MARINE ECOLOGY

Long photoperiods sustain high pH in Arctic kelp forests

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Concern on the impacts of ocean acidification on calcifiers, such as bivalves, sea urchins, and foraminifers, has led to efforts to understand the controls on pH in their habitats, which include kelp forests and seagrass meadows. The metabolism of these habitats can lead to diel fluctuation in pH with increases during the day and declines at night, suggesting no net effect on pH at time scales longer than daily. We examined the capacity of subarctic and Arctic kelps to up-regulate pH in situ and experimentally tested the role of photoperiod in determining the capacity of Arctic macrophytes to up-regulate pH. Field observations at photoperiods of 15 and 24 hours in Greenland combined with experimental manipulations of photoperiod show that photoperiods longer than 21 hours, characteristic of Arctic summers, are conducive to sustained up-regulation of pH by kelp photosynthesis. We report a gradual increase in pH of 0.15 units and a parallel decline in pCO_2 of 100 parts per million over a 10-day period in an Arctic kelp forest over midsummer, with ample scope for continued pH increase during the months of continuous daylight. Experimental increase in CO_2 concentration further stimulated the capacity of macrophytes to deplete CO_2 and increase pH. We conclude that long photoperiods in Arctic summers support sustained up-regulation of pH in kelp forests, with potential benefits for calcifiers, and propose that this mechanism may increase with the projected expansion of Arctic vegetation in response to warming and loss of sea ice.

INTRODUCTION

Ocean acidification (OA) is predicted to affect marine calcifiers (*1*), and Arctic ecosystems are argued to be at particular risk because low temperatures increase CO_2 solubility and freshwater inputs dilute the buffering capacity of seawater (*2*, *3*). The potential threat to calcifiers has led to efforts to understand the controls on pH in their habitats, which include kelp forests and seagrass meadows (*4*). Calcifiers, such as bivalves, brittle stars, and sea urchins, thrive in coastal vegetated ecosystems (*5*), which not only provide them with food and shelter but also affect their pH environment (*4*, *6*).

Marine macrophytes are autotrophic communities, where photosynthesis exceeds respiration (*7*), and their metabolism can create marked pH fluctuations in seagrass meadows (*8*) and kelp forests (*9*, *10*) with increases of up to 1 pH unit during the day (*11*), depending on plant biomass, activity (*8*, *12*, *13*), and flow attenuation (*14*, *15*). Vegetated habitats may therefore provide refugia for calcifiers from future OA (*4*, *15*). However, pH in these habitats typically shows diurnal oscillations with elevated pH during daytime, due to CO_2 uptake by photosynthesis, and reduced pH at night when community respiration prevails. Evaluation of the role of vegetated habitats as refugia from OA, through the associated diurnal pH fluctuations, has reached a contradictory conclusion. Some studies conclude that these diel fluctuations lead to an overall buffering of OA (*8*, *13*, *16*, *17*) due to reductions in CO_2 and increases in pH during the day, whereas others highlight the idea that they may amplify negative effects of OA (*18*–*20*), because increases in CO_2 and reductions in pH during the night have particularly important adverse effects on calcifiers. Available research therefore shows that the potential role depends on the balance between positive effects in the daytime and negative effects during the night. Hence, it follows that the relative duration of the positive (day) versus the negative (night) period, that is, the photoperiod, should determine the overall effect. We therefore hypothesized that photoperiod should constrain diurnal fluctuations in pH and CO_2 and, hence, the potential role of vegetated habitats as buffers or amplifiers of OA effects (*6*, *21*). However, the role of photoperiod in constraining the effects of marine macrophytes on pH and CO_2 remains untested. Although all experiments and observations thus far reported have been conducted in temperate or tropical areas, it is in the Arctic where long summer photoperiods should create optimal conditions for marine vegetated habitats to sustain elevated pH throughout the months of continuous daylight, for example, 4 months and 10 days at 80°N. This would render Arctic vegetated habitats potential refugia for calcifiers during summer when calcifiers are most susceptible to low pH (*1*, *22*). Moreover, the predicted poleward expansion of macroalgal forests and seagrass meadows with warming would increase the potential for pH up-regulation during summer in Arctic coastal ecosystems in the future (*6*, *23*). On the other hand, the long polar nights should result in a down-regulation of pH, potentially amplifying negative effects of OA during winter, when calcifiers are likely less susceptible to low pH (*1*, *22*).

The high daytime pH of dense macrophyte beds also implies low CO_2 concentration and the possibility that macrophyte metabolism could be CO_2-limited (*13*, *24*). CO_2 levels in coastal Arctic ecosystems are particularly low [<200 parts per million (ppm)] during the light period, largely driven by intense drawdown by the spring phytoplankton bloom (*25*–*28*), and macrophyte photosynthesis would further reduce the levels. Hence, future increases in CO_2 may lead to a positive feedback, promoting Arctic photosynthesis and further enhancing the capacity of macrophytes to up-regulate pH. The same

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may be the case for planktonic primary production (*27*), leading to potential pH up-regulation by phytoplankton (*29*).

