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Ecophysiological Responses of the Intertidal Seaweed *Fucus Distichus* to Temperature Changes and Reduced Light Driven by Tides and Glacial Input

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Abstract
Climate change is influencing the performance and distribution of macroalgae in the marine environment. Although intertidal seaweeds successfully adapt to extreme and rapid abiotic changes, exposure to persistent or prolonged potentially stressful conditions can affect their vitality and productivity. Rapid glacial melt can severely alter seawater physicochemical characteristics for shallow and intertidal seaweed communities on the Alaskan coasts. Understanding how intertidal macroalgae respond to this complex mosaic of stressors is key to assessing their ability to adapt to a climate change scenario. This study assessed whether specific stress responses and acclimation mechanisms were exhibited by the intertidal brown seaweed *Fucus distichus* subsp. evanescence may enable it to cope with changing temperatures and reduced light availability linked to tides and glacial inputs. We analyzed its physiological performance, including photobiological variables, nutrient content, nitrate uptake, and oxidative stress descriptors under strictly controlled laboratory conditions. Results show that this subspecies of *Fucus distichus* may be relatively unaffected by changes in light and temperature driven by glacial melt due to the presence of pre-adapted strategies that collectively express wide physiological tolerances. Outcomes provide insights into some of the mechanisms of stress tolerance of this major structuring seaweed across the Alaskan coast. Nonetheless, glacial melt would also lower salinity in coastal water, potentially resulting in osmotic stress and other physiological effects not explored here.

Keywords Fucoid · Glacial melt · Electron transfer rate · Nitrate uptake · Non-photochemical quenching

Introduction

Shifts in environmental conditions because of ongoing global climate change are negatively affecting the distribution and abundance of a variety of seaweeds, while facilitating the prevalence of others (Wernberg et al. 2010; Weslawski et al. 2010; Muth et al. 2019). These impacts may be noticeable in intertidal and shallow coastal habitats where seaweeds must already overcome extreme and rapid abiotic changes at relatively small spatial (cm to m) and temporal scales (Valdivia et al. 2011; Umanzor et al. 2019). Seaweeds inhabiting these environments can cope with intermittent stresses and still thrive. However, when stress increases in magnitude, frequency, or time and includes the interaction of multiple stressors, seaweed physiological tolerance thresholds may eventually be exceeded. Here, stress (or "distress," sensu Lichtenthaler 1998) is defined as the impacts of abiotic or biotic factors that unfavorably affect seaweeds' performance and could ultimately diminish their growth rates, reproduction, and survival (Vinebrooke et al. 2004). Whether or not a factor is stressful depends on the physiology of the seaweed, the nature of the stressful event, and whether interactions among multiple factors increase stress. In this sense, stress is ubiquitous, and seaweeds inhabiting extreme environments or at the edges of their normal ranges are highly vulnerable to increasingly stressful conditions.

Intertidal zones at high latitudes, such as those in Alaska, are particularly extreme habitats with large diurnal changes in temperature and light where seaweeds can be exposed to

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freezing and overheating conditions and dark periods that can extend for more than 16 h over a 24-h period. Seaweeds here are also intermittently and seasonally exposed to excessive desiccation (Lindstrom 2009). Nearby glacial discharge introduces colder freshwater and silt inputs that reduce water clarity depending on air temperature, rainfall, and season (Hood and Berner 2009). Intertidal zones in Southeast Alaska are experiencing significant regime changes driven by the increasing influence of glacial meltwater and runoff. Historical maps and satellite imagery show that coastal glaciers in Alaska have retreated since the end of the Little Ice Age, with accelerated retreat after the mid-2000s (Maraldo 2020). A robust and growing body of evidence shows that changes in watershed glacial coverage and increasing meltwater input affect downstream water properties, influencing estuarine dynamics at different trophic levels (Arimitsu et al. 2016). These changes may create favorable or unfavorable conditions for intertidal seaweeds depending on their phenotypic plasticity to a wide range of conditions and their capacity to mitigate through ecosystem engineering processes or adapt to new environmental conditions (Bertness and Leonard 1997; Bertness et al. 1999; Weslawski et al. 2010; McCabe and Konar 2021).

Fucus distichus is the dominant perennial seaweed in intertidal communities of wave-protected sites in coastal Alaska, able to adapt to widely varying environmental conditions (Lindeberg and Lindstrom 2010). Like other macroalgal species, *F. distichus* is a foundation species and ecosystem engineer, adding roughness and tridimensionality to the intertidal seascape and providing refuge and food for many species (Stekoll and Deysher 2000; Watt and Scrosati 2013). Intertidal ecosystem engineers are typically dominant space holders, able to ameliorate localized stressful physical conditions such as extreme temperatures and increased particle flow, thus facilitating biofilm formation and the recruitment, survival, and growth of macroinvertebrates (Bertness et al. 1999; Umanzor et al. 2017).

Some species and populations of *Fucus* appear to be relatively unaffected or even benefit from climate change-driven events, including glacial melt, presumably because of preadapted ecotypes that collectively express a wide range of physiological tolerance (Weslawski et al. 2010; Wahl et al. 2011). A recent study conducted in southeast and south-central Alaska showed greater biomass of *F. distichus* at sites influenced by more heavily glaciated watersheds with colder temperatures and lower light availability than at sites with less glacial influence (McCabe and Konar 2021). These results suggest that increased glacial influence may be advantageous to *F. distichus*. However, the physiological basis behind this apparent physiological resistance or preadaptation remains unknown.

