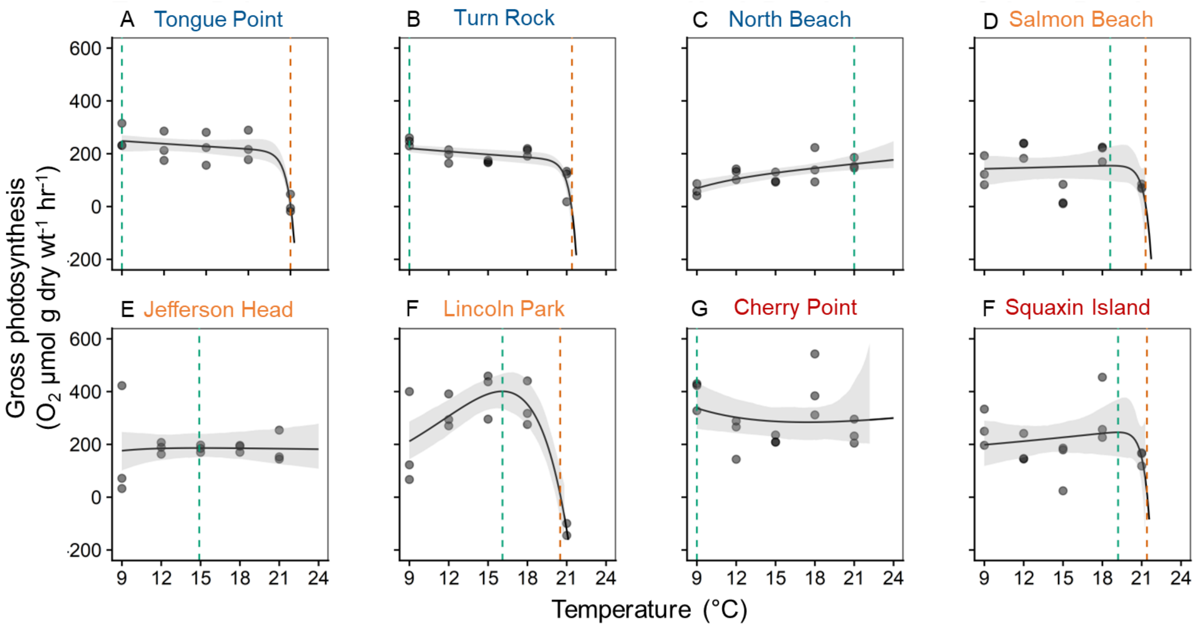

Figure 5. Thermal performance curves fit with a Spain model for gross photosynthesis. Points represent biological replicates and gray shading indicates 95% confidence interval. T_{opt} is represented by green dashed lines and CT_{max} by orange dashed lines Colors correspond to Fig. 1 with cold (blue), moderate (orange), and warm (red) temperature regimes. It was not possible to estimate CT_{max} at North Beach, Jefferson Head, or Cherry Point.

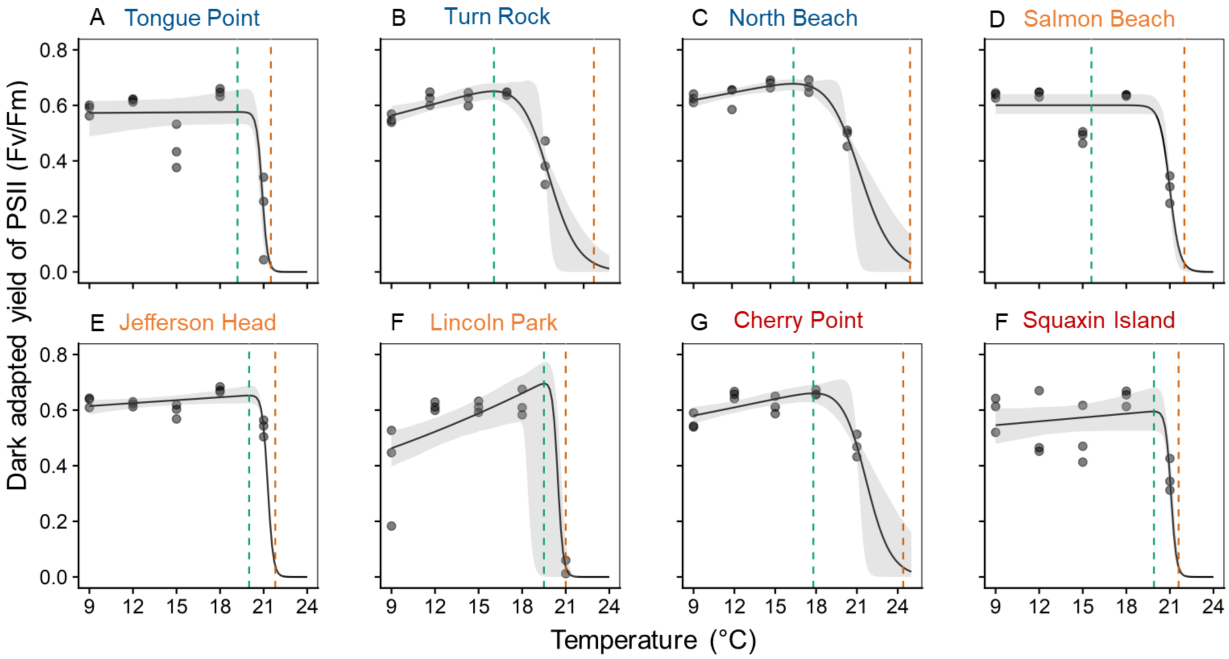

Figure 6. Thermal performance curves fit with a Johnson-Lewin model for dark adapted yield of photosystem II (Fv/Fm). Points represent biological replicates and gray shading indicates 95% confidence interval. T_{opt} is represented by green dashed lines and CT_{max} by orange dashed lines. Colors correspond to Fig. 1 with cold (blue), moderate (orange), and warm (red) temperature regimes.

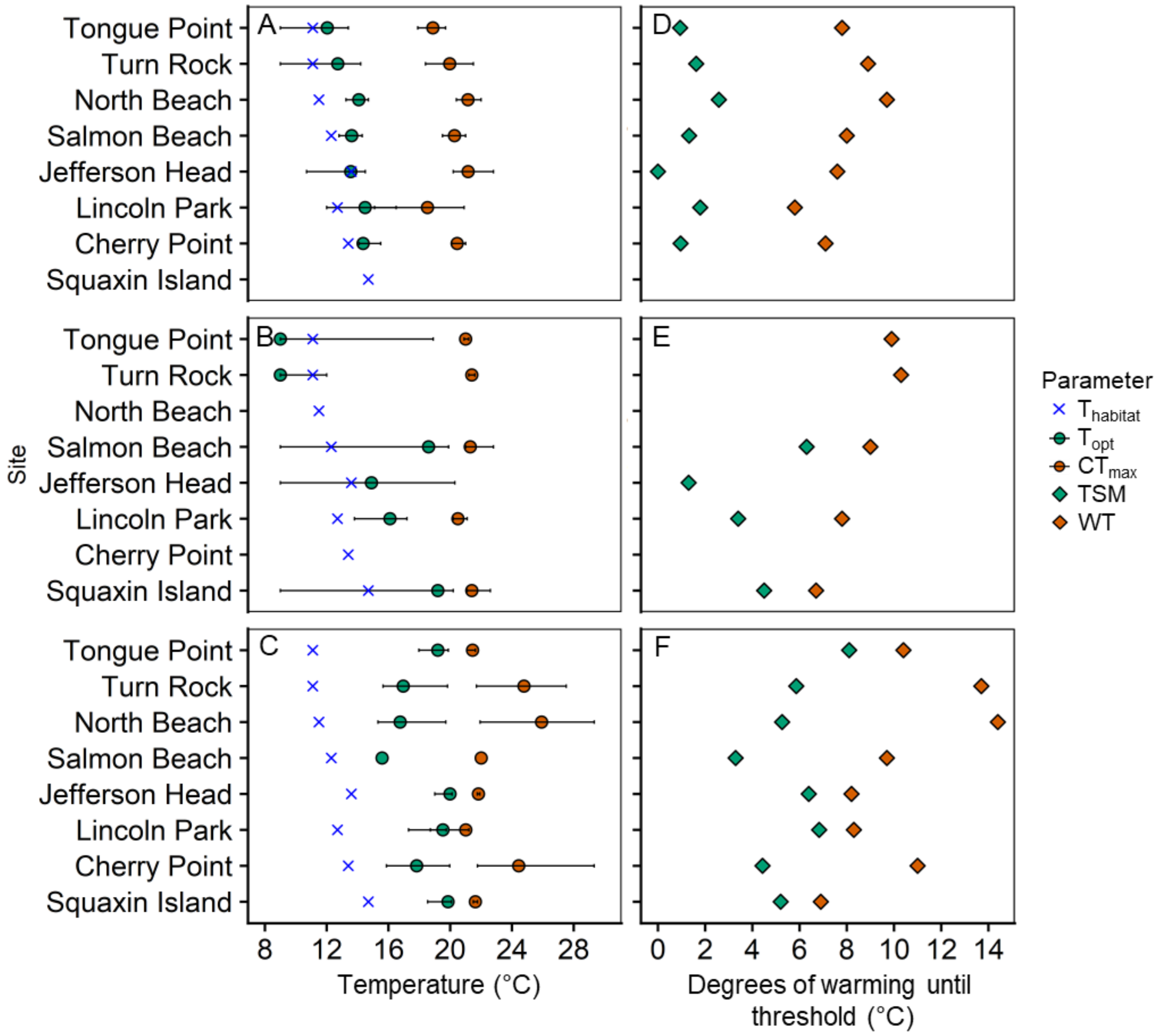

Figure 7. Parameter estimates (T_{opt} and CT_{max}) from TPCs and relationship to habitat temperature though thermal safety margins (TSM) and warming tolerances (WT) for (A & D) growth, (B & E) gross photosynthesis, and (C & F) Fv/Fm. Circles with 95% confidence intervals represent T_{opt} (green) and CT_{max} (orange), blue x represents $T_{habitat}$, and diamonds represent TSM (green) and WT (orange).

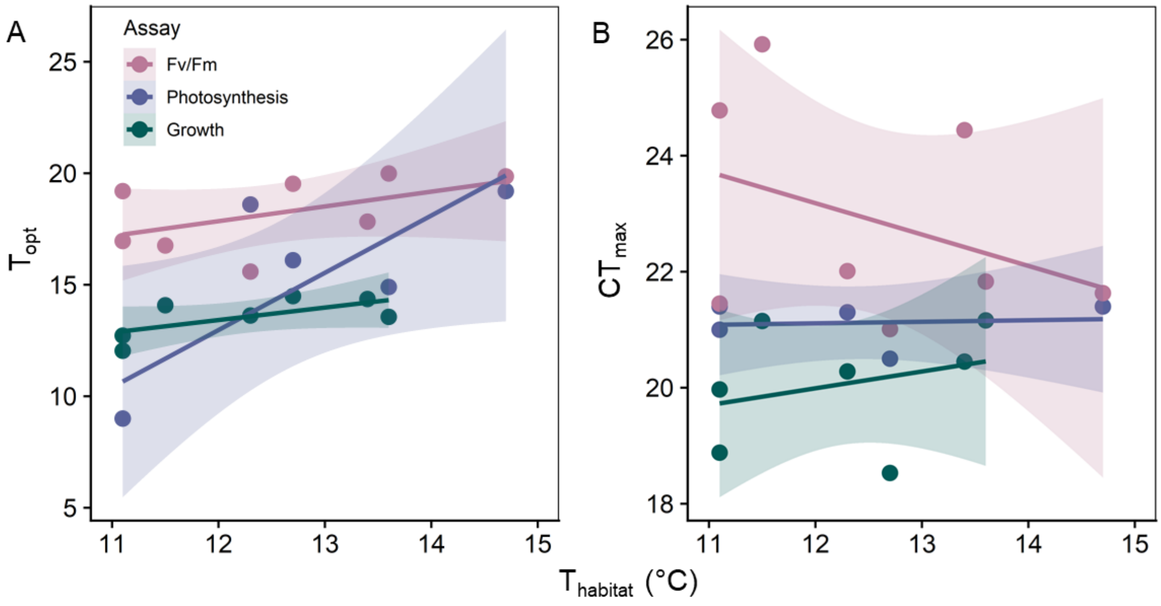

Figure 8. Linear regression comparing the relationship between (A) T_{opt} and (B) CT_{max} with $T_{habitat}$. Colors represent the three different assays measured: Fv/Fm (pink), photosynthesis (purple), and growth (green). Shading represents 95% confidence intervals.

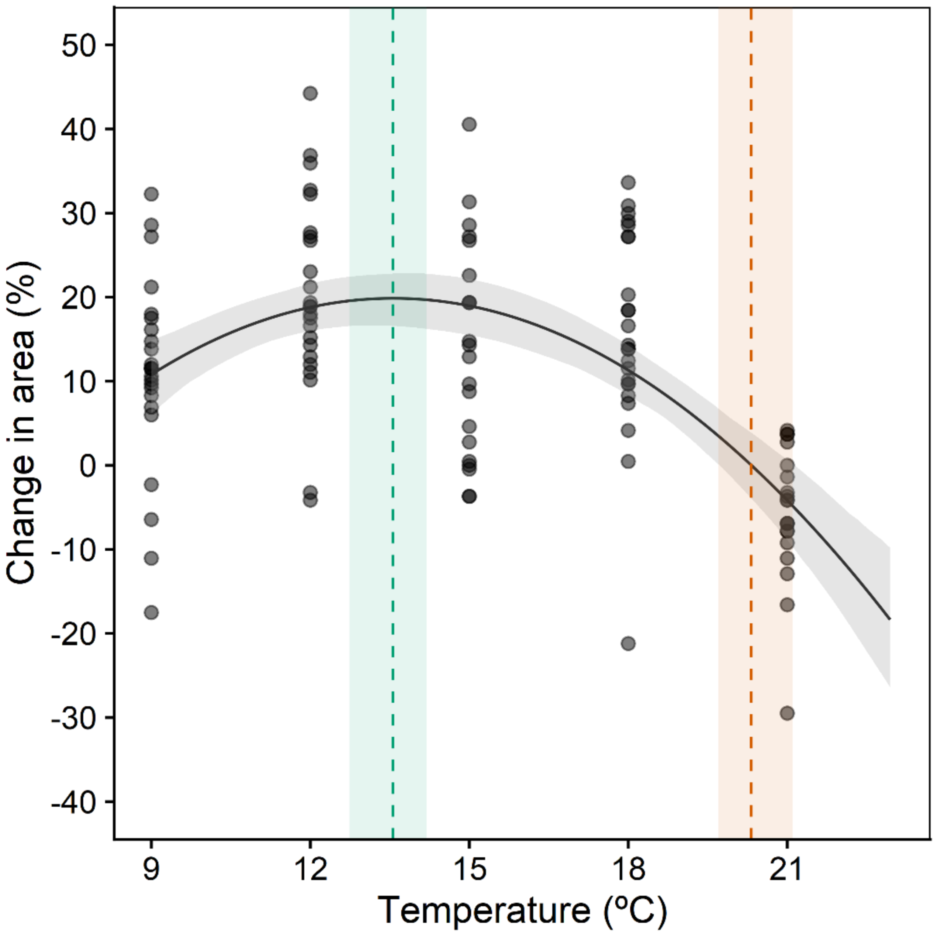

Figure 9. Salish Sea growth (percent change in area) thermal performance curve fit with a quadratic model. Points represent biological replicates across all sites and gray shading indicates 95% confidence interval. T_{opt} is represented by green dashed lines and CT_{max} by orange dashed lines with shaded 95% confidence intervals. There was no growth data from Squaxin Island.

