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Interactive effects of temperature and nitrogen on the physiology of kelps (*Nereocystis luetkeana* and *Saccharina latissima*)

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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 climate change. 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 temperature stress, including alleviating warming stress. We compared the physiological responses of kelp sporophytes to high temperature stress and nitrogen limitation between two populations of *N. luetkeana* 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 (8–9 day) exposure of kelp blades to different temperatures: low (9, 13°C), moderate (15, 16°C), and warm (21°C) at two different nitrogen concentrations: low (1–3 μM) vs. high (>10 μM). We examined a wide array of physiological 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 in the warmest temperature treatment (21°C), at both high and low nitrogen levels, suggesting that *N. luetkeana* and *S. latissima* are susceptible to thermal stress over short time periods. 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 may actually reduce kelp performance at supra-optimal temperatures, and a thorough understanding of kelp responses to coastal temperature and nutrient dynamics is needed to guide conservation and restoration actions.

KEYWORDS

thermal stress, foundation species, Laminariales, physiology, metabolism, photosynthesis, respiration, nutrient uptake

1 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 (Riche et al., 2014; Khangaonkar et al., 2019). 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 (Wernberg et al., 2018; Liesner et al., 2020; Vranken et al., 2021). Knowledge of population-level variability in thermal tolerance is critical to determine 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 in kelp sporophytes (Gerard, 1997; Staehr and Wernberg, 2009; Biskup et al., 2014; Umanzor et al., 2021). Photosynthesis rates usually decline above optimum temperatures while respiration rates continue to increase, leading to decreased growth rates (Umanzor et al., 2021). These 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 (Gerard, 1997; Fernández et al., 2020; Umanzor et al., 2021). Nitrogen-containing

chlorophyll pigments decline with nitrogen deficiency, which can reduce photosynthesis and growth rates (Korb and Gerard, 2000; Roleda and 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 result 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. In the macroscopic sporophyte phase, 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, gametophytes, 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., 2023). These studies highlight the complexity of kelp responses to interactive stressors, including the potential for nitrogen availability to reduce negative impacts of high temperatures, 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; Starko et al., 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 and 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 and 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 grow in the shallow subtidal; *S. latissima* grows at depths up to 20 m (Lamb and Hanby, 2005), while *N. luetkeana* can be found deeper (Abbott & Hollenberg, 1976). Buoyant pneumatocysts keep the majority of *N. luetkeana* blade biomass 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 *N. luetkeana* experiences. However, thermal tolerances and nitrogen requirements often differ among kelp species (Lüning and Freshwater, 1988; Roleda and 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.

2 Methods

2.1 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 two different spatially separated populations: 1) Cherry Point, Strait of Georgia, USA (48.855194, -122.730944), a warm and nutrient poor site, and 2) Turn Rock, San Juan Islands, USA (48.535333, -122.964778), a cool and nutrient rich site (Weigel et al., 2023). We collected blades of *N. luetkeana* ($n = 48$ per population from distinct individuals) from the surface by carefully detaching the blade from the pneumatocyst at the narrowest point. Individuals were randomly chosen, and multiple areas within the kelp bed were sampled to achieve a representative sample. 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. Individuals were randomly collected as they were encountered during a scuba dive. We also collected extra kelp blade tissue samples from each population and species to compare the C:N content with that 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 removed visible 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 (*N. luetkeana*) or the stipe (*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.

2.2 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 (O'Donnell et al., 2013; Guenther et al., 2022). 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). 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 ($<3 \mu\text{M}$), kelp blades drew down dissolved nitrogen from starting mean ($\pm$ SD) NO_3 levels of $23.40 \pm 0.22 \mu\text{M}$ (population exp.) and $18.14 \pm 0.01 \mu\text{M}$ (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. The two lower temperatures span the normal range of cooler areas in the Salish Sea, while temperatures of $15\text{--}16^\circ\text{C}$ are often experienced during the summer and fall in warmer areas, and 21°C represents a marine heatwave or extreme event ([Weigel et al., 2023](#)). 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 ($\pm 0.1^\circ\text{C}$) 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 ([Figure S1](#)). LED lights (5000K daylight) provided $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 ([Figure S1](#)).

After a 2 day period of initial nutrient drawdown, which occurred at the start of the 5-day acclimation period ([Figure S1](#)), we manipulated nutrient concentrations in recirculating tanks with daily nutrient additions of sodium nitrate (NaNO_3), ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$), 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 and 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), which are reflective of oceanic seawater entering the Salish Sea ([Pfister et al., 2007](#)), vs. 5 NO_3 , 0.5 NH_4 and 0.3 PO_4 (low N treatment), which are reflective of nutrient poor conditions in Puget Sound in summer ([Berry et al., 2021](#)). Starting seawater N availability ([Table S2](#)) was high (23 $\mu\text{mol NO}_3$) 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 ([Figure 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 $0.7 \mu\text{m}$ 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 ([Intergovernmental Oceanography Commission, 1994](#)).

