Continuous photoperiod of the Artic summer stimulates the photosynthetic response of some marine macrophytes

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Abstract

Subarctic macrophytes are predicted to expand in the Arctic as a result of on-going global climate change. This will expose them to 24 hours of light during the Arctic summer while pCO_2 levels are predicted to rise globally. Here, we tested the photosynthetic activity of two brown macroalgae (*Ascophyllum nodosum, Fucus vesiculosus*) and one seagrass (*Zostera marina*) from subarctic Greenland, measuring their relative maximum electron transport rate ($rETR_{max}$), photosynthetic efficiency (α) and saturating irradiance (I_k) after 3 days of incubation at different photoperiods (12:12h, 15:09h, 18:06h, 21:03h and 24:00h, light:dark) with ambient values of pCO_2 (200 ppm, characteristic of current subarctic surface waters) and increased pCO_2 (400 and 1000 ppm). The photosynthetic parameters $rETR_{max}$ and I_k increased significantly with longer photoperiods and increased, however insignificantly, with increased pCO_2. Responses differed between species. *A. nodosum* and *Z. marina* showed the highest increase of $rETR_{max}$ and I_k from 12 h to 24 h while the increase of *F. vesiculosus* was smaller. Our results suggest that as subarctic macrophytes expand in the Arctic in response to retracting sea ice, the long summer days will stimulate the productivity of the species tested here, while the effect of high-CO_2 environment needs further research.

Highlights

- Long photoperiods increase the photosynthetic activity of certain subarctic macrophytes
- Increased CO_2 had no effect on tested macrophytes
- Highest increases of photosynthetic activity of *Ascophyllum nodosum* and *Zostera marina* at long day lengths; smaller increase for *Fucus vesiculosus*
- Subarctic macrophytes, expanding as sea ice retreats, will benefit from long summer days

Keywords

Macrophytes; subarctic; electron transport rate; continuous photoperiod and carbon dioxide.

1 Introduction

The presence of macroalgae assemblages in the Arctic is often limited by extended sea-ice cover that reduces submarine light penetration (Krause-Jensen et al., 2012; Krause-Jensen and Duarte, 2014; Wulff et al., 2009). However, an Arctic largely free of sea ice in summer, as predicted by the year 2037 (IPCC Panel, 2014; Wang and Overland, 2009), may favour the entrance of new species into the Arctic and the expansion of existing Arctic vegetation (Krause-Jensen and Duarte, 2014). Indeed, the northward extension range of macroalgae, mainly Phaeophyta, but also Chlorophyta and Rhodophyta has been observed around 78 ºN in Svalbard (Fredriksen et al., 2014). An increase of macrophyte biomass has also been detected together with the upward movement of sublittoral algal species to the littoral zone and a biodiversity increase in this Arctic region, likely related to climate warming (Weslawski et al., 2010). Arctic warming has already led to the reappearance of the blue mussel, *Mytilus edulis*, in Svalbard after 1,000 year absence (Berge et al., 2005) as well as an increase of the Arctic cod fishery due to the poleward expansion of cod habitat (Kjesbu et al., 2014). At a global scale, 75 % of the range shifts studied in marine species, including macrophytes and phytoplankton among other taxa, are in poleward direction due to climate warming (Sorte et al., 2010).

Atmospheric carbon dioxide concentration has increased ~40% since the beginning of the industrial era (Zeebe, 2012), currently exceeding 400 ppm. Yet, pCO_2 in the Arctic Ocean ranks amongst the lowest values reported across the open ocean (Takahashi et al., 2009), presenting characteristically low pCO_2 (< 200 µatm) due to intense spring phytoplankton blooms (Bates et al., 2006; Fransson et al., 2009; Holding et al., 2015) and the influence of sea ice melting during summer (Rysgaard et al., 2009).

Marine macrophyte communities tend to be autotrophic, where photosynthesis dominates over respiration (Duarte and Cebrián, 1996), thereby leading to a depletion of CO_2, potentially reaching limiting pCO_2 levels (Krause-Jensen et al., 2016; Mercado and Gordillo, 2011). In parallel, atmospheric CO_2 is predicted to increase, reaching 1000 ppm before the end of the century under some scenarios (IPCC Panel, 2014). Such levels may further stimulate submerged sub-arctic vegetation (Krause-Jensen and Duarte, 2014).

Most habitat-forming macroalgae in the Arctic are originally from North Atlantic and North Pacific regions (Wilce and Dunton, 2014; Wulff et al., 2009). *Zostera marina* has been reported in the Arctic up to 70ºN, although its presence is scarce (reviewed by Olesen et al., 2015). Intertidal belts of the macroalgae *Ascophyllum nodosum* are present up to 69.7º N (reviewed by Marbà et al., 2017) and *Fucus vesiculosus* has been found as far north of 75 °N as the Svalbard Archipelago (Florczyk and Latala, 1989; Hansen and Haugen, 1989).

