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Impact of elevated pH on succession in the Arctic spring bloom

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ABSTRACT: The development of pH during the spring bloom of 2011 and 2012 was investigated in Disko Bay, West Greenland. During the spring phytoplankton bloom, pH reached 8.5 at the peak of the bloom and subsequently decreased to 7.5. Microcosm experiments were conducted on natural assemblages sampled at the initiation of the spring bloom each year and pH levels were manipulated in the range of 8.0–9.5 to test the immediate tolerance of Arctic protist plankton to elevated pH under nutrient-limiting (2011) and nutrient-rich conditions (2012). The most pronounced effect of elevated pH was found for heterotrophic protists, whereas phytoplankton proved more robust. Two out of 3 heterotrophic protist species were significantly affected if pH increased above 8.5, and all heterotrophic protists had disappeared at pH 9.5. Based on chl *a* measurements from the 2 sets of experiments, phytoplankton community growth was significantly reduced at pH 9.5 during nutrient-rich conditions, while pH had little impact on nutrient-limited phytoplankton growth. The results were supported by cell counts which revealed that phytoplankton growth during nutrient-rich conditions was significantly reduced from an average of 0.49 d^{-1} at pH 8.0 to an average of 0.27 d^{-1} at pH 9.5. In comparison, only 1 out of 4 tested phytoplankton species was significantly affected by elevated pH under nutrient-limited conditions. Sudden pH fluctuations, such as those occurring during phytoplankton blooms, will most likely favour pH-tolerant species, such as diatoms.

KEY WORDS: pH · Arctic phytoplankton · Spring bloom · Growth rates · *Phaeocystis pouchetii* · Heterotrophic protists

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INTRODUCTION

The phytoplankton spring bloom is a key event in Arctic marine ecosystems, where it plays a central role in the fixation and sequestering of carbon dioxide during the short productive season (Pabi et al. 2008). Carbon dioxide is a weak acid when dissolved in water, and any reductions in the concentrations of inorganic carbon will be compensated by a shift in the chemical equilibrium, making the water more alkaline. Seawater contains high amounts of bicarbonate ions, which act as buffers against changes in pH; consequently, pH in the open ocean is relatively stable (Skirrow 1975). However, in brackish coastal waters, where the buffering capacity is lower and phytoplankton blooms are more intense, pH may be highly variable (Hansen 2002, Hinga 2002, Provoost et al. 2010, Brutemark et al. 2011) and may affect phytoplankton growth and species succession (Hansen 2002, Hinga 2002, Pedersen & Hansen 2003a,b, Søderberg & Hansen 2007). There are only a few studies on the role of pH in Arctic marine ecosystems, and these studies indicate that pH may also

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play a central role in plankton dynamics in these high-latitude ecosystems (Charalampopoulou et al. 2011, Søgaard et al. 2011, Silyakova et al. 2013).

Disko Bay is an important fishing and hunting area in Greenland, located just south of the southern limit of Arctic winter sea ice. The sea ice cover in the bay shows substantial inter-annual variability (Hansen et al. 2006), and most of the new primary production is confined to a 2–4 wk long phytoplankton bloom occurring in spring (Dünweber et al. 2010). The fate of the bloom, due to grazing by the dominating heterotrophs, has been investigated over the past 2 decades (Levinsen et al. 1999, Madsen et al. 2001), but the role of pH as a regulating factor has not yet been investigated. Heterotrophic dinoflagellates and ciliates are major grazers in Disko Bay during spring, and their gross growth rates are coupled to the phytoplankton biomass (Levinsen et al. 1999). However, heterotrophic protists may be more sensitive to changes in pH than the dominant phytoplankton species (Pedersen & Hansen 2003a,b). In this way, sudden changes in pH may uncouple the grazers from the succession. This tendency could be strengthened by the high tolerance of ice algae, which are known to thrive in brine channels where pH can reach 10.0 (Gleitz et al. 1995).

We hypothesise that both phytoplankton and heterotrophic protists are sensitive to changes in pH and that pH may play a role in protist growth rates and, thus, in the succession of the Arctic spring bloom. The aim of our study was to study fluctuations in pH during, before and after the phytoplankton spring bloom in Disko Bay, Greenland. In addition, we investigated the impact of elevated pH on the growth and succession of the phytoplankton and heterotrophic protist community. Because nutrient concentrations most likely affect the pH tolerance and growth of the organisms (Li et al. 2012), 2 independent microcosm experiments were conducted under controlled laboratory conditions. In the first set of experiments (2011), the response of a pre-bloom plankton assemblage was studied as the phytoplankton depleted the nutrients during the course of the experiment at different levels of pH. In the second set of experiments (2012), frequent dilutions with filtered pre-bloom water adjusted to different pH levels allowed us to study the growth response of a natural pre-bloom phytoplankton assemblage to elevated pH under nutrient-rich conditions. We hypothesised that the growth rates of the phytoplankton and heterotrophic protists would decrease with increasing pH and that the relative change in growth between pH treatments and controls would be the same regardless of the nutrient level.

MATERIALS AND METHODS

Sampling and hydrography

The study was conducted from April to May in both 2011 and 2012 in Disko Bay, West Greenland (experiments were conducted at Arctic Station, Copenhagen University). Water was collected from the RV Porsild (Copenhagen University) at a 300 m deep monitoring station, approx. 1 nautical mile off the coast (69° 14′ N, 53° 23′ W), using a 30 l Niskin bottle. Water samples for inorganic carbon, chl *a*, nutrients and pH measurements were taken from the fluorescence maximum depth (15–20 m) and from 7 additional depths (1, 50, 75, 100, 150, 200, 250 m) throughout the investigated period at intervals of 2 to 7 d. Conductivity, temperature, density and fluorescence were measured with a CTD, SBE25-01 (Seabird).

Chl *a* was estimated from 50–200 ml sub-samples, which were filtered on GF/F filters. The filters were extracted in 5 ml 96% ethanol in darkness for 12–24 h and measured fluorometrically before and after HCl (1 M) addition on a fluorometer TD-700 (Turner Designs) calibrated against a chl *a* standard.

Samples (30 ml) for measuring inorganic nutrients were kept frozen (−18°C) for later analysis on a Skalar autoanalyser (Breda) following the procedures of Hansen & Koroleff (1999). The precision (analytical reproducibility) of the nutrient analyses was 0.06, 0.1, and 0.2 µM for phosphorus, nitrate+nitrite, and silicate, respectively.

Samples for measuring pH *in situ* were transferred to 50 ml airtight plastic bottles, which were kept cold and dark until pH was measured immediately after transfer or a few hours later, using either a pH meter (Hanna) or a 3210i (WtW), both with a detection level of 0.01. The pH meters were calibrated using a 2 point calibration (NBS scale) with NIST buffers 7.01 and 10.01.