2.3 Response variables

We blotted and weighed field-collected blades to determine the initial wet biomass, photographed each with a reference scale at 1 mm resolution to determine the initial surface area and length, and measured initial dark adapted photosystem II yield with a PAM fluorometer (described below). We measured growth (wet weight, length, surface area) and PAM response variables after the acclimation period and at the conclusion of the treatment period ([Figure S1](#)). We measured length and surface area from photographs with a reference scale at 1 mm resolution by outlining each blade in ImageJ ([Schneider et al., 2012](#)). The blade area of *S. latissima* was underestimated by this method because of the bullate morphology, compared to the smooth blades of *N. luetkeana*. We calculated change in wet mass (hereafter biomass) as percent change from beginning to end of the treatment period ([Figure S1](#)). Finally, we calculated growth of blade length and area as the difference between final and initial measurements and divided by the number of days in treatment. We considered blades to be dead if they were bleached in color and the tissue was too fragmented to be manipulated as a whole blade.

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 measurements 10 cm from the base of the blade at the same time each 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 ([Figure S1](#)).

After 6 to 8 days of exposure to experimental treatments ([Figure 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 treatment temperatures 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 mixing.

We measured dark respiration from 75 min incubations under dark tarps with lights off, then photosynthesis from 45 min incubations in $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 ([Figure 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 ($\text{mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$) as:

$$\text{metabolic rate} = \left(\frac{\Delta \text{O}_2 - C}{t} \right) * \left(\frac{V}{m} \right)$$

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 (Figure S1), we quantified kelp blade nutrient uptake rates on a random subset of blades ($n = 4$ 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 $0.7 \mu\text{m}$ glass-fiber filters and froze the samples until analysis of NO_3 , NH_4 , and PO_4 concentrations. We calculated nutrient uptake rates ($\mu\text{mol g}^{-1} \text{ hr}^{-1}$) as:

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

Where ΔN is the change in NO_3 or NH_4 concentration ($\mu\text{mol L}^{-1}$) 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.

2.4 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 and 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).

3 Results

3.1 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 : $1.8 \mu\text{M}$) and low at Cherry Point (NO_3 : 0.8 , NH_4 : 0.4 , PO_4 : $0.8 \mu\text{M}$; Table S2). From July 07 to Sept. 27, 2022, Cherry Point experienced elevated water temperatures (mean $\pm$ SD: $13.3 \pm 1.8^\circ\text{C}$, min: 10.3°C , max: 20.5°C), while Turn Rock (mean $\pm$ SD: $11.3 \pm 0.8^\circ\text{C}$, min: 10.0°C , max: 15.2°C) remained cool (Figure S3; Weigel et al., 2023). 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 : $1.7 \mu\text{M}$; Table S2).

Temperature treatments in the population and species experiments were stable and within 1°C of the target. In the population exp., temperatures as mean $\pm$ SD and ranges as (min, max) were $12.7 \pm 0.2^\circ\text{C}$ (12.1, 13.4), $16.2 \pm 0.1^\circ\text{C}$ (16.0, 16.9), and $20.6 \pm 0.1^\circ\text{C}$ (20.2, 20.8) during the full treatment period (Figure S1A). In the species exp., temperatures were $9.0 \pm 0.3^\circ\text{C}$ (8.3, 9.2), $14.6 \pm 0.1^\circ\text{C}$ (14.4, 15.4), and $20.7 \pm 0.2^\circ\text{C}$ (20.1, 21.6) during the full treatment period (Figure 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 $1.0 \mu\text{M}$ (species exp.) and below $3 \mu\text{M}$ (population exp.; Table S2; Figure S2). In the population exp., NO_3 levels in the high N treatments increased gradually over time from 25 up to $100 \mu\text{M}$, with a mean of $\sim 80 \mu\text{M}$ (Table S2; Figure S2). We reduced daily nutrient additions for the species exp., so mean NO_3 levels in the high N treatments were between 10 and $20 \mu\text{M}$ (Table S2; Figure 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 (Figure S2).

3.2 Survival and condition

In both experiments, we observed mortality or visual tissue damage and decay only at the highest temperature of 21°C (Figure 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. 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.