Warming and consequently retreating sea-ice is increasing the available habitat, leading to a forecasted expansion of subarctic and Arctic vegetation into the Arctic Ocean that would increasingly expose the vegetation to the extended daylight period of the Arctic summer (Krause-Jensen et al., 2016; Krause-Jensen and Duarte, 2014). Potentially, continuous daylight increases the hours of C-fixation through photosynthesis, increasing its primary productivity. However, the excess of light energy and long photoperiods might induce photoinhibition damage and prevent growth (Velez Ramirez et al., 2011). Therefore, studying the physiology and the photosynthetic machinery of subarctic marine macrophytes under a continuous photoperiod, characteristic of the Arctic summer, is key to evaluate the probabilities of a forecasted poleward expansion. Research on the effect of continuous light on photosynthetic organisms is scarce and has mainly been related to use in greenhouse food production (Velez Ramirez et al., 2011).

The physiological responses of a canopy-forming species to different levels of light intensity, CO_2 and temperature has been evaluated in the Antarctica (Iñiguez et al., 2017). In the same region, the transition from late winter to summer on phytoplankton physiology has been evaluated focussing on variable light intensities (McMinn et al., 2010) in combination with increased CO_2 (Hoppe et al., 2015). In the Arctic Ocean, the effect of increased CO_2 has been physiologically tested in macroalgae (Iñiguez et al., 2016) and phytoplankton in combination with light intensity and temperature (Hoppe et al., 2018, 2017). The physiological responses of polar macro and microphytes to continuous photoperiod remains insufficiently explored. Krause-Jensen et al. (2016) concluded that day lengths longer than 21 hours, characteristic of Arctic summers, were conducive to sustain a high pH in seawater as a result of photosynthesis by macro-autotrophs. They also concluded that experimental increase in CO_2 concentration stimulated the capacity of macrophytes to deplete CO_2. However, they analysed an assemblage of three species (*Z. marina*, *A. nodosum* and *F. vesiculosus*) and did not explore the species-specific response of photosynthetic physiology to longer photoperiods and increased CO_2, neither the interactive effect between the day length and enhanced CO_2, which are the aims of our study.

Marine macrophytes are often limited by CO_2 because the boundary layers formed around the blades limit the entrance of CO_2 by diffusion due to its thickness (Hendriks et

al., 2017), which together with high primary productivity rates in densely vegetated areas result in low CO_2 levels close to the blade surface (Bowes and Salvuci, 1989; Holbrook et al., 1987; Hurd et al., 2009). The low CO_2 concentration characteristic of Arctic and subarctic waters due to strong biological uptake in spring and melting sea ice in summer (Bates et al., 2006; Fransson et al., 2009; Meire et al., 2015; Takahashi et al., 2009) may cause CO_2 limitation, - with the risk being particularly likely in vegetated coastal habitats that represent hot spots of productivity and CO_2-draw down. Productivity of subarctic macrophytes could, hence, be limited by CO_2 similar to the limitation of phytoplankton productivity in the Arctic (Engel et al., 2014; Hein and Sand-Jensen, 1997; Holding et al., 2015).

Key to understanding how marine vegetation will respond to predicted increased levels of dissolved inorganic carbon (DIC) and longer photoperiods is their species-specific photosynthetic physiology (Koch et al., 2013). The majority of autotrophs have a higher photosynthetic affinity for CO_2 than bicarbonate (HCO_3^-) (Bowes, 1985; Madsen and Sand-Jensen, 1991). In the oceans, an increase in CO_2 concentration will increase the total DIC and hydrogen ion concentration (H^+) leading to a decrease in pH and a change in the carbon species. The increase in CO_2 availability will probably enhance photosynthesis and growth of seagrasses and many macroalgae as well as the competition between species and would benefit those with a greater ability to rapidly sequester CO_2 (Koch et al., 2013).

The majority of marine macroalgae and seagrass are supposed to possess Rubiscos that are not saturated at the current ocean DIC concentration, thus these organisms have Carbon Concentrating Mechanisms (CCMs), which finally increase CO_2 around Rubisco relative to the CO_2 concentration in seawater, through an active transport of CO_2 and/or HCO_3^- inside the cell (Giordano et al., 2005). Despite the widespread presence of CCMs, different species-specific responses to increased levels of CO_2 has been observed (Beer et al., 2014; Beer and Rehnberg, 1997; Sand-Jensen and Gordon, 1984; Koch et al., 2013; Surif and Raven, 1989). Many marine macrophytes possess the capacity to utilize HCO_3^- for photosynthesis (Giordano and Maberly, 1989; Koch et al., 2013; Mercado et al., 2009; Surif and Raven, 1989), although it does not indicate that their photosynthesis might be saturated at present CO_2 conditions. In fact, all analysed seagrass and most brown macroalgae showed that an increase in DIC concentrations promotes higher photosynthetic and growth rates (Koch et al., 2013). In conditions of severe CO_2 limitation, the high availability of HCO_3^- increases C acquisition, but it is energetically expensive and CO_2 remains preferable for photosynthesis as compared to HCO_3^- due to lower photosynthetic $K_{0.5}$ values for CO_2 than for HCO_3^- in seagrasses and macroalgae (Sand-Jensen and Gordon, 1984).