Chapter 2 – Supplementary Materials

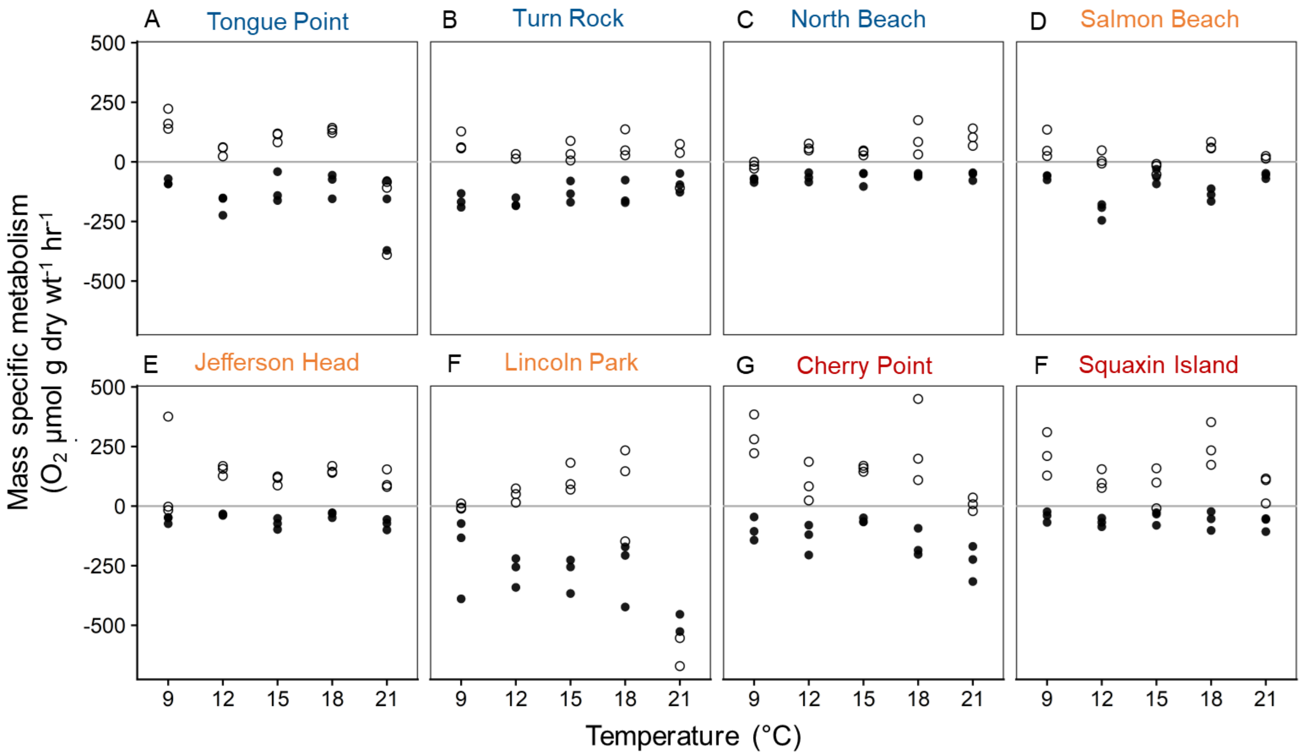

Figure S1. Mass specific metabolism with net photosynthesis (open circles) and dark respiration (closed circles) at each site. Colors correspond to Fig. 1 with cold (blue), moderate (orange), and warm (red) temperature regimes.

Interactive effects of temperature and nitrogen on the physiology of two kelps in the Salish Sea

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Abstract

Kelp forest declines have been linked to warming ocean temperatures worldwide. Ocean warming rarely occurs in isolation, so multiple stressor studies are necessary to understand the physiological responses of kelp to rapidly changing ocean conditions. The canopy-forming bull kelp, *Nereocystis luetkeana*, is going locally extinct in areas of the Salish Sea that are seasonally warm and nutrient poor, while the understory kelp, *Saccharina latissima*, persists at those sites. Further, nitrogen availability can alter physiological responses of kelps to high temperature stress, including alleviating warming stress. Thus, there is an urgent need to understand the interactive effects of these correlated stressors on kelp physiology among species and populations which can differ due to thermal history or other factors. We compared the physiological responses of kelp to high temperature stress and nitrogen limitation between two *N. luetkeana* populations with different environmental histories (warm and nutrient poor vs. cold and nutrient rich) and between two species, *N. luetkeana* and *S. latissima*. Using laboratory mesocosms, we tested the interactive effects of short term (9 day) exposure to temperatures (9-21°C) and nitrogen concentrations (low (1-3 μM) vs. high (>10 μM)) on a comprehensive suite of kelp responses: blade growth, photosynthesis, respiration, photosynthetic yield, nutrient uptake, and tissue C:N. Both kelp species responded negatively to elevated temperatures but not to low nitrogen levels. Blades of both species showed signs of metabolic stress and reduced growth at 21°C, regardless of nitrogen levels, suggesting *S. latissima* cannot outperform *N. luetkeana* under thermal stress in either high or low nutrient conditions. Populations of *N. luetkeana* from warm, nutrient poor and cool, nutrient rich areas were equally susceptible to the effects of ocean warming. Our results suggest that nutrient additions are unlikely to enhance kelp performance at supra-optimal temperatures, and a thorough understanding of coastal temperature dynamics is needed to guide conservation and restoration actions.

Introduction

The ocean is warming rapidly due to the thermal impacts of greenhouse gases, as it has absorbed more than 90% of the global excess heat generated since 1971 (Zanna et al., 2019). Global mean sea surface temperatures have increased by 0.88°C over the last century, and marine heatwaves are twice as frequent (IPCC, 2022). Some coastal regions are warming more rapidly – for example, the Salish Sea in the Northeast Pacific is projected to warm by 1.5-3°C by 2100 (Khangaonkar et al., 2019; Riche et al., 2014). Temperature exerts a major influence on organismal physiology due to the temperature dependence of biochemical reactions, including metabolic rates (Brown et al., 2004; Dillon et al., 2010). Ectothermic organisms, whose metabolism is controlled by environmental temperatures, are especially susceptible to exceeding their thermal tolerance limits. Marine ectotherms experience temperatures closer to their upper limits than terrestrial ectotherms, leading to greater vulnerability in the face of global climate change (Pinsky et al., 2019).

To persist in the face of climate change, species can acclimate, adapt, or migrate to less stressful conditions. Immobile organisms such as primary producers may be more susceptible to climate change, but they also experience high selection pressure in response to changing environmental conditions (Bennett et al., 2019). Populations can differ in their thermal tolerance limits due to acclimation to local environmental conditions or adaptive genetic differentiation (King et al., 2017). Previous thermal exposure, which tends to differ across populations in heterogeneous environments, can modify physiological responses to warming (Becheler et al., 2022; Gauci et al., 2022). For example, macroalgae sampled across latitudinal gradients that spanned a range of thermal environments displayed variable responses to heat stress (Liesner et al., 2020; Vranken et al., 2021; Wernberg et al., 2018). Knowledge of population-level variability in thermal tolerance is critical to determining how a species will respond to global climate change, as certain populations may go locally extinct.

Kelp, marine macroalgae in the order Laminariales, are foundation species that create underwater forests in temperate and subpolar regions. Kelp forests have been declining rapidly in response to warming ocean temperatures and marine heatwaves (Arafeh-Dalmau et al., 2019; Smale, 2020), with more than one-third of global kelp forests in decline over the past 50 years (Krumhansl et al., 2016). Supra-optimal temperatures are physiologically stressful leading to higher respiration rates and greater metabolic demand, which has also been shown in kelp sporophytes (Biskup et al., 2014; Gerard, 1997; Staehr & Wernberg, 2009; Umanzor et al., 2021). Photosynthesis rates usually decline above optimum temperatures while respiration rates continue to increase, leading to decreased growth rates at supra-optimal temperatures (Umanzor et al., 2021). These physiological responses to elevated temperatures can lead to decreased size and survival of sporophytes, and thus kelp forest losses. In addition to supra-optimal temperatures, low dissolved inorganic nitrogen concentrations can be stressful for marine primary producers, as they require nitrogen to sustain rapid growth rates (Fernández et al., 2020; Gerard, 1997; Umanzor et al., 2021). Nitrogen-containing chlorophyll pigments decline with nitrogen deficiency, which can reduce photosynthesis and growth rates (Korb & Gerard, 2000; Roleda & Hurd, 2019).

In temperate marine ecosystems, sea surface temperatures and dissolved inorganic nitrogen levels are often negatively correlated due to the effects of upwelling and stratification (Palacios et al., 2013). Upwelling brings cool, nutrient rich water to the surface, while thermal stratification of the water column can lead to nutrient depletion by phytoplankton in the warmer surface waters. In estuarine systems such as the Salish Sea, temperature and nutrients are driven partly by mixing associated with tidal currents, but thermal stratification and nutrient reduction still results from sustained high temperatures (Khangaonkar et al., 2021). As sea surface temperatures continue to rise and stratification increases, surface nitrate concentrations are predicted to decline (IPCC, 2022), thus there is an urgent need to understand the interactive effects of these correlated stressors on kelp physiology.

Prior experimental studies have found positive or neutral effects of nitrogen availability on kelp performance and thermal tolerance. High nitrogen concentrations improved the growth and photosynthetic rates of *Macrocystis pyrifera* blades at elevated temperatures (Fernández et al., 2020). Gerard (1997) found that the photosynthetic performance of juvenile *S. latissima* improved at high temperatures only when N reserves were maintained. Survival, photosynthesis, and growth of juvenile sporophytes of *Ecklonia cava* improved with additional nitrogen across all temperatures (Gao et al., 2016). Photosynthetic responses to simulated marine heatwaves improved with higher nitrate availability, but pigment content decreased at high temperatures despite high nitrate levels (Umanzor et al., 2021). Franco et al. (2018) found that nitrogen had no effect on the survival of *Laminaria ochroleuca* at elevated temperatures, but additional nitrogen increased growth under optimal temperatures. Studies on the interactive effects of temperature and nitrogen on early life history stages of kelp (zoospores, gametophyte, and microscopic sporophytes) found that elevated temperatures were much more limiting than low nitrogen concentrations (Mabin et al., 2013; Martins et al., 2017; Muth et al., 2019; Weigel et al., in review). These studies highlight the complexity of kelp responses to interactive stressors, including the potential for nitrogen availability to reduce the stress of high temperature, and the importance of assessing nitrogen and temperature interactions across multiple species and populations.

The annual bull kelp, *Nereocystis luetkeana*, is one of the largest canopy-forming kelp species in the Northeast Pacific. Bull kelp forests have been declining rapidly in some areas, including warmer, wave-sheltered coastlines in British Columbia (Starko et al., 2019, 2022), warm and nutrient-poor regions of South Puget Sound (Berry et al., 2021), and in northern California following the 2013 marine heatwave and sea urchin population increases (Rogers-

Bennett & Catton, 2019). Additionally, a decade-long temperature increase of 3.5°C induced by the thermal outflow from a power plant led to a 97% decline in the abundance of *N. luetkeana* in California (Schiel et al., 2004). Despite the correlation of bull kelp losses with elevated summer water temperatures and low nutrient concentrations, no studies to date have examined the interactive effects of these two stressors on the physiology of sporophytes of *N. luetkeana*.

Kelp forests often contain diverse assemblages of understory species that inhabit deeper subtidal environments and make important year-round contributions to kelp forest productivity (Bell & Kroeker, 2022). These forests also provide many important ecosystem services, including habitat for rockfish (Williams et al., 2010). In Washington state (USA), the understory kelp *S. latissima* has persisted in areas where the *N. luetkeana* canopy has disappeared (Berry et al., 2021). As an understory kelp, *S. latissima* greatly differs from *N. luetkeana* in morphology and habitat and may compete with *N. luetkeana* for space and light. Both species have benthic holdfasts and grow in the shallow subtidal; *S. latissima* grows at depths up to 20 m (Lamb & Hanby, 2005), while *N. luetkeana* can be found deeper (Abbott & Hollenberg, 1976). However, the buoyant pneumatocysts keep the majority of the blade biomass *N. luetkeana* at the surface, while blades of *S. latissima* remain prostrate near the benthos. As nitrogen concentrations are generally higher and temperatures are cooler in deeper waters beneath the thermocline due to stratification (Khangaonkar et al., 2019), it is possible that *S. latissima* has a refuge from the stressful surface conditions that surface floating sporophytes of *N. luetkeana* experience. However, thermal tolerances and nitrogen requirements often differ among kelp species (Lüning & Freshwater, 1988; Roleda & Hurd, 2019), and it is not known the extent to which the persistence of *S. latissima* at warm, nutrient poor sites is due to higher thermotolerance or nutrient acquisition abilities or stress avoidance due to its benthic habitat.

We conducted two controlled mesocosm experiments to determine the interactive effects of temperature and nitrogen on the physiology of *N. luetkeana* and *S. latissima* sporophytes. We first compared the physiological responses of *N. luetkeana* from two populations with different environmental histories (warm and nutrient poor vs. cold and nutrient rich) to test the hypothesis that a population with a history of stressful environmental conditions will perform better when challenged with thermal and/or nutrient stress, due to local adaptation or acclimation. We then compared the physiological responses of *N. luetkeana* and *S. latissima* collected from the same location to test the hypothesis that the persistence of the understory species is due to superior performance under temperature stress and nitrogen limitation. By measuring diverse response variables including growth, photosynthesis and respiration rates, photosynthetic physiology via pulse amplitude modulated (PAM) fluorometry, blade tissue C:N, and nitrogen (NO_3, NH_4) uptake rates, we aimed to gain a comprehensive mechanistic understanding of the effects of these stressors on kelp physiology. We hypothesized that photophysiology and growth would be negatively impacted by warmer temperatures, but greater nitrogen availability could mitigate the effects of high temperature stress. Additionally, we expected blade tissue nitrogen to increase with nitrogen availability, but if nutrient uptake rates are temperature-dependent, then blade tissue nitrogen may be affected by temperature as well.

Methods

Kelp blade collection

We conducted two separate experiments to examine the interactive effects of temperature and nutrients on 1) sporophyte blades of *Nereocystis luetkeana* collected from two populations with different thermal histories (hereafter referred to as “population exp.”), and 2) sporophyte blades of *N. luetkeana* and *Saccharina latissima* collected from the same location ("species exp."). For the population exp., we collected non-reproductive blades of *N. luetkeana* on July 06, 2022 from 48 distinct individuals from the population at each of two sites: 1) Cherry Point, Strait of Georgia, USA (48.855194, -122.730944) and 2) Turn Rock, San Juan Islands, USA (48.535333, -122.964778). We collected blades of *N. luetkeana* (n = 48 per population) from the surface by carefully detaching the blade from the pneumatocyst at the narrowest point. For the species exp., we collected non-reproductive blades of *N. luetkeana* from the surface and whole sporophytes of *S. latissima* (n = 42 per species) via scuba diving at Deadman Island, San Juan Islands, USA (48.457833, -122.942111) on August 04, 2022. We collected sporophytes of *S. latissima* (30-40 cm in length) from a depth of 5-9 m (relative to mean lower low water) by carefully detaching the holdfast from the substrate. We also collected extra kelp blade tissue samples from each population and species from the field for comparison with C:N content of experimental kelp (n = 8 for population exp., n = 7 for species exp.).

We transported kelp immediately back to Friday Harbor Laboratories (FHL) in coolers with seawater and ice packs, and placed samples into flow-through seawater tanks at 11-12°C. On the day of collection, we cleaned each replicate of epiphytes, cut the blades to a standard length, and tagged each replicate by sewing fishing line with small numbered beads into the thick narrow tissue at the proximal end of the blade of *N. luetkeana* or the stipe of *S. latissima*. We cut the blades of *N. luetkeana* to a length of 40 cm from the blade base in the population exp., and 30 cm from the blade base in the species exp. to better match the size of *S. latissima*. In addition, we removed the holdfasts from *S. latissima* and cut blades to 30 cm so both species had equivalent tissue types and initial wounding levels.

Experimental design

We manipulated seawater temperatures and nutrient concentrations using 100 L (91 x 34 x 30 cm; L x W x H) recirculating tanks in the FHL Ocean Acidification Environmental

Laboratory (Guenther et al., 2022; O'Donnell et al., 2013). Natural seawater was UV-sterilized to deter phytoplankton and diatom growth prior to starting experiments. For both experiments, treatments included 3 static temperatures crossed with 2 nitrogen levels (n = 7-8 blades per treatment and population). Our biological replicates were individual blades that were mixed between both populations or species within tanks; replication for each treatment and response variable can be found in Table S1. To establish low N levels (<3 μM), kelp blades drew down dissolved nitrogen from starting mean (±SD) NO_3 levels of 23.40 ± 0.22 μM (population exp.) and 18.14 ± 0.01 μM (species exp.) over 2 days. Temperatures were 13, 16 and 21°C in the population exp. and 9, 15 and 21°C in the species exp. Temperatures in the species exp. were slightly lower to mimic relatively cooler conditions likely experienced by *S. latissima* in the understory of a stratified water column. We manipulated temperatures in each tank following the methods in O'Donnell et al. (2013) and Guenther et al. (2022) and measured temperature (±0.1°C) with HOBO Pendant Temperature/Light Data Loggers (Onset model #MX2202, Bourne, MA) every 5 min. We slowly changed temperatures (by 2°C per day) over 5 days until final treatment temperatures were reached (Fig. S1). LED lights (5000K daylight) provided 100 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation to the water surface on a 16:8 light-dark cycle. Additionally, we added two submersible micro-pumps (Aquaneat BP-50, CLL Pet Supplies, Madison, WI) in each tank to move blades around and reduce boundary layers. Finally, we exposed kelp blades to temperature and nutrient treatments (described below) for 8 days (population exp.) and 9 days (species exp.), after which we measured response variables (Fig. S1).