3.3 Growth rates

In both experiments, blade growth rates responded to temperature and nutrient treatments, and were also influenced by source population or species (Table 1). All aspects of blade growth, whether measured as change in biomass, length, or blade area, were positive at the two cooler temperatures (population exp: 13 and 16°C, species exp: 9 and 15°C) in each experiment (Figures 1, 2) and significantly lower at 21°C ($P < 0.05$). At 21°C, change in biomass was negligible in the population exp. and negative in the species exp., indicating a net loss of tissue (Figure 1). 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 (Figures 1, 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 ($P < 0.001$, Figure 2B). At these cooler temperatures, blades in both experiments grew ~15–30% in overall biomass (Figure 1), 1.0–1.5 cm in length per day, and 5–10 cm² in area per day (Figure 2), demonstrating that laboratory conditions were suitable to both species.

Kelp blade growth metrics responded to nitrogen manipulations 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 (Figure 1A). Additionally, kelp from the low N treatment elongated by 0.5 cm more per day at 13°C ($P > 0.05$, Figure 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. On average, *N. luetkeana* blades in low N treatments grew 7% more in biomass, 0.4 cm/day in length, and 4 cm²/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 cm²/day in area in low N compared to high N treatments (Figures 1B, 2B, D). However, change in biomass was low to negative at 21°C in both experiments at both high and low nitrogen levels, with an average loss of 9% biomass at 21°C (Figure 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% (Figure 1A), while *N. luetkeana* from Cherry Point grew significantly more in total blade area than those from Turn Rock by 3 cm² per day, averaged across treatments (Figure 2C), yet the two populations had equivalent changes in length (Figure 2A). In the species exp., *N. luetkeana* increased in biomass by 13% more than *S. latissima*

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: biomass as wet weight (percent change), change in length per day, and change in area per day.

Factor	Biomass			Length		Area	
	Df	F value	P value	F value	P value	F value	P value
Population exp.							
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

The population and species experiments were analyzed separately.

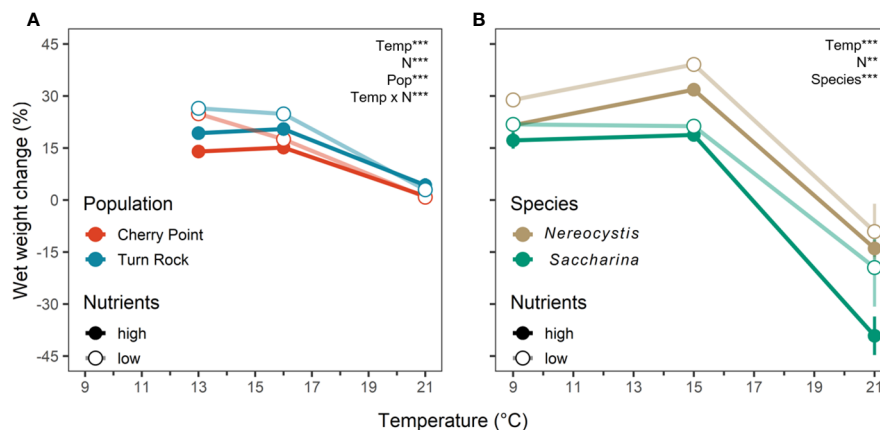


FIGURE 1

Change in wet weight (%; mean $\pm$ SE) during the experimental treatment period across temperature and nutrient levels as either high N (closed circle) or low N (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.

across all temperatures (Figure 1B), while *N. luetkeana* elongated more only at 15°C (Figure 2B) and *N. luetkeana* grew more in blade area compared to *S. latissima* at 15°C and 21°C (Figure 2D).

3.4 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. (Figures 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 ($P < 0.05$, Figure 3A). At high N, net photosynthesis was $0.7 \text{ mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ lower at the coldest temperature ($P < 0.0001$, Figure 3A), but did not differ between 15 and 21°C ($P = 0.4$, Figure 3A). Additionally, net photosynthesis was $0.1 \text{ mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ higher at low N than high N at 13 and 21°C than at 16°C ($P < 0.05$, Figure 3A), but nutrient treatments did not differ at 16°C ($P > 0.05$, Figure 3A). In the species exp., net photosynthesis did not differ with temperature in the low N treatments ($P > 0.05$), but was $0.9 \text{ mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ higher at 15°C compared to 9 and 21°C in the high N treatments ($P < 0.05$, Figure 3B). Finally, net photosynthesis of both species only responded to nutrients at 21°C, where low N kelp had $0.8 \text{ mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ greater net photosynthesis compared to high N kelp ($P < 0.001$, Figure 3B).