The brown macroalgae *F. vesiculosus* is an exception, with photosynthetic rates being saturated at current DIC levels (Koch et al., 2013; Raven and Osmond, 1992) and has a high affinity and capacity for DIC uptake by non-diffusive mechanisms (Mercado et al., 2009). Conversely, photosynthesis of *A. nodosum* is not CO_2-saturated, even though its capacity for HCO_3^--use has been demonstrated (Koch et al., 2013; Surif and Raven, 1989). The utilization of HCO_3^- in seagrasses is generally less efficient than in macroalgae (Beer et al., 2014; Beer and Rehnberg, 1997; Sand-Jensen and Gordon, 1984) although for *Z.*

marina it is not confirmed whether photosynthesis is saturated at current CO_2 levels (Koch et al., 2013). *A. nodosum, F. vesiculosus* and *Z. marina* therefore represent 3 test organisms for which different responses to increased CO_2 could be expected.

Polar macroalgae are generally hypothesized to have a competitive advantage in a future high CO_2 environment (Raven et al., 2002a, 2002b). In cold regions the entry of CO_2 by diffusion into the cell seems to be more widespread for brown and red macroalgae, than the same species of macroalgae in temperate regions, avoiding the need of CCMs and making them more prone to stimulation by increased CO_2 than temperate macroalgae (Mercado and Gordillo, 2011; Raven et al., 2002a, 2002b), although higher CO_2 dependence has been attributed to red polar macroalgae (Raven et al., 2002b).

Here we experimentally test the hypothesis that increased CO_2 supply (400 and 1000 ppm) relative to the low ambient CO_2 levels of Arctic waters (200 ppm) increase photosynthesis of subarctic marine macrophytes. Such an increase would support the projected expansion of marine vegetation in the future high Arctic given that photosynthetic activity of individual leaves is related with estimates of macrophyte productivity at a community-level (Silva et al., 2005). We also evaluate the effect of longer photoperiods (12:12, 15:09, 18:06, 21:03 and 24:00 light:dark hours) on photosynthetic activity to assess whether long days/continuous daylight, as resulting from longer open water periods in the future Arctic, hamper or stimulate photosynthesis. We use two macroalgae species (*A. nodosum* and *F. vesiculosus)* and one seagrass species (*Z. marina*) as test organisms.

2 Materials and methods

2.1. Experimental set-up and sampling

Brown macroalgae (*A. nodosum*, *F. vesiculosus*) fronds were collected in the lower intertidal zone and seagrass (*Z. marina*) shoots, sparse in this region (Olesen et al., 2015), were collected from shallow waters (1 to 3 m depth) at the central part of Kobbefjord (64°10'N and 51°29'W, Godthåbsfjord, Greenland) in early June 2014.

They were kept cool and humid during the 30 h transport by airplane to the laboratories of the Mediterranean Institute for Advanced Studies (IMEDEA, Mallorca, Spain). In the laboratory facilities they were acclimated in artificial seawater to the photoperiod to which they were exposed at collection (21:03h, light:dark hours, in the Godthåbsfjord in early June), during 10 days in a climate controlled room at 4 ^{o}C.

After the acclimation period, undamaged tips of *A. nodosum* and *F. vesiculosus* and shoots of *Z. marina* were transferred into nine aquaria (6 L), with controlled photoperiod, temperature and pCO_2 levels. The total biomass of macrophytes in each aquarium was 2.7-3.7 g dry weight (DW), yielding a biomass density of 0.45-0.61 g DW L^{-1}, mimicking dense vegetation. The aquaria were exposed to three pCO_2 levels (200, 400 and 1000 ppm in the absence of macrophytes) with three replicated aquaria per level. 200 ppm reflected contemporary pCO_2 levels in subarctic surface waters in spring/summer in Godthåbsfjord, close to Kobbefjord (Meire et al., 2015). The highest pCO_2 manipulation (1000 ppm) was set to the predicted scenario of atmospheric pCO_2 by 2100 (IPCC Panel,

2014), and 400 ppm represented an intermediate level (Fig. 1). To mimic the photosynthetic active radiation (PAR) within *in situ* canopies, the aquaria were illuminated with 111 ± 5 µmol photons m^{-s} s^{-1} of PAR at the water surface using 2 lamps with 54 W fluorescent tubes. This light level was about half of the maximum PAR reported in Greenland fjords close to the Godthåbsfjord (204-289 µmol photons m^{-2} s^{-1}) at 2m depth during May and June 2013 (Olesen et al., 2015). This experiment also provided the results described in Krause-Jensen et al. (2016).