Understanding how *F. distichus* responds to single and combined stress factors is necessary to assess its potential physiological limits and the risks to intertidal communities shaped by this species if its limits are exceeded due to climate change effects. Here, we summarize our results on the physiological adjustments showed by *Fucus distichus* subsp. evanescence, including photobiology, nutrient content, nitrate uptake, and oxidative stress, in response to environmental factors linked to glacial inputs in Southeast Alaska, i.e., temperature and light availability. Understanding such mechanisms will provide insights into the stress tolerance and resiliency of a major structuring seaweed in Alaska and contribute to understanding how global climate change may affect subarctic intertidal and subtidal habitats.

Materials and Methods

Collection of Targeted Seaweed

Complete juvenile sporophytes of 10–15 cm length with exactly two branches were collected from the intertidal zone at Lemon Creek estuary, Juneau (58.339737, – 134.516992), in July 2020. The intertidal zone at Lemon Creek is a mudflat fed by a watershed with approximately 25% glacial coverage (Hood and Berner 2009). This provides sufficient glacial input from meltwater to prevent stream water temperatures from rising with summer air temperatures and maintains consistently elevated turbidity levels during that season. Discharge for Lemon Creek mudflats ranged from < 1 to 71 $m^3 s^{-1}$ throughout the study period. The substrate is characterized by small cobbles interspersed throughout the glacial mud and silt, in which single *F. distichus* thalli occupy separate cobbles, allowing for the collection of discrete experimental units without any physical disturbance (Fig. 1).

Fig. 1 An individual juvenile *F. distichus* thallus growing on a small cobble. Collected from the Lemon Creek mudflats, Juneau, Alaska

Forty non-reproductive individuals with complete dark green tissue were collected at roughly the same intertidal elevation on a spring low tide (− 1.1 m MLLW) without detaching them from their substrate. They were promptly stored in coolers without water and transported to the Mariculture Lab at the Juneau College of Fisheries and Ocean Sciences. Upon arrival, individuals were placed inside a 1000 L holding tank with flow-through seawater. Thalli were acclimated for 5 days to average low reference field values of temperature (6 °C) and maximum irradiance (210 µmol photons m^{-2} s^{-1}). Salinity was maintained at 22 ppt, as at high tide on the day of collection. Experimental parameters were monitored using submersible temperature loggers (HOBO MX2202; ONSET, USA) and a full spectrum and cosine-corrected quantum sensor (MQ-510; Apogee, USA). After the acclimation period, thalli were moved to 4 L aquaria, such that each of the 12 aquaria contained two individuals.

Experimental Design

The treatments consisted of four combinations of temperatures (6 ± 0.5 °C or 18 ± 0.5 °C) and light regimes ($L = 210 \pm 15$ µmol quanta m^{-2} s^{-1} or $D = 70 \pm 15$ µmol photons m^{-2} s^{-1}) reflecting average low and high extremes of these variables recorded at high tide between June and September when glacial discharge is elevated. Experimental conditions were established using values of temperature and light collected around the same period the year before. At this time of the year, the photoperiod at Lemon Creek was close to 18 h of light and 6 h of darkness. Temperature and irradiance were measured continuously using a data logger (HOBO MX2202; ONSET, USA) and point measurements were made using a multi-parameter reader (YSI, USA). Field measurements were filtered to extract and average those values collected during times when thalli would be submerged completely due to high tide.

Experimental tanks ($n = 4$ with 2 individuals each) were exposed to 6D, 6L, 18D, or 18L following the categorical factors above. Thalli were maintained fully submerged in their experimental treatments for three consecutive days resembling conditions during neap tides when *Fucus* growing in the low intertidal remain submerged throughout the tidal cycle. All physiological descriptors were measured in both individuals per aquaria (i.e., pseudo replicates). Before the beginning of the experiment, photochemistry descriptors and tissue absorptance were measured in eight thalli randomly selected among the aquaria to determine if differences in baseline physiological status among thalli existed.

Chlorophyll-a Fluorescence

Chlorophyll-a fluorescence emission of PSII was measured using a portable fluorometer (Junior-PAM; Walz, Germany). Photochemistry measurements were acquired from the mid-section of one of the dichotomous branches on each thallus to standardize measurements among individuals and avoid within-tissue variability. The branch and sections to be measured were assessed a priori and selected according to the highest values of F_v/F_m (maximum quantum yield) obtained. Each blade segment was clipped to delimit this position and held to the fluorometer using DCL-8 leaf clip holders to ensure a constant distance between the tissue and the fiber optic. Values of F_v/F_m were obtained after applying a saturating pulse (~ 5000 µmol photons m^{-2} s^{-1}, 0.8 s) on thalli fully acclimated to darkness overnight (Schreiber 2004; Larkum et al. 2006). The clipped segments were then exposed to actinic light intensities provided by the fluorometer of either 210 or 70 µmol photons m^{-2} s^{-1} for 3 min, to ensure a steady-state of fluorescence. These actinic irradiances match those applied for the experimental treatments. Saturating pulses were then applied to these illuminated tissues to calculate Φ_{PSII} (effective quantum yield), electron transport rate (ETR), and non-photochemical quenching (NPQ). Absolute ETR was calculated as $ETR = \Phi_{PSII} \times I \times A \times 0.5$ where Φ_{PSII} is the effective quantum yield, I is the corresponding irradiance, A is the blade absorptance (see below), and 0.5 is a correction factor for absorption of quanta per photosystem (Schreiber and Neubauer 1990; Beer et al. 2014). Values of NPQ (non-photochemical quenching) were calculated as $NPQ = (F_m - F_{m'})/F_{m'}$, where F_m is the maximum fluorescence and $F_{m'}$ is the fluorescence measured when blades were exposed to each intensity of actinic light.