In both experiments, respiration increased with temperature and was highest at 21°C (Table 2; Figures 3C, D, $P < 0.05$). 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 $0.3 \text{ mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$ greater respiration rates at 21°C than low N kelp ($P < 0.001$, Figure 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 $0.1 \text{ mg O}_2 \text{ g}^{-1} \text{ hr}^{-1}$, respectively (Table 2; Figures 3A, C).

3.5 Dark-adapted Yield of PSII (Fv/Fm)

Change in Fv/Fm responded to temperature in the species exp. (Figure 4B), but not in the population exp. (Table 2), where change in Fv/Fm during the treatment period was close to 0% (Figure 4A; S4). Nutrients did not affect photosynthetic yield in either experiment (Table 2). In the population exp., photosynthetic yield at high temperatures was 3.1% lower in kelp from Turn Rock than Cherry Point (Table 2; Figures 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 ($P < 0.0001$, Figure 4B).

3.6 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 in the high N treatments compared to the low N treatments across both experiments, reflecting the difference in nitrogen availability (Table 3; Figures 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 $1.6 \mu\text{mol NO}_3 \text{ g}^{-1} \text{ hr}^{-1}$ and $0.1 \mu\text{mol NH}_4 \text{ g}^{-1} \text{ hr}^{-1}$ ($P < 0.05$, Figures 5A, C). In the species exp., NO_3 and NH_4 uptake rates increased significantly at high N by $0.3 - 1.4 \mu\text{mol NO}_3 \text{ g}^{-1} \text{ hr}^{-1}$ and $0.4 - 0.6 \mu\text{mol NH}_4 \text{ g}^{-1} \text{ hr}^{-1}$ (Table 3; Figures 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 temperature effects.

Nitrate uptake rates generally increased with temperature across experiments (Figures 5A, B), but there were significant interactive