Experimental conditions

Two experiments were conducted in the thermoregulated shipping container at the Arctic Station. The first set of experiments (Expt 1) was initiated on 23 April 2011, and the second set of experiments (Expt 2) on 15 April 2012. Water for the experiments was collected at 3–5 m depth and gently siphoned into dark carboys. Additional water for dilution between the sampling events was collected from the photic zone and below the pycnocline at 200 m depth

because there was no phytoplankton at this depth and pH was close to 8.0. This water was filtered through a 0.45 µm capsule filter (Whatman Polycap) and thereafter stored under cold (4°C) and dark conditions.

A total of 12 transparent 2.5 l polycarbonate bottles (Expt 1) or 15 transparent 1.2 l bottles (Expt 2) were filled with sea water via a silicon tube equipped with a 250 µm mesh to remove mesozooplankton. Two additional bottles were filled under the same procedures, and water samples were taken from these bottles when the experiment was initiated (Day 0). The rest of the experimental bottles were incubated on a plankton wheel (1 rpm) and exposed to a 12:12 h light:dark cycle of cool white fluorescence of 100 µmol photons m^{-2} s^{-1}, corresponding to the light intensity in the water when it was collected. Average temperatures during incubation were 3.5°C (Expt 1) and 3.0°C (Expt 2).

The pH was adjusted in the bottles by gradually adding 0.1 M NaOH. The increase in pH was ≤0.5 pH units per 12 h. Three or 4 pH treatments (pH 8.5, 8.8, 9.0, and 9.5) were maintained during the experimental period. Treatment bottles were accompanied by control bottles in which pH was not manipulated but remained at approx. 8.0. Controls and treatments were run in triplicate.

During Expt 1 (2011), the effect of high pH under late bloom conditions, i.e. nutrient-limited growth conditions for the phytoplankton and sufficient phytoplankton for their heterotrophic grazers, was investigated. These conditions were ensured by replacing 20% of the bottle volume at each sampling event (every 2–3 d) with 0.45 µm filtered low-nutrient surface seawater pre-adjusted to the respective pH. In Expt 2 (2012), an early bloom situation was simulated to investigate the effect of elevated pH on the maximum growth rates of Arctic phytoplankton. Nutrient-rich conditions were produced by replacing approximately 50% of the cultures with 0.45 µm filtered nutrient-rich deep water pre-adjusted to pH at each sampling event (every 24 h). The appropriate dilution of the cultures after each sampling event was ensured by keeping chl *a* in the range 5–8 µg l^{-1} after dilution. The duration of the experiments was 8–14 d.

In both sets of experiments, bottles were sampled prior to the dilution for inorganic nutrients, chl *a* and pH (using the same procedures as described above). Sub-samples for counting phytoplankton and heterotrophic protists were fixed in acetic Lugol's solution at a final concentration of 2%. Samples were kept cold and dark until examination, which was conducted 1–2 mo after the experiment. Depending on the density of the cultures, 10–50 ml samples were settled for 24 h in Utermöhl chambers (Hydro-Bios). Phytoplankton and heterotrophic protists were counted on an inverted microscope at ×100 or ×200 magnification. Five transects or a minimum of 400 cells were counted for each of the investigated species. For the prymnesiophyte *Phaeocystis pouchetii*, only cells in colonies were counted (a colony was defined as ≥4 cells grouped together).

Growth rates (µ, d^{-1}) were calculated as the increase in cell concentration according to the formula:

$$\mu\,(\mathrm{d}^{-1}) = \frac{\ln N_1 - \ln N_0}{t_1 - t_0} \quad (1)$$

where N_0 and N_1 are the number of cells before dilution (t_1) and after previous dilution (t_0), respectively.

Cumulative cell abundance (N_{cum}) was estimated according to the formula:

$$N_{cum}(\text{cells l}^{-1}) = N^{t-1} \times e^{\mu(t_1 - t_0)}. \quad (2)$$

Growth rates in treatments and controls were calculated as the slope of the log-transformed N_{cum} as a function of time during Days 2–8 (2011) and Days 4–9 (2012), i.e. 4 and 5 sampling events, respectively. The changes in log-transformed cumulated concentration ($\ln N_{cum}$) for each replicate were fitted a linear regression. The slope of the regression was accepted as significant, i.e. $p < 0.05$.

Dissolved inorganic carbon (DIC)

Dissolved inorganic carbon (DIC) was only measured in Expt 2, where it was measured twice in each of the treatments and controls. Subsamples (10 ml) were taken from each experimental bottle and fixed with 100 µl Hg_2Cl_2 in airtight glass vials (12 ml), not allowing any headspace to prevent CO_2 from leaking out of the water phase. Samples were stored under dark and cold conditions (ca. 5°C) until analysis 1 mo later. DIC concentrations of triplicate 60 µl subsamples were measured on an IRGA (infrared gas analyser) following the procedures of Tor Nielsen et al. (2007). The data were analysed with the computer program Prologger®, and concentrations of DIC, depending on pH, temperature and salinity, were determined for 3 carbon species (HCO_3^-, CO_3^{2-} and CO_2) using the program CO_2Sys EXCEL Macro (Lewis & Wallace 1998), with the following available inputs: set of constants: K_1, K_2 from Mehrbach et al. (1973) refit by Dickson and Millero (1987), $KHSO_4$ Dickson (1990), pH scale: NBS scale (mol kg^{-1} seawater).

RESULTS

In situ variation in pH and other environmental parameters during the spring blooms

At the onset of the sampling period, sea ice covered 15 and 100% of the bay in 2011 and 2012, respectively (Fig. 1A,B). The bay was ice-free from 4 May 2011, and from 28 May 2012. The water column was stratified in both years, and the depth of the mixed layer remained relatively constant throughout the study periods. A sub-surface fluorescence maximum was observed at depths of 15–40 m throughout the study period in both years. By late April, a phytoplankton bloom had developed, with chl *a* concentrations reaching 14 and 18 μg chl *a* l^{-1} in 2011 and 2012, respectively (Fig. 1C,D). At the depth of the fluorescence maximum, pH ranged from 7.9 to 8.3 in 2011 and from 7.6 to 8.3 in 2012 (Fig. 1E,F). The peak values coincided with the phytoplankton spring bloom on 19 May 2011 and 2 May 2012. In 2011, the highest pH (pH 8.5) was measured in the surface water at 1 m