The aquaria were filled with artificial seawater made from distilled water, Reef Crystals® and NaCl to obtain a similar salinity (30.2 ± 0.42, subarctic range: 28.9-31.7) and alkalinity (2241 µmol kg SW^{-1}± 31.3, measured in the sampled area: 1980-2240 µmol kg SW^{-1}) as conditions in the field. The artificial seawater was pre-exposed to UV to limit the growth of microorganisms during the experiment. To reach targeted pCO_2 levels, air was circulated through soda lime tubes to remove the CO_2 present and it was mixed with pure CO_2 gas in a bottle with marbles to maximize the mixing surface area. The concentration of air and pure CO_2 in the mixing bottle was regulated with mass flow controllers (MFCs, AALBORG GFC-17, US). The targeted pCO_2 concentration was continuously supplied to the aquaria through porous bubble curtains. pH was measured *in continuum* with pH electrodes (Omega, PHE-1411) and recorded at 15 min intervals (IKS Aquastar, Germany; for more details see Krause-Jensen et al., 2016). When seawater in the aquaria had reached a stabilized pH and targeted pCO_2 was reached, the same number of tips of *A. nodosum* and *F. vesiculosus* and shoots of *Z. marina* (6 individuals of each species) were attached to the base of the aquaria, with every individual identified with a label. The macrophytes in each of the six aquaria were cycled through a series of random photoperiods (12:12h, 15:9h, 18:6h, 21:3h and 24:0h, light:dark hours), that were run as a whole experimental unit and maintained 3 days at each photoperiod treatment (Fig. 1). Total alkalinity (TA) was measured at the start and end of each photoperiod and interpolated linearly over the 3-days period. Sampled seawater was poisoned with 0.02 ml of a saturated solution of $HgCl_2$ (Merck, Analar) in order to avoid biological alteration of the sample during storage. TA was determined with a Titrando 808 (Metrohm) by open cell titration as described in Dickson et al., (2007). Certified CO_2 seawater reference material (CRM Batch136) from the Andrew Dickson lab at UC San Diego was used to warrant the accuracy of results. TA and pH_{NBS} were used to calculate the CO_2 concentrations using CO2SYS Pierrot et al., (2006), with dissociation constants from Mehrbach et al., (1973) refitted by Dickson and Millero, (1987) and HSO_4^- using Dickson, (1990). In between photoperiod treatments, seawater was changed and replaced by seawater pre-treated with CO_2 gas at the targeted pCO_2 levels.

The photosynthetic capacity of the three macrophyte species was measured through photosynthesis-irradiance curves (PI curves), also known as rapid light curves (RLC), showing the response of the chlorophyll *a* fluorescence to a range of light intensities (Falkowski and Raven, 2013; Ralph and Gademann, 2005). The measurements were done after 3 days exposure to an experimental photoperiod on two of the six individuals of each species present in two out of three replicated aquaria using Pulse Amplitude Modulation, with a diving-PAM fluorometer (Walz, Germany). The measurements were always done on the same individuals. *Z. marina* measurements were conducted on the second youngest

leaf and 4 cm above the meristem. On *A. nodosum* and *F. vesiculosus* measurements were done below the youngest bladder.

To avoid excessive manipulation of macrophytes, the measurements were done inside the aquaria. Using a PAM leaf-clip, the tissue, was dark-adapted for 5 min as done in previous studies (e. g. Anton et al., 2018; Apostolaki et al., 2014; Hendriks et al., 2017) and was illuminated with a series of nine increasing actinic light intensities (from 0 to maximum 616 μmol PAR m^{-s} s^{-1}) at intervals of 10s to produce a RLC. The maximum relative electron transport rate ($rETR_{max}$, n = 12 per species and per photoperiod) was calculated by fitting the RLC data with the non-linear model (Harrison and Platt, 1986; Platt et al., 1982; Ralph and Gademann, 2005):

$$rETR = rETR_{max} \times (1 - e^{(-\alpha \times PAR_{incident}/rETR_{max})}) \quad \text{(Eq. 1)}$$

The photosynthetic parameters $rETR_{max}$, photosynthetic or quantum efficiency (α) and saturating irradiance (Ik, $Ik = rETR_{max}/\alpha$) were estimated by fitting the data of the RLC to Eq. 1 using R version 1.0.44 (R Core Team, 2014). Even though *A. nodosum* and *Z. marina* showed no asymptotic approximation to the $rETR_{max}$ in some cases due to the limited range of actinic light intensities above light saturation (Fig. A1 and A3), $rETR_{max}$ could still be estimated from the curvature of the RLC.

2.2. Statistical analysis

We evaluated if CO_2 (3 levels) and photoperiod (5 levels) had an effect on photosynthetic responses, as represented by $rETR_{max}$, α and Ik, and if such effects were similar or different for the three macrophyte species tested. The experiment was a split-plot design due to restricted randomization for photoperiod, i.e. each level of photoperiod was run separately (whole plot) and without replication. Experimental units (whole plots) consisted of six aquaria that were grouped into 3 levels of CO_2 with 2 replicates for each CO_2 treatment at each photoperiod (Fig. 1). The experimental units were sub-divided or split into different periods (sub-plots) to test the effects of varying photoperiod. However, aquaria were nested within CO_2 treatment, as the same CO_2 treatment was applied to the same aquarium for all photoperiods. All three species were randomly sampled for each sub-plot and, consequently, species variation was a factor fully crossed with the two treatment factors. Finally, individual macrophyte specimens within each aquarium were not replaced between different photoperiod levels and constituted a random factor nested within CO_2 treatment and aquarium. The following split-plot model (using capital letters for random factors in Eq. 2) was employed for the three photosynthesis variables separately ($rETR_{max}$ and Ik were log-transformed):

$$Y_{ijkl} = \mu + \underbrace{c_i + A_l(c_j) + I_m\left(A_l(c_j)\right)}_{whole-plot} + \underbrace{p_j + p_j \times c_i + P_j \times A_l(c_j) + P_j \times I_m\left(A_l(c_j)\right)}_{split-plot}$$
$$+ \underbrace{s_k + s_k \times c_i + s_k \times p_j + s_k \times p_j \times c_i}_{species\ variations\ fixed\ effects}$$

(Eq. 2)

where μ is the overall mean, c_j= CO_2 treatment, p_i= photoperiod, s_k=species, A_l= aquarium and I_m= individual macrophyte specimen. The three main effects (p_i, c_j, and s_k) and their interaction were fixed effects, whereas variation among aquaria (A_l) and macrophyte individuals (I_m) and interactions derived from these were random. Marginal means were calculated for all the fixed effects and back-transformed to the geometric mean for the two log-transformed response variables.