Blade absorptance (A) was estimated and measured in the same tissue used for photochemistry, following the method proposed by Beer et al. (1998), Beer and Bjork (2000), and Beer and Axelsson (2004). Samples were placed over the cosine-corrected quantum sensor and at a constant distance below a halogen light source. Transmitted light was measured through intact pigmented tissues and tissues bleached with a 10% bleach solution in seawater, and A was then calculated according to the equations in Vásquez-Elizondo et al. (2017).

Pigment Content

Thallus tissue was processed following the method described by Seely et al. (1972) and modified by Wheeler (1980). A total of 0.03 g of fresh tissue was placed into a tube containing dimethyl sulfoxide (DMSO). The solution of DMSO with extracted pigments, including fucoxanthin, Chl-*a*, Chl-*c*, and others, was diluted at a 4:1 ratio DMSO:water. Pigment content in the dilution was estimated from absorbance measurements at wavelengths of 470, 581, 631, and 664 nm in a spectrophotometer (Cary, 50 Bio, USA), using the equations and specific molar extinction coefficients from Seely et al. (1972). The remaining tissue was stored in darkness

at 4 °C for a second extraction. A second extraction was conducted by placing the remaining tissue in a 15 ml conical tube with 2 ml of 100% acetone for 24 h at 4 °C in darkness. After extraction, the solution was diluted at a 3:1:1 ratio extract:methanol:water. Pigment content in the dilution was also calculated using the equations and specific molar extinction coefficients from Seely et al. (1972). Final pigment content (i.e., fucoxanthin, Chl-a, and Chl-c) was calculated as the sum of all pigments obtained from the two sequential extractions.

Soluble Carbohydrates

Soluble carbohydrates were measured using the colorimetric phenol–sulfuric acid method, with glucose as the standard (Dubois et al. 1956). Thalli were first dried and ground to a fine powder from which 0.02–0.025 g were digested in conical tubes containing 2 mL of 0.2 M HCl maintained at 60 °C for 3 h. Samples were agitated every 30 min to facilitate digestion. Each sample was then centrifuged for 5 min at 1000 × g. A mixture of 0.025 mL of supernatant, 1 mL of distilled water, and 0.25 mL of 3% phenol was placed in a new conical tube and cooled in an ice bath. Then 2.5 mL of concentrated sulfuric acid were added directly into the mix and vortexed vigorously. After exactly 30 min, absorbance was measured at 490 nm.

Nitrate Uptake and Total Nitrogen and Carbon Content

Entire thalli were incubated for 30 min in 2 L Plexiglas transparent chambers filled with artificial seawater (Instant Ocean®) enriched with 15 µM of labeled nitrate $K^{15}NO_3$ (99 atoms % 15N, Cambridge Isotope Laboratories). Seaweed biomass:volume ratio of 1:4 (g FW/ml) was selected to prevent a substantial decline of nitrate concentration during the incubation. All chambers (2 L) were submersed in their respective experimental tanks (4 L) to maintain the temperature and light conditions of each treatment while incubation occurred. Chambers were continuously agitated to ensure that the cultivation media mixed continuously to homogenize the ^{15}N tracer during incubation and reduce the boundary layer around the thalli (Cornelisen and Thomas 2004). Following the 30 min, thalli were removed from the chambers and rinsed thoroughly with deionized water to remove the tracer from the surface of the thalli. All samples were dried at 55 ± 0.2 °C for at least 8 h. Samples were ground finely and prepared for isotope enrichment analyses. Isotopic ^{15}N determinations were carried out at the Alaska Stable Isotope Facility (ASIF) using an elemental analyzer interfaced with a continuous flow isotope ratio mass spectrometer. Also, elemental N and C were determined in samples not exposed to the tracer.

Nitrate uptake rates (V), expressed as µmol $N \cdot g^{-1}$ DW $\cdot h^{-1}$ (DW = dry weight) were calculated as $V = [(^{15}N_{treatment} - ^{15}N_{background}) \times N_{content}] / (M_N \times t)$, where the difference ($^{15}N_{treatment} - ^{15}N_{background}$, as %) is the ^{15}N enrichment relative to natural (i.e., background) ^{15}N thalli levels, $N_{content}$ is the nitrogen content (g $N \cdot g^{-1}$ DW) measured in thalli not exposed to the ^{15}N tracer, while M_N is the molar mass of the labeled nitrogen (15 g mol^{-1}), and t is the incubation period in hours.

Total Phenolic Content

A total of 0.03–0.035 g DW of dried and finely ground thalli tissue was mixed with 0.75 mL 80% methanol and kept in darkness for 24 h. The extract was then centrifuged at 10 rpm for 10 min. The phenolic compound content was measured from the supernatant following a modified Folin–Ciocalteu assay with a curve of gallic acid as reference (Singleton and Rossi 1965). In brief, an 0.1 ml aliquot of the methanolic extract was diluted in 1 mL distilled water. Then, 0.1 mL of Folin–Ciocalteu reagent and 0.3 mL of saturated $NaCO_3$ were added, and the solution was homogenized and heated at 40 °C for 3 min. The absorbance of each sample was read at 765 nm using a spectrophotometer. The rest of the methanolic extract was stored for further analysis.