Here, we examine the role of photoperiod in modulating the effect of plant photosynthesis on pH dynamics by combining in situ observations of pH dynamics in Arctic kelp forests under long photoperiods with an experiment that tests the role of photoperiod in driving the observed up-regulation of pH in these ecosystems. Specifically, we examined pH fluctuations over diel cycles in natural kelp forests at day lengths of 15 hours in early September in subarctic Greenland (Kobbefjord, Nuuk, 64°N) and at 24-hour light per day during midsummer in Arctic Greenland (Fortuna Bay, Disko Bay, 69°N) (fig. S1). We then manipulated photoperiod for Arctic macroalgae and seagrass communities in controlled aquarium experiments and examined how photoperiod modulated the effect of plant photosynthesis on pH and pCO_2. We follow Cornwall *et al.*'s (*18*) proposal to assess the effect of macrophytes on pH using ecologically realistic assemblages, and biomass densities of macroalgae to alter pH within the experimental setup themselves. The aquarium experiments were conducted at various levels of CO_2 supply to also test the potential interacting effect of photoperiod and CO_2 supply on controlling pH levels in Arctic macrophyte communities (see Materials and Methods). The in situ observations and experimental results yield consistent results supporting the notion that photoperiods longer than 21 hours, characteristic of Arctic summers, are conducive to sustained up-regulation of pH by kelp photosynthesis.

RESULTS

Photoperiod control of pH dynamics and pH up-regulation in Arctic macrophytes

In the subarctic kelp forest exposed to a photoperiod of 15-hour daylight, in situ pH varied within a range of 0.18 pH units around a mean value of pH 8.10 without any distinctive trend over the 10-day observation period (Fig. 1). By contrast, the kelp forest north of the Arctic Circle exposed to a photoperiod of 24-hour daylight showed a steady increase in pH by 0.15 units over 10 days during midsummer (0.0154 ± 0.0001 pH unit day^{-1}), with oscillations driven by tidal regimes and variable solar radiation (Fig. 1). The metabolic nature of the changes was confirmed by the strong relationship between pH and oxygen concentrations at both locations (Fig. 1 and fig. S2).

This pattern toward steadily increasing pH at long photoperiods was also observed when manipulating photoperiod in aquaria populated with Arctic macrophytes at densities representative of dense communities in the field (see Materials and Methods). At short day lengths of 12-hour light, pH oscillated with an amplitude of 0.11 pH units around a slightly increasing mean value in aquarium experiments (Fig. 2). At longer photoperiods, the pattern in pH oscillations gradually changed toward a steady increase in pH, whereas the range of diel oscillations vanished in the absence of darkness (Fig. 2).

CO_2 removal and photosynthetic activity of Arctic macrophytes

The changes in pH observed in macrophyte communities in the field and in the laboratory were driven by CO_2 depletion associated with photosynthetic uptake in the light and respiratory CO_2 release in the dark (Figs. 1 and 2). The continuous increase in pH in the Arctic kelp forest was paralleled by a steady decline in pCO_2 by about 100 ppm over the 10-day study period. Maximum pH observed during the study period was 8.33 with an associated minimum pCO_2 of 158 ppm (Fig. 1). The observed changes in temperature and salinity over the deployment period even tended to buffer the decline in pH, which would have been about 0.01 pH units larger if temperature and salinity had remained constant [calculated using CO2SYS (*30*)]. In contrast to the sustained decline of CO_2 over time, O_2 concentrations showed diurnal oscillations around a base level, reaching maxima at peak irradiance and minima at lowest irradiance (Fig. 1).

The photosynthesis-induced increase in pH further acted to shift the speciation of the carbonate system toward less CO_2. Hence, sustained photosynthesis resulted in declining minimum CO_2 concentration in the macrophyte community with increasing day length (Fig. 3A). The net rate of CO_2 depletion by the macrophyte community during the day increased 10-fold with experimental increases in day length but somewhat declined in the absence of night (Fig. 3B). For a given photoperiod, the rate of CO_2 depletion also increased with increasing CO_2 supply (Fig. 3B), from 200 ppm, characteristic of contemporary Arctic conditions during spring and summer (*25*, *27*, *28*) and exceeding the minimum levels observed in situ within the Arctic kelp canopy (Fig. 1), to 400 and 1000 ppm, reflecting a CO_2-enriched situation possibly met in the future. The rate of increase in CO_2 concentration at night, reflecting respiration rates, declined from 10.4 ± 0.8 μM $hour^{-1}$ at low CO_2 supply to 5.7 ± 0.8 μM $hour^{-1}$ at high CO_2 supply (fig. S3).

In aquarium experiments, relative electron transport rates (rETRs) of photosystem II per unit CO_2 increased with a photoperiod of up to 21 hours and declined in the absence of night (Fig. 3C). This response pattern was observed for each of the three tested species and matches that of CO_2 depletion as a function of photoperiod. Field measurements of rETR in brown macroalgae from the Arctic demonstrated that the photosystem was active on a diurnal basis but showed some differences in diurnal variability among species (fig. S4). Diel rETR was relatively uniform for *Agarum clathratum* (maximum/minimum rETR ratio over 24 hours = 1.7), rETR was much reduced during the lowest light levels in *Fucus vesiculosus* (maximum/minimum rETR ratio over 24 hours = 5.1), and rETR of *Saccharina latissima* exhibited intermediate diel variability (maximum/minimum rETR ratio over 24 hours = 3.7). Hence, for all species, diel variability in rETR was much lower than diel variability in photosynthetically active radiation (PAR) (fig. S4; PAR maximum/minimum ratio over the 24-hour sampling events = 12.7).