The split-plot model is a mixed linear model and was analysed using PROC MIXED in SAS version 9.3. Statistical testing for fixed effects (F-test with Satterthwaite approximation for denominator degrees of freedom) and random effects (Wald Z-test) were carried out at a 5% significance level. The F-test for fixed effects was partial, i.e. considering the specific contribution of the given effect in addition to all other factors.

3 Results

The RLCs (Fig. A1, A2, A3) typically flattened out for all three species around 200 µmol photons m^{-2} s^{-1}, but light saturation (Ik) was reached at lower light levels for *F. vesiculosus* and *Z. marina* than *A. nodosum*. Photoinhibition, lowering of photosynthetic rates at higher irradiances, was not detected.

$rETR_{max}$ and saturating irradiance (Ik) were significantly different between photoperiods with values increasing progressively up to the 24h-light photoperiod (Table 1). The largest differences in $rETR_{max}$ were found between the 21 h and 24 h photoperiod with increases from 20.4 up to 30 µmol electrons m^{-2} s^{-1}, when all species data was pooled. At the photoperiods of 12 h, 15 h and 18 h the $rETR_{max}$ was variable (21.8, 23.5 and 23.5 µmol electrons m^{-2} s^{-1}, respectively when all species data were pooled).

The highest average values of $rETR_{max}$ and Ik were found for *A. nodosum* (Fig. 2a, c, Table 1). $rETR_{max}$ increased 15%, on average, from 200 ppm of pCO_2 to 1000 ppm (from 21.2 to 24.4 µmol electrons m^{-2} s^{-1}) although the effect of the CO_2 treatment was not significant (Table 1). The photosynthetic efficiency, α, was significantly different between species with higher values for both macroalgae compared to *Z. marina* (Fig. 2b, Table 1). The CO_2 effect on α was not significant but a gradual increase of 10% was observed from 200 to 1000 ppm (Table 1), while Ik did not show an effect of CO_2 (Table 1). The interaction effect between the different photoperiods and the species was significant for α and Ik, but not for $rETR_{max}$ (Fig. 3, Table 1). *Z. marina* showed the highest increase in $rETR_{max}$ on average from the photoperiod of 12 h to 24 h (10.7 µmol electrons m^{-2} s^{-1}); followed by *A. nodosum* (9.9 µmol electrons m^{-2} s^{-1}) and the lowest increase was observed in *F. vesiculosus* (3.2 µmol electrons m^{-2} s^{-1}, Fig. 3a).

The interaction effect between CO_2 treatments and species was not significant for any of the photosynthetic responses, but *A. nodosum* showed the highest $rETR_{max}$ and Ik at 1000 ppm (Fig. 4). The three photosynthetic parameters ($rETR_{max}$, α and Ik) showed significant residual variation only while other random effects were not significant, which

indicates that variations associated with using different aquarium and analysis on individual macrophyte specimen were relatively small compared to variations associated with the photosynthetic activity.

4 Discussion

Our results showed a significant increase in photosynthetic capacity in response to longer photoperiod, since $rETR_{max}$ increased from 21 h to 24 h for the three species tested after 3 days of incubation. During shorter photoperiods (12 h to 18 h) $rETR_{max}$ was variable and lower than at 24 h for the three species tested. The CO_2-effect tended to be positive but non-significant. Our results showed maximum $rETR_{max}$ at continuous daylight for all three species, indicating an increase of their photosynthetic capacity. The responses were variable among species: *Z. marina* and *A. nodosum* showed the highest increases in $rETR_{max}$ with increasing daylight hours whereas the increase of *F. vesiculosus* was smaller. The photosynthetic efficiency, α, in both macroalgae was similar and higher than in *Z. marina* up until the photoperiod of 21 h was reached. At continuous light, α showed variations both in *A. nodosum* and *F. vesiculosus*.

Along with the increase of its photosynthetic capacity at continuous daylight, our results indicated no stress, as Yield (Fv/Fm) in 24 h light for *A. nodosum, F. vesiculosus*, nor *Z. marina* was no different from other photoperiods and showed no decreasing trend over time. These results indicate that the three studied macrophytes species could potentially benefit from increased day length, at least on short time scales (as interfered from 3 days of incubation), however *Z. marina* might encounter more difficulties than macroalgae as they expand northward. Indeed, the presence of *Z. marina* in the subarctic is scarce while the two macroalgae form intertidal subarctic belts. Our results for *A. nodosum* agree with those of Strömgren, (1978), using specimen from the North Sea and Norwegian Sea where *A. nodosum*, *Fucus serratus* and *Pelvetia canaliculate* increased its growth up to 24 h whereas *F. vesiculosus* only increased up to 20 h of daylight. The small increase in $rETR_{max}$ of *F. vesiculosus* compared with the increase of *A. nodosum* and *Z. marina* at continuous daylight could explain their current presence in the high-Arctic (Florczyk and Latala, 1989; Hansen and Haugen, 1989). Other species such as *Ulva lactuca* and *Porphyra umbilicalis* from the North Sea did not increase their growth at photoperiods greater than 16 h (Fortes and Lüning, 1980). Reduced photosynthetic rates from 21 to 24 h daylight in species from the North Sea and Norwegian Sea are likely caused by high photon flux rates leading to photoinhibition, light-induced reduction in the photosynthetic capacity of the macrophytes, to protect the photosynthetic apparatus, suggesting that macrophytes from different latitudes might regulate their photosynthetic rates differently. Moreover, the Antarctic brown alga (*Adenoscystis utriculares*) showed faster photoinhibition than brown macroalgae of temperate zones (Hanelt, 1996). Photoinhibition might be produced by continuous high irradiances, low temperatures and CO_2 limitation (Beer et al., 2014). Before photoinhibition occurs, there are defence mechanisms that down-regulate photosynthesis to dissipate the excess light energy such as the xanthophyll cycle, the Mehler reaction or the use of Carbon Concentration