Antioxidant Capacity

The radical scavenging activity of the extracts was determined, using the same methanolic extracts prepared to measure the total phenolic content, with the stable free radical, DPPH, and ascorbic acid as standard (Farvin and Jacobsen 2013). The reactive solution was prepared by mixing 0.1 mL of diluted extract (1:4 with aqueous methanol at 80%) and 1 mL of 30 µM DPPH freshly prepared in aqueous methanol at 90%. Exactly 30 min after the DPPH was added, the absorbance was measured at 517 nm. The absorbance was also measured in a blank control solution with the same proportions of DPPH and aqueous methanol without the algal extract.

Statistical Analysis

Temperature (hereafter 6 or 18) and light availability (hereafter D or L) were considered categorical independent factors. Differences in all response variables as a function of both factors and their interactions were explored using two-way factorial ANOVA at an alpha of 0.05 and after fulfillment of assumptions. Data transformation was not required. Tukey HSD analyses were conducted where significant differences were found. Analyses were conducted using R (R Core Team 2020).

Table 1 Reference values of physiological descriptors measured in thalli of *Fucus distichus* before the experiment

	Mean (SD), $N = 8$	Min–Max
Photochemistry		
F_v/F_m	0.63 (0.02)	0.59–0.67
Φ_{PSII}	0.32 (0.06)	0.20–0.40
ETR (at 210 µmol photon m^{-2} s^{-1})	20.54 (3.98)	13.44–25.46
Bio-optical properties		
$A_{400\text{-}700}$	0.62 (0.02)	0.59–0.64
Nitrogen and carbon		
% N	2.57 (0.27)	2.41–2.72
% C	36.19 (1.66)	34.21–39.19

Results

The initial physiological status was similar in all the studied thalli (Table 1) showing that physiological changes observed at the end of the experimental period were due to the treatments and not preconditioned physiological differences. Values of maximum quantum yield, F_v/F_m, showed differences as a function of temperature only, with thalli exposed to 6 °C and low light showing the lowest values (Fig. 2a, Table 2). Non-photochemical quenching (NPQ) values showed significant interactive effects of temperature and light availability (Fig. 2b, Table 2), with 6L-6D (Tukey $P < 0.05$), 18L-18D (Tukey $P < 0.001$), and 18L-6L ((Tukey $P < 0.001$), showing significant differences. Electron transport rate, ETR, showed significant differences due to light availability but not temperature, with thalli exposed to reduced light showing a reduced rate (Fig. 2c, Table 2). No differences were observed in effective quantum yield, Φ_{PSII} (Fig. 2d, Table 2).

Overall, pigment content was higher in thalli exposed to reduced light availability. These differences were mainly driven by Chl-a and were only significant for light treatments in Chl-a and Fx but not in Chl-c (Fig. 3a–c; Table 2). Only Chl-c showed a significant response to temperature (Fig. 3b, Table 2). For Chl-c, an interaction between light and temperature did show significance in the posthoc comparison,