DISCUSSION

Photoperiod control of pH dynamics and pH up-regulation in Arctic macrophytes

Our results confirm, through field evidence and experimental manipulation, that the capacity of Arctic macrophytes to up-regulate pH depends on photoperiod. We showed that balanced light and dark cycles lead to diurnal oscillations in pH, whereas long photoperiods (>21 hours) lead to a continuous increase in pH. The results are consistent with diel pH oscillations reported for dense subpolar macroalgal forests (*9*, *21*) and temperate macrophyte beds (*8*), as well as with summer peaks in pH reported from seasonal studies in these habitats (*10*, *11*). To the best of our knowledge, the records from Disko Bay represent the first report of pH dynamics in polar (that is, >66 latitude) macrophyte stands and confirm that the absence of night supports pH up-regulation in kelp forests during the Arctic summer. The combined evidence from field observations and laboratory manipulations yields consistent and conclusive results. Hence, the field data document ecosystem-scale differences in the capacity of subarctic and Arctic kelp forests to sustain high pH despite tidal variability and

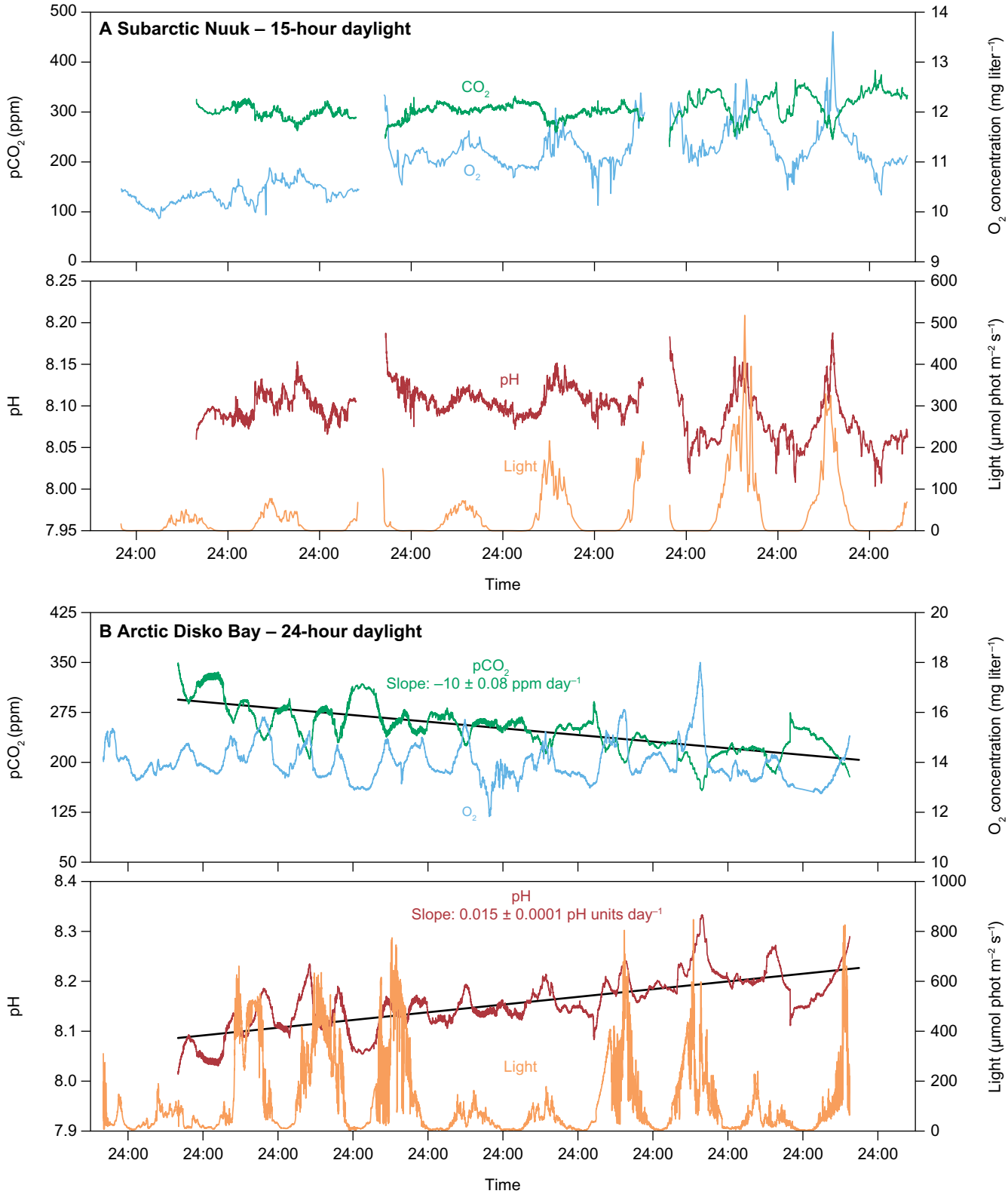

Fig. 1. CO_2, O_2, pH, and light in subarctic and Arctic kelp forests. Changes in pCO_2, O_2, pH, and light during field deployments in kelp forests in subarctic Nuuk at a 15-hour daylight photoperiod (**A**) (27 August to 5 September 2013) and in the Arctic Disko Bay at a 24-hour daylight photoperiod during midsummer (**B**) (16 to 25 June 2014). The slopes of linear regressions of the steady decline in pCO_2 and increase in pH in the Arctic are indicated.