After a 2 day period of initial nutrient drawdown to allow kelp to deplete nitrogen in the low N tanks, which occurred at the start of the 5-day acclimation period (Fig. S1), we manipulated nutrient concentrations in recirculating tanks with daily nutrient additions of sodium nitrate ($NaNO_3$), ammonium sulfate ($(NH_4)_2SO_4$), and sodium phosphate (NaH_2PO_4).

We calculated targeted nutrient additions based on tank volume, expected starting concentrations of dissolved inorganic nutrients, and established nutrient uptake rates for *N. luetkeana* (Weigel & Pfister, 2021) and *S. latissima* (Forbord et al., 2021). We aimed to manipulate nitrogen levels, so we added phosphate in a 16:1 N:P ratio to ensure that phosphorus did not become limiting. For the population exp., we aimed to maintain dissolved nutrient concentrations (μM) of 35 NO_3, 2 NH_4 and 2.2 PO_4 (high N treatment) vs. 5 NO_3, 0.5 NH_4 and 0.3 PO_4 (low N treatment). Starting seawater N availability (Table S2) was high (23 μmol NO_3) for the San Juan Islands (Murray et al., 2015), and nitrogen uptake rates were lower than expected in the population exp., causing a slow increase in N availability over the experiment that led to higher than targeted N levels (Fig. S2). Therefore, in the species exp. we targeted lower dissolved nutrient concentrations (μM) of 15 NO_3, 2 NH_4 and 1.9 PO_4 (high N treatment) vs. 1 NO_3, 0.1 NH_4 and 0.06 PO_4 (low N treatment).

To monitor whether nutrients were at targeted levels, we collected seawater samples from each tank 2 hours after daily nutrient addition, samples were filtered through 0.7 μm glass-fiber filters and frozen until analysis. The University of Washington Marine Chemistry Lab quantified seawater inorganic nutrient concentrations (NO_3, NH_4, PO_4) using standard methods (UNESCO 1994).

Response variables

After collecting sporophyte blades, we weighed blotted blades to determine the initial wet biomass, photographed each individual with a reference scale to determine the initial surface area and length, and measured initial dark adapted photosystem II yield with a PAM fluorometer (described in detail below). We also collected measurements of growth (wet weight, length, surface area) and PAM response variables after the acclimation period and at the conclusion of the treatment period (Fig. S1). We measured length and surface area from photographs with a reference scale at 1 mm resolution by outlining each kelp blade in ImageJ

(Schneider et al., 2012). The blade area of *S. latissima* was underestimated by this method because of the bullate blade morphology, compared to the smooth blades of *N. luetkeana*. We calculated change in wet mass (hereafter biomass) as percent change from the beginning to the end of the treatment period (Fig. S1). Finally, we calculated growth rates (of blade length and area) as the difference between final and initial measurements and divided by the number of days in treatment.

We measured dark adapted yield of photosystem II (Fv/Fm) with a Pocket PEA Chlorophyll Fluorimeter (Hansatech Instruments, Norfolk, England) following 15 min of dark adaptation under leaf clips. We collected one measurement 10 cm from the base of the blade at the same time of day. We collected Fv/Fm measurements daily in the population exp., and only on days 1, 6, and 14 in the species exp. As with the growth rates, we calculated percent change in Fv/Fm from the beginning to the end of the treatment period (Fig. S1).

After 6 to 8 days of exposure to experimental treatments (Fig. S1), we quantified mass-specific metabolic rates (net photosynthesis and dark respiration) from changes in dissolved oxygen (DO) over time. We measured DO (in mg/L) optically with a FireSting-GO2 pocket oxygen meter equipped with a robust oxygen probe and a temperature sensor (Pyroscience, Aachen, Germany). We incubated individual blades in 1.6 L clear Nalgene bottles filled with seawater from the corresponding tank and maintained the treatment temperature by submerging the bottles. Bottles were sealed water-tight with an 8 mm rubber stopper to allow for quick access with minimal disturbance during DO measurements. A large (1,000 GPH) aquarium pump shook the bottles around in the tanks to ensure that the seawater inside was well-mixed.

We measured dark respiration from 75 min incubations under dark tarps with lights off, then photosynthesis from 45 min incubations in 100 μmol $m^{-2}s^{-1}$ of light. Prior to the measurements for respiration, we acclimated blades to dark conditions for 15 mins to allow oxygen to equilibrate in the bottles. During each run, we had a seawater control to account for potential plankton metabolism. To accommodate all replicate blades in the experiment, we split measurements into multiple runs over 2 days (Fig. S1). We measured the biomass of the blades immediately after metabolic rates, and converted to dry mass based on the relationship between wet mass and dry mass from blades sampled at the end of the experiment. We calculated photosynthesis and respiration rates (mg O_2 g^{-1} hr^{-1}) as:

$$metabolic\ rate = \left(\frac{\Delta\, O_2 - C}{t}\right) * \left(\frac{V}{m}\right)$$

Where ΔO_2 is the change in DO in mg L^{-1} between the start and end of the incubation, C is the seawater correction factor due to metabolism by plankton, t is the incubation time in hours, V is the volume of the bottle in liters, and m is the dry mass of the blade in grams.

On the final day of each experiment (Fig. S1), we quantified kelp blade nutrient uptake rates on a random subset of blades in each treatment (n = 4 per treatment). Each kelp blade was placed into a sealed 1.6 L clear Nalgene bottle filled with seawater from the corresponding tank and maintained at the treatment temperature. Therefore, low N treatments had lower nutrient availability at the start of the nutrient uptake assay. We collected initial seawater nutrient samples from the well-mixed tank prior to filling bottles and final seawater nutrient samples from each individual chamber after an incubation period of 100 mins. We included seawater control bottles to account for possible nutrient uptake by plankton in the seawater. We filtered seawater samples through 0.7 µm glass-fiber filters and froze the samples until analysis of NO_3, NH_4, and PO_4 concentrations. We calculated nutrient uptake rates (µmol $g^{-1}hr^{-1}$) as:

$$uptake\ rate = \left(\frac{\Delta N - C}{t}\right) * \left(\frac{V}{m}\right)$$

Where ΔN is the change in NO_3 or NH_4 concentration (µmol L^{-1}) between the start and end of the incubation, C is the seawater correction factor due to uptake by microalgae, V is the volume of the chamber in liters, *m* is the dry mass of the blades in grams, and *t* is incubation period in hours.

At the end of each experiment, we dried kelp blade tissues to constant mass in a drying oven. We pulverized dried kelp blade tissues (field-collected and experimental) with a Retsch Mixer Mill 400 tissue grinder. The Washington State University Stable Isotope Core Laboratory analyzed these samples for percent carbon and nitrogen on an elemental analyzer.

Data Analyses

We used R version 4.1.2 for all data analyses and visualizations (R Core Team 2021). Using the “car” package in R, we ran multi-factor ANOVAs with temperature, nutrients, and population (population exp.) or species (species exp.), and their interactions, as fixed effects (Fox & Weisberg, 2019). We ran separate models on each response variable for each experiment. We used Type III Sum of Squares ANOVAs to account for our unbalanced design (in the species exp., as some kelp died at the highest temperature, Table S1) as well as interactions among factors. Following significant ANOVA outcomes, we performed Tukey post hoc tests with the package “emmeans” (Lenth et al., 2023).

Results

Site differences and efficacy of treatments

On the day of the kelp collections, seawater dissolved inorganic nutrient levels were high at Turn Rock (NO_3: 23.4, NH_4: 0.2, PO_4: 1.8 μM) and low at Cherry Point (NO_3: 0.8, NH_4: 0.4, PO_4: 0.8 μM; Table S2). In the summer of 2022, from July 07 to Sept. 27, Cherry Point experienced elevated water temperatures (mean ± SD: 13.3 ± 1.8°C, min: 10.3°C, max: 20.5°C), while Turn Rock (mean ± SD: 11.3 ± 0.8°C, min: 10.0°C, max: 15.2°C) remained cool (Fig. S3, Weigel et al. in review). Deadman Island temperatures are generally cool, and likely similar to Turn Rock due to their close proximity (8 km). Nutrient levels at Deadman Island were high on the day of collection (NO_3: 19.1, NH_4:0.7, PO_4: 1.7 µM; Table S2).

Temperature treatments in the population and species experiments were stable and within 1°C of the targeted range. In the population exp., temperatures as mean ± SD and ranges as (min, max) were 12.7 ± 0.2°C (12.1, 13.4), 16.2 ± 0.1°C (16.0, 16.9), and 20.6 ± 0.1°C (20.2, 20.8) during the full treatment period (Fig. S1A). In the species exp., temperatures were 9.0 ± 0.3°C (8.3, 9.2), 14.6 ± 0.1°C (14.4, 15.4), and 20.7 ± 0.2°C (20.1, 21.6) during the full treatment period (Fig. S1B). Meanwhile, nutrient treatments were within targeted range for the species exp. but were higher than expected in the population exp. Following nutrient draw down, mean NO_3 levels in the low N treatments remained around 1.0 µM (species exp.) and below 3 µM (population exp.; Table S2, Fig. S2). In the population exp., NO_3 levels in the high N treatments increased gradually over time from 25 up to 100 µM, with a mean of ~80 µM (Table S2, Fig. S2). We reduced daily nutrient additions for the species exp., so mean NO_3 levels in the high N treatments were between 10 and 20 µM (Table S2, Fig. S2). Despite the difference in NO_3 concentrations in the high N treatments between the two experiments, there were clear differences between low and high N treatments in each experiment (Fig. S2).

Survival and condition

In both experiments, we observed mortality or visual tissue damage and decay only at the highest temperature of 21°C (Fig. S4). In the population exp., survival was 100% across all treatments, but signs of damage to kelp blades were visible in both high and low nutrient treatments at 21°C. In the species exp., survival was 100% across nutrient treatments in the 9 and 15°C treatments, but not in the 21°C treatments. Specifically, at the conclusion of the species exp., the 21°C high N treatment had high mortality (*N. luetkeana*: 71%, *S. latissima*: 57%) while the 21°C low N treatment had low mortality (*N. luetkeana*: 0%, *S. latissima*:

14%). Mortality prevented measurement of some response variables at the highest temperature.

Growth rates

In both experiments, blade growth rates responded to temperature and nutrient treatments, as well as source population or species (Table 1). All aspects of blade growth, whether measured as change in biomass, length, or blade area, were significantly lower at 21°C compared to the two cooler temperatures in each experiment (Figs. 1 & 2, $P < 0.05$). At 21°C, change in biomass was negligible in the population exp. and negative in the species exp., indicating a net loss of tissue (Fig. 1). All aspects of growth (wet biomass, length, area) were positive at the two cooler temperatures (population exp: 13 and 16°C, species exp: 9 and 15°C) in each experiment (Figs. 1 & 2). Further, for all metrics of growth, there were no differences between growth rates at the cool and moderate temperatures ($P > 0.05$), indicating that kelp grew equally well at 13 vs. 16°C and at 9°C vs. 15°C (Fig. 1, Fig. 2). The only exception was that in the species exp., *N. luetkeana* displayed a 0.5 cm/day higher elongation rate at 15°C compared to 9°C ($P < 0.001$, Fig. 2B). At these cooler temperatures, blades in both experiments grew ~15-30% in overall biomass (Fig. 1), 1.0-1.5 cm in length per day, and 5-10 cm^2 in area per day (Fig. 2), demonstrating that laboratory conditions were suitable to both species.

Kelp blade growth metrics responded to nitrogen in both experiments (Table 1). In the population exp., change in biomass and length responded significantly to the interaction between temperature and nitrogen ($P < 0.001$, Table 1). Change in biomass in the low N relative to the high N treatment was 11% higher at 13°C, and 2% higher at 16°C (Fig. 1A). Additionally, kelp from the low N treatment elongated by 0.5 cm more per day at 13°C ($P > 0.05$, Fig. 2A). In the species exp., there were no interactions between temperature and nitrogen, but there were significant effects of nitrogen on all growth metrics ($P < 0.05$, Table

1). Kelp from the low N treatments displayed higher growth rates compared to kelp from high N treatments in the species exp. On average, *N. luetkeana* blades in low N treatments grew 7% more in biomass, 0.4 cm/day in length, and 4 cm^2/day in area compared to blades in high N treatments. Likewise, *S. latissima* grew 9% more in biomass, 0.1 cm/day in length, and 1.2 cm^2/day in area in low N compared to high N treatments (Figs. 1B, 2B & D). However, change in biomass was low to negative at 21°C in both experiments regardless of nitrogen levels, with an average loss of 9% biomass while cooler temperatures had increases (Fig. 1).

Finally, there were significant effects of source population and species on kelp growth metrics (Table 1). In the population exp., blades of *N. luetkeana* from Turn Rock grew significantly more in biomass compared to those from Cherry Point by 4% (Fig. 1A), while *N. luetkeana* from Cherry Point grew significantly more in total blade area than those from Turn Rock by 3 cm^2 per day, averaged across treatments (Fig. 2C), yet the two populations had equivalent changes in length (Fig. 2A). In the species exp., *N. luetkeana* increased in biomass by 13% more than *S. latissima* across all temperatures (Fig. 1B), while *N. luetkeana* elongated more only at 15°C (Fig. 2B) and *N. luetkeana* grew more in blade area compared to *S. latissima* at 15°C and 21°C (Fig. 2D).

Photosynthesis and respiration rates

In both experiments, net photosynthesis responded to temperature and nutrients, as well as their interaction (Table 2). Net photosynthesis rates increased with temperature in the population exp., but were highest at the moderate temperature in the species exp. (Fig. 3A & B); however, these temperature effects interacted with nutrients. In the population exp., net photosynthesis increased significantly at each temperature under low N conditions, with the greatest net photosynthesis at 21°C ($P < 0.05$, Fig. 3A). At high N, net photosynthesis was 0.7 mg O_2 g^{-1} hr^{-1} lower at the coldest temperature ($P < 0.0001$, Fig. 3A), but did not differ between 15 and 21°C ($P = 0.4$, Fig. 3A). Additionally, net photosynthesis was 0.1 mg O_2 g^{-1}

hr^{-1} higher at low N than high N at 13 and 21°C than at 16°C ($P < 0.05$, Fig. 3A), but nutrient treatments did not differ at 16°C ($P > 0.05$, Fig. 3A). In the species exp., net photosynthesis did not differ with temperature in the low N treatments ($P > 0.05$), but net photosynthesis was 0.9 mg O_2 g^{-1} hr^{-1} higher at 15°C compared to 9 and 21°C in the high N treatments ($P < 0.05$, Fig. 3B). Finally, net photosynthesis of both species only responded to nutrients at 21°C, where low N kelp had 0.8 mg O_2 g^{-1} hr^{-1}greater net photosynthesis compared to high N kelp ($P < 0.001$, Fig. 3B).

In both experiments, respiration increased with temperature and kelp blades consumed the most oxygen via respiration at 21°C (Table 2, Fig. 3C & 3D, $P < 0.05$). Respiration was not affected by nutrients in the population exp, but responded significantly to the interaction between temperature and nutrients in the species exp. (Table 2). In the species exp., high N kelp had 0.3 mg O_2 g^{-1} hr^{-1} greater respiration rates at 21°C than low N kelp ($P < 0.001$, Fig. 3D). Finally, we found a significant effect of population but not species on net photosynthesis and respiration rates (Table 2). Kelp from Cherry Point had higher net photosynthesis and respiration rates compared to Turn Rock by 0.25 and 0.1 mg O_2 g^{-1} hr^{-1}, respectively (Table 2, Figs. 3A & C).

Dark-adapted yield of PSII (Fv/Fm)

Change in Fv/Fm only responded to temperature in the species exp. (Fig. 4B), but not in the population exp. (Table 2), where change in Fv/Fm during the treatment period was close to 0% (Fig. 4A, Fig. S4). Nutrients did not affect photosynthetic yield in either experiment (Table 2). In the population exp., the decline photosynthetic yield at high temperatures was 3.1% more in kelp from Turn Rock than Cherry Point (Table 2, Figs. 4A & S5). In the species exp., Fv/Fm responded significantly to the interaction between temperature and species, with 14.5% greater declines in Fv/Fm for *N. luetkeana* than *S. latissima* at 21°C ($P < 0.0001$, Fig. 4B).

Nitrate and ammonium uptake rates

As expected, uptake rates of nitrate (NO_3) and ammonium (NH_4) were concentration dependent. NO_3 and NH_4 uptake rates were significantly higher and increased in the high N treatments compared to the low N treatments across both experiments, reflecting the difference in nitrogen availability (Table 3, Fig. 5A-D). However, in the population exp., NO_3 and NH_4 uptake rates were higher in the high N treatment only at 16 and 21°C by 1.6 µmol NO_3 $g^{-1}hr^{-1}$ and 0.1 µmol NH_4 $g^{-1}hr^{-1}$ ($P < 0.05$, Fig. 5A, C). In the species exp., NO_3 and NH_4 uptake rates increased significantly at high N by 0.3 – 1.4 µmol NO_3 $g^{-1}hr^{-1}$ and 0.4 – 0.6 µmol NH_4 $g^{-1}hr^{-1}$ (Table 3, Fig. 5B, D). However, we note that nutrient uptake rates were not measured for the warmest high N treatment (21°C high N) in the species exp. due to blade mortality in that treatment, making it difficult to evaluate interactive effects across temperatures.