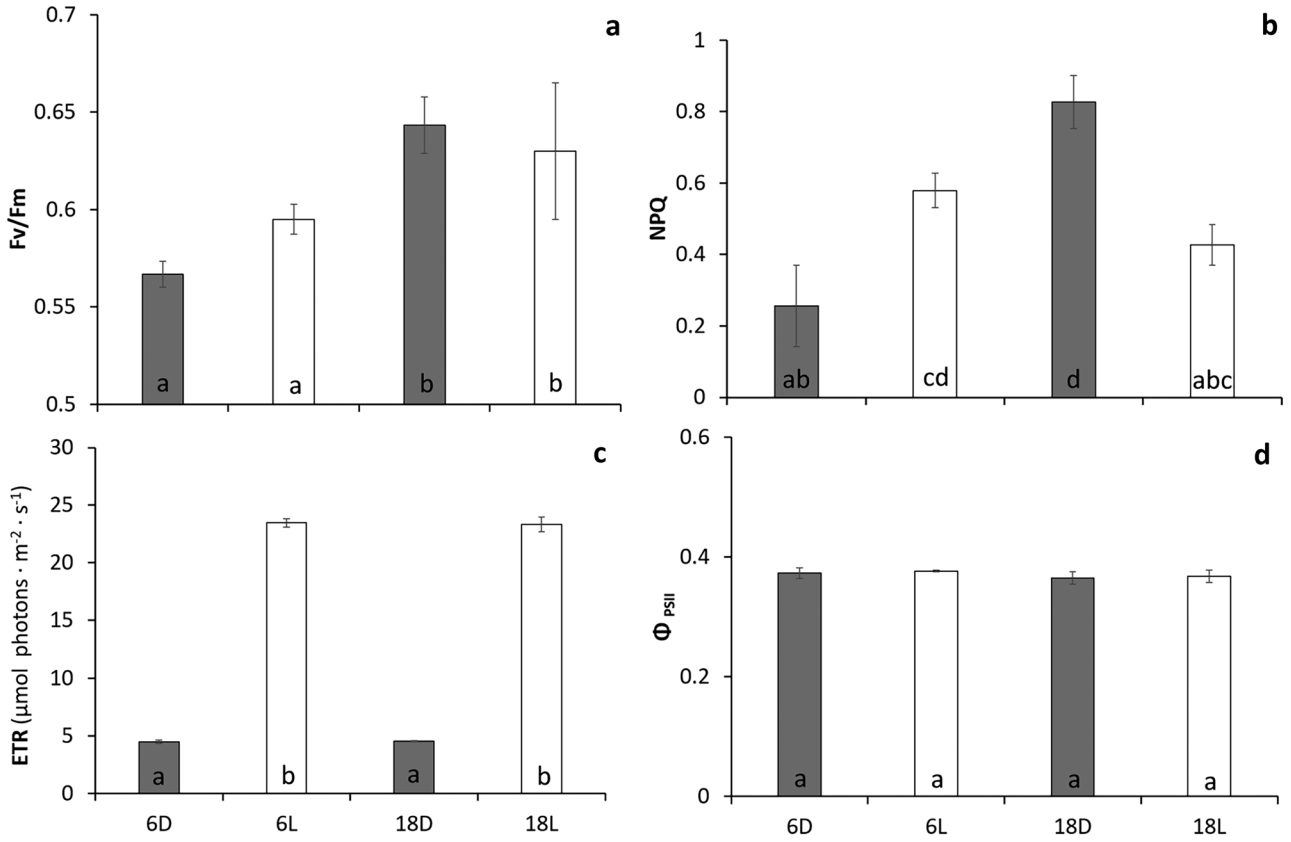

Fig. 2 Photochemical descriptors measured in thalli of *Fucus distichus* (mean ± SE, $N = 4$) exposed to 6 °C (denoted as 6) or 18 °C (denoted as 18) and 210 µmol photons m^{-2} s^{-1} (denoted as L with light-filled bars) or 70 µmol photons m^{-2} s^{-1} (denoted as D with dark-filled bars) for three consecutive days. Maximum quantum yield (**a**), non-photochemical quenching (**b**), electron transport rate (**c**), effective quantum yield (**d**)

Table 2 Two-way ANOVA showing the main and interactive effects of temperature (6 and 18 °C) and light availability (210 or 70 µmol photons m^{-2} s^{-1}) on physiological descriptors for *Fucus distichus* after a 3-day exposure period

		Temperature			Light			T x L		
	df	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*
Photochemistry										
F_v/F_m	1	0.02	13.07	**0.001**	0.0003	0.24	0.632	0.00	1.82	0.192
NPQ	1	0.26	7.59	**0.012**	0.01	0.26	0.617	0.78	22.71	**< 0.001**
ETR	1	0.00	0.005	0.944	2145	1661	**< 0.001**	0.00	0.04	0.846
ΦPSII	1	< 0.001	0.98	0.335	0.0001	0.14	0.717	0.00	0.00	0.985
Pigments										
Chl-a	1	6691	0.11	0.754	23,841	37.43	**< 0.001**	21	0.000	0.986
Chl-c	1	56,723	11.77	**0.009**	20,235	4.20	0.075	2961	0.62	0.456
FX	1	5671	0.74	0.415	14,984	19.52	**0.002**	755	0.10	0.762
C, N, ratio, uptake										
Carbon %	1	1.56	0.30	0.594	3.07	0.58	0.458	0.000	0.000	0.999
Nitrogen %	1	0.01	0.07	0.796	0.04	0.41	0.533	0.31	3.03	0.101
C:N	1	0.37	0.13	0.722	0.10	0.04	0.851	11.57	4.16	0.058
Nitrate uptake rate	1	0.15	61.69	**< 0.001**	0.02	8.72	**0.018**	0.01	4.56	0.065
Phenols, antioxidants										
Phenols	1	6.57	20.71	**0.002**	0.25	0.80	0.398	0.003	0.01	0.922
Antioxidant capacity	1	0.002	0.13	0.727	0.07	5.50	**0.030**	0.02	1.64	0.214
Soluble carbohydrates	1	0.06	5.05	**0.036**	0.01	0.95	0.343	0.000	0.000	0.994

Bold numbers indicate significant differences. Underscore value denote a *P*-value close to significance

where Chl-c content in 6D was significantly higher than in 18L (Tukey $p = 0.019$, Fig. 3b).

Neither light nor temperature affected C:N ratio, but light x temperature interaction was nearly significant (Fig. 4c; $p = 0.0581$, Table 2). However, the differences between treatments were relatively small (lower than 12.2%). Nitrogen uptake was significantly influenced by temperature and light (Fig. 4d), and their interactive effect was marginal ($p = 0.0653$, Table 2).

Changes in total phenolic content were statistically significant in response to the two temperature treatments, but not to light levels, with cold temperature resulting in higher phenolic content (Fig. 5a, Table 2). Significant differences in total antioxidant capacity appeared in response to light availability but not to temperature (Fig. 5b, Table 2). The tissue content of total soluble carbohydrates showed a significant difference in response to temperature (Fig. 6, Table 2).

Discussion

Summer growth of *Fucus distichus* in glaciated regions of coastal Alaska is influenced by colder water temperatures and reduced light availability due to siltation compared to winter when water is clear, but days are shorter. Short-term physiological adjustments after the 3-day exposure period were observed in response to light and temperature experimental treatments. Such responses included both stress responses and acclimation mechanisms, and the interaction of their metabolic costs and benefits is critical to elucidate how *F. distichus* withstand short-term stressful conditions caused by the glacial discharge.

Some works have documented that temperature and light stress in seaweeds can lead to a decrease in their maximum quantum yield (F_v/F_m), possibly reflecting a weakening of the PSII functioning (Mabin et al. 2019; Sánchez-Barredo et al. 2020). In this study, values of F_v/F_m were slightly lower (~ 0.55 vs. ~ 0.65) in thalli exposed to low temperature but showed no significant change in response to light availability. This difference may not be biologically significant, with results possibly reflecting that our highest light treatment (i.e., ~ 210 ± 15 µmol photons m-2 s-1) is non-saturating for the experimental *Fucus*. Typically, only high excitation pressures and overreduction of photosynthetic apparatus would cause photodamage. Another alternative could be that reduced values of Fv/Fm due to temperature reflect adjustments in the photosynthetic apparatus related to the slowdown of enzyme-mediated processes acting as sinks of metabolic energy (ATP and NADPH) and electrons from the light phase of photosynthesis (Takolander 2022).