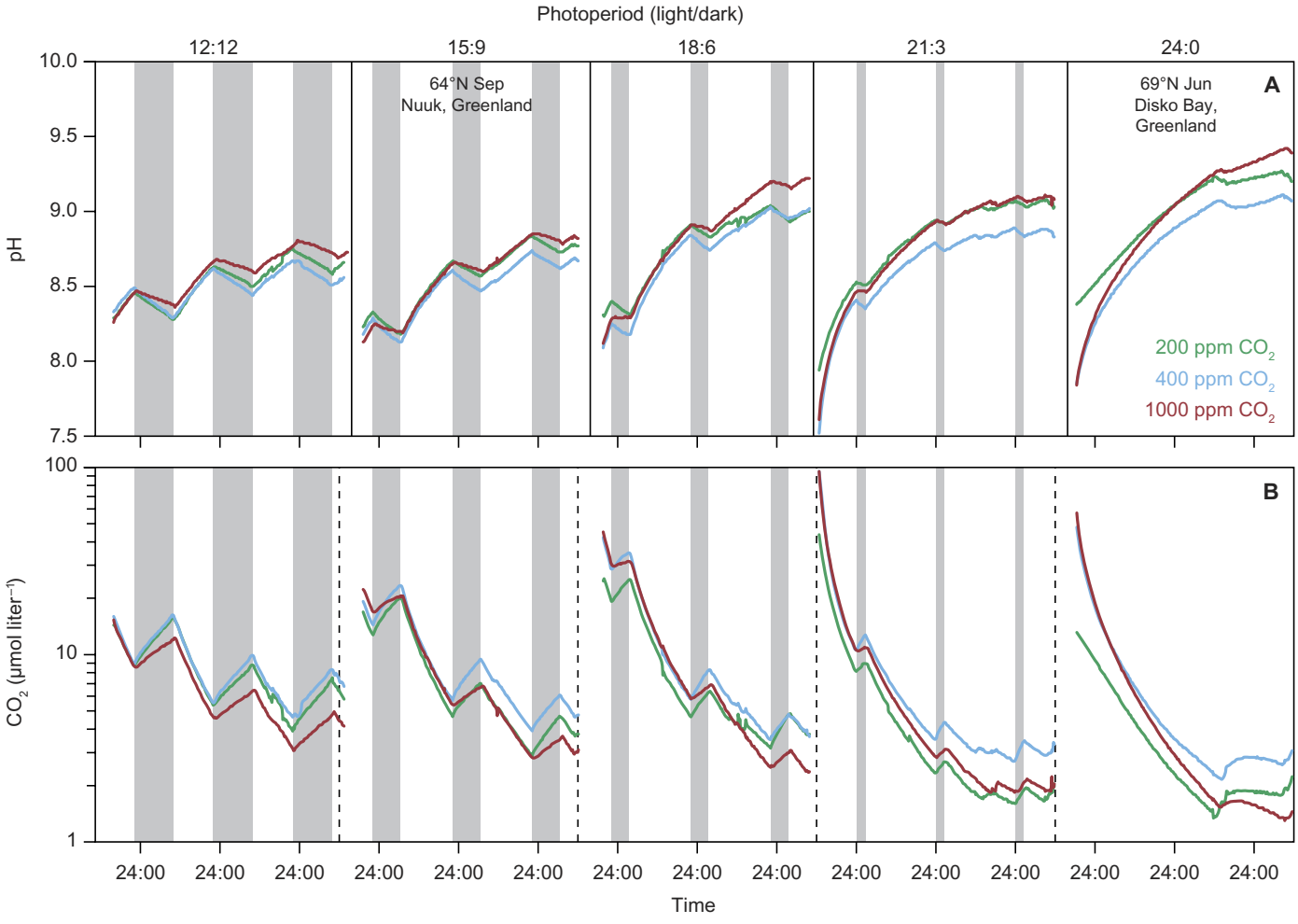

Fig. 2. pH and CO_2 levels in aquaria. Average pH (**A**) and CO_2 concentration (**B**) over time in aquaria ($n = 3$) exposed to different photoperiods (left to right: 12-, 15-, 18-, 21-, and 24-hour light; shading illustrates dark periods) and treatments with different CO_2 supply (~200, 400, and 1000 ppm). Latitude/season equivalents of the tested photoperiods are shown at the top of the relevant photoperiod panel; that is, the 15:9 and the 24:0 photoperiods represent the field studies in Nuuk and Disko Bay, respectively.