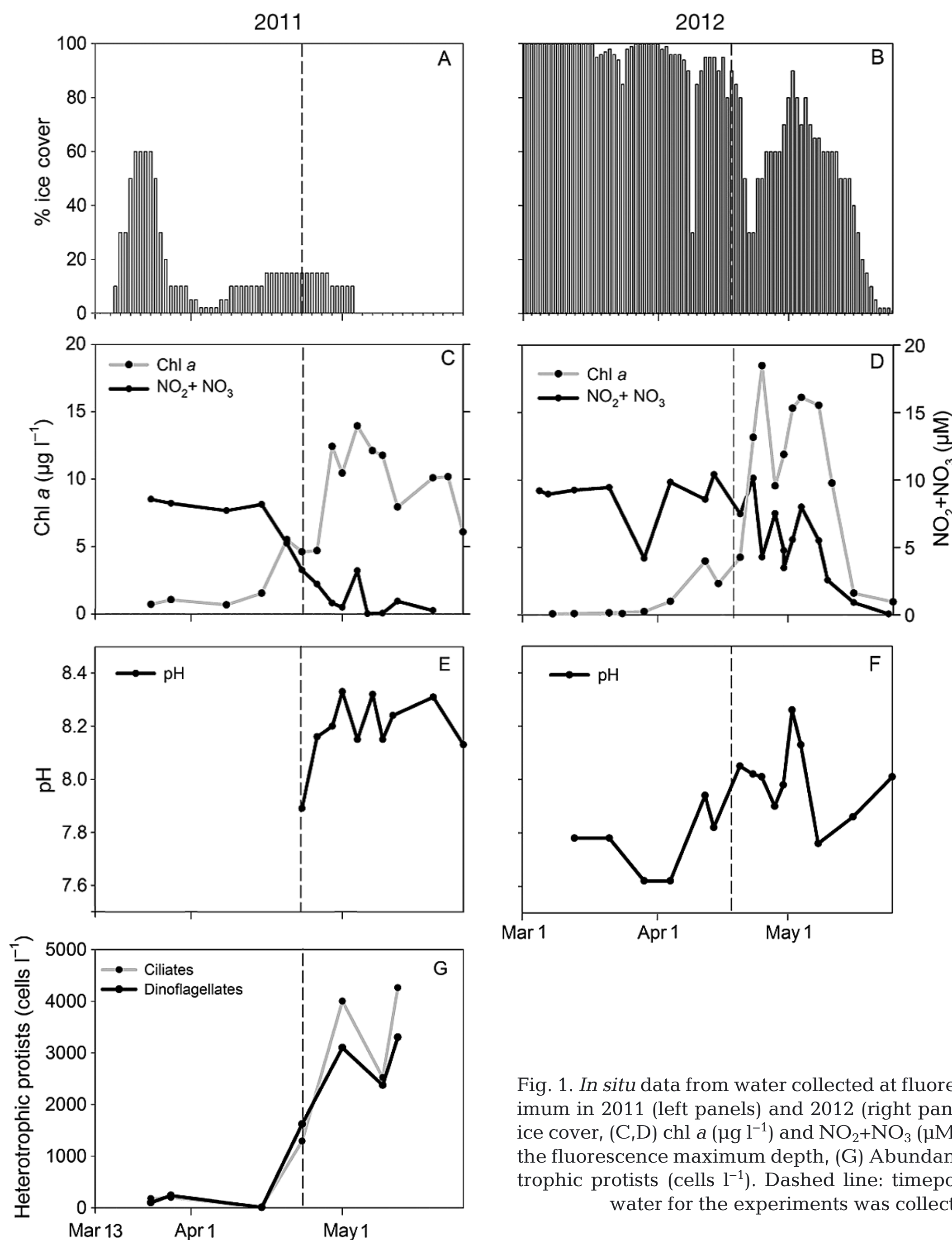

Fig. 1. *In situ* data from water collected at fluorescence maximum in 2011 (left panels) and 2012 (right panels). (A,B) % ice cover, (C,D) chl *a* (μg l^{-1}) and NO_2+NO_3 (μM), (E,F) pH at the fluorescence maximum depth, (G) Abundance of heterotrophic protists (cells l^{-1}). Dashed line: timepoint at which water for the experiments was collected

depth, while the highest pH in 2012 was found at 10 m depth (Fig. 2). The lowest values were measured at a depth of 250 m in both years, reaching values of pH 7.9 and 7.5 on 9 May 2011 and 8 May 2012, respectively (Fig. 2). The pH values were generally higher in 2011 than 2012. This difference may have been due to the lower % ice cover in 2011. The succession of heterotrophic protists in 2011 followed the development of chl *a*, reaching a maximum on 12 May with 7560 cells l^{-1} (ciliates and heterotrophic dinoflagellates contributed equally) (Fig. 1G). The ciliates were dominated by oligotrich species, primarily *Strombidium* spp., *Strobilidium* spp., *Strobilidium oviformis* and *Mesodinium rubrum*. The dinoflagellates were dominated by the thecate *Protoperidinium bipes* and naked gymnodinid species including *Gyrodinium spirale*.

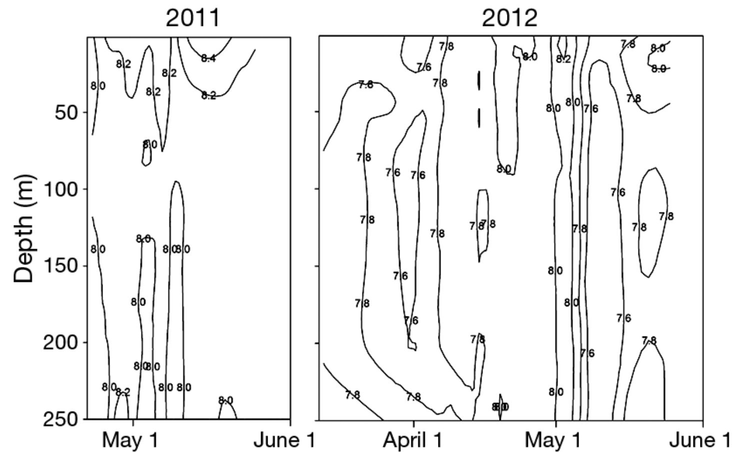

Fig. 2. Vertical distribution of *in situ* pH in 2011 (left panel) and 2012 (right panel)

Expt 1: protist community response to elevated pH under nutrient limitation

In Expt 1, chl *a* was allowed to reach a maximum of 22 µg chl *a* l^{-1} in the experimental bottles, corresponding to the chl *a* concentration during a spring bloom (Fig. 3). During the following days, chl *a* declined but remained within a narrow range (5–12 µg chl *a* l^{-1}) during the rest of the period (Table 1). Total phytoplankton (chl *a*) growth rates were generally low (<0.05 d^{-1}, Figs. 4 & 5) and the slopes of the linear regressions in log-transformed cumulated chl *a* were not significantly different from zero ($p > 0.05$). Furthermore, there was no significant difference between treatments and controls (Table 2). NO_2^- + NO_3^-, and PO_4^{-3} concentrations were low but