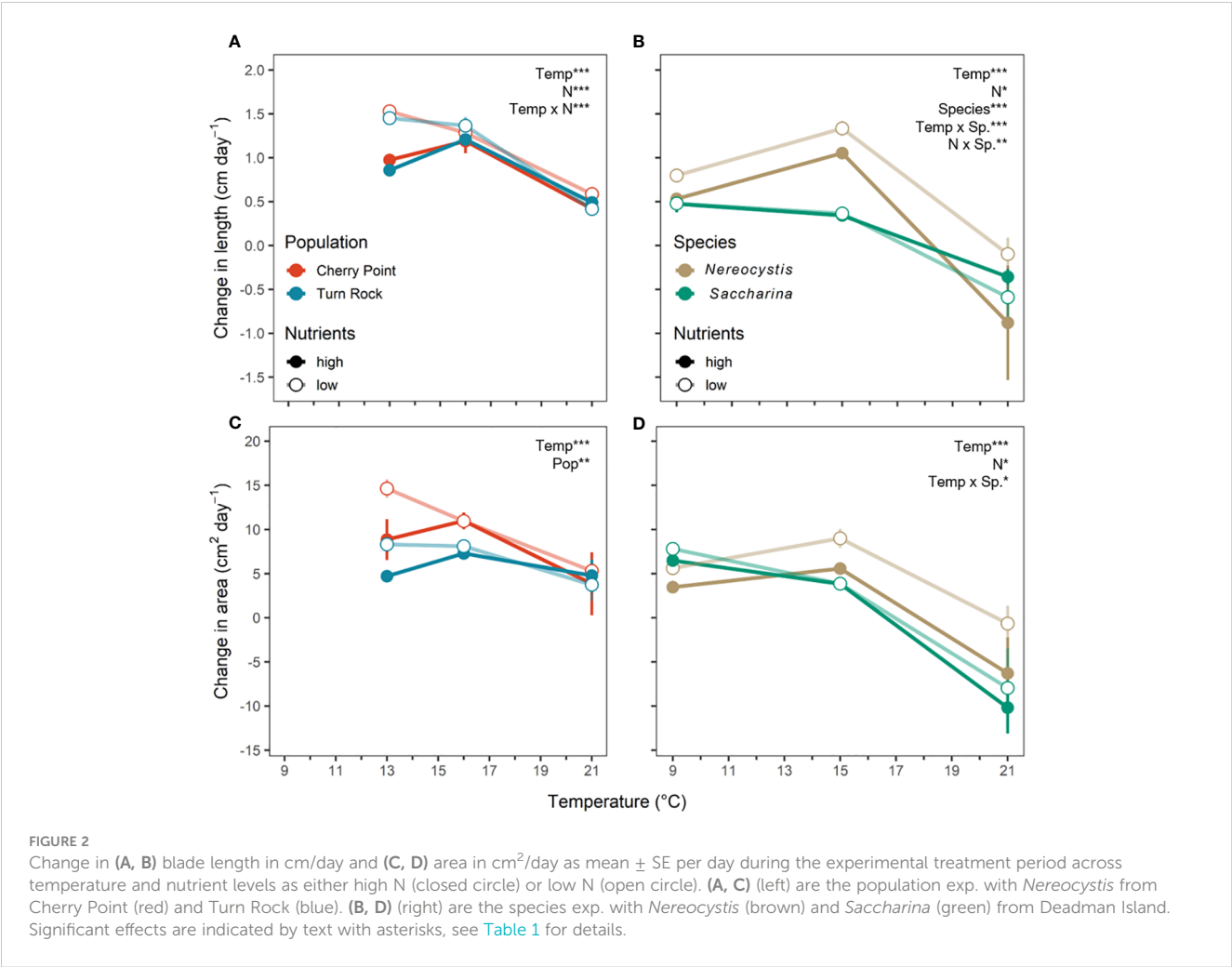


TABLE 2 Summary of two-factor ANOVAs of the fixed and interactive effects of temperature, nutrients and population or species on kelp photophysiology (net photosynthesis, dark respiration, and change in Fv/Fm).

Factor		Net Photosynthesis		Respiration		Fv/Fm change	
	Df	F value	P value	F value	P value	F value	P value
Population exp.							
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

(Continued)

TABLE 2 Continued

Factor	Df	Net Photosynthesis		Respiration		Fv/Fm change	
		F value	P value	F value	P value	F value	P value
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

The population and species experiments were analyzed separately.

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; Figures 5B, D). In the population exp., there was an interactive effect of temperature and nutrients (Table 3); 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 $0.2 \mu\text{mol NH}_4^+ \text{g}^{-1}\text{hr}^{-1}$ ($P < 0.05$, Figure 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 $0.2 \mu\text{mol NH}_4^+ \text{g}^{-1}\text{hr}^{-1}$) than those from Cherry Point ($P < 0.01$, Figure 5C). In the species exp., *S. latissima* had higher NH_4^+ uptake rates than *N. luetkeana* at 9 and 15°C by $0.1 \mu\text{mol NH}_4^+ \text{g}^{-1}\text{hr}^{-1}$ (Table 3; Figure 5D).

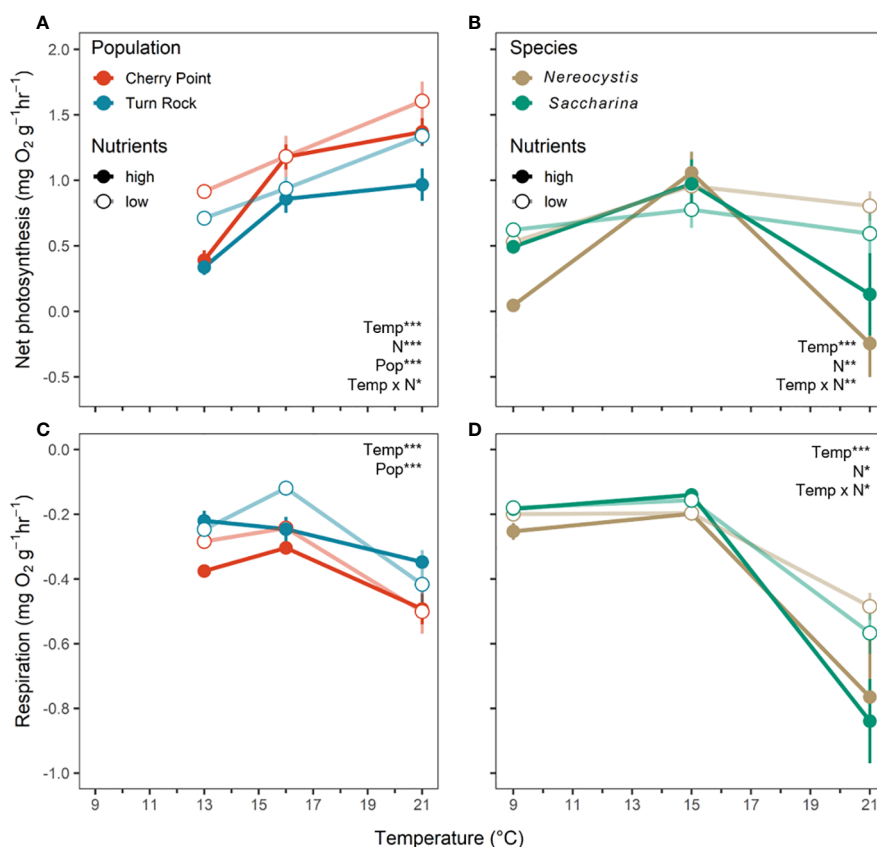


FIGURE 3

Metabolism measured as change in dissolved oxygen (mean $\pm$ SE) for (A, B) net photosynthesis and (C, D) dark respiration at the end of the experiments across temperature and nutrient levels as either high N (closed circle) or low N (open circle). Note that the respiration y-axis is negative, indicating oxygen consumption, with higher respiration rates at more negative values. (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 2 for details.