Nitrate uptake rates generally increased with temperature across experiments (Fig. 5 A, B), but there were significant interactive effects of temperature and nitrogen in the population exp. (Table 3). In the species exp., both NO_3 and NH_4 uptake rates were greater at higher temperatures and nutrient levels, and there were no significant interactions (Table 3, Fig. 5B, D). In the population exp., there was an interactive effect of temperature and nutrients (Table 3), specifically NO_3 uptake rates increased with temperature only in the high N treatment ($P < 0.001$) and did not differ across temperatures at low N ($P > 0.05$). In contrast, NH_4 uptake rates in the population exp. only differed across temperatures at low N, where NH_4 uptake rates were greater at 13°C compared to 16°C by 0.2 µmol NH_4 $g^{-1}hr^{-1}$ ($P < 0.05$, Fig. 5C). Finally, NH_4 uptake rates responded significantly to the interaction between nutrients and population (Table 3); in the low N treatment, kelp from Turn Rock had higher NH_4 uptake rates (by 0.2 µmol NH_4 $g^{-1}hr^{-1}$) than those from Cherry Point ($P < 0.01$, Fig. 5C).

In the species exp., *S. latissima* had higher NH_4 uptake rates than *N. luetkeana* at 9 and 15°C by 0.1 µmol NH_4 $g^{-1}hr^{-1}$ (Table 3, Fig. 5D).

Blade tissue percent carbon and nitrogen

At the end of each experiment, blade tissue % nitrogen was significantly higher in the high N treatment compared to the low N treatment (Table 3, Fig. 6E, F), demonstrating that kelp blade nitrogen content was successfully manipulated in both experiments. Compared to field-collected blade tissue samples, the nitrogen content of the high N kelp in the population exp. was 0.7-1.0% higher ($P < 0.05$), while the nitrogen content of the low N kelp did not differ from field-collected samples ($P > 0.05$, Fig. 6E). In the species exp., neither treatment differed significantly from the nitrogen content of field-collected samples (Fig. 6F).

Kelp blade nitrogen content also changed significantly within the experimental treatments. In the species exp., nutrient treatment was the only factor that affected kelp nitrogen content (Table 3), while in the population exp., blade tissue % nitrogen also responded to temperature, population and the interaction between temperature and nitrogen (Table 3). Surprisingly, despite lower seawater NO_3 levels at Cherry Point (Table S2), the blade tissue nitrogen content of *N. luetkeana* from Cherry Point was significantly higher than from Turn Rock at the time of collection (Table 3, Fig. 6E). At high nutrient levels in the population exp., percent nitrogen at 16°C was lower by 0.3% compared to 13 and 21°C ($P < 0.05$, Fig. 6E), while at low nutrient levels, % nitrogen was 0.4% lower at 13°C ($P < 0.05$, Fig. 6E).

In the population exp., the carbon content of kelp blades differed significantly with temperature, nitrogen, and by population (Table 3, Fig. 6C). The carbon content of kelp in the species exp. did not differ with any experimental factor (Table 3, Fig. 6D). In the population exp., *N. luetkeana* from Cherry Point had 2.7% higher carbon content than those from Turn Rock (Table 3, Fig. 6E). In addition, kelp from the high N treatment had 4.6% greater percent carbon compared to kelp from the low N treatment (Table 3, Fig. 6E). Finally, kelp blade carbon content responded significantly to temperature; percent carbon was 2.9% higher at 21°C compared to 13°C ($P=0.046$), but neither treatment differed from 16°C (Fig. 6E).

Correspondingly, there were also differences in the C:N ratio of kelp blade tissues in both experiments with temperature, nitrogen, and species or population (Table 3, Fig.6A, B). In both experiments, kelp from the low N treatment had a higher C:N compared to kelp from the high N treatment, by 1.1 for the population exp. and 3.1 for the species exp. (Fig. 6A, B), reflecting the differences in nitrogen content between treatments. In the population exp., *N. luetkeana* from Turn Rock had higher blade tissue C:N by 1.1 compared to those from Cherry Point (Table 3, Fig. 6A). There also was a significant effect of temperature on blade tissue C:N in the population exp. (Table 3), where kelp from 16°C had a 0.7 higher C:N compared to kelp from 13 and 21°C ($P < 0.05$, Fig. 6A). In the species exp., blade tissue C:N responded significantly to the interaction between temperature and species (Table 3), where the C:N of *N. luetkeana* was 1.2 higher at 9 than 21°C ($P = 0.046$), while the C:N of *S. latissima* was 1.6 higher at 15 than 21°C ($P = 0.0024$, Fig. 6B). Finally, the two species only differed in blade tissue C:N at 15°C ($P = 0.0003$), where *S. latissima* had a 1.5 higher C:N than *N. luetkeana* (Fig. 6B).

Discussion

We found that despite their different morphologies and habitats, the canopy-forming kelp *Nereocystis luetkeana* and the understory kelp *Saccharina latissima* responded strongly and similarly to elevated temperatures, showing signs of metabolic stress and low to negative growth rates at 21°C. We found interactive effects of temperature and nitrogen stress, but surprisingly, no negative impacts of low NO_3 concentrations (1-3 μM) on the growth, metabolism, or photophysiology of either species. Our findings suggest that physiological damage from exposure to supra-optimal temperatures (21°C) can be greater than stress from short-term nutrient limitation, thus temperature should be considered a primary stressor. Additionally, we found few differences in the physiological responses of *N. luetkeana* from two populations with different environmental histories, suggesting that kelp from warm and cool areas are equally susceptible to the effects of ocean warming. Finally, we did not find evidence that *S. latissima* could outperform *N. luetkeana* under temperature stress and nitrogen limitation.

Kelps require nitrogen to sustain rapid growth rates, as both amino acids (the building blocks of proteins) and chlorophyll pigments are nitrogen rich (Korb & Gerard, 2000). In some cases, nitrogen-replete conditions can alleviate the effects of high temperature stress (Fernández et al., 2020), and most studies report higher physiological stress under warm temperatures combined with low nitrogen levels (Endo et al., 2020; Gao et al., 2016; Gerard, 1997; Umanzor et al., 2021). In contrast, we found that low nitrogen levels were not physiologically stressful for *N. luetkeana* or *S. latissima* sporophytes and high nitrogen levels did not improve survival, growth, or photophysiology at high temperatures. We found that nitrate levels as low as 1- 3 μM supported high growth rates at cold to moderate temperatures of 9 to 16 °C for up to 9 days. These low nitrogen concentrations are similar to the nutrient-poor conditions experienced during the summer by declining kelp populations in southern Puget Sound (Berry et al., 2021), yet were not limiting to kelp growth at any temperature in our experiments.

While our study indicated strong physiological effects of elevated temperatures and no deleterious effects of low nitrogen or rescue from thermal stress with the availability of nitrogen, the experimental duration (8-9 days) was short. Other studies testing the interactive effects of temperature and nitrogen stress have also occurred over short durations of 3 to 5 days (Fernández et al., 2020; Umanzor et al., 2021). In the Salish Sea, stressful conditions can span hours, days, weeks or even months in response to tidal cycles, mixing, wind, bathymetry, and other physical factors. For example, daily maximum seawater temperatures exceeded 16°C for 50 consecutive days in a kelp forest in southern Puget Sound (Weigel et al., in review), and subtidal daily maximum temperatures at Cherry Point exceeded 18°C on multiple days in the summer (Fig. S3). We hypothesize that longer periods of elevated temperatures would exacerbate blade tissue damage, which was already present after 8-9 days of exposure to 21°C (Fig. S4). In contrast, the effects of longer duration nutrient limitation are harder to predict. Blade tissue nitrogen declined significantly after 8-9 days in low N treatments, suggesting that nitrogen stores would become depleted with persistent nutrient limitation. Gerard (1997) found that holding juvenile *S. latissima* for 1.5 to 6 weeks with no nutrient enrichment depleted internal N reserves to growth-limiting levels of 1.8% nitrogen. In our experiments, high vs. low N treatments had a mean of 3.0 vs. 2.3% and 2.5 vs. 2.0% nitrogen in the population and species exp., respectively. It is possible that the blades could have drawn from internal reserves during the limited duration of our study, as kelps are known to store nitrogen for later use (Gerard, 1982; Korb & Gerard, 2000; Pueschel & Korb, 2001). Our experiments, as well as others, show that nutrient uptake rates can increase with temperature (Harlin & Craigie, 1978; Nishihara et al., 2005; Roleda & Hurd, 2019). Both NO_3 and NH_4 uptake rates increased with temperature, which could increase nitrogen supply, but only if there is sufficient dissolved inorganic nitrogen available. Over longer time periods, nutrient limitation likely plays an increasingly important role in kelp physiological performance as reserves are depleted. The magnitude and duration of elevated temperatures and limited nutrients will ultimately drive kelp responses. A more nuanced understanding of stress thresholds related to magnitude and duration may explain, in part, observed patterns of kelp loss and persistence in the Salish Sea and other regions.

Counterintuitively, we found that some aspects of kelp performance were higher in the low N treatment in both experiments. The nutrient concentrations in the species exp. were on target, representing high (10-20 µM) and low (1 µM) NO_3 levels in the Salish Sea (Berry et al., 2021; Murray et al., 2015). We note that the population exp. had higher than targeted NO_3 levels in the high N treatments (~80 µM), which elevated blade percent nitrogen above field conditions, but both experiments successfully manipulated kelp blade nitrogen content. There are many other kelp studies using excess nitrogen availability that do not report deleterious effects (eg. 240 µM NO_3 in Pueschel and Korb 2001, 80 µM NO_3 Fernández et al. 2020). Because we found similar counterintuitive effects of low N in both experiments, it is unlikely that the excessive nitrogen levels in the population exp. altered our conclusions. Both species grew significantly more biomass in low N treatments in the species exp., and *N. luetkeana* in the population exp. that were exposed to cool and moderate temperatures grew more in low N treatments. One possible explanation for this counterintuitive growth trend is that kelp grew more to increase blade area for nutrient uptake under nitrogen limited conditions, as they did in the species exp. Analogously, terrestrial plants can increase their root-to-shoot ratio in nitrogen-poor soil (Lopez et al., 2023). In response to nitrate enhancement in the field, blades of *M. pyrifera* increased in thickness and tissue percent N, but did not elongate faster (Stephens & Hepburn, 2016). Another possible explanation is that high temperatures are usually correlated with low, and not high, nitrogen levels (Palacios et al., 2013), thus the combination of warm temperatures and elevated nitrogen conditions is unnatural.

Saccharina latissima (sugar kelp) is a cosmopolitan species with one of the widest distributions of the Laminariales (Bartsch et al., 2008), found across multiple oceans and a wide range of temperatures. This kelp is also an important food source that is prominent in seaweed aquaculture (Forbord et al., 2012; Visch et al., 2020). There are few studies on *S.*

latissima from the Northeast Pacific, but studies in other locations also found negative physiological responses to warming above 20°C. For example, pigment concentrations and photosynthetic capacity decreased and respiration increased under high temperatures (Andersen et al., 2013; Nepper-Davidsen et al., 2019). Similar to our observations, *S. latissima* tissue damage and weakness increased at 21°C compared to cooler temperatures (Simonson et al., 2015). We hypothesize that the understory stature of *S. latissima*, relative to canopy-forming *N. luetkeana*, may play a role in its ability to persist where *N. luetkeana* has been lost in areas of the southern Salish Sea (Berry et al., 2021), since it inhabits cooler and deeper water. Additionally, *S. latissima* had higher nitrogen uptake rates and blade tissue percent nitrogen compared to *N. luetkeana*, which could be beneficial in nutrient poor waters.

Nereocystis luetkeana is an endemic monotypic genus which is only distributed along the west coast of North America (Abbott & Hollenberg, 1976). Optimal growth of *N. luetkeana* sporophyte blades occurs at 12°C in British Columbia, Canada (Supratya et al., 2020), which is lower than the temperature range of highest growth rates from 13 to 16°C in our study. Supratya et al. (2020) also found that *N. luetkeana* blades were able to elongate at temperatures as high as 20 and 22°C after exposure for 7 days. We found that temperatures up to 16°C had no negative impacts on the physiology of *N. luetkeana*, but at 21°C, respiration was significantly elevated, and growth was low to negative. Increased metabolic demand, via elevated respiration, may have prevented kelp from allocating carbon into new biomass. However, while *N. luetkeana* in the species exp. had low or even negative net photosynthesis rates at 21°C, both populations of *N. luetkeana* still had positive net photosynthesis at 21°C in the population exp. It is possible that kelp in the species exp. experienced more stressful field conditions prior to the experiment, as the species exp. was conducted later in the growing season (early August).

Other physiological responses to high temperatures, such as lipid degeneration and oxidative damage (Schmid et al., 2020; Umanzor et al., 2021), likely contributed to the low growth rates despite positive net photosynthesis. We also saw signs of tissue degradation at 21°C in both species (Fig. S4). Interestingly, we observed blade tissue degradation at 21°C in both nitrogen treatments, and in the species exp., we observed kelp mortality in the 21°C high N treatment, suggesting that tissue damage is a result of high temperature, and not low nutrient levels. In the Salish Sea, other studies report *N. luetkeana* blade tissue loss, degradation, and negative growth rates at warm, nutrient poor sites during the summer (Berry et al., 2021; Starko et al., 2022). Portions of the Salish Sea experience extremely warm conditions (up to 20°C, Weigel et al. in review) relative to other areas within the range of *N. luetkeana*, such as coastal Pacific Ocean populations (Hamilton et al., 2020). Given our results, recent declines *N. luetkeana* within the Salish Sea are likely due to elevated temperatures, and prolonged sea surface temperatures of 21°C and above are likely to lead to further kelp forest declines in the warmest areas.

Strong negative effects of ocean warming have been well established for many taxa, including other foundation species (Anthony, 2016; Newcomb et al., 2019; Smale, 2020). But warming rarely occurs in isolation, therefore studies of multiple interacting stressors are necessary to understand rapidly changing environments (Crain et al., 2008; Gunderson et al., 2016; Newcomb et al., 2019). It is important to gain an understanding of the physiological mechanisms for kelp forest declines and responses to multiple stressors, which will help determine priorities for management and restoration actions. Hollarsmith et al. (2022) identified multiple stressor pathways that may be contributing to kelp declines in the Salish Sea, including temperature, nutrients, salinity, water clarity, benthic sedimentation, and algal competition, among others. While we focused on temperature and nutrients, it is likely that other factors such as decreased light availability to benthic life cycle stages are also important in urbanized environments. For example, poor water clarity combined with elevated temperatures exacerbated the loss of *Macrocystis pyrifera* in New Zealand (Tait et al., 2021). Future studies in the Salish Sea should examine the interactions among temperature, nutrients, and light, which together may affect competitive outcomes between benthic and canopy species.

We found sporophytes of *S. latissima* and *N. luetkeana* responded similarly and strongly to high temperature, which were much more stressful than low nitrogen levels. Our results support the prioritization of management actions that limit temperature increases, especially in the coastal oceans and inland seas where temperatures are rising more rapidly (IPCC, 2022; Khangaonkar et al., 2019; Riche et al., 2014). In addition to curtailing emissions, management actions to maintain and restore water circulation (Khangaonkar & Wang, 2013; Khangaonkar & Yang, 2011) could support cooler water temperatures in portions of the Salish Sea. Further, our results demonstrate that management actions involving nutrient additions are unlikely to enhance kelp performance at elevated temperatures. In fact, anthropogenic nutrient additions can exacerbate other kelp stressors, including light limitation and competition with other primary producers (Filbee-Dexter & Wernberg, 2018; Tait et al., 2021). Therefore, important policies to limit eutrophication should remain in place.

Our results highlight the importance of considering elevated temperature in kelp forest conservation. We found that the deleterious effects of warming to 21°C were consistent across populations of *N. luetkeana* and between species, suggesting that kelp from different thermal environments are equally susceptible to the effects of ocean warming. Other studies on multiple populations of *N. luetkeana* in Salish Sea indicated a lack of local adaptation or acclimation with similar critical temperatures of 16 – 18°C (microscopic sporophytes and gametophytes, respectively) and 22°C (sporophytes) across populations (Weigel et al., in review; Fales et al., in prep). The lack of local adaptation to rising ocean temperatures highlights the need to understand more about genetic connectivity and differences across populations when selecting populations for restoration efforts. Furthering our understanding of the interactive effects of temperature and nutrient stressors on kelp physiology is critical to guide stressor identification and target conservation and restoration.