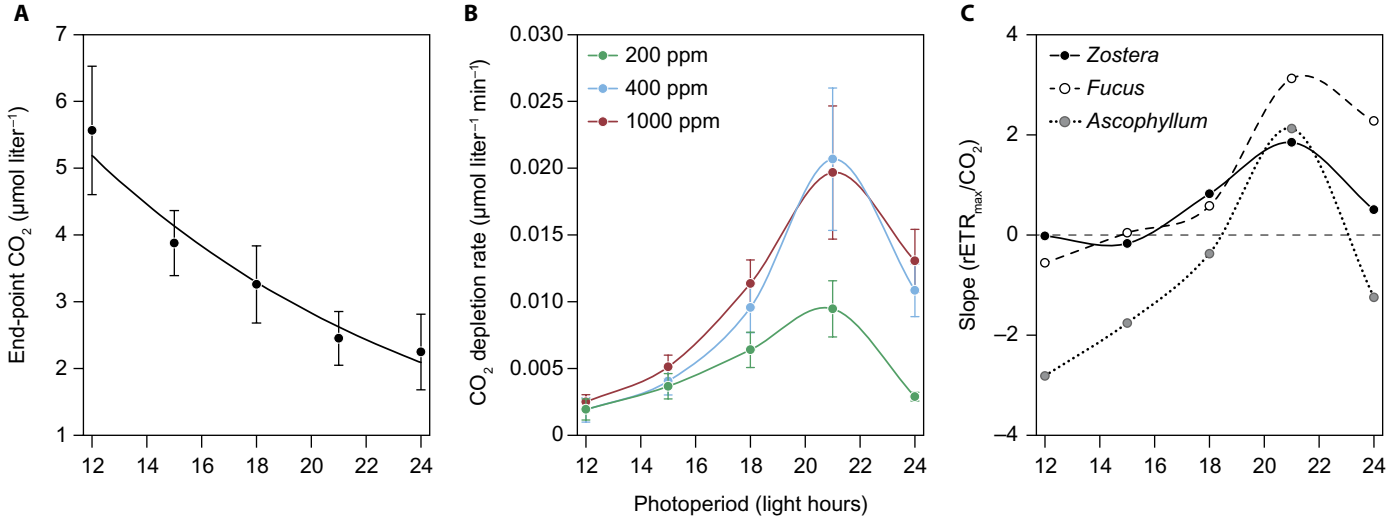

Fig. 3. Macrophyte effects on CO_2 in relation to photoperiod. (**A**) CO_2 end point as a function of photoperiod (mean ± SE across pCO_2 treatments) with an exponential fit ($y = 12.82e^{-0.076x}$, $R^2 = 0.96$). (**B**) Mean CO_2 removal rate (in micromolar per hour) as a function of photoperiod (mean ± SE across CO_2 treatments). (**C**) Linear regression slopes of mean relative maximum electron transport rate ($rETR_{max}$) per unit of CO_2 as a function of photoperiod for the three tested species.

variations in solar radiation. The complementary evidence from the highly controlled but simplified aquarium experiments supports the conclusion that photoperiod is the major mechanism controlling the observed pH dynamics.

CO_2 removal and photosynthetic activity of Arctic macrophytes

The steady increase in pH of 0.15 units and the parallel decline in pCO_2 concentration in the Arctic kelp forest over the 10-day study period during midsummer suggest a large capacity for sustained pH up-regulation by kelp photosynthesis in this environment. Moreover, the maximum pH of 8.33 and the associated minimum pCO_2 of 158 ppm, recorded by the end of the study period, indicated that the kelp forest still had ample potential to further remove CO_2 and increase pH over the remaining month of continuous daylight at this latitude. According to the parallel photoperiod experiment, the kelps should potentially be able to increase pH levels to >9, as observed in subarctic tidal pools (*21*).

The contrasting behavior of CO_2 and O_2 in the Arctic kelp forest, with CO_2 concentrations exhibiting a sustained decline over time as opposed to O_2 concentrations showing daily oscillations around a base level, reflects the almost 30-fold difference in Henry's law constant for O_2 and CO_2, resulting in much slower air-sea equilibration for CO_2 (*31*). Hence, the fast air-sea equilibration for O_2 allows this gas to maintain the same base level over time, whereas the slow CO_2 supply from the atmosphere does not keep pace with the photosynthetic CO_2 consumption rate by the kelp community.

The sustained rETRs of photosystem II in aquarium experiments and in the field confirmed that the macroalgae remained photosynthetically active throughout the 24-hour daylight photoperiod of the Arctic midsummer. Long photoperiods of up to 21 hours even stimulated $rETR_{max}$ per unit CO_2 as well as the capacity for CO_2 drawdown of all tested species, whereas a further increase in photoperiod triggered some decline in the rates. This response is consistent with the reported stimulated growth of fucoids (*32*) and *S. latissima* (*33*) at longer photoperiods. The finding of relatively low diel variability in rETR over the diurnal cycle indicated that diel acclimation of photosynthesis to prevalent light buffered the changes in rETR relative to those in PAR.