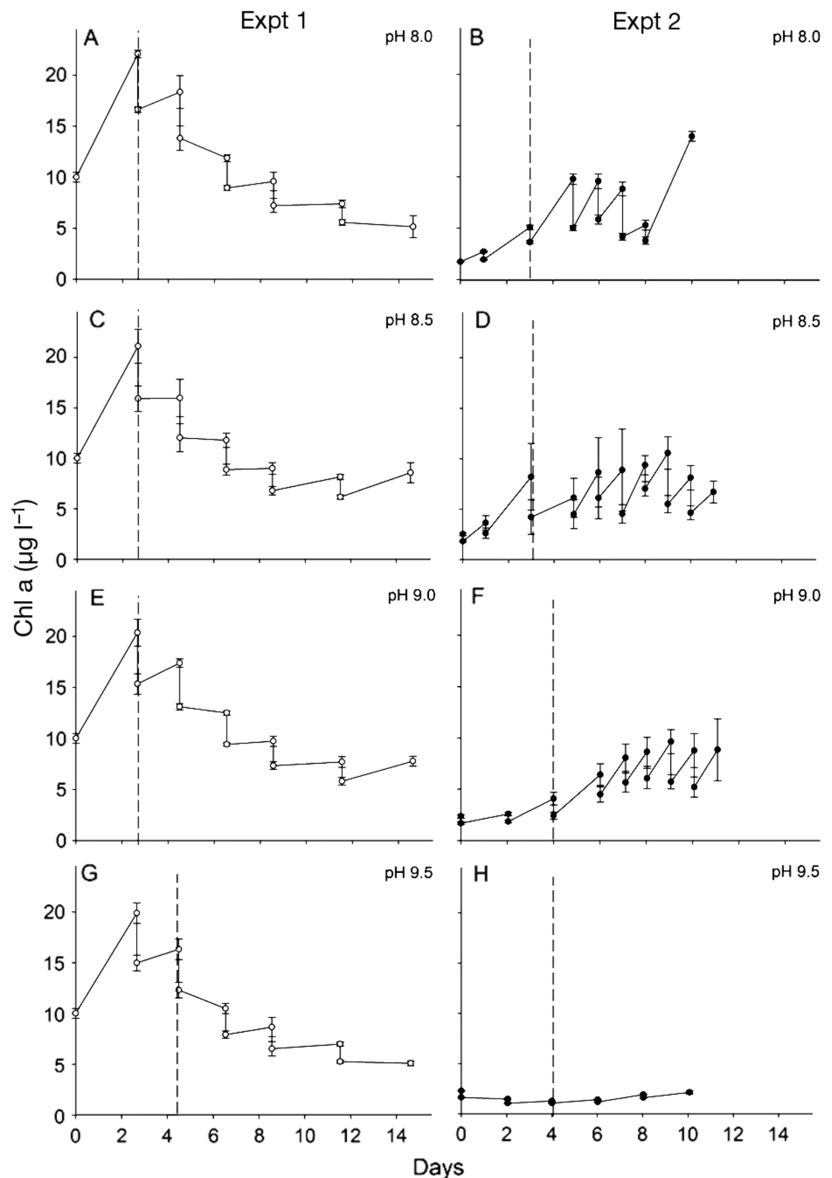

Fig. 3. Development in chl *a* (mean ± SD, n = 3) during Expt 1 (left panels) and Expt 2 (right panels) in controls (pH 8.0) and 3 pH treatments (pH 8.5, 9.0 and 9.5) before and after dilution of the cultures where the diluted samples are always given as the lowest concentration on a specific day. Dashed lines: end of acclimation period, i.e. timepoint when experiments were initiated

Table 1. Concentrations (mean ± SD, n = 15–18) of chl *a* and nutrients (N, P, Si) during the experimental periods (Expt 1: 2011, nutrient-limited conditions; Expt 2: 2012, nutrient-replete conditions) for controls (pH: 8.0) and pH treatments

pH	chl *a* (µg l^{-1})	N (µM)	P (µM)	Si (µM)
Expt 1				
8.00 ± 0.02	7.46 ± 2.53	0.05 ± 0.03	0.49 ± 0.20	1.38 ± 0.72
8.50 ± 0.01	8.23 ± 1.81	0.09 ± 0.07	0.38 ± 0.12	3.68 ± 1.98
9.00 ± 0.01	8.26 ± 2.11	0.08 ± 0.13	0.38 ± 0.17	15.8 ± 4.34
9.52 ± 0.03	6.84 ± 2.07	0.16 ± 0.22	0.32 ± 0.18	39.6 ± 5.76
Expt 2				
8.00 ± 0.02	7.12 ± 3.88	8.16 ± 1.57	0.72 ± 0.13	10.0 ± 1.12
8.50 ± 0.02	7.27 ± 3.29	8.36 ± 1.75	0.75 ± 0.18	16.5 ± 4.49
8.77 ± 0.05	6.59 ± 2.96	8.72 ± 1.74	0.73 ± 0.15	16.7 ± 5.04
9.03 ± 0.05	6.60 ± 3.06	9.26 ± 0.75	0.75 ± 0.14	20.4 ± 7.73
9.51 ± 0.06	1.74 ± 0.39	11.4 ± 0.43	0.88 ± 0.08	49.6 ± 6.92

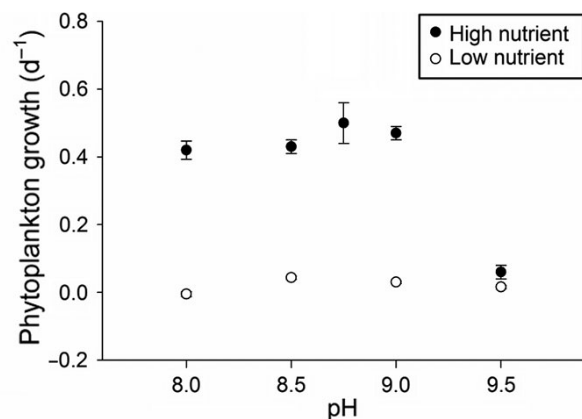

Fig. 4. Growth rate for the total phytoplankton community (chl *a*) as a function of pH when grown under nutrient-rich (●) and nutrient-limited (○) conditions. Data points represent the means ± SD (n = 3; SD for low nutrients too small to be visible)

remained relatively stable throughout the experimental period (Table 1). Since glass contains Si, Si increased with pH in the experimental bottles as an artefact caused by the storage of NaOH in a glass bottle. However, according to the Si:C:N ratio of diatoms of 15:106:16 (Brezinski 1985), N was the limiting nutrient in the treatment and the controls.