Mechanisms (CCMs). Under conditions of CO_2-limitation and excess light energy, the Mehler reaction diverts the excess of electrons towards the production of H_2O_2 and ultimately water. However, the Mehler reaction cannot be detected by PAM fluorometry (Beer et al., 2014). CO_2-limitation and excess light energy also might lead into the use of CCMs, increasing CO_2 concentration inside the cell and raising the use of energy. In our study, CO_2 concentration was not limiting, and neither were light or temperature. We chose to use PAM fluorometry, despite its shortcomings of only assessing part of the photosynthetic response, because it is a non-invasive technique that does not overly stress or damage the macrophyte, which is crucial in experiments with repeated measures over some period of time.

$rETR_{max}$ and Ik of *A. nodosum* and *Z. marina* increased slightly, yet insignificantly, in response to increasing CO_2, while *F. vesiculosus* did not show any response. Other studies show that the arctic macroalgae *Alaria esculenta* was positively affected by increased CO_2, showing an increase of $rETR_{max}$, α and Ik along with increasing growth rates, while the species *Desmarestia aculeata* responded by decreasing $rETR_{max}$ and growth rates (Iñiguez et al., 2016). The small response of *A. nodosum* agrees with the notion that this macroalgae is not CO_2-saturated at ambient levels and diffusive entry of CO_2 seemed to be required (Koch et al., 2013; Surif and Raven, 1989), while the absence of response of *F. vesiculosus* might indicate that it is CO_2-saturated at ambient levels (Koch et al., 2013; Raven and Osmond, 1992) and had high capacity for DIC uptake by non-diffusive mechanisms (Mercado et al., 2009). Increased CO_2 might benefit subarctic *A. nodosum* because it is not currently CO_2-saturated and the diffusive entry of CO_2 or CCMs based on HCO_3^- usage are required (Johnston and Raven, 1986; Koch et al., 2013; Surif and Raven, 1989), while *F. vesiculosus* was not affected by increased CO_2 probably due to its currently CO_2-saturated stage and its capacity for non-diffusive mechanisms (Koch et al., 2013; Raven and Osmond, 1992). The seagrass *Z. marina* can be either CO_2-saturated or -limited at ambient levels (Koch et al., 2013). We observed a positive response, though non-significant of *Z. marina* to increased CO_2, in line with CO_2-stimulation of seagrass photosynthesis observed in other regions (Koch et al., 2013).

Our results show a non-significant interaction between increased CO_2 concentration and increased photoperiods. CO_2-limitating environments generally increase the use of CCMs, producing photoinhibition and decreasing the photosynthetic activity and growth. Excess of light irradiance also might produce photoinhibition and a decrease of photosynthetic activity. Our results indicate that continuous daylight at their natural submerged irradiance (111 ± 5 μmol photons m^{-s} s^{-1} of PAR) lead to a positive photosynthetic response of the three species tested while no significant response was observed to increased CO_2.

5 Conclusions

Our results suggest that a continuous photoperiod, characteristic of Arctic summers, may benefit the subarctic macrophytes tested in this study, increasing their photosynthetic activity. *A. nodosum*, *F. vesiculosus* and *Z. marina*, hence, take advantage of increased daylength during the Arctic summer as they migrate poleward with decreasing ice cover.

As they expand northward, they will probably be able to successfully cope with 24 h of darkness during the Arctic winter since they are already present at 69.7º N (*A. nodosum*, reviewed by Marbà et al., 2017), 75º N (*F. vesiculosus*, Florczyk and Latala, 1989; Hansen and Haugen, 1989) and 70º N (*Z. marina*, reviewed by Olesen et al., 2015), and thus exposed to photoperiods of long daylight and darkness. Nevertheless, further research is needed on the interactive effects of day length and increased CO_2 with nutrients availability, salinity and light intensities on photosynthesis of arctic and subarctic macrophyte. Melting ice and coastal erosion leads to reduced light penetration due to increased turbidity altering how macrophytes are distributed (Traiger and Konar, 2018) and been negatively affected (Bonsell and Dunton, 2018; Filbee-Dexter et al., 2019). Freshwater accumulation is reducing nutrient inventories in the arctic water column (Coupel et al., 2015; Yun et al., 2016) probably due to strong stratification (McLaughlin and Carmack, 2010; Post et al., 2013). Gordillo *et al.*, (2006) showed a high resilience to nutrient-limitation of many arctic macrophytes. Our results demonstrate that continuous photoperiod stimulates the photosynthesis of the subarctic macrophytes tested and support the forecasted poleward expansion of subarctic vegetation into the high-Arctic (Krause-Jensen and Duarte, 2014). However, the spread of existing Arctic macroalgae with climate change needs to be further studied focussing on the interactive effects of long day length with CO_2, nutrients availability, salinity and light intensities.