Potential CO_2 limitation of macroalgal production

The minimum CO_2 concentration observed in the laboratory at a 24-hour photoperiod was 2.5-fold lower than that at a 12-hour photoperiod. This suggests that the presence of a dark period, which ensures resupply of CO_2, prevents macrophyte photosynthesis from reaching severe CO_2 limitation, whereas continuous photosynthetic CO_2 uptake during the Arctic midsummer may lead to CO_2 limitation. The end-point pH under continuous photosynthesis reflects the efficiency of inorganic carbon utilization, providing a natural analog to pH drift experiments used to assess inorganic carbon utilization in aquatic plants (*34*, *35*). Hence, the decline in CO_2 removal rate and $rETR_{max}$ when pH reached a maximum of about 9.5 under the 24-hour photoperiod likely reflects increasing CO_2 limitation. pH end points for photosynthesis by seaweed genera present in the Arctic have been established at pH 9.5 to 9.8 [pH 9.7 to 9.8 for fucoids and pH 9.0 to 9.5 for kelps (*34*, *36*)]. However, in natural kelp stands, CO_2 is resupplied through tidal advection and community respiration. Hence, although dense macrophyte stands could deplete CO_2 within a few days under experimental conditions, mechanisms resupplying CO_2 in nature imply that macrophytes in the Arctic may be able to photosynthesize at maximum rates throughout the Arctic summer. However, the experimental upper limit of pH of about 9.5 matches the maximum pH observed in situ in shallow dense mixed macroalgal beds (*11*) and that in subarctic tidal pools densely populated by fucoids (*21*), suggesting that it represents a threshold for photosynthesis in both the laboratory and the field. Hence, although differences in flow conditions affect pH in vegetated meadows (*14*, *15*), the conditions in the aquaria do not deviate greatly from field settings of Arctic tidal pools, where the hourly rates of change in CO_2 and pH driven by seaweed photosynthesis match those observed in our experimental systems (*21*).

Evidence for CO_2 limitation at long photoperiods is provided by the observation that the $rETR_{max}$ per unit CO_2 increased with day length but declined in the absence of a dark period for all tested species. The parallel observation of reduced net removal of CO_2 in the absence of a dark period strengthens the evidence. Current CO_2 concentrations are far below atmospheric saturation in the Arctic macroalgal forest, which further suggests potential CO_2 limitation of photosynthesis in those communities during summer. However, the scope for increased photosynthesis with increased CO_2 may be modest as community photosynthesis of dense kelp beds tends to be light-limited (*24*).

In conclusion, the results presented here confirm that the capacity of macrophytes to regulate pH depends on photoperiod and that long photoperiods characteristic of Arctic summers are conducive to sustained up-regulation of pH by Arctic vegetation. An implication of this is potential benefits for Arctic calcifiers during summer (*6*) when organisms reproduce and are most vulnerable to OA (*1*, *22*). This up-regulation of pH during the Arctic summer may gain increased importance in the future because of the predicted large scope for macrophytes to expand along the extensive Arctic coastline with climate change (*6*), as OA may approach thresholds affecting Arctic calcifiers (*3*).

MATERIALS AND METHODS

Experimental design

We tested the hypothesis by examining pH fluctuations over diel cycles in natural kelp forests exposed to a photoperiod of 15-hour daylight in subarctic Greenland (Kobbefjord, Nuuk, 64°N, 51°W) and a photoperiod of 24-hour daylight in Arctic Greenland (Fortuna Bay, Disko Bay, 69°N, 53°W) (fig. S1). We further manipulated photoperiod at various levels of CO_2 supply in aquarium experiments with Arctic macroalgae and seagrass and recorded resulting diel pH fluctuations in the plant communities.

Field study

We examined pH fluctuations over diel cycles in natural kelp forests on Greenland's west coast under a 15-hour photoperiod in the subarctic Kobbefjord near Nuuk in early fall (27 August to 5 September 2013; average temperature, 5.2°C) and under a 24-hour photoperiod in the Arctic Fortuna Bay in Disko Bay during midsummer (16 to 25 June 2014; average temperature, 5.1°C; fig. S1). Kobbefjord is located in the extensive Godthåbsfjord system at Nuuk and exposed to a tidal range of 1 to 4.5 m. Subtidal macroalgae form dense and productive benthic habitats along the shores to water depths of ca. 40 m (*37*) interspaced with communities of benthic microalgae and with scarce occurrence of eelgrass meadows at 1 to 3 m depth (*38*) and rich intertidal communities (*21*). More details on Kobbefjord are available (*26*, *28*), including data acquired in parallel to those reported here in neighboring kelp sites (*21*). Fortuna Bay is a shallow (ca. 1 to 3 m deep), more or less circular cove of about 350 m in diameter exposed to a tidal range of 1 to 2.5 m and protected from ice scouring by rocks at the entrance blocking

the passage of icebergs. Dense kelp forests dominated by *S. latissima* and *A. clathratum* cover most subtidal areas of the cove, whereas fucoid algae are abundant in the intertidal zone.