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References

Abbott, I. A., & Hollenberg, G. J. (1976). *Marine algae of California*. Stanford University Press.

Andersen, G. S., Pedersen, M. F., & Nielsen, S. L. (2013). Temperature acclimation and heat tolerance of photosynthesis in Norwegian *Saccharina latissima* (Laminariales, Phaeophyceae). *Journal of Phycology, 49*(4), 689–700. https://doi.org/10.1111/jpy.12077

Anthony, K. R. N. (2016). Coral reefs under climate change and ocean acidification: Challenges and opportunities for management and policy. *Annual Review of Environment and Resources, 41*(1), 59–81. https://doi.org/10.1146/annurev-environ-110615-085610

Arafeh-Dalmau, N., Montaño-Moctezuma, G., Martínez, J. A., Beas-Luna, R., Schoeman, D. S., & Torres-Moye, G. (2019). Extreme marine heatwaves alter kelp forest community near its equatorward distribution limit. *Frontiers in Marine Science, 6*, 1–18. https://doi.org/10.3389/fmars.2019.00499

Bartsch, I., Wiencke, C., Bischof, K., Buchholz, C. M., Buck, B. H., Eggert, A., Feuerpfeil, P., Hanelt, D., Jacobsen, S., Karez, R., Karsten, U., Molis, M., Roleda, M. Y., Schubert, H., Schumann, R., Valentin, K., Weinberger, F., & Wiese, J. (2008). The genus *Laminaria sensu lato*: Recent insights and developments. *European Journal of Phycology, 43*(1), 1–86. https://doi.org/10.1080/09670260701711376

Becheler, R., Haverbeck, D., Clerc, C., Montecinos, G., Valero, M., Mansilla, A., & Faugeron, S. (2022). Variation in thermal tolerance of the giant kelp's gametophytes: Suitability of habitat, population quality or local adaptation? *Frontiers in Marine Science*, *9*. https://www.frontiersin.org/articles/10.3389/fmars.2022.802535

Bell, L. E., & Kroeker, K. J. (2022). Standing crop, turnover, and production dynamics of *Macrocystis pyrifera* and understory species *Hedophyllum nigripes* and *Neoagarum fimbriatum* in high latitude giant kelp forests. *Journal of Phycology, 58*(6), 773–788. https://doi.org/10.1111/jpy.13291

Bennett, S., Duarte, C. M., Marbà, N., & Wernberg, T. (2019). Integrating within-species variation in thermal physiology into climate change ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1778), 20180550. https://doi.org/10.1098/rstb.2018.0550

Berry, H. D., Mumford, T. F., Christiaen, B., Dowty, P., Calloway, M., Ferrier, L., Grossman, E. E., & VanArendonk, N. R. (2021). Long-term changes in kelp forests in an inner basin of the Salish Sea. *PLOS ONE, 16*(2), e0229703. https://doi.org/10.1371/journal.pone.0229703

Biskup, S., Bertocci, I., Arenas, F., & Tuya, F. (2014). Functional responses of juvenile kelps, *Laminaria ochroleuca* and *Saccorhiza polyschides,* to increasing temperatures. *Aquatic Botany*, *113*, 117–122. https://doi.org/10.1016/j.aquabot.2013.10.003

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Crain, C. M., Kroeker, K., & Halpern, B. S. (2008). Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters*, *11*(12), 1304–1315. https://doi.org/10.1111/j.1461-0248.2008.01253.x

Dillon, M. E., Wang, G., & Huey, R. B. (2010). Global metabolic impacts of recent climate warming. *Nature*, *467*(7316), Article 7316. https://doi.org/10.1038/nature09407

Endo, H., Inomata, E., Gao, X., Kinoshita, J., Sato, Y., & Agatsuma, Y. (2020). Heat stress promotes nitrogen accumulation in meristems via apical blade erosion in a brown macroalga with intercalary growth. *Frontiers in Marine Science*, *7*. https://www.frontiersin.org/articles/10.3389/fmars.2020.575721

Fernández, P. A., Gaitán-Espitia, J. D., Leal, P. P., Schmid, M., Revill, A. T., & Hurd, C. L. (2020). Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: Implications for acclimation under thermal stress. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-60104-4

Filbee-Dexter, K., & Wernberg, T. (2018). Rise of turfs: A new battlefront for globally declining kelp forests. *BioScience*, *68*(2), 64–76. https://doi.org/10.1093/biosci/bix147

Forbord, S., Etter, S. A., Broch, O. J., Dahlen, V. R., & Olsen, Y. (2021). Initial short-term nitrate uptake in juvenile, cultivated *Saccharina latissima* (Phaeophyceae) of variable nutritional state. *Aquatic Botany*, *168*, 103306. https://doi.org/10.1016/j.aquabot.2020.103306

Forbord, S., Skjermo, J., Arff, J., Handå, A., Reitan, K. I., Bjerregaard, R., & Lüning, K. (2012). Development of *Saccharina latissima* (Phaeophyceae) kelp hatcheries with year-round production of zoospores and juvenile sporophytes on culture ropes for kelp aquaculture. *Journal of Applied Phycology*, *24*(3), 393–399. https://doi.org/10.1007/s10811-011-9784-y

Fox, J., & Weisberg, S. (2019). *An {R} Companion to Applied Regression, Third Edition*. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Franco, J. N., Tuya, F., Bertocci, I., Rodríguez, L., Martínez, B., Sousa-Pinto, I., & Arenas, F. (2018). The 'golden kelp' *Laminaria ochroleuca* under global change: Integrating multiple eco-physiological responses with species distribution models. *Journal of Ecology*, *106*(1), 47–58. https://doi.org/10.1111/1365-2745.12810

Gao, X., Endo, H., Nagaki, M., & Agatsuma, Y. (2016). Growth and survival of juvenile sporophytes of the kelp *Ecklonia cava* in response to different nitrogen and temperature regimes. *Fisheries Science*, *82*(4), 623–629. https://doi.org/10.1007/s12562-016-0998-4

Gauci, C., Bartsch, I., Martins, N., & Liesner, D. (2022). Cold thermal priming of *Laminaria digitata* (Laminariales, Phaeophyceae) gametophytes enhances gametogenesis and thermal performance of sporophytes. *Frontiers in Marine Science*, *9*, 862923. https://doi.org/10.3389/fmars.2022.862923

Gerard, V. A. (1982). Growth and utilization of internal nitrogen reserves by the giant kelp *Macrocystis pyrifer*a in a low-nitrogen environment. *Marine Biology*, *66*(1), 27–35. https://doi.org/10.1007/BF00397251

Gerard, V. A. (1997). The role of nitrogen nutrition in high-temperature tolerance of the kelp, *Laminaria saccharina* (Chromophyta). *Journal of Phycology*, *33*(5), 800–810. https://doi.org/10.1111/j.0022-3646.1997.00800.x

Guenther, R., Porcher, E., Carrington, E., & Martone, P. (2022). Effects of temperature and pH on the growth, calcification, and biomechanics of two species of articulated coralline algae. *Marine Ecology Progress Series*, *700*, 79–93. https://doi.org/10.3354/meps14166

Gunderson, A. R., Armstrong, E. J., & Stillman, J. H. (2016). Multiple stressors in a changing world: The need for an improved perspective on physiological responses to the dynamic marine environment. *Annual Review of Marine Science*, *8*(1), 357–378. https://doi.org/10.1146/annurev-marine-122414-033953

Hamilton, S. L., Bell, T. W., Watson, J. R., Grorud-Colvert, K. A., & Menge, B. A. (2020). Remote sensing: Generation of long-term kelp bed data sets for evaluation of impacts of climatic variation. *Ecology*, *101*(7), e03031. https://doi.org/10.1002/ecy.3031

Harlin, M. M., & Craigie, J. S. (1978). Nitrate uptake by *Laminaria longicruris* (Phaeophyceae). *Journal of Phycology, 14*(4), 464–467. https://doi.org/10.1111/j.1529-8817.1978.tb02470.x

Hollarsmith, J. A., Andrews, K., Naar, N., Starko, S., Calloway, M., Obaza, A., Buckner, E., Tonnes, D., Selleck, J., & Therriault, T. W. (2022). Toward a conceptual framework for managing and conserving marine habitats: A case study of kelp forests in the Salish Sea. *Ecology and Evolution*, *12*(1), e8510. https://doi.org/10.1002/ece3.8510

IPCC. (2022). *Climate Change 2022: Impacts, Adaptation and Vulnerability Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. https://www.ipcc.ch/report/ar6/wg2/downloads/report/IPCC_AR6_WGII_Citation.pdf

Khangaonkar, T., Nugraha, A., Xu, W., & Balaguru, K. (2019). Salish Sea response to global climate change, sea level rise, and future nutrient loads. *Journal of Geophysical Research: Oceans*, *124*(6), 3876–3904. https://doi.org/10.1029/2018JC014670

Khangaonkar, T., Nugraha, A., Yun, S. K., Premathilake, L., Keister, J. E., & Bos, J. (2021). Propagation of the 2014–2016 Northeast Pacific marine heatwave through the Salish Sea. *Frontiers in Marine Science*, *8,* 1836. https://doi.org/10.3389/fmars.2021.787604

Khangaonkar, T., & Wang, T. (2013). Potential alteration of fjordal circulation due to a large floating structure—Numerical investigation with application to Hood Canal basin in Puget Sound. *Applied Ocean Research, 39*, 146–157. https://doi.org/10.1016/j.apor.2012.11.003

Khangaonkar, T., & Yang, Z. (2011). A high-resolution hydrodynamic model of Puget Sound to support nearshore restoration feasibility analysis and design. *Ecological Restoration*, *29*(1–2), 173–184. https://doi.org/10.3368/er.29.1-2.173

King, N. G., McKeown, N. J., Smale, D. A., & Moore, P. J. (2017). The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. *Ecography*. https://doi.org/10.1111/ecog.03186

Korb, R., & Gerard, V. (2000). Nitrogen assimilation characteristics of polar seaweeds from differing nutrient environments. *Marine Ecology Progress Series, 198,* 83–92. https://doi.org/10.3354/meps198083

Krumhansl, K. A., Okamoto, D. K., Rassweiler, A., Novak, M., Bolton, J. J., Cavanaugh, K. C., Connell, S. D., Johnson, C. R., Konar, B., Ling, S. D., Micheli, F., Norderhaug, K. M., Pérez-Matus, A., Sousa-Pinto, I., Reed, D. C., Salomon, A. K., Shears, N. T., Wernberg, T., Anderson, R. J., … Byrnes, J. E. K. (2016). Global patterns of kelp forest change over the past half-century. *Proceedings of the National Academy of Sciences*, *113*(48), 13785–13790. https://doi.org/10.1073/pnas.1606102113

Lamb, A., & Hanby, B. P. (2005). Brown algae phylum Ochrophyta. In *Marine Life of the Pacific Northwest: A Photographic Encyclopedia of Invertebrates, Seaweeds and Selected Fishes* (pp. 29–36). Harbour Publishing.

Lenth, R. V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2023). *emmeans: Estimated Marginal Means, aka Least-Squares Means* (1.8.6). https://cran.r-project.org/web/packages/emmeans/index.html

Liesner, D., Shama, L. N. S., Diehl, N., Valentin, K., & Bartsch, I. (2020). Thermal plasticity of the kelp *Laminaria digitata* (Phaeophyceae) across life cycle stages reveals the importance of cold seasons for marine forests. *Frontiers in Marine Science, 7*. https://www.frontiersin.org/articles/10.3389/fmars.2020.00456

Lopez, G., Ahmadi, S. H., Amelung, W., Athmann, M., Ewert, F., Gaiser, T., Gocke, M. I., Kautz, T., Postma, J., Rachmilevitch, S., Schaaf, G., Schnepf, A., Stoschus, A., Watt, M., Yu, P., & Seidel, S. J. (2023). Nutrient deficiency effects on root architecture and root-to-shoot ratio in arable crops. *Frontiers in Plant Science*, *13*. https://www.frontiersin.org/articles/10.3389/fpls.2022.1067498

Lüning, K., & Freshwater, W. (1988). Temperature tolerance of Northeast Pacific marine algae. *Journal of Phycology*, *24*(3), 310–315. https://doi.org/10.1111/j.1529-8817.1988.tb04471.x

Mabin, C., Gribben, P., Fischer, A., & Wright, J. (2013). Variation in the morphology, reproduction and development of the habitat-forming kelp *Ecklonia radiata* with changing temperature and nutrients. *Marine Ecology Progress Series*, *483*, 117–131. https://doi.org/10.3354/meps10261

Martins, N., Tanttu, H., Pearson, G. A., Serrão, E. A., & Bartsch, I. (2017). Interactions of daylength, temperature and nutrients affect thresholds for life stage transitions in the kelp *Laminaria digitata* (Phaeophyceae). *Botanica Marina*, *60*(2), 109–121. https://doi.org/10.1515/bot-2016-0094

Murray, J. W., Roberts, E., Howard, E., O'Donnell, M., Bantam, C., Carrington, E., Foy, M., Paul, B., & Fay, A. (2015). An inland sea high nitrate-low chlorophyll (HNLC) region with naturally high pCO2. *Limnology and Oceanography*, *60*(3), 957–966. https://doi.org/10.1002/lno.10062

Muth, A. F., Graham, M. H., Lane, C. E., & Harley, C. D. G. (2019). Recruitment tolerance to increased temperature present across multiple kelp clades. *Ecology*, *100*(3), e02594. https://doi.org/10.1002/ecy.2594

Nepper-Davidsen, J., Andersen, D., & Pedersen, M. (2019). Exposure to simulated heatwave scenarios causes long-term reductions in performance in *Saccharina latissima*. *Marine Ecology Progress Series*, *630*, 25–39. https://doi.org/10.3354/meps13133

Newcomb, L. A., George, M. N., O'Donnell, M. J., & Carrington, E. (2019). Only as strong as the weakest link: Structural analysis of the combined effects of elevated temperature and pCO2 on mussel attachment. *Conservation Physiology*, *7*(1), coz068. https://doi.org/10.1093/conphys/coz068

Nishihara, G. N., Terada, R., & Noro, T. (2005). Effect of temperature and irradiance on the uptake of ammonium and nitrate by *Laurencia brongniartii* (Rhodophyta, Ceramiales). *Journal of Applied Phycology*, *17*(5), 371–377. https://doi.org/10.1007/s10811-005-5519-2

O'Donnell, M. J., George, M. N., & Carrington, E. (2013). Mussel byssus attachment weakened by ocean acidification. *Nature Climate Change*, *3*(6), Article 6. https://doi.org/10.1038/nclimate1846

Palacios, D. M., Hazen, E. L., Schroeder, I. D., & Bograd, S. J. (2013). Modeling the temperature-nitrate relationship in the coastal upwelling domain of the California Current. *Journal of Geophysical Research: Oceans*, *118*(7), 3223–3239. https://doi.org/10.1002/jgrc.20216

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., & Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*, *569*(7754), Article 7754. https://doi.org/10.1038/s41586-019-1132-4

Pueschel, C. M., & Korb, R. E. (2001). Storage of nitrogen in the form of protein bodies in the kelp *Laminaria solidungula*. *Marine Ecology Progress Series*, *218*, 107–114. https://doi.org/10.3354/meps218107

Riche, O., Johannessen, S. C., & Macdonald, R. W. (2014). Why timing matters in a coastal sea: Trends, variability and tipping points in the Strait of Georgia, Canada. *Journal of Marine Systems*, *131*, 36–53. https://doi.org/10.1016/j.jmarsys.2013.11.003

Rogers-Bennett, L., & Catton, C. A. (2019). Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. *Scientific Reports*, *9*(1), 15050. https://doi.org/10.1038/s41598-019-51114-y

Roleda, M. Y., & Hurd, C. L. (2019). Seaweed nutrient physiology: Application of concepts to aquaculture and bioremediation. *Phycologia*, *58*(5), 552–562. https://doi.org/10.1080/00318884.2019.1622920

Schiel, D. R., Steinbeck, J. R., & Foster, M. S. (2004). Ten years of induced ocean warming causes comprehensive changes in marine benthic communities. *Ecology*, *85*(7), 1833–1839. https://doi.org/10.1890/03-3107

Schmid, M., Fernández, P. A., Gaitán-Espitia, J. D., Virtue, P., Leal, P. P., Revill, A. T., Nichols, P. D., & Hurd, C. L. (2020). Stress due to low nitrate availability reduces the biochemical acclimation potential of the giant kelp *Macrocystis pyrifera* to high temperature. *Algal Research*, *47*, 101895. https://doi.org/10.1016/j.algal.2020.101895

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), Article 7. https://doi.org/10.1038/nmeth.2089