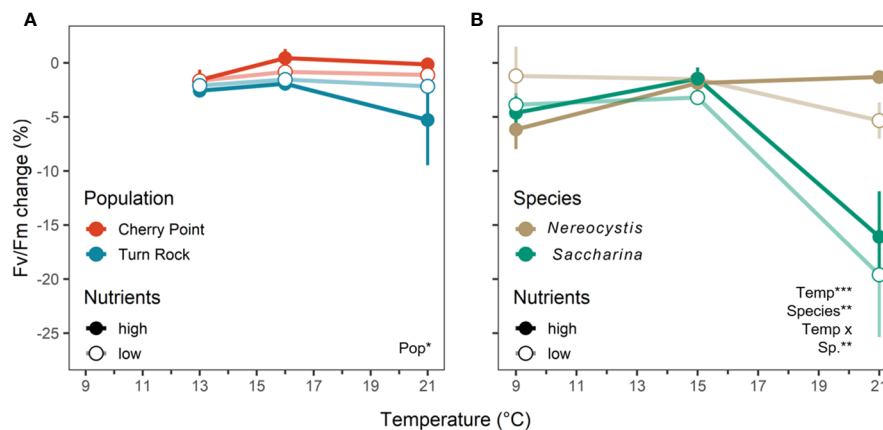


FIGURE 4

Change of dark-adapted yield of photosystem II (Fv/Fm) (%; mean $\pm$ SE) during the experimental treatment period across temperature and nutrient levels as either high N (closed circle) or low N (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.

3.7 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; Figures 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 ($P > 0.05$, Figure 6E). In the species exp., neither treatment differed significantly from the nitrogen content of field-collected samples (Figure 6F).

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.

Factor		NO ₃ uptake		NH ₄ uptake		C:N		% Carbon		% Nitrogen	
	Df	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value
Population exp.											
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

The population and species experiments were analyzed separately.

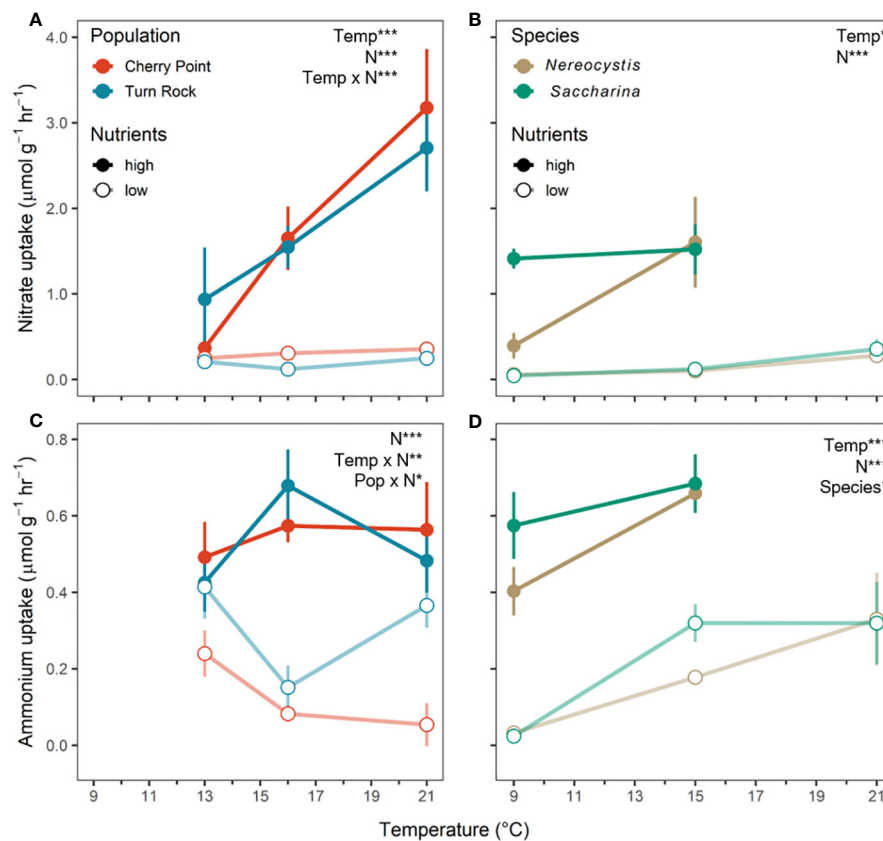


FIGURE 5

Nutrient uptake rates of (A, B) nitrate (NO₃) and (C, D) ammonium (NH₄) as mean ± SE across temperature and nutrient levels as either high N (closed circle) or low N (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.