Light availability influences electron transport in the thylakoid membranes downstream of photosystem II (PSII; Falkowski and Raven 2007). As expected, the maximum electron transport rate (ETR) was significantly lower in

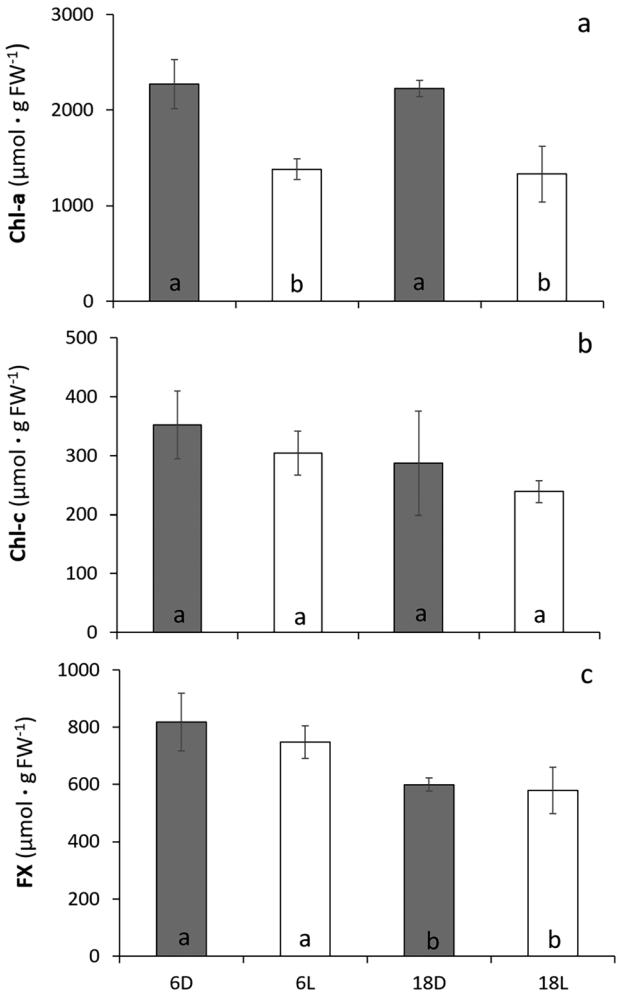

Fig. 3 Chlorophyll-a (**a**), chlorophyll-c (**b**), and fucoxanthin (**c**) content (µg per gram of fresh weight) measured in thalli of *Fucus distichus* (mean ± SE, $N=4$) exposed to 6 °C (denoted as 6) or 18 °C (denoted as 18) and 210 µmol photons m^{-2} s^{-1} (denoted as L with light-filled bars) or 70 µmol photons m^{-2} s^{-1} (denoted as D with dark-filled bars) for three consecutive days

thalli exposed to low light availability. Energy dissipation as heat by the xanthophylls cycle (i.e., NPQ) was the highest in thalli growing at 18 °C and exposed to low light availability. Heat release is a photoprotective mechanism activated to avoid the over-reduction of electron transport carriers (Niyogi 2000; Sanchez-Barredo et al. 2020). Such a photoprotective mechanism has been demonstrated in other seaweeds, particularly when assessing light acclimation strategies (Colombo-Pallotta et al. 2006; Umanzor et al. 2020). Therefore, it was not expected to measure the highest values in thalli exposed to dark conditions. However, increments in NPQ are not always related to saturating or over-saturating light conditions. These increments could also relate to high excitation pressure conditions (a measure of the relative redox state of Q_A- first stable quinone) generated by environmental stress, such as nutrient limitation (Hüner et al. 2012). We did not find significant differences in ΦPSII despite the differences in ETR and NPQ, indicating that the efficiency to process light at thylakoid level remained optimum in periods of colder temperature and lower light simulating conditions of high glacial meltwater input.

In our study, the concentration of Fx in 6D and Chl-*a* in 6D and 18D were higher than in thalli with more light availability. Pigment concentrations are consistent with the general patterns described in other studies where pigment concentration, including Chl-*a*, Chl-*c*, fucoxanthin, and carotenoids, increases in individuals exposed to light-limiting conditions, regardless of temperature. Changes in pigment concentration are a photoacclimation strategy widely recognized in subtidal and intertidal seaweeds (Schiel and Foster 2015; Mabin et al. 2019; Sanchez-Barredo et al. 2020), as higher pigment content can counteract low light levels by increasing light harvesting capacities and, in most cases, tissue absorptance (Beer et al. 2014).

The percentage of tissue carbon, nitrogen, and the C: N ratio is consistent with values obtained for other *Fucus* species, with carbon contents of 30–40% and upper nitrogen levels of 2.5–3% dry weight (Brenchley et al. 1998) These values suggest that neither the experimental temperatures nor stress due to irradiance below the compensation point for photosynthesis are compromising the internal nutrient resources of *Fucus* (as well as soluble carbohydrates), at least during short term periods tested in this study simulating environmental conditions influenced by glacial discharge (Lehvo et al. 2001; Middelboe et al. 2006; Wahl et al. 2011). However, nitrogen uptake was affected by temperature and light availability independently. Assimilation of inorganic nitrogen into organic compounds requires energy and carbon skeletons from photoassimilates (Hurd et al. 2014) so we expected an effect on carbon skeletons particularly link to the 18 °C treatments. In this experiment, light limitation at 18 °C seemed to have reduced the capacity of F. distichus thalli to uptake N to less than half the uptake measured at 6 °C, regardless of the irradiance level. The diminished nitrate uptake rates in thalli exposed to 18 °C, particularly of those exposed to lower light levels, can be a consequence of different factors, such as the scarcity of metabolic energy required to incorporate (and assimilate) nitrate, the alteration in the functionality of its transmembrane active transport system, or even the metabolic decoupling between nitrate incorporation and the consumption of internal non-structural C and N by respiration (Sanchez-Barredo et al. 2020; Hurd et al. 2014).