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8 Figures and tables

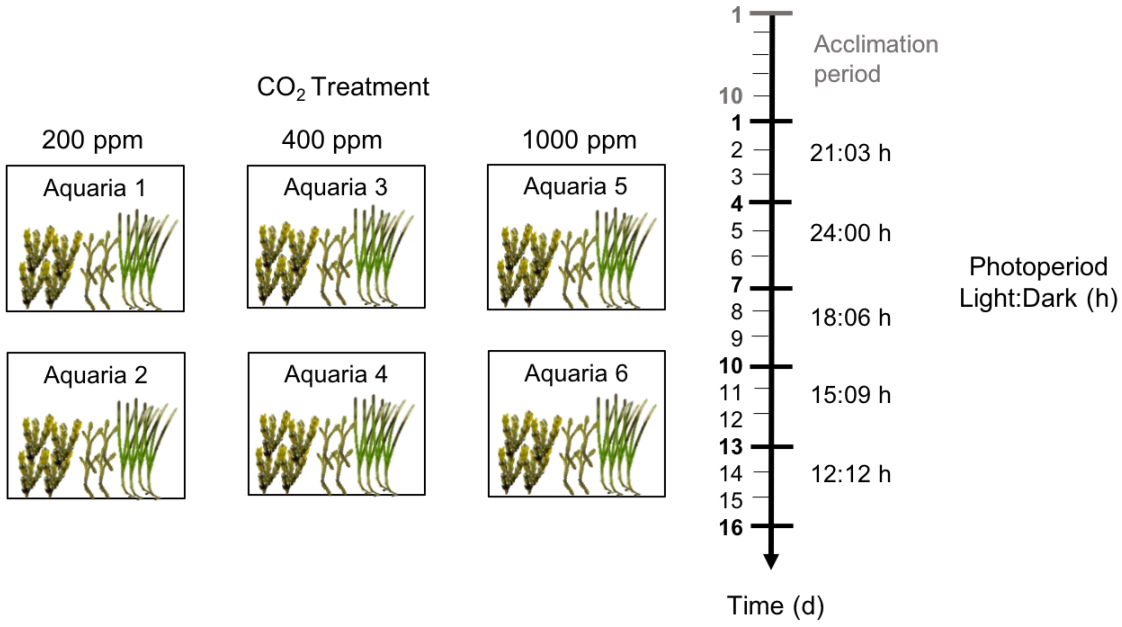

Figure 1: Experimental set-up, analysed with a split-plot model, where specimens of *Fucus vesiculosus*, *Ascophyllum nodosum* and *Zostera marina* were placed into replicated aquaria. After the acclimation period, the CO_2 treatments were applied throughout the experiment and light treatments (i.e. photoperiods) were changed every three days.

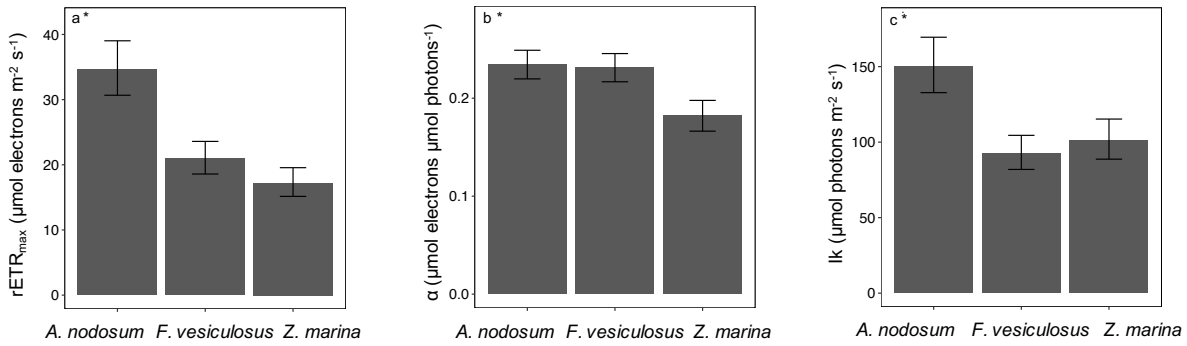

Figure 2: Estimated means of $rETR_{max}$, and saturating irradiance, Ik (a, b, c, respectively) as calculated for different species (*Ascophyllum nodosum*, *Fucus vesiculosus* and *Zostera marina*) as the average of the levels of daylight and the pCO_2 treatments. Significance is indicated with an asterisk ($p < 0.05$). Error bars mark standard errors of the means.