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 NO₃ levels at Cherry Point (Table S2), the blade tissue nitrogen content of *N. luetkeana* from Cherry Point was significantly higher than the nitrogen content of blades from Turn Rock at the time of collection (Table 3; Figure 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 ($P < 0.05$, Figure 6E), while at low nutrient levels, % nitrogen was 0.4% lower at 13°C ($P < 0.05$, Figure 6E).

In the population exp., the carbon content of kelp blades differed significantly with temperature, nitrogen, and by population (Table 3; Figure 6C). The carbon content of kelp in the species exp. did not differ with any experimental factor (Table 3; Figure 6D). In the population exp., *N. luetkeana* from Cherry Point had 2.7% higher carbon content than those from Turn Rock (Table 3; Figure 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; Figure 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 (Figure 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; Figures 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. (Figures 6A, B), reflecting the different nitrogen 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; Figure 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 ($P < 0.05$, Figure 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 ($P = 0.0024$, Figure 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* (Figure 6B).

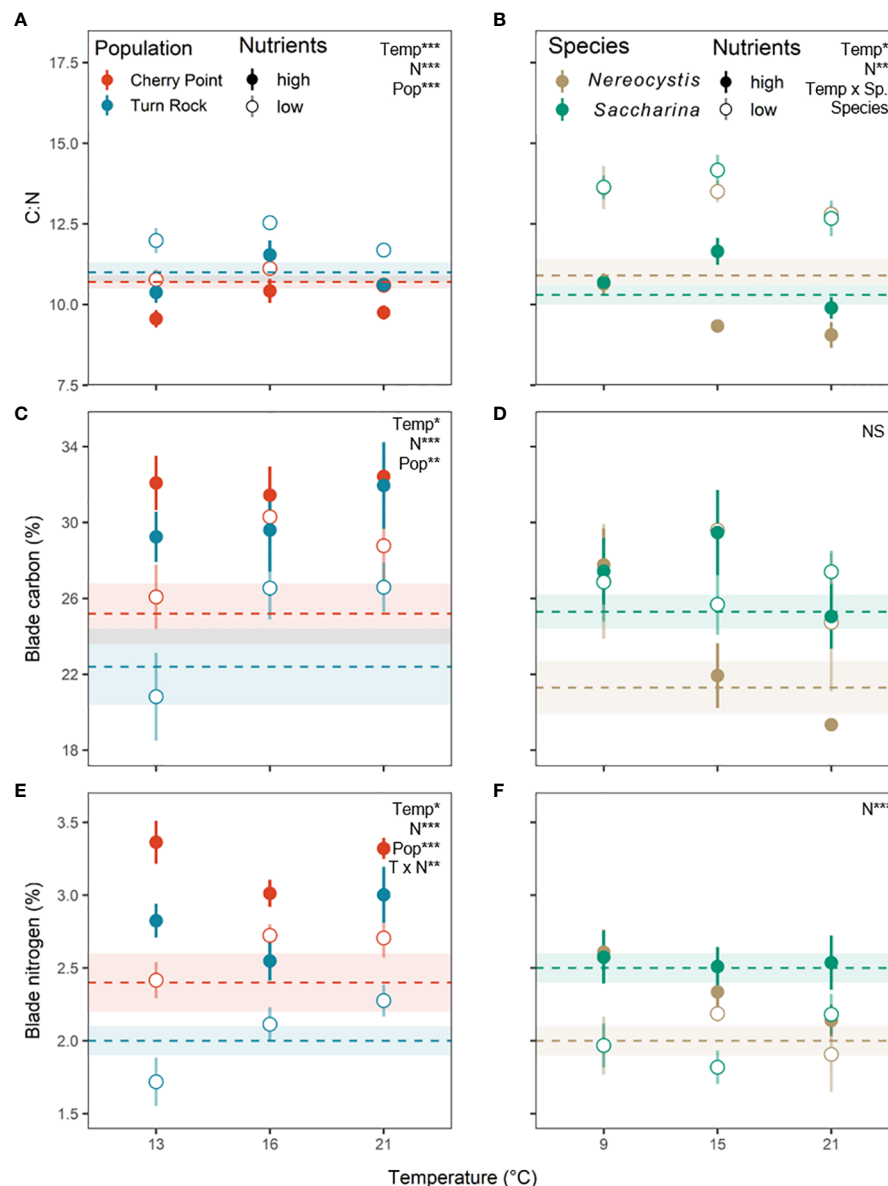


FIGURE 6

Kelp blade tissue (A, B) carbon to nitrogen ratios, (C, D) percent carbon, and (E, F) percent nitrogen at the conclusion of the experiments across temperature and nutrient levels as either high N (closed circle) or low N (open circle). Circles are mean $\pm$ SE of experimental blades. Horizontal dashed lines with shading are mean $\pm$ SE of field collected blades representing natural conditions. (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.