In our study, the lower temperature treatment triggered an increase in total phenolic content but not an increase in total antioxidant activity. This trend in total phenolic levels contrasts with results obtained for *Ascophyllum nodosum* collected in the northeastern US and west Scotland, which

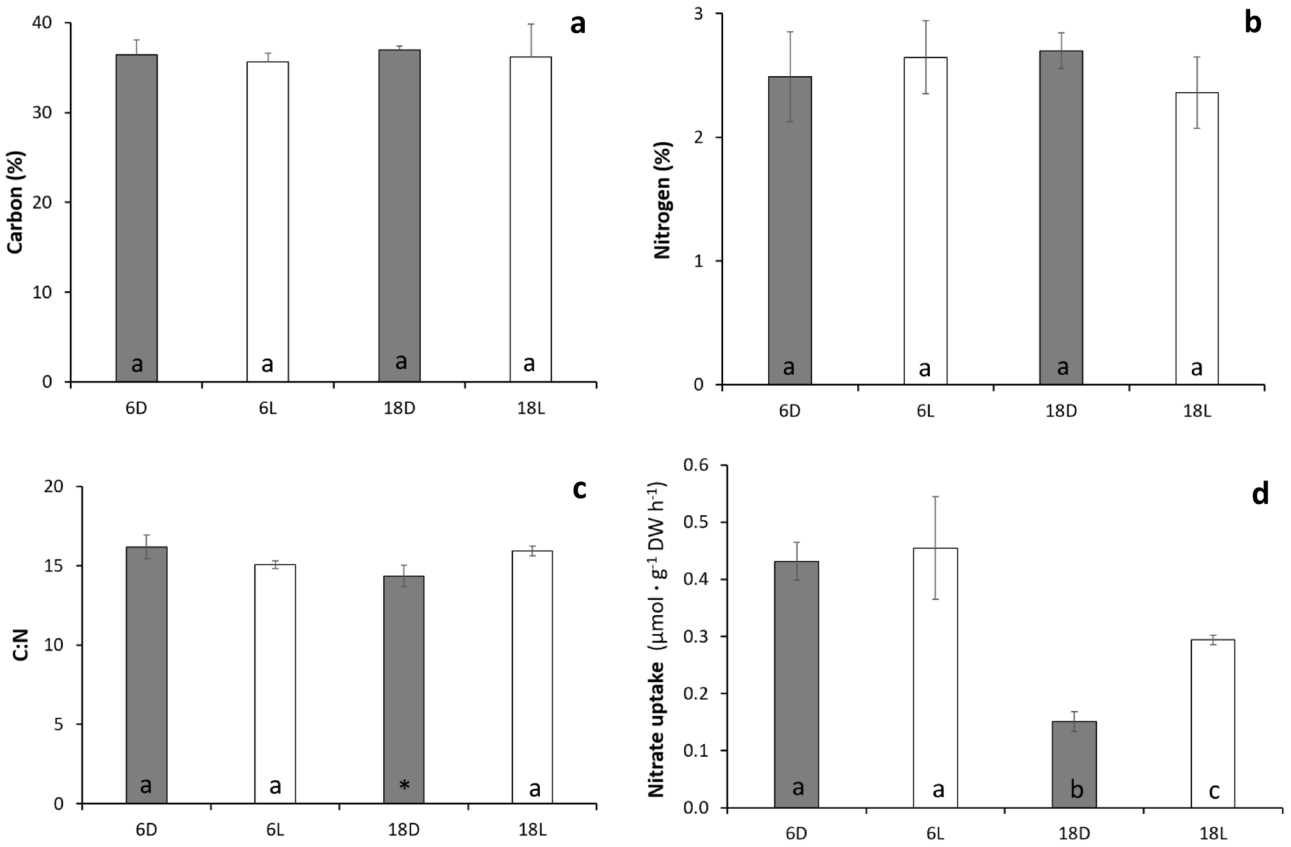

Fig. 4 **A** Tissue carbon, **B** tissue nitrogen, **C** carbon to nitrogen ratio, and **D** nitrate uptake rates measured in thalli of *Fucus distichus* (mean ± SE, $N = 4$) exposed to 6 °C (denoted as 6) or 18 °C (denoted as 18) and 210 µmol photons m^{-2} s^{-1} (denoted as L with light-filled bars) or 70 µmol photons m^{-2} s^{-1} (denoted as D with dark-filled bars) three consecutive days. *Close to significance

showed the highest phenolic contents in summer, mainly due to the presence of herbivory (Parys et al. 2009; Apostolidis et al. 2011). However, they coincide with the seasonality described for the Fucales *Ascophyllum nodosum*, *Cystoseira amentacea*, and *Fucus vesiculosus* collected in the southern Mediterranean Sea, which showed higher levels in colder (winter) versus warmer (summer) temperatures (Mannino et al. 2016) when a great proportion of energy is invested in growth and reproduction. Finally, low light but not temperature triggered an increase in scavenging activity mediated by antioxidant compounds. In an oxygenated environment, metabolic processes, such as photosynthesis, involving the transfer of electrons have the potential to facilitate oxygen radical formation. Oxidative stress is a major component in the cellular stress response of organisms exposed to environmental stress, and in intertidal seaweeds, it is typically induced under excessive irradiance conditions (Bischof and Rautenberger 2012). However, our outcomes show a different trend. It is likely that the low irradiance treatment caused a carbon imbalance triggering an increment in ROS by a respiratory activity stimulated to produce metabolic energy in absence of energy from photosynthesis. Nonetheless, in this study, we only measured total antioxidant components and not antioxidant enzymes or other specific compounds that could contribute greater specificity to our results.