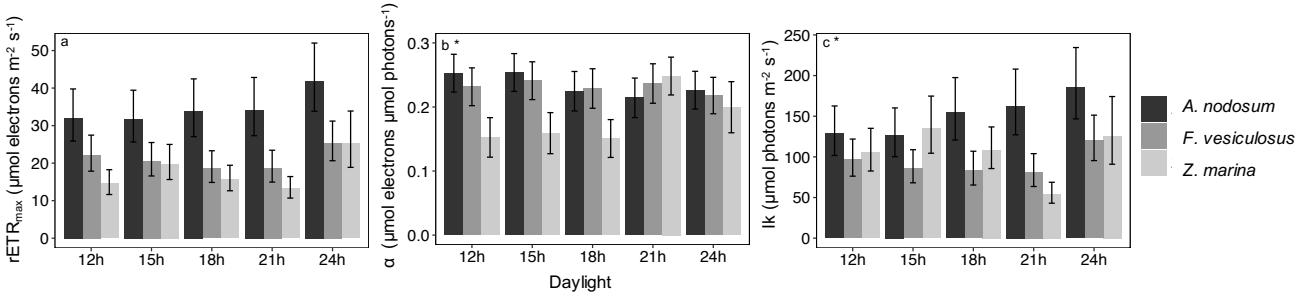

Figure 3: Estimated means of $rETR_{max}$, (a) α (b) and saturating irradiance, Ik (c) for different combinations of photoperiod and species (*Ascophyllum nodosum*, *Fucus vesiculosus* and *Zostera marina*), i.e. $s_k \times p_j$ in the mixed model. Significance of the interaction is indicated with an asterisk ($p < 0.05$). Error bars mark standard errors of the means.

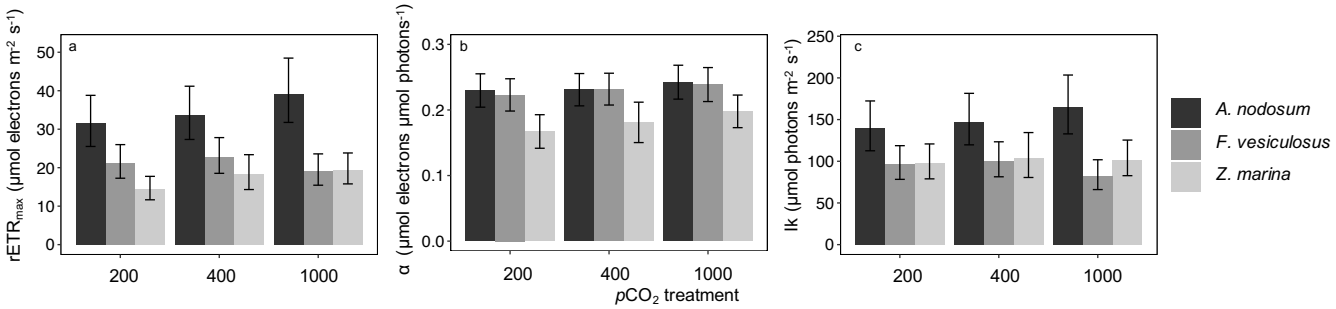

Figure 4: Estimated means of $rETR_{max}$ (a), α (b) and saturating irradiance, Ik (c) for different combinations of pCO_2 treatments and species (*Ascophyllum nodosum* in black, *Fucus vesiculosus* in dark grey and *Zostera marina* in light grey), i.e. $s_k \times c_i$ in the mixed model. Significance of the interaction is indicated with an asterisk ($p < 0.05$) (none of them). Error bars mark standard errors of the means.

Table 1: Statistical tests of the fixed effects for the three photosynthetic responses. Denominator degrees of freedom was calculated with Satterthwaite's approximation. Asterisk on the effect column indicates the interaction between factors while asterisk on the p-value column indicate the significance ($p < 0.05$)

Parameter	Effect	Numerator Df	Denominator Df	F-value	p-value	
$rETR_{max}$	CO_2 treatment	2	9.0	1.4	0.30	
$rETR_{max}$	Photoperiod	4	14.6	4.6	0.01	*
$rETR_{max}$	Photoperiod*CO_2 treatment	8	14.2	0.7	0.72	
$rETR_{max}$	Species	2	8.6	34.9	7.7E-05	*
$rETR_{max}$	CO_2 treatment*Species	4	8.4	1.3	0.34	
$rETR_{max}$	Photoperiod*Species	8	87.5	1.3	0.27	
$rETR_{max}$	Photoperiod*CO_2 Treatment*Species	16	85.9	0.6	0.88	
α	CO_2 treatment	2	9.9	1.8	0.22	
α	Photoperiod	4	33.4	1.7	0.17	
α	Photoperiod*CO_2 treatment	8	32.7	0.7	0.68	
α	Species	2	9.8	14.1	1.3E-03	*
α	CO_2 treatment*Species	4	9.7	0.2	0.95	
α	Photoperiod*Species	8	32.8	3.8	2.9E-03	*
α	Photoperiod*CO_2 Treatment*Species	16	32.1	1.5	0.14	
Ik	CO_2 treatment	2	3.0	0.1	0.90	
Ik	Photoperiod	4	13.0	4.8	0.01	*
Ik	Photoperiod*CO_2 treatment	8	12.6	1.4	0.28	
Ik	Species	2	23.6	23.9	2.1E-06	*
Ik	CO_2 treatment*Species	4	23.1	1.0	0.41	
Ik	Photoperiod*Species	8	91.4	3.8	7.3E-04	*
Ik	Photoperiod*CO_2 Treatment*Species	16	90.2	1.1	0.38	