At each site, we deployed loggers in shallow dense kelp forests collecting continuous data (every minute) of PAR, O_2 concentration, pH, temperature, salinity, and water level approximately 50 cm above the seafloor. At the subarctic site, we conducted three consecutive deployments each lasting about 48 hours, and at the Arctic site, we conducted one continuous deployment over 10 days. PAR was measured using Odyssey PAR loggers (Dataflow Systems Pty Limited) calibrated to a LI-COR PAR logger (LI-1000, Bad Homburg). Oxygen concentration was measured using miniDOT oxygen loggers (Precision Measurement Engineering) calibrated to O_2 concentrations measured by a multimeter (HQ40D, Hach Lange). pH_T was measured with SeaFET pH loggers (Satlantic) newly calibrated at the Satlantic facility. Conductivity, temperature, and water level were recorded with a MicroCAT (SBE 37, Sea-Bird). On several occasions, triplicate point samples for determination of total inorganic carbon C_T and total alkalinity A_T were collected to allow calculation of carbonate chemistry. A_T was measured on an alkalinity titrator (AS-ALK2, Apollo SciTech Inc. or Metrohm Titrando 808) by open-cell titration (*39*), whereas C_T was measured on a C_T analyzer (AS-C3, Apollo SciTech Inc.). Results were verified against certified reference material with an average accuracy of 2.9 μmol kg^{-1} for A_T and 2.4 μmol kg^{-1} for C_T. pCO_2 was calculated on the basis of pH and C_T [on the basis of a relationship between C_T and salinity (s): for Nuuk waters, $CT = 87.237s - 823.55$, $R^2 = 0.89$; for Disko waters, $C_T = 51.63s + 97.95$, $R^2 = 0.69$, as established from the point samples] in CO2SYS, as described for the laboratory experiment, but using total pH scale.

The photosynthetic performance of *S. latissima*, *A. clathratum*, and *F. vesiculosus* ($n = 3$ per species) was measured as chlorophyll fluorescence in the field over a midsummer diurnal cycle (23 to 24 June 2014) using a portable pulse amplitude modulation fluorometer (Diving-PAM, Walz, Model S/N 0109) (*40*). Using a PAM leaf chip, the tissue was dark-adapted for 5 min and then illuminated by a series of nine increasing actinic light intensities (0 to 348 μmol photons m^{-2} s^{-1}) at intervals of 10 s to produce rapid light curves (RLCs). The rETR, reflecting the activity of photosystem II, was plotted against I (PAR) and fitted by Eilers and Peeters' photosynthesis model (*41*), using the software JMP (version 11.1.1) to estimate the model constants b_0, b_1, and b_2 based on their relationships with rETR, maximum rETR ($rETR_{max}$), the photosynthetic efficiency (α), and the saturating irradiance ($I_k = rETR_{max}/\alpha$)

$$\mathrm{rETR} = \frac{I}{(b_0 \times I^2 + b_1 \times I + b_2)} \quad (1)$$

where

$$\alpha = 1/b_2, \ \mathrm{rETR_{max}} = \frac{1}{b_1 + 2(b_0 \times b_2)^{0.5}}$$

and

$$I_k = \frac{b_2}{b_1 + 2(b_0 \times b_2)^{0.5}}$$

In situ photosynthetic activity (rETR) was calculated for each of the sampling events on the basis of PAR measured at the kelp canopy at the time of sampling and using the identified constants b_0, b_1, and b_2. No single formula fitted all rETR-*I* curves; thus, different equations were used for field data, which displayed strong photoinhibition, and laboratory data (see details below).

Laboratory experiments

The aquarium experiments were simplified representations of the field situations of dense vegetation, allowing controlled manipulations of photoperiod and pCO_2 concentration while keeping everything else equal. The experiments were designed with ecologically realistic assemblages and densities of marine vegetation to alter pH within the experimental setup themselves.

Brown macroalgae (*Ascophyllum nodosum*, *F. vesiculosus*, and *Saccharina longicruris*) and *Zostera marina* were collected in shallow waters of the subarctic site in early June 2014. The same species, except *Z. marina*, also occurred at the Arctic site. Tips of *A. nodosum* and *F. vesiculosus*, fragments of new blades of *S. latissima*, and shoots of *Z. marina* were transferred to cooler bags where they were kept cold and humid during the 30-hour transportation to the experimental facilities at the Mediterranean Institute for Advanced Studies (IMEDEA), Mallorca.

Experiments were conducted in temperature-controlled climate rooms set at 4°C. Artificial seawater (Reef Crystals) was adjusted to resemble alkalinity (A_T) at the study sites by adding distilled water and NaCl to obtain the desired salinity [30 parts per thousand (ppt)] [salinity range at field sites: subarctic, 28.9 to 31.7; Arctic, 29.6 to 33.1; alkalinity-salinity relationship for the area, $A_T = 159 + 63S$ (*28*)], that is, 1980 to 2240 μmol per kilogram of seawater for the range of salinities measured at the study sites. The resulting A_T of the artificial water was 2241 ± 31.3 μmol per kilogram of seawater, and salinity was 30.2 ± 0.42 ppt. Seawater was mixed in a 200-liter tank and circulated through an ultraviolet lamp to mix and reduce microalgal growth. Three different CO_2 supply treatments were applied to three replicated 6-liter aquaria, yielding a total of nine experimental units. The pH of the artificial seawater was manipulated by aeration with pCO_2 concentrations of 200, 400, and 1000 ppm, resulting in stable pH values of about 8.2, 8.1, and 7.8, respectively, in the absence of algae (fig. S5). The 200 ppm value was chosen as representative of the current spring and summer levels in the Arctic (*25*, *27*, *28*), matching the choice of current Arctic pCO_2 levels applied in the European Project on Ocean Acidification experiments (*42*–*45*). The 400- and 1000-ppm treatments were chosen to reflect possible future scenarios of elevated pCO_2 in the Arctic Ocean. For all treatments, air was stripped of CO_2 by passing it through soda lime tubes and mixed to the desired concentration with pure CO_2 gas using mass flow controllers (AALBORG GFC-17) for air and CO_2. Gases were mixed in a mixing bottle and led to the aquaria through tubes, with microporous bubble curtains secured to the base of each tank. Measurement of pH in the aquaria was conducted using National Institute of Standards and Technology buffer (*4*, *7*, *10*)–calibrated electrodes connected to a data logger (IKS Aquastar), collecting data every 15 min. In addition, to warrant correct functioning of the electrodes, pH was measured using a spectrophotometer (Jasco 7800), following the standard operating procedure 6b (Department of Energy, 1994).