It is key to highlight that distinct subspecies of *Fucus distichus* may show different physiological responses than those obtained in our experiment. In fact, an assessment of thermal stress responses conducted on two distinct populations (i.e., Arctic vs. subarctic) of *Fucus distichus* from Norway showed population-level differences in thermal tolerance. Specifically, samples collected from the Arctic showed a lesser decrease in photosynthesis performance but a greater activation of molecular defense mechanisms than those from the subarctic (Smolina et al. 2016). A meta-analysis conducted on the genus indicates that some *Fucus* may be relatively unaffected by global warming due to the presence of pre-adapted ecotypes that collectively express wide physiological tolerances (Wahl et al. 2011). *F. distichus* in Southeast Alaska seems to be no exception. At least at the experimental temperatures tested here, which are close to the average winter (3 °C) and summer (18.3 °C) temperatures in SE Alaska. These findings may align with modeled predictions for arctic *Fucus* that would not impact its southern distribution range

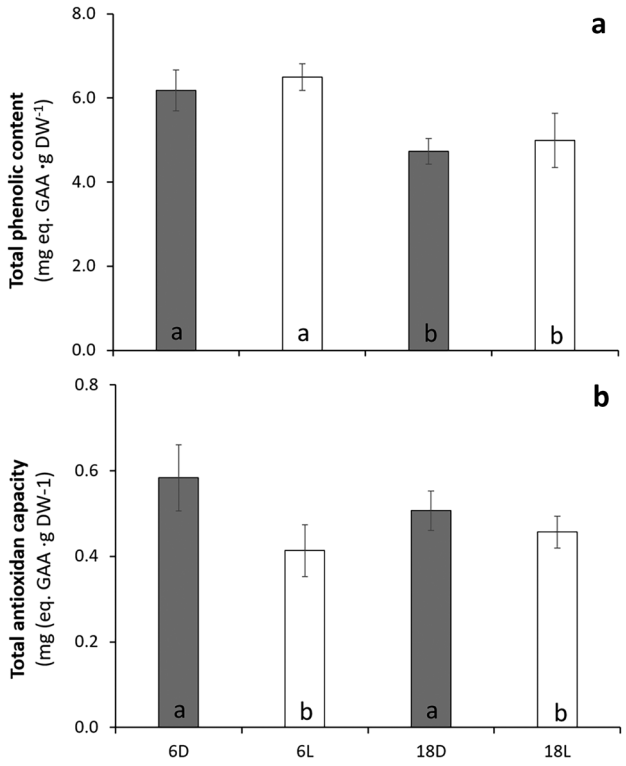

Fig. 5 Total phenolic content (**a**) and total antioxidant capacity (**b**) as mg equivalent to GAA measured in thalli of *Fucus distichus* (mean ± SE, $N=4$) exposed to 6 °C (denoted as 6) or 18 °C (denoted as 18) and 210 µmol photons m^{-2} s^{-1} (denoted as L with light-filled bars) or 70 µmol photons m^{-2} s^{-1} (denoted as D with dark-filled bars) three consecutive days

by raising temperatures driven by climate change (Jueterbock et al. 2016). Despite the relatively optimistic scenario for *Fucus distichus* in SE Alaska (this study) and northern Norway (Jueterbock et al. 2016; Smolina et al. 2016), the full extent of glacial melt's effects on the species cannot be interpreted adequately without understanding the interactive effects that simultaneous and multiple factors (aside from temperature and light) have on *F. distichus* ecophysiology. Future studies assessing the resilience of nearshore species at higher latitudes, particularly in areas influenced by glacial discharge, should include additional factors, such as salinity and extended periods of turbidity driven by silt, expected to play a significant role with increased glacial melt.

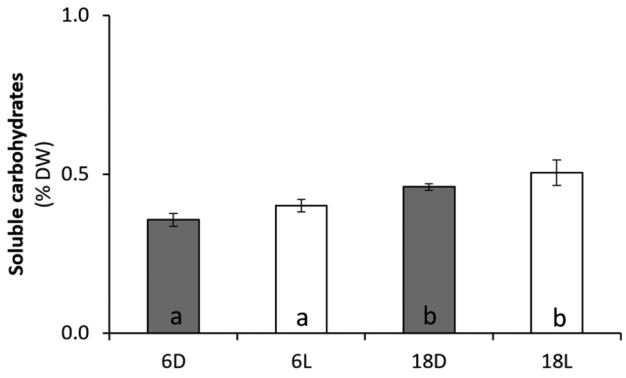

Fig. 6 Soluble carbohydrate content measured in thalli of *Fucus distichus* (mean ± SE, $N=4$) exposed to 6 °C (denoted as 6) or 18 °C (denoted as 18) and 210 µmol photons m^{-2} s^{-1} (denoted as L with light-filled bars) or 70 µmol photons m^{-2} s^{-1} (denoted as D with dark-filled bars) three consecutive days

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Data Availability Data collected from this project is available upon request.

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