4 Discussion

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. Surprisingly, we did not find any negative impacts of low NO₃ concentrations (1–3 μ M) 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. 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 and Gerard, 2000). In some cases, nitrogen-replete conditions can alleviate the effects of high temperature stress (Fernández et al., 2020). Most studies report higher physiological stress under warm temperatures combined

with low nitrogen levels (Gerard, 1997; Gao et al., 2016; Endo et al., 2020; 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 over short time periods (8–9 days). Surprisingly, in the species exp., mortality at 21°C was higher for both species in the high N treatment compared to the low N treatment. 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). While they were not limiting to kelp growth at any temperature in our experiments, the time span of the experiment (8–9 days) was shorter than the duration of low nutrient levels in South Puget Sound, which can last for months (Berry et al., 2021).

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 was short. Other studies testing the interactive effects of temperature and nitrogen stress were also conducted over even shorter periods (3 to 5 days: (Fernández et al., 2020; Umanzor et al., 2021). However, after only 8–9 days of exposure to 21°C, kelp in both experiments experienced tissue damage and decay. 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, subtidal daily maximum seawater temperatures exceeded 16°C for 50 consecutive days in a kelp forest in southern Puget Sound, but warm temperatures occurred at other sites for just a few days (Weigel et al., 2023), and daily maximum temperatures at Cherry Point exceeded 18°C on multiple days in the summer (Figure 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 (Figure 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 and Gerard, 2000; Pueschel and Korb, 2001). Our experiments, as well as others, show that nutrient uptake rates can increase with temperature (Harlin and Craigie, 1978; Nishihara et al., 2005; Roleda and Hurd, 2019). Both NO_3 and NH_4 uptake rates increased with temperature, which could increase blade tissue nitrogen, but only if there is sufficient dissolved inorganic nitrogen available in the environment. 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 (Murray et al., 2015; Berry et al., 2021). 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 (e.g. 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 and Hepburn, 2016). It is clear that further physiological work is needed to understand the negative impacts of high N on both kelp species at elevated temperatures, but our experiments strongly suggest that low N levels in the Salish Sea are not a cause of kelp declines.

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 a monotypic genus endemic to the west coast of North America (Abbott and Hollenberg, 1976). Optimal growth of *N. luetkeana* sporophyte blades occurred at 12°C in British Columbia, Canada (Supratya et al., 2020), while in our study, the highest growth rates occurred at 13 to 16°C. 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 for 8–9 days 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. *N. luetkeana* is an annual species, with sporophyte growth peaking in late spring to early summer. 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 (Figure 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 bottom temperatures (up to 20°C, Weigel et al., 2023) 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 > 21°C are likely to lead to further kelp forest declines.

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). 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). Yet studies on *Ecklonia radiata* found that nutrient enrichment may alleviate the stress of low light availability due to turbidity (Blain and Shears, 2020), highlighting the complexities of multiple stressors. Future studies in the Salish Sea should examine the interactions among temperature, nutrients, light, and duration of stressors which together may affect competitive outcomes between benthic and canopy species.

We found that sporophytes of *S. latissima* and *N. luetkeana* responded similarly and strongly to high temperatures, 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 (Riche et al., 2014; Khangaonkar et al., 2019; IPCC, 2022). In addition to curtailing emissions, management actions to maintain and restore water circulation (Khangaonkar and Yang, 2011; Khangaonkar and Wang, 2013) 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 and Wernberg, 2018; Tait et al., 2021). Therefore, important policies to limit eutrophication should remain in place.

Our results highlight the importance of considering elevated temperatures in kelp forest conservation. 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 critical temperatures of 16–18°C (microscopic sporophytes and gametophytes, respectively) (Weigel et al., 2023). 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.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://doi.org/10.5061/dryad.9p8cz8wpn>.

Author contributions

RF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. BW: Conceptualization, Data curation, Formal analysis,

Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing – original draft, Writing – review & editing. EC: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. HB: Conceptualization, Funding acquisition, Resources, Writing – review & editing. MD: Conceptualization, Funding acquisition, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1281104/full#supplementary-material>

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