After ensuring stable pH in the aquaria in the absence of macrophytes, we established a community of the four collected macrophyte species [six specimens from each species, yielding a total biomass of 2.7 to 3.7 g dry weight (DW); table S1] in each of the aquaria by attaching

them to a net at the base of the aquaria. This biomass represents a biomass density of 0.45 to 0.61 g DW liter^{-1}, characteristic of dense vegetation. Hence, a study of Greenland kelp forests reported an average biomass of 740 g DW m^{-2} (*46*), corresponding to a biomass density of 0.74 g DW liter^{-1} (assuming a canopy height of 1 m), and a Canadian study reported an average biomass of 6.3 kg fresh weight (FW) m^{-2} (*47*), corresponding to a biomass density of 0.6 g DW liter^{-1} (assuming 10% DW and a canopy height of 1 m).

The experimental units were then exposed to a series of photoperiods representing 12-, 15-, 18-, 21-, and 24-hour light, each lasting 3 days, to record a clear pH response pattern for all photoperiods. The aquaria were illuminated by two T5 lamps with two 54-W fluorescent bulbs delivering PAR light levels at an intensity of 111 ± 5 µmol photons m^{-2} s^{-1} at the water surface, to represent PAR levels within canopies in situ. Hence, the maximum daily PAR levels recorded near the top of Greenland eelgrass canopies during summer were 17.6 and 25 mol photons day^{-1}, corresponding to 204 and 289 µmol photons m^{-2} s^{-1} (*38*), and average PAR levels recorded near the top of kelp canopies during light hours of the 10-day sampling periods at the subarctic and the Arctic site were 58 and 119 µmol photons m^{-2} s^{-1}, respectively, with peaks of up to ca. 500 and 800 µmol photons m^{-2} s^{-1}, respectively (Fig. 1), and with light levels attenuating rapidly within the canopy.

After each photoperiod incubation, seawater was sampled for measurement of A_T. Subsequently, photosynthetic performance was measured for *A. nodosum*, *F. vesiculosus*, and *Z. marina* using a Diving-PAM, as described above. To avoid excessive manipulation of plant tissue, we conducted all measurements in the aquaria. Using a PAM leaf chip, the tissue was dark-adapted for 5 min and then illuminated by a series of nine increasing actinic light intensities (0 to 616 µmol photons m^{-2} s^{-1}) at intervals of 10 s to produce RLCs. rETR was recorded (n = 12 per species) and fitted against PAR by a nonlinear model (*40*, *48*)

$$\mathrm{rETR} = \mathrm{rETR}_{\max} * \left(1 - e^{(-\alpha * \text{ incident PAR}/\mathrm{ETRmax})}\right) \quad (2)$$

The parameters $\mathrm{rETR}_{\max}$, photosynthetic efficiency (α), and saturating irradiance ($I_k = \mathrm{rETR}_{\max}/\alpha$) were estimated by separately fitting Eq. 2 to each of the RLCs. The effects of photoperiod and CO_2 supply on maximum ETR of the three species (*A. nodosum*, *F. vesiculosus*, and *Z. marina*) were subsequently compared among treatments using analysis of variance ($P < 0.05$) after ensuring normality (Shapiro-Wilk test). The analyses were carried out using RStudio 0.98.945.

Between each photoperiod incubation, the aquaria were emptied and filled with clean seawater pretreated with the treatment CO_2 gas to speed up equilibration to the treatment CO_2 level. The remaining carbonate chemistry parameters were calculated using CO2SYS (*30*) [with dissociation constants (*49*) refitted (*50*) on the basis of $\mathrm{pH}_{\mathrm{NBS}}$ sensor measurements and measured alkalinity at the start and end of the experiment interpolated linearly over time].

Changes in pH and pCO_2 based on experiments and field observations were fitted by linear regression, whereas CO_2 end points as functions of photoperiod were fitted by an exponential model. In addition, respiration rates were modeled from the change in CO_2 concentration during the night in each of the nine experimental units (the three replicates of each CO_2 supply treatment) over the experimental period, taking into account the constant CO_2 supply.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/2/12/e1501938/DC1

fig. S1. Map of Greenland field locations.
fig. S2. pH and O_2 concentrations in subarctic and Arctic kelp forests.
fig. S3. Respiration rates of macrophytes in aquaria.
fig. S4. rETR of macroalgae in Arctic midsummer.
fig. S5. pH in experimental treatments in the absence of macrophytes.
table S1. FW biomass of each species in each aquarium.

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Long photoperiods sustain high pH in Arctic kelp forests

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