The increase in log-transformed cumulated phytoplankton cell abundance fitted a significant linear regression for controls ($p < 0.05$), but not for the pH treatments ($p > 0.05$). The examination of the specific dominant phytoplankton species revealed that the growth rates of the tested phytoplankton species were only significantly reduced for *Skeletonema* sp. (Table 2).

The log-transformed cumulated cell abundance of all 3 species of heterotrophic protists as a function of time (Days 2–12) fitted a positive linear regression ($p < 0.05$). An exception was pH treatment 9.5, where no heterotrophic protists were found after Day 4. The

Table 2. Results of 1-way ANOVA (Holm-Sidak) for effect of elevated pH on growth rates (µ, d^{-1}). Significant effects are marked for 99% (**) or 95% (*) confidence levels. ns: non-significant; nd: no data

	Nutrient level	pH level 8.0 vs. 8.5	8.0 vs. 8.8	8.0 vs. 9.0	8.0 vs. 9.5	8.5 vs. 8.8	8.5 vs. 9.0	8.5 vs. 9.5	8.8 vs. 9.0	8.8 vs. 9.5	9.0 vs. 9.5
Phytoplankton											
Chaetoceros socialis	Low	ns	nd	ns	ns	nd	ns	ns	nd	nd	ns
Chaetoceros socialis	High	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fragilariopsis sp. 1	High	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Fragilariopsis sp. 2	High	ns	ns	ns	ns	ns	ns	*	ns	ns	ns
Navicula sp.	High	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Phaerocystis pouchetii	Low	ns	nd	ns	ns	nd	ns	ns	nd	nd	ns
Phaerocystis poucheti	High	ns	ns	**	**	ns	ns	*	ns	ns	ns
Skeletonema sp.	Low	ns	nd	ns	**	nd	ns	*	nd	nd	*
Thalassiosira spp.	Low	ns	nd	ns	ns	nd	ns	ns	nd	nd	ns
Thalassiosira spp.	High	ns	ns	ns	**	ns	ns	**	ns	*	**
Total phytoplankton	Low	ns	nd	ns	ns	nd	ns	ns	nd	nd	ns
Total phytoplankton	High	ns	ns	ns	**	ns	ns	**	ns	**	**
Heterotrophic protists											
Protoperidinium bipes	Low	ns	nd	*	nd	nd	*	nd	nd	nd	nd
Gyrodinium spirale	Low	ns	nd	*	nd	nd	*	nd	nd	nd	nd
Strobilidium oviformis	Low	ns	nd	ns	nd	nd	ns	nd	nd	nd	nd

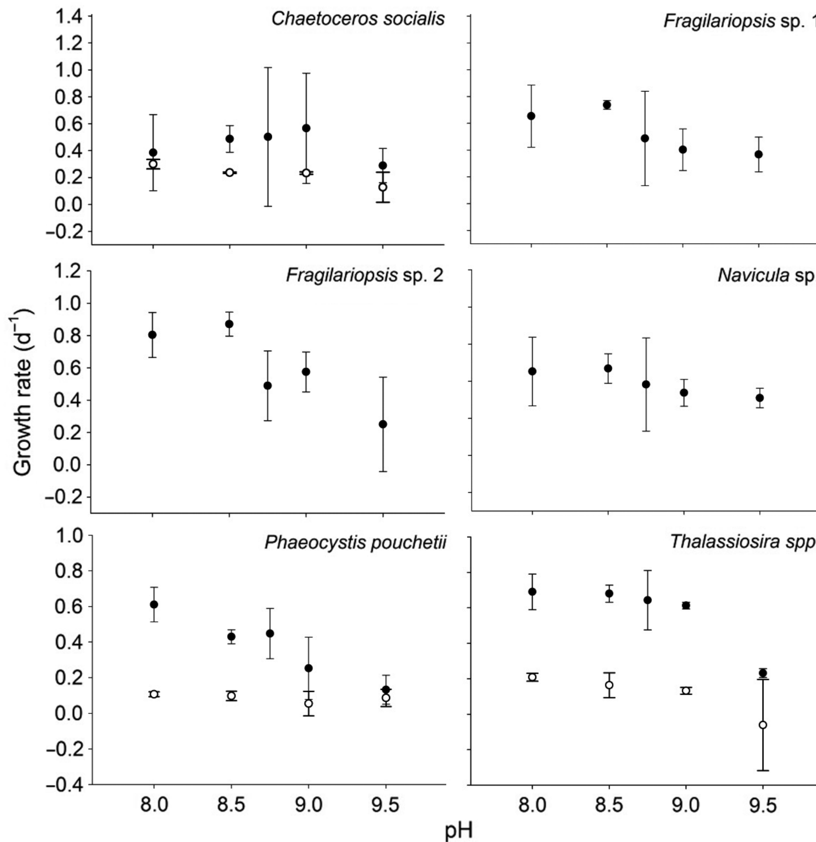

Fig. 5. Growth rate (mean ± SD, n = 3) as a function of pH shown for different phytoplankton species cultured under nutrient-rich (●) and nutrient-limited (○) conditions

growth rates of *G. spirale* and *P. bipes* were significantly affected by pH in the range from 8.0 to 9.0, and growth rates changed from 0.30 to 0.07 d^{-1} and 0.31 to 0.12 d^{-1} for *G. spirale* and *P. bipes*, respectively (Holm-Sidak; $p < 0.05$) (Table 2, Fig. 6). The growth rate of *S. oviformis* was not significantly different from pH 8.0 to pH 9.0 (Holm-Sidak; $p > 0.05$) (Table 2, Fig. 6), but no *S. oviformis* were found in the pH 9.5 treatment after 2 d of incubation under the given pH.

Expt 2: protist community response to elevated pH under nutrient-rich conditions

Expt 2 was carried out under nutrient replete conditions, which due to the high dilution rates, included very low concentrations of heterotrophic grazers throughout the experiment (<0.4 cells ml^{-1}). The high dilution rate kept the chl *a* level in all treatments and controls in the range 3–12 µg chl *a* l^{-1} throughout the experimental period (Fig. 3, right panel). The increase in log-transformed cumulated chl *a* concentrations as a function of time fitted a significant linear regression ($p < 0.05$) in almost all treatments. An exception was the pH 9.5 treatment, where a significant reduction in chl *a* occurred as soon as the flasks had been adjusted to pH 9.5. Hereafter, there was no significant increase in log-transformed cumulated chl *a* concentration (linear regression; $p > 0.05$).