Simonson, E. J., Scheibling, R. E., & Metaxas, A. (2015). Kelp in hot water: I. Warming seawater temperature induces weakening and loss of kelp tissue. *Marine Ecology Progress Series*, *537*, 89–104. https://doi.org/10.3354/meps11438

Smale, D. A. (2020). Impacts of ocean warming on kelp forest ecosystems. *New Phytologist*, *225*(4), 1447–1454. https://doi.org/10.1111/nph.16107

Staehr, P. A., & Wernberg, T. (2009). Physiological responses of *Ecklonia radiata* (Laminariales) to a latitudinal gradient in ocean temperature. *Journal of Phycology*, *45*(1), 91–99. https://doi.org/10.1111/j.1529-8817.2008.00635.x

Starko, S., Bailey, L. A., Creviston, E., James, K. A., Warren, A., Brophy, M. K., Danasel, A., Fass, M. P., Townsend, J. A., & Neufeld, C. J. (2019). Environmental heterogeneity mediates scale-dependent declines in kelp diversity on intertidal rocky shores. *PLOS ONE*, *14*(3), e0213191. https://doi.org/10.1371/journal.pone.0213191

Starko, S., Neufeld, C. J., Gendall, L., Timmer, B., Campbell, L., Yakimishyn, J., Druehl, L., & Baum, J. K. (2022). Microclimate predicts kelp forest extinction in the face of direct and indirect marine heatwave effects. *Ecological Applications*, e2673. https://doi.org/10.1002/eap.2673

Stephens, T. A., & Hepburn, C. D. (2016). A kelp with integrity: *Macrocystis pyrifera* prioritises tissue maintenance in response to nitrogen fertilisation. *Oecologia*, *182*(1), 71–84. https://doi.org/10.1007/s00442-016-3641-2

Supratya, V. P., Coleman, L. J. M., & Martone, P. T. (2020). Elevated temperature affects phenotypic plasticity in the bull kelp (*Nereocystis luetkeana*, Phaeophyceae). *Journal of Phycology*, *56*(6), 1534–1541. https://doi.org/10.1111/jpy.13049

Tait, L. W., Thoral, F., Pinkerton, M. H., Thomsen, M. S., & Schiel, D. R. (2021). Loss of giant kelp, *Macrocystis pyrifera*, driven by marine heatwaves and exacerbated by poor water clarity in New Zealand. *Frontiers in Marine Science*, *8*. https://www.frontiersin.org/articles/10.3389/fmars.2021.721087

Umanzor, S., Sandoval-Gil, J. M., Sánchez-Barredo, M., Ladah, L. B., Ramírez-García, M.-M., & Zertuche-González, J. A. (2021). Short-term stress responses and recovery of giant kelp (*Macrocystis pyrifera*, Laminariales, Phaeophyceae) juvenile sporophytes to a simulated marine heatwave and nitrate scarcity. *Journal of Phycology*. https://doi.org/10.1111/jpy.13189

Visch, W., Kononets, M., Hall, P. O. J., Nylund, G. M., & Pavia, H. (2020). Environmental impact of kelp (*Saccharina latissima*) aquaculture. *Marine Pollution Bulletin*, *155*, 110962. https://doi.org/10.1016/j.marpolbul.2020.110962

Vranken, S., Wernberg, T., Scheben, A., Severn-Ellis, A. A., Batley, J., Bayer, P. E., Edwards, D., Wheeler, D., & Coleman, M. A. (2021). Genotype–Environment mismatch of kelp forests under climate change. *Molecular Ecology*, *30*(15), 3730–3746. https://doi.org/10.1111/mec.15993

Weigel, B. L., & Pfister, C. A. (2021). The dynamics and stoichiometry of dissolved organic carbon release by kelp. *Ecology*, *102*(2), e03221. https://doi.org/10.1002/ecy.3221

Weigel, B. L., Small, S. L., Berry, H. D., & Dethier, M. N. (in review). Effects of temperature and nutrients on microscopic stages of the bull kelp (*Nereocystis luetkeana*, Phaeophyceae).

Wernberg, T., Coleman, M. A., Bennett, S., Thomsen, M. S., Tuya, F., & Kelaher, B. P. (2018). Genetic diversity and kelp forest vulnerability to climatic stress. *Scientific Reports*, *8*(1), Article 1. https://doi.org/10.1038/s41598-018-20009-9

Williams, G. D., Levin, P. S., & Palsson, W. A. (2010). Rockfish in Puget Sound: An ecological history of exploitation. *Marine Policy*, *34*(5), 1010–1020. https://doi.org/10.1016/j.marpol.2010.02.008

Zanna, L., Khatiwala, S., Gregory, J. M., Ison, J., & Heimbach, P. (2019). Global reconstruction of historical ocean heat storage and transport. *Proceedings of the National Academy of Sciences*, *116*(4), 1126–1131. https://doi.org/10.1073/pnas.1808838115

Tables

Table 1. Summary of two-factor ANOVAs of the fixed and interactive effects of temperature, nutrients and population or species on three kelp growth metrics: wet weight (percent change), change in length per day, and change in area per day. The population and species experiments were analyzed separately.

Factor		Biomass		Length		Area	
Population exp.	Df	F value	P value	F value	P value	F value	P value
Temperature	2	185.00	< **0.001**	114.02	< **0.001**	12.22	< **0.001**
Nutrients	1	19.02	< **0.001**	27.69	< **0.001**	3.69	**0.058**
Population	1	21.75	< **0.001**	0.46	0.50	10.26	**0.002**
Temp: Nutrients	2	10.29	< **0.001**	12.10	< **0.001**	2.54	0.08
Temp: Pop	2	1.59	0.21	0.84	0.44	2.42	0.10
Nutrients: Pop	1	0.28	0.60	0.25	0.62	0.48	0.49
Temp: Nutrients: Pop	2	0.86	0.43	1.08	0.34	0.34	0.71
Species exp.							
Temperature	2	91.95	< **0.001**	67.26	< **0.001**	39.44	< 0.001
Nutrients	1	7.30	**0.009**	4.78	**0.03**	4.66	**0.03**
Species	1	20.71	< **0.001**	15.51	< **0.001**	3.53	0.06
Temp: Nutrients	2	0.53	0.59	0.21	0.81	0.33	0.72
Temp: Species	2	1.86	0.16	9.77	< **0.001**	4.92	**0.01**
Nutrients: Species	1	0.19	0.67	8.92	**0.004**	1.25	0.27
Temp: Nutrients: Sp.	2	0.95	0.39	1.74	0.18	0.17	0.85

Table 2. Summary of two-factor ANOVAs of the fixed and interactive effects of temperature, nutrients and population or species on kelp photophysiology (net photosynthesis, dark respiration, and change in Fv/Fm). The population and species experiments were analyzed separately.

Factor		Net Photosynthesis		Respiration		Fv/Fm change	
Population exp.	Df	F value	P value	F value	P value	F value	P value
Temperature	2	55.20	< **0.001**	21.45	< **0.001**	0.89	0.41
Nutrients	1	18.33	< **0.001**	1.11	0.29	0.12	0.73
Population	1	16.15	< **0.001**	13.13	< **0.001**	5.06	**0.03**
Temp: Nutrients	2	3.38	**0.04**	1.82	0.17	0.31	0.74
Temp: Pop	2	1.14	0.33	0.07	0.93	0.77	0.47
Nutrients: Pop	1	0.03	0.87	0.46	0.50	1.74	0.19
Temp: Nutrients: Pop	2	0.56	0.57	0.87	0.42	0.43	0.65
Species exp.							
Temperature	2	16.48	< **0.001**	59.98	< **0.001**	9.66	< **0.001**
Nutrients	1	10.31	**0.002**	5.82	**0.02**	0.13	0.72
Species	1	0.59	0.44	0.01	0.95	11.90	**0.001**
Temp: Nutrients	2	7.48	**0.001**	4.56	**0.01**	1.51	0.23
Temp: Species	2	1.54	0.22	1.17	0.32	7.50	**0.001**
Nutrients: Species	1	3.32	0.07	0.07	0.80	0.40	0.53
Temp: Nutrients: Sp.	2	0.55	0.58	0.04	0.96	0.18	0.83

Table 3. Summary of two-factor ANOVAs of the fixed and interactive effects of temperature, nutrients and population or species on kelp for five nutrient assays: nitrate uptake rates, ammonium uptake rates, blade C:N, blade percent carbon, and blade percent nitrogen. The population and species experiments were analyzed separately.

Factor		NO_3 uptake		NH_4 uptake		C:N		% Carbon		% Nitrogen	
Population exp.	Df	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value
Temperature	2	12.79	**< 0.001**	0.14	0.87	8.78	**< 0.001**	3.37	**0.04**	4.55	**0.01**
Nutrients	1	59.54	**< 0.001**	53.28	**< 0.001**	41.86	**< 0.001**	22.49	**< 0.001**	86.04	**< 0.001**
Population	1	0.09	0.77	3.84	0.058	42.77	**< 0.001**	7.87	**0.006**	47.44	**< 0.001**
Temp: Nutrients	2	11.15	**< 0.001**	6.26	**0.005**	1.07	0.35	2.31	0.11	6.74	**0.002**
Temp: Pop	2	0.74	0.48	0.17	0.85	0.31	0.73	0.65	0.52	0.94	0.39
Nutrients: Pop	1	0.08	0.78	5.21	**0.03**	0.86	0.36	1.07	0.30	0.88	0.35
Temp: Nutrients: Pop	2	0.53	0.59	2.07	0.14	0.02	0.98	0.01	0.99	0.01	0.99
Species exp.											
Temperature	1	5.11	**0.03**	29.91	**< 0.001**	5.75	**0.005**	1.43	0.25	0.27	0.76
Nutrients	1	52.51	**< 0.001**	143.58	**< 0.001**	150.67	**< 0.001**	1.39	0.24	17.43	**< 0.001**
Species	1	2.21	0.15	5.03	**0.03**	5.77	**0.02**	1.82	0.18	0.48	0.49
Temp: Nutrients	1	3.54	0.07	0.27	0.61	0.25	0.78	0.84	0.44	0.81	0.45
Temp: Species	1	2.83	0.11	0.00	0.98	3.75	**0.03**	0.70	0.50	1.26	0.29
Nutrients: Sp.	1	2.12	0.16	0.18	0.67	2.91	0.09	2.70	0.11	0.96	0.33
Temp: Nutrients: Sp.	1	3.18	0.09	4.03	0.056	1.08	0.35	1.95	0.15	0.86	0.43

Figures

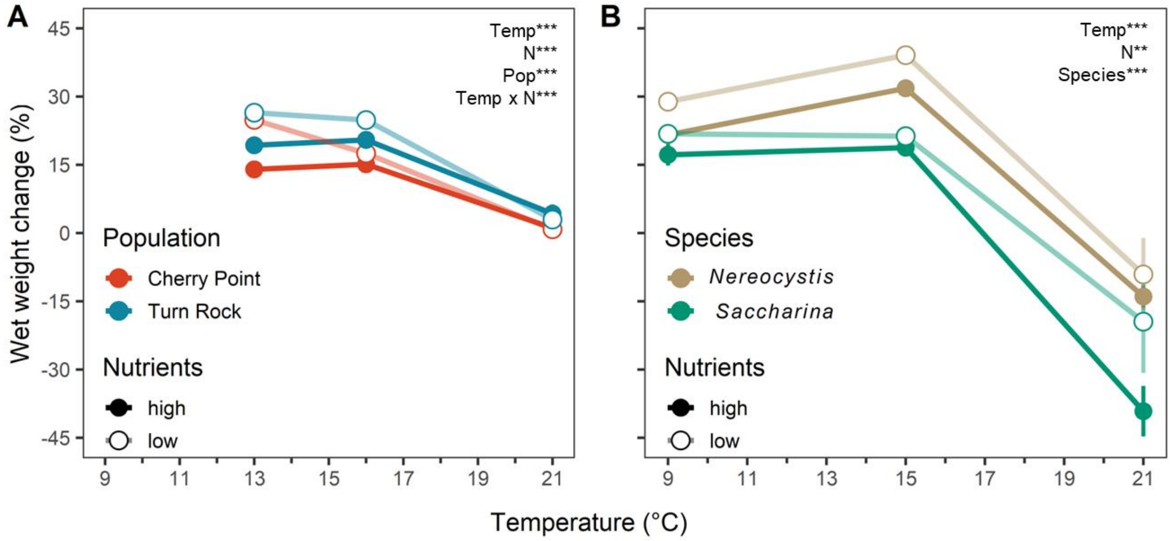

Figure 1. Change in wet weight (%, mean ± SE) during the experimental treatment period across temperature and nutrient levels as either high (closed circle) or low (open circle). A (left) is the *Nereocystis* population exp. from Cherry Point (red) and Turn Rock (blue). B (right) is the species exp. with *Nereocystis* (brown) and *Saccharina* (green) from Deadman Island. Significant effects are indicated by text with asterisks, see Table 1 for details.

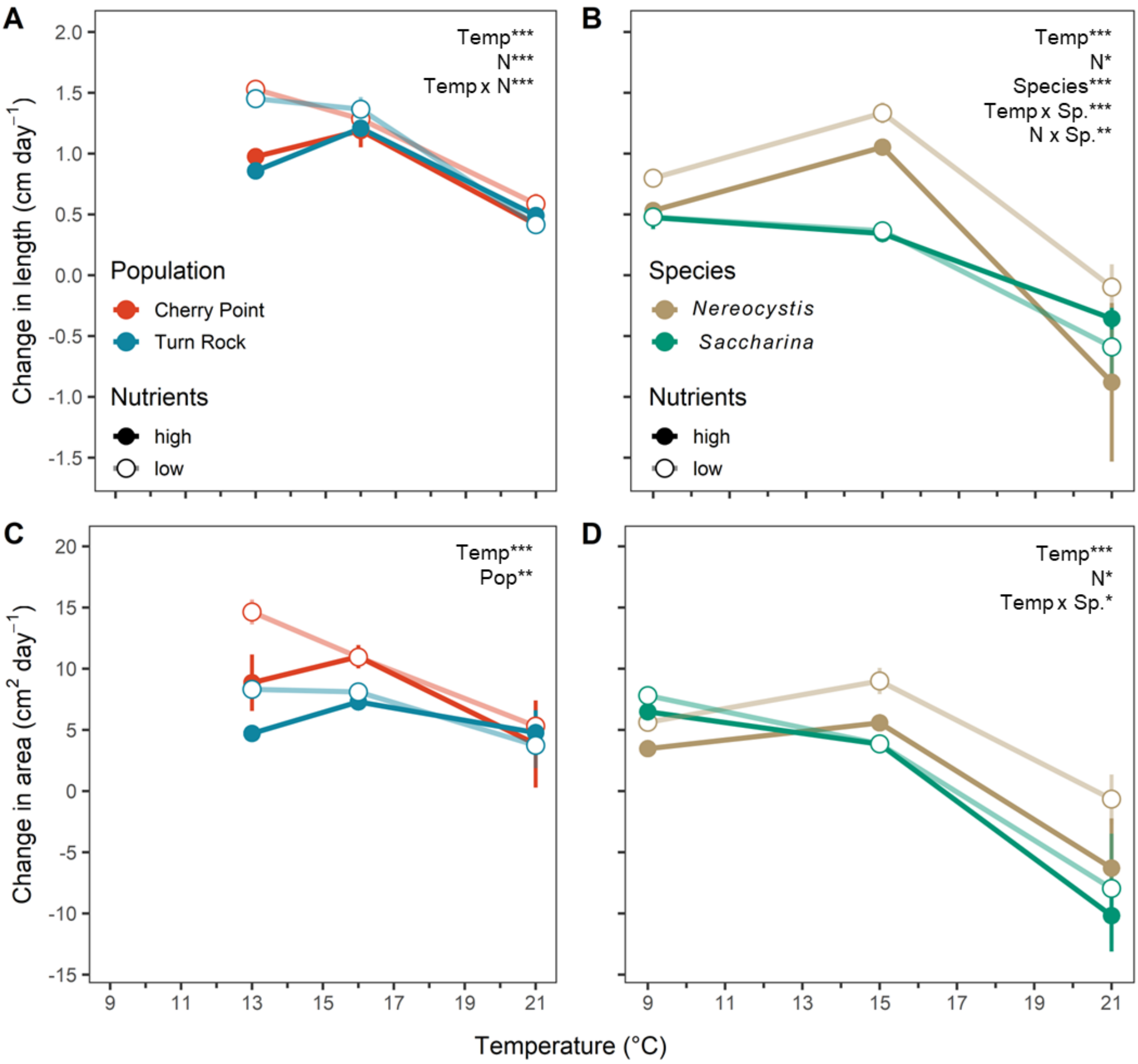

Figure 2. Change in blade length in cm/day (A & B) and area (C & D) in cm^2/day as mean ± SE per day during the experimental treatment period across temperature and nutrient levels as either high (closed circle) or low (open circle). A & C (left) are the population exp. with *Nereocystis* from Cherry Point (red) and Turn Rock (blue). B & D (right) are the species exp. with *Nereocystis* (brown) and *Saccharina* (green) from Deadman Island. Significant effects are indicated by text with asterisks, see Table 1 for details.

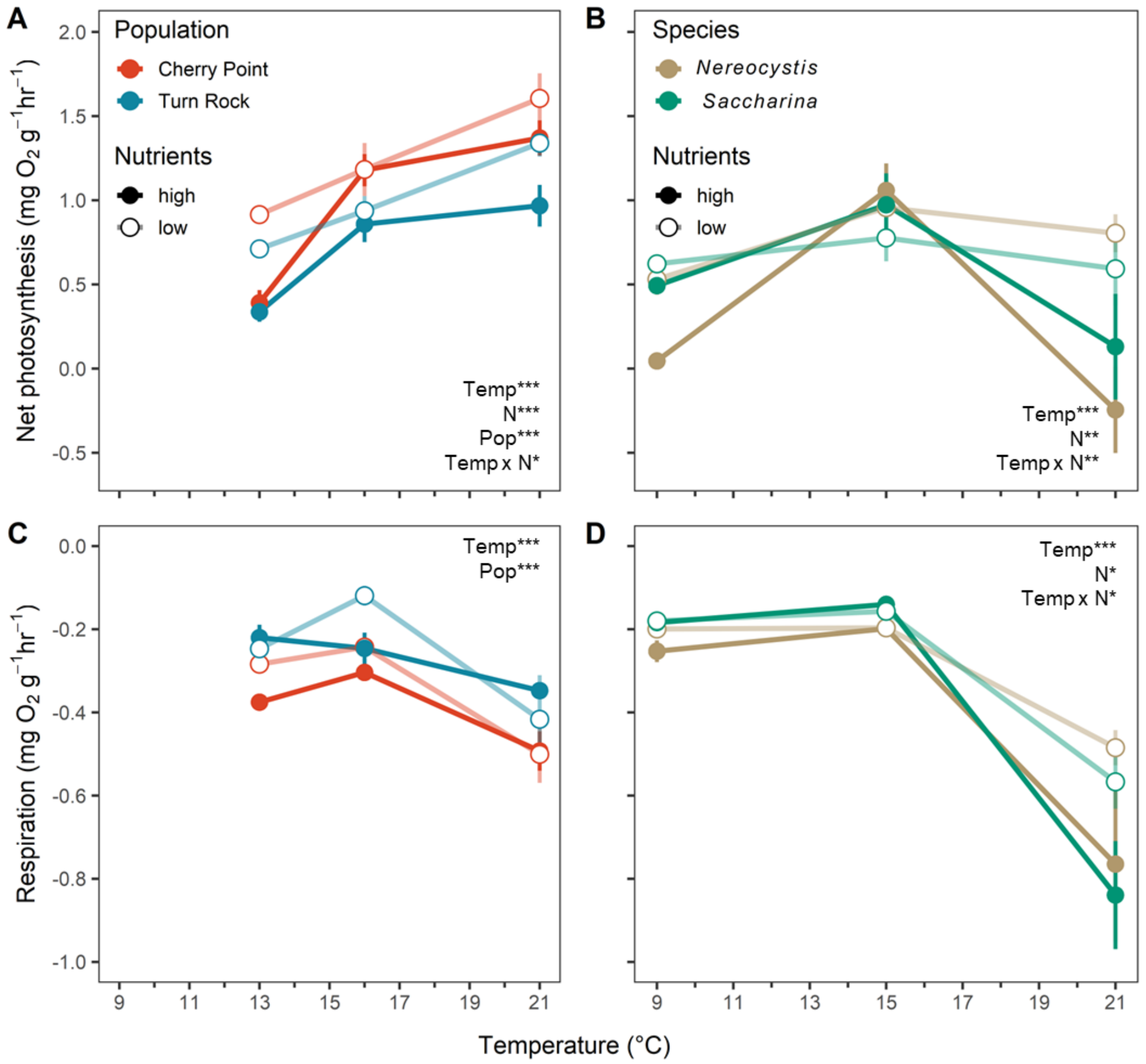

Figure 3. Net photosynthesis (A & B) and dark respiration (C & D) measured as change in dissolved oxygen (mean ± SE) at the end of the experiments across temperature and nutrient levels as either high (closed circle) or low (open circle). A & C (left) are the population exp. with *Nereocystis* from Cherry Point (red) and Turn Rock (blue). B & D (right) are the species exp. with *Nereocystis* (brown) and *Saccharina* (green) from Deadman Island. Negative values in C & D are a result of oxygen consumed by respiration. Significant effects are indicated by text with asterisks, see Table 2 for details.

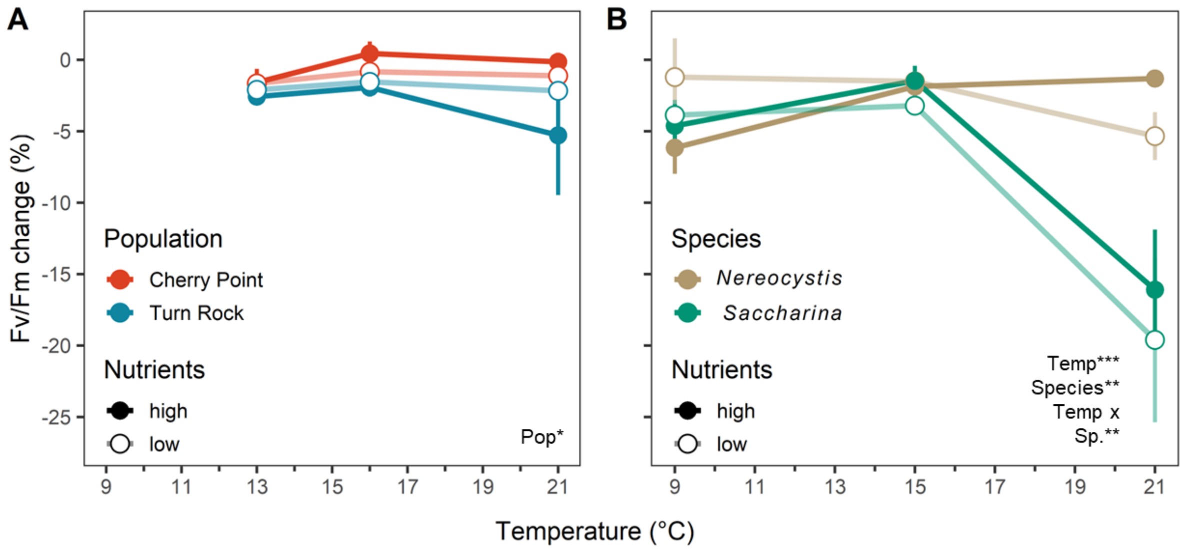

Figure 4. Change of dark-adapted yield of photosystem II (Fv/Fm) (%, mean ± SE) during the experimental treatment period across temperature and nutrient levels as either high (closed circle) or low (open circle). A (left) is the population exp. with *Nereocystis* from Cherry Point (red) and Turn Rock (blue). B (right) is the species exp. with *Nereocystis* (brown) and *Saccharina* (green) from Deadman Island. In the species exp., replication for the 21°C high N treatment was low (n = 2) due to mortality (Table S1). Significant effects are indicated by text with asterisks, see Table 2 for details.

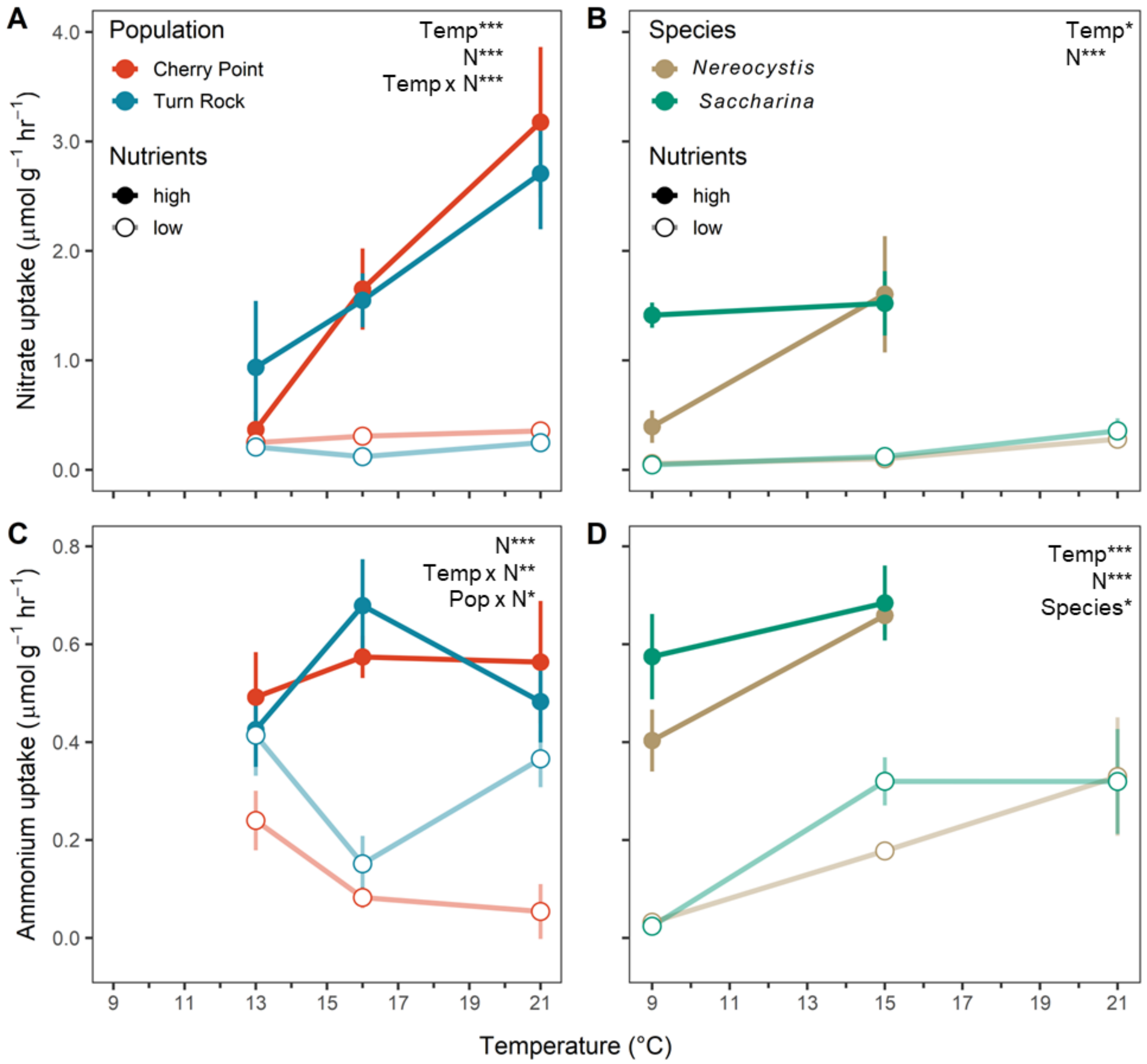

Figure 5. Nutrient uptake rates of nitrate (NO_3, A & B) and ammonium (NH_4, C & D) as mean ± SE across temperature and nutrient levels as either high (closed circle) or low (open circle). A & C (left) are the population exp. with *Nereocystis* from Cherry Point (red) and Turn Rock (blue). B & D (right) are the species exp. with *Nereocystis* (brown) and *Saccharina* (green) from Deadman Island. In the species exp., nutrient uptake was not measured for the 21°C high N treatment due to low replication associated with kelp mortality. Significant effects are indicated by text with asterisks, see Table 3 for details.

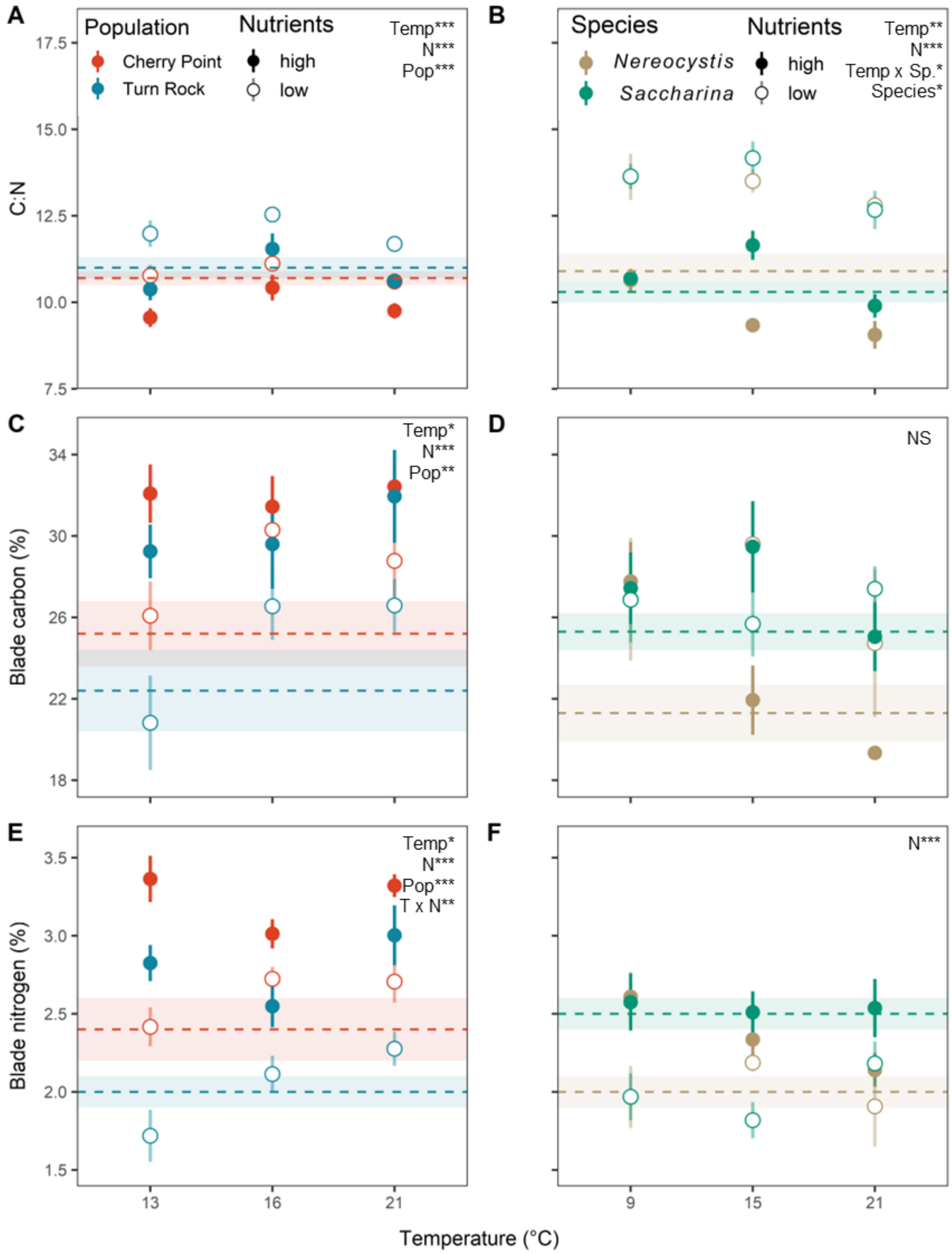

Figure 6. Kelp blade tissue carbon to nitrogen ratios (A & B), blade tissue percent carbon (C & D) and percent nitrogen (E & F) at the conclusion of the experiments across temperature and nutrient levels as either high (closed circle) or low (open circle). Circles are mean ± SE of experimental blades and horizontal lines are mean ± SE of field collected blades. A, C, & E (left) are the population exp. with *Nereocystis* from Cherry Point (red) and Turn Rock (blue). B, D, & F (right) are the species exp. with *Nereocystis* (brown) and *Saccharina* (green) from Deadman Island. Significant effects are indicated by text with asterisks for both high and low nutrient treatments, see Table 3 for details.

Chapter 3 – Supplementary Materials

Table S1. Sample sizes of biological replicates for each response variable and treatment in the population and species experiments.