The phytoplankton community growth rates (based on chl *a*) in these experiments were higher than in the first set of experiments, reaching ~0.4 d^{-1}, and were unaffected by pH in the range of pH 8.0–9.0 (Holm-Sidak; $p > 0.05$) (Table 2, Fig. 4). At pH 9.5, however, the total phytoplankton growth rate dropped to 0.06 d^{-1}. When considering the individual phytoplankton species, the trends were the same as for the community growth rates (Fig. 5) with significant linear increase in log-transformed cumulated cell abundance for all replicates in controls and pH treatments pH 8.5, 8.8 and 9.0 ($p < 0.05$). Log-transformed cumulated cell abundance in treatment pH 9.5 did not fit a linear regres-

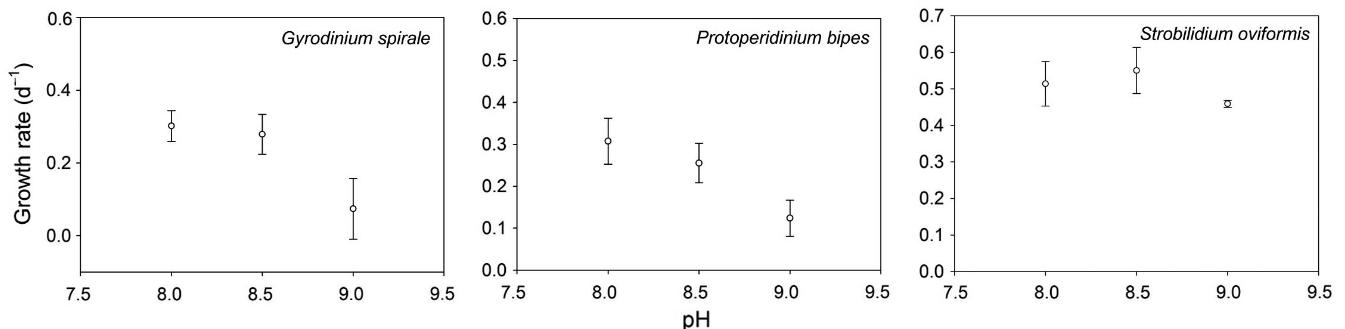

Fig. 6. Growth rate (mean ± SD, n = 3) as a function of pH for 3 heterotrophic protists

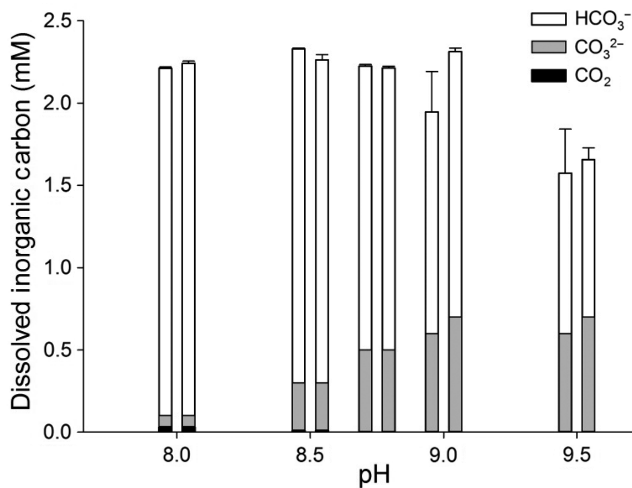

Fig. 7. Mean ± SD values (n = 3) for concentrations of total dissolved inorganic carbon (DIC), and the relative proportions of DIC (mM) for species in Expt 2. Bars are paired and represent concentrations at Day 7 (left bars) and Day 11 (right bars)

sion ($p > 0.10$). Average growth rates dropped from 0.49 d^{-1} to 0.27 d^{-1} at pH 8.0 and 9.5, respectively. Four of the 6 phytoplankton species studied were significantly negatively affected when pH was elevated to 9.5 (Holm-Sidak; $p < 0.05$) (Table 2), and 5 out of 6 species had maximum growth at pH 8.0 and tended to show a lower growth rate as pH increased (Fig. 5).

DIC was measured twice for each treatment and control; at Day 7 and at the end of the experiment, respectively (Fig. 7). The DIC level stayed close to 2 mM at pH 8.0, 8.5, 8.8 and 9.0 and was not significantly different between the start and the end of the experiment (Holm-Sidak; $p > 0.05$) (Table 2). At pH 9.5, the DIC level (1.7 mM) was significantly lower than at the other pH levels (Holm-Sidak; $p < 0.05$). The DIC pool was dominated by HCO_3^- at pH 8.0 but changed towards a higher proportion of CO_3^{2-} relative to HCO_3^- as the pH increased. The proportion of CO_2 was <1% in the treatments (<0.01 mM) as well as in the controls (0.03 mM).

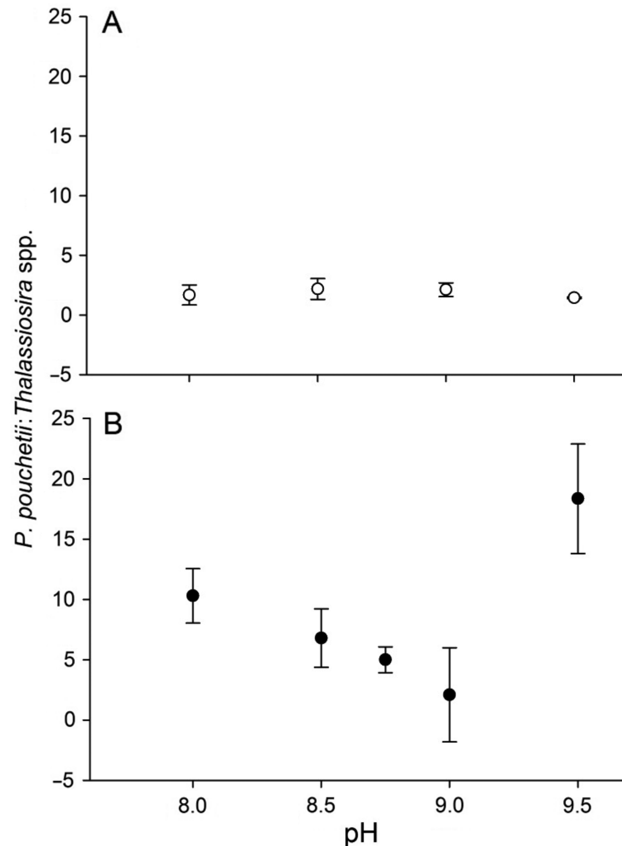

Fig. 8. Ratio between abundance (means ± SD, n = 3) of *Phaeocystis pouchetii* and *Thalassiosira* spp. as a function of pH. Ratios are estimated at the last sampling day under (A) nutrient-limited, and (B) nutrient-rich conditions

Impact of nutrients and elevated pH on community composition

Phaeocystis pouchetii and *Thalassiosira* spp. dominated the phytoplankton community in both years. The ratio between the cell concentrations of the 2 species (*P. poucheti*:*Thalassiosira*) was initially approx. 11:1 and 13:1 in 2011 and 2012, respectively. At the termination of Expt 1 in 2011, under nutrient-limiting growth conditions, the ratio had changed to approx. 2:1 in the treatments and the controls (Holm-Sidak; $p < 0.05$). The ratio between the 2 species did not differ significantly between the controls and the treatments at the end of the experiment (Holm-Sidak; $p > 0.05$) (Table 2, Fig. 8A).