Treatment	Biomass	Length	Area	Net photosynthesis	Respiration	Fv/Fm change	NO_3 uptake	NH_4 uptake	C:N	% C	% N
Population exp. (Cherry Point, Turn Rock)											
21°+N	8, 8	8, 8	8, 8	8, 8	8, 8	8, 8	4, 4	4, 4	8, 8	8, 8	8, 8
21°-N	8, 8	8, 8	8, 8	8, 8	8, 8	8, 8	4, 4	4, 4	8, 8	8, 8	8, 8
16°+N	8, 8	8, 8	8, 8	4, 4	4, 4	8, 8	4, 4	4, 4	8, 8	8, 8	8, 8
16°-N	8, 8	8, 8	8, 8	8, 8	8, 8	8, 8	4, 4	4, 4	8, 8	8, 8	8, 8
13°+N	8, 8	8, 8	8, 8	8, 8	8, 8	8, 8	4, 4	4, 4	8, 8	8, 8	8, 8
13°-N	8, 8	8, 8	8, 8	8, 8	8, 8	8, 8	4, 4	4, 4	8, 8	8, 8	8, 8
Species exp. (*Nereocystis, Saccharina*)											
21°+N	2, 3	2, 3	2, 3	6, 6	6, 6	2, 3	0, 0	0, 0	2, 3	2, 3	2, 3
21°-N	7, 6	7, 6	7, 6	7, 7	7, 7	7, 7	4, 4	4, 4	7, 5	7, 5	7, 5
15°+N	7, 7	7, 7	7, 7	7, 7	7, 7	7, 7	4, 4	4, 4	7, 7	7, 7	7, 7
15°-N	7, 7	7, 7	7, 7	7, 7	7, 7	7, 7	4, 4	4, 4	7, 7	7, 7	7, 7
9°+N	7, 7	7, 7	7, 7	7, 7	7, 7	7, 7	4, 4	4, 4	7, 7	7, 7	7, 7
9°-N	7, 7	7, 7	7, 7	7, 7	7, 7	7, 7	4, 4	4, 4	7, 7	7, 7	7, 7

Table S2. Daily nutrient concentrations as mean ± SD (species exp. n = 8, population exp. n = 9) in each treatment tank during the treatment period of each experiment, as well as nutrient concentrations of field-collected water samples of from each collection site (n = 3), and initial concentrations in the Friday Harbor Laboratories (FHL) ocean acidification environmental laboratory tanks (n = 2).

Temperature	Nutrient	NO_3	NH_4	PO_4
Population exp.				
13	-N	2.58 ± 0.34	0.34 ± 0.08	0.54 ± 0.07
16	-N	2.30 ± 0.55	0.23 ± 0.09	0.54 ± 0.05
21	-N	2.69 ± 0.64	0.24 ± 0.10	0.49 ± 0.07
13	+N	77.95 ± 28.66	1.36 ± 0.15	2.24 ± 0.19
16	+N	76.43 ± 29.65	0.84 ± 0.09	2.10 ± 0.10
21	+N	81.48 ± 37.51	1.04 ± 0.26	1.97 ± 0.10
Cherry Point (field)		0.80 ± 0.03	0.44 ± 0.03	0.79 ± 0.01
Turn Rock (field)		23.40 ± 1.02	0.15 ± 0.11	1.84 ± 0.08
FHL (tank initial)		23.40 ± 0.22	0.68 ± 0.01	1.88 ±0.01
Species exp.				
9	-N	0.59 ± 0.23	0.22 ± 0.11	0.59 ± 0.10
15	-N	1.04 ± 1.83	0.17 ± 0.10	0.46 ± 0.09
21	-N	0.87 ± 1.37	0.12 ± 0.08	0.69 ± 0.17
9	+N	10.56 ± 0.60	1.46 ± 0.16	2.68 ± 0.36
15	+N	16.42 ± 5.49	1.37 ± 0.49	2.65 ± 0.21
21	+N	20.93 ± 5.62	1.20 ± 0.43	2.94 ± 0.89
Deadman Island (field)		19.17 ± 0.12	0.68 ± 0.06	1.73 ± 0.01
FHL (tank initial)		18.14 ± 0.01	1.15 ± 0.01	1.66 ± 0.02

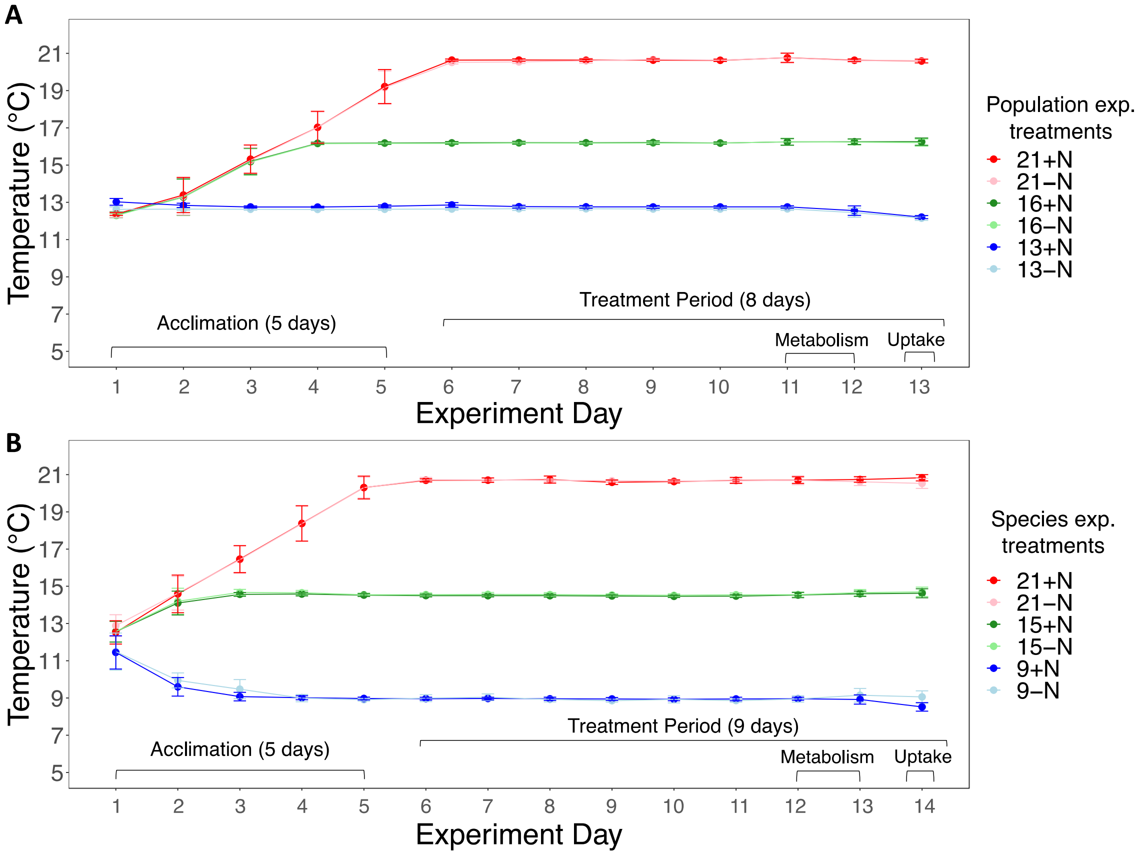

Figure S1. Daily mean temperatures (± SD) in each treatment tank, measured every 5 min, for the (A) population exp., and (B) species exp. During the initial 5-day acclimation period tank temperatures were slowly changed to final treatment levels (2°C/day). The treatment period was the 8 or 9-day period when experimental temperatures and nutrient levels were maintained. Treatments temperatures were 21, 16, 15, 13, and 9°C and nutrients were either high (+N, dark colors) or low (-N, light colors). Measurements for metabolism, including photosynthesis and dark respiration rates, were carried out over 2 days, and nutrient uptake measurements were carried out on the last day of the experiment. Day 1 of the population exp. was July 07 and Day 1 of the species exp. was August 05, 2022.

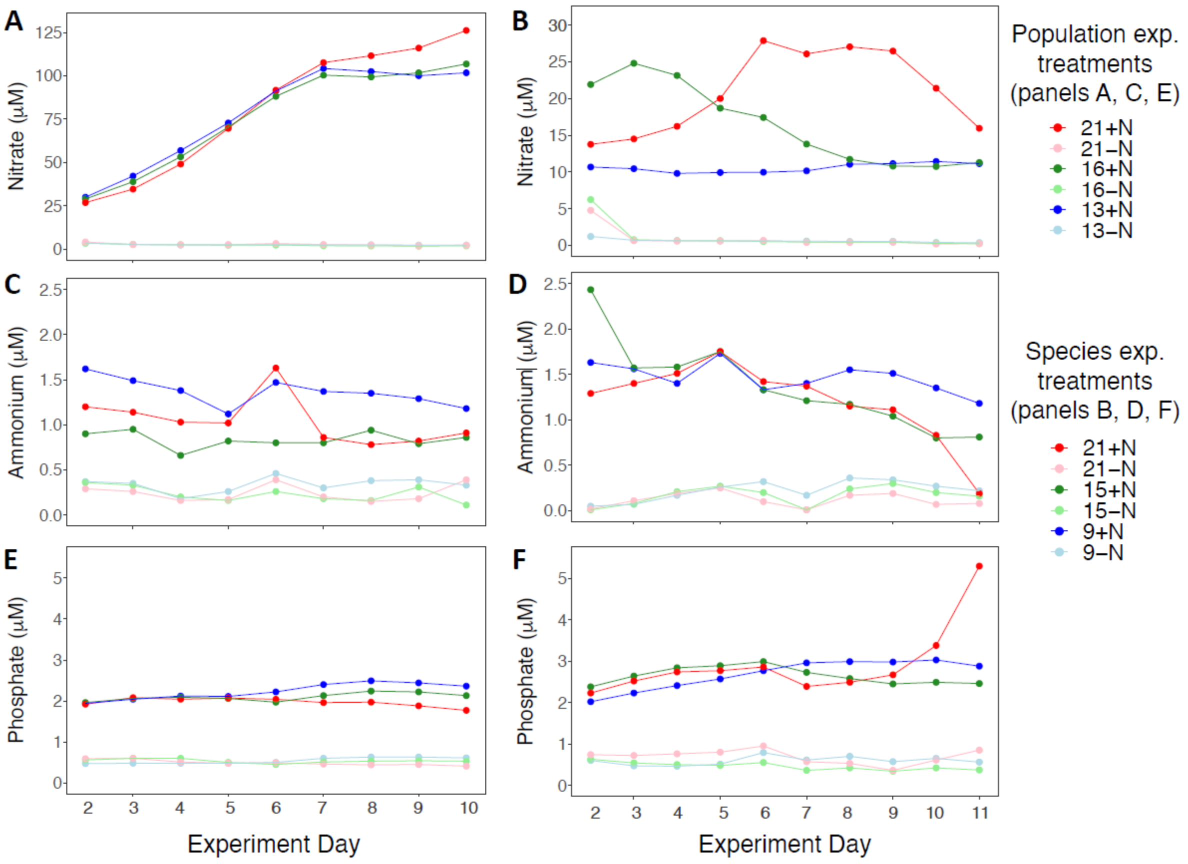

Figure S2. Daily nutrient concentrations (n = 1) in each treatment tank of nitrate (A, B), ammonium (C, D), and phosphate (E, F) in each experiment. Panels on left (A, C, & E) are from the population exp. and panels on right (B, D, & F) are from the species exp. Treatments temperatures were 21, 16, 15, 13, and 9°C and nutrients were either high (+N, dark colors) or low (-N, light colors).

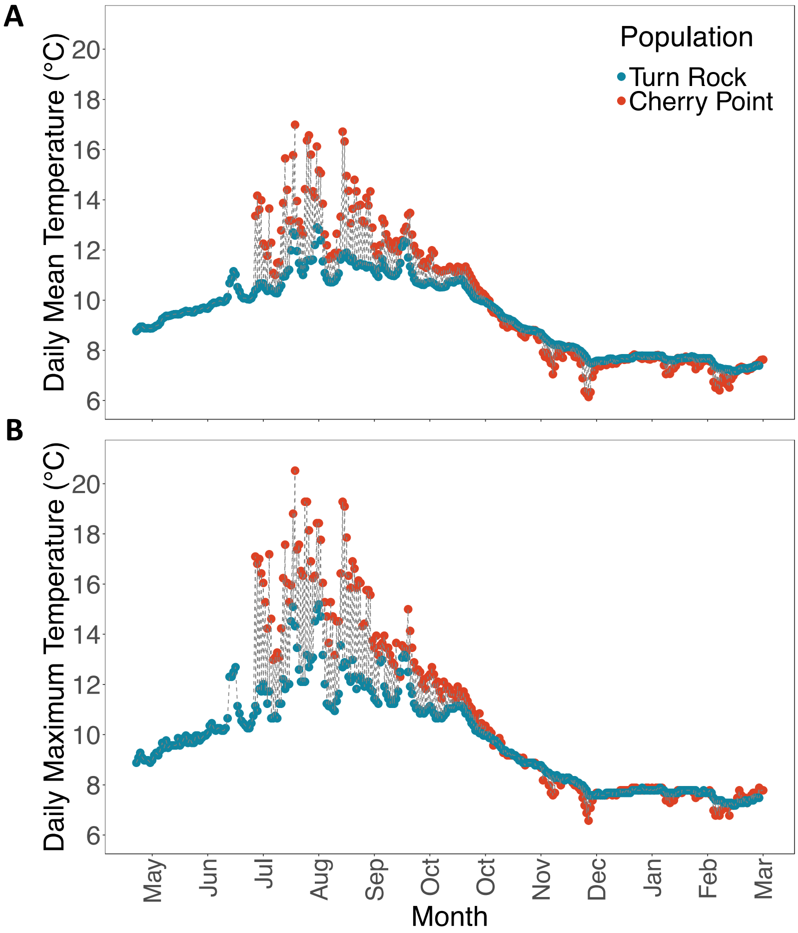

Figure S3. Daily thermal dynamics of Turn Rock (blue) and Cherry Point (red) in 2022. (A) Mean temperature (B) and maximum temperature collected in the subtidal within *N. luetkeana* forests every 15 minutes at Cherry Point (-3 m depth) and Turn Rock (-3.5 m).

Figure S4. Representative photographs of kelp blades at the conclusion of the experiment for the (A) population exp. and (B) species exp. in each nutrient and temperature treatment. In the population exp, *N. luetkeana* blades from Cherry Point (left) and Turn Rock (right). In the species exp., *S. latissima* (left) and *N. luetkeana* (right). Scales are not the same and photos intend to show condition of blades not the relative size.

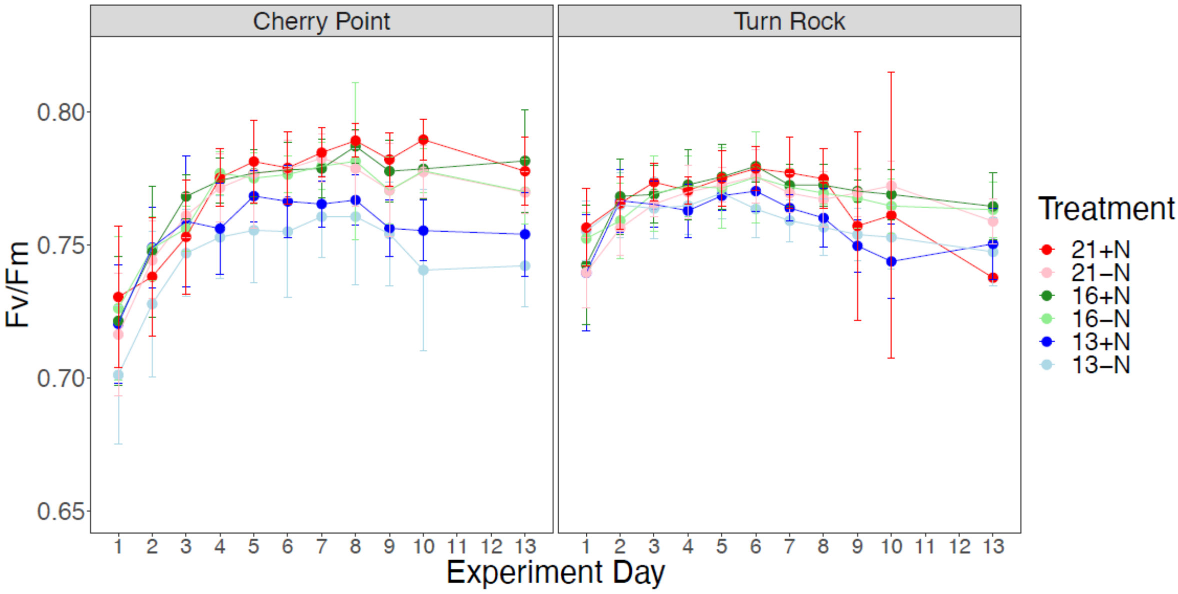

Figure S5. Daily measurements of *N. luetkeana* dark-adapted yield of photosystem II (Fv/Fm) in each treatment (n = 8 per treatment) as mean ± SE in the population exp. Panels separate populations: Cherry Point (left) and Turn Rock (right). Treatments temperatures were 21, 16, and 13°C and nutrients were either high (+N) or low (-N). Treatments temperatures were 21, 16, and 13°C and nutrients were either high (+N, dark colors) or low (-N, light colors).