In Expt 2, conducted under nutrient-rich conditions, the ratio between the 2 species remained unchanged at pH 8.0 (Fig. 8B). However, as pH increased towards pH 9.0, a relative dominance of *Thalassiosira* spp. was apparent and the ratio decreased to 2:1 (Holm-Sidak; $p < 0.05$) (Fig. 8B). At pH 9.5, *Thalassiosira* spp. was so strongly affected by the elevated pH that the abundance decreased to <10% at pH 8.0, resulting in a ratio of 18:1. This shift in species composition with increasing pH was observed not only between *P. pouchetii* and *Thalassiosira* spp. but also between *P. pouchetii* and the other diatom species. A possible explanation for this sudden shift in the ratio could be caused by a high pH optimum for *Thalassiosira*, after which the population collapses when the threshold is reached.

DISCUSSION

Fluctuations of pH in Arctic coastal waters

The temporal fluctuations in pH in Arctic waters are primarily caused by high respiration rates during the dark Arctic winter and high primary production rates as the day length increases and sea-ice cover disappears during spring. During the 2011 and 2012 spring blooms in Disko Bay, pH ranged from 7.6 to 8.5. This range in pH is similar to what has been observed in many temperate and tropical coastal waters (Provoost et al. 2010, Brutemark et al. 2011, Praveena & Aris 2013) and is consistent with the few existing measurements from Arctic and Antarctic waters (Charalampopoulou et al. 2011, Yakushev & Sørensen 2013, Mattsdotter Björk et al. 2014). Although pH did not exceed 8.5 in Disko Bay, pH may exceed this value in other parts of the Arctic region where high accumulations of phytoplankton biomass occur, e.g. during intense phytoplankton blooms, which periodically develop under sea ice/pack ice (Gradinger 1996, Spilling 2007, Arrigo et al. 2012), inside sea ice (Legendre et al. 1992) or in marginal ice zones (Falk-Petersen et al. 2000). In the Arctic region, sea-ice primary productivity is estimated to represent 7.5% of primary production (Dupont 2012), and because pH has been reported to reach 9.0 under sea ice (Spilling 2007) and 10.0 in sea-ice brine channels (Gleitz et al. 1995), elevated pH may be a potentially controlling factor within these habitats. For the same reason, we chose to include treatments where pH was elevated as high as pH 9.5, although pH values exceeding 8.5 must be considered rare in the Arctic marine environment.

Does nitrate+nitrite affect phytoplankton tolerance to elevated pH?

The spring phytoplankton bloom in Disko Bay is characterised by a peak in phytoplankton biomass, accompanied by a reduction in nitrate+nitrite as the phytoplankton depletes the surface water of nutrients (Dünweber et al. 2010). In Expt 1, nitrate+nitrite levels were close to the level found during the decline of the bloom. The low phytoplankton concentration during the experiments in 2011 was most likely a result of the low nitrate+nitrite levels, although grazing also played a role. In order to evaluate if the latter had a major impact on the low growth rates, we inspected the log-transformed cumulated cell abundances as a function of time, and experiments were terminated when the log-transformed cumulated cell abundance was no longer increasing (data not shown). For all phytoplankton species in the controls the increase followed a significant linear regression until Day 8 (linear regression; $p < 0.05$), and based on this, grazing mortality was assumed to stay relatively constant despite an increasing number of grazers.

The changes in the chl *a* measurements from the 2 experiments showed that community phytoplankton growth was only affected when pH increased to 9.5 under nutrient-rich conditions. Although growth rates were low under nutrient-limited conditions, we could have expected an increased mortality with elevated pH. This scenario would have been even more marked if the effects of nutrient limitation and pH sensitivity were interacting; for example, Li et al. (2012) showed that elevated CO_2 increased the photosynthetic capacity in a diatom when cultured under N-rich conditions but not when cultured under N-limited conditions. Other factors such as UV radiation and nutrients have also been shown to interact and thereby modulate phytoplankton sensitivity to changes in these factors (Beardall et al. 2009).

Arctic coastal waters are generally nutrient-limited during the summer. However, climate-related changes in water mass exchange are expected to increase the nutrient input in the Arctic Ocean, e.g. the input of nutrient-rich water into the euphotic coastal zones is expected to increase as a consequence of intensified runoff from glacial melting and increased upwelling of nutrient-rich water due to retreating ice cover on the continental shelves (Pabi et al. 2008, IPCC 2013). In addition, increased human activities may produce eutrophication in certain areas of the Arctic. Our data suggest that a higher nutrient level triggers diatom growth despite elevations in pH.

Impact of elevated pH on plankton community composition

The microcosm experiments demonstrated that heterotrophic protists are tolerant to changes in pH within the natural pH range of 8.0–8.5. This finding is in accordance with previous studies demonstrating that heterotrophic protists are robust to 0.5 unit reductions in pH relative to an *in situ* pH of 8.33 (Aberle et al. 2013). Nevertheless, the heterotrophic protists were generally more sensitive to elevated pH than the diatoms. Two out of 3 heterotrophic protists were significantly affected at pH >8.5, and none of the heterotrophic protists survived at pH 9.5. The upper growth limit for heterotrophic protists is in

accordance with previously published data showing that heterotrophic protists from temperate marine waters are negatively affected when pH exceeds 9.0 (Pedersen & Hansen 2003a,b). In comparison, diatoms have been shown to maintain their growth even at pH 10 (Spilling et al. 2013). All species responded to the elevated pH within the first 24–48 h; thereafter, the growth rate was rather constant. However, because different species respond differently, short-term increases in pH may favour some species rather than others. If grazing is a major bloom limiting factor we could, for example, expect to see a decoupling of heterotrophic protists from the succession, thus expanding the length and increasing the magnitude of the bloom.

Unfortunately, we could not test the pH tolerance of heterotrophic protists grown under high-nutrient conditions due to their lower growth rates compared with the phytoplankton, which meant that they were outgrown by the rapidly growing phytoplankton. For this reason, we cannot conclude whether the higher sensitivity of heterotrophic protists was caused by 'cascading' trophic effects (e.g. due to poor food quality and/or less food availability as pH increased) or whether the effect was caused directly by elevated pH.

The tolerance of elevated pH to the tested phytoplankton species revealed substantial interspecific variation. *Thalassiosira* spp. were unaffected by modest elevations in pH, while *Phaeocystis pouchetii* reduced its relative abundance by approx. 66% at pH 8.5 after only 6 days of incubation. This tendency towards higher ratios of diatoms with elevated pH contrasts with previous findings in the Equatorial Pacific, where the abundance of colony-forming *P. pouchetii* increased relative to diatoms when CO_2 was reduced from 750 ppm (pH ~7.9) to 150 ppm (pH ~8.5) (Tortell et al. 2002). Since *P. pouchetii* and *Thalassiosira* spp. are both important components of Northern temperate and Arctic phytoplankton blooms (Schoemann et al. 2005, Degerlund & Eilertsen 2010, Dünweber et al. 2010), even small changes in pH will affect the succession of the bloom. Note that only *P. pouchetii* cells in colonies were counted, and pH could thus potentially have affected the ability to form colonies but not cell growth.

Among the most tolerant species was *Navicula* sp., which grew at 66% of its maximum growth rate at pH 9.5. This pennate diatom is an ice alga, and one hypothesis could be that ice algae are more tolerant to high pH than true pelagic species. This hypothesis is supported by Søgaard et al. (2011), who found similar high tolerances to elevated pH for 3 cultivated ice-algae species (*Fragilariopsis nana*, *Fragilariopsis* sp. and *Clamydomonas* sp.). Despite these few exceptions, almost all phytoplankton species were affected at pH 9.5. Physical stress was also evident from the morphology of the cell; many of the Lugol-stained cells became darker, colonies decreased in size and the cell structures changed. These morphological changes were most evident for *P. pouchetii*, *Fragelariopsis* spp. and *Thalassiosira* spp.

Is inorganic carbon limiting phytoplankton growth?

In the present study, we manipulated the carbon system by adding NaOH to a plankton assemblage with minor CO_2 exchange. Manipulating the carbon system in this way causes pH and total alkalinity (TA) to increase, whereas DIC concentrations remain largely unchanged. The DIC levels in the present study were, therefore, higher than would be expected during a natural bloom, where DIC concentrations decrease (e.g. Hansen et al. 2007). It is possible that the phytoplankton becomes carbon limited during a natural phytoplankton bloom situation as CO_2 is depleted from the surface. We argue that this was not the case in the present study because bloom-forming phytoplankton species have been demonstrated to be limited by pH rather than CO_2 at pH levels up to 9.0 (Hansen et al. 2007). Moreover, most marine phytoplankton species have evolved highly effective CO_2 concentration mechanisms (CCMs) to avoid carbon limitation (Johnston & Raven 1996, Korb et al. 1997, Price et al. 2004, Nakajima et al. 2013). The efficiency of CCMs is species specific, but it is generally high for marine species, including diatoms and *Phaeocystis* spp. (Tortell et al. 1997, Trimborn et al. 2013). In fact, even at DIC concentrations as low as 1.6 mM, diatoms have been shown to maintain >90% of their growth rate relative to natural seawater DIC of 2 mM (Clark & Flynn 2000). This means that the pH 9.5 treatments in which DIC concentrations were 1.6 mM were the only ones in which DIC was limited. Major CCMs in diatoms are HCO_3^- transporters in the cell plasma membrane, which enable the cell to directly or indirectly use the high amounts of HCO_3^- in seawater for their production (Nakajima et al. 2013). Other mechanisms include direct CO_2 transporters, which have also been found to increase intracellular CO_2 levels of some diatoms (Hopkinson 2014).

Although carbon is generally not limiting for the growth of marine phytoplankton, this might be differ-

ent at extreme pH levels, where the DIC speciation changes drastically. The relative percentage of CO_2 to total DIC decreased from 1.1% at pH 8.0 to 0.08% at pH 9.0. Thus, the phytoplankton would almost certainly be limited by CO_2 at pH >9.0. However, at pH ≤9.0 the phytoplankton have theoretically had sufficient HCO_3^- and were most likely limited directly by pH rather than by carbon. It is still unknown how exactly autotrophic and heterotrophic protists are affected by pH, but intracellular changes in pH could, for example, affect nutrient uptake, ion transport or enzyme functioning. The cells could also be affected indirectly by changes in the solubility/precipitation of metals, nutrients or other vital elements that are known to be affected by pH. In treatment pH 9.5 where the DIC level was close to 1.6 mM, some of these changes could have influenced phytoplankton growth.

pH of Arctic coastal waters in the future

Over the past 3 decades, the extent of Arctic perennial sea ice has decreased at a rate of ~12% per decade (Comiso 2012). The shrinking ice cover has resulted in increased annual primary production in large parts of the Arctic along with significant increases in annual net CO_2 fixation rates (Arrigo et al. 2008, Pabi et al. 2008, Slagstad et al. 2011). Changes in seawater carbon chemistry are known to affect natural plankton communities, but until now most studies in the polar regions have focused on the impact of CO_2 enrichment (ocean acidification) caused by increased atmospheric pCO_2 (Rost et al. 2008, Aberle et al. 2013, Trimborn et al. 2013, Mattsdotter Björk et al. 2014). By the end of 2100, surface water pH in the Arctic region is predicted to decrease from an average of ~8.2 at present to ~7.6 (IPCC 2013). However, we must be aware that pH regulation in coastal ecosystems is largely disconnected from the open ocean (Duarte et al. 2013) and that the reductions in pH in the coastal areas are also regulated by drivers other than atmospheric pCO_2. These other drivers include processes affecting the input of nutrients, terrestrial organic matter, and freshwater discharge.

It is unknown how increased human activities in the Arctic will impact pH, but human activities have historically resulted in eutrophication. On a global scale, nitrogen has increased >3-fold since 1860 due to human activities (Passow & Carlson 2012). In temperate coastal waters, this has resulted in higher photosynthetic rates and increased pH (Hansen 2012). Because predicted estimates of net primary production in the Arctic also suggest an increase (Arrigo & van Dijken 2011), we suggest that biologically related changes in pH will exceed the impact caused by elevated atmospheric CO_2, and that particular coastal zones will be associated with temporal increases in pH.

This and previous studies demonstrate that a wide range of protist species respond negatively when pH is elevated during algal blooms. However, certain species, including many diatoms, are tolerant to temporary elevations in pH, which gives them a competitive advantage to more sensitive species during phytoplankton blooms. Higher frequencies in bloom events will thus favour species such as diatoms that are tolerant to elevated pH. However, ocean acidification could reduce the pH maximum reached during the blooms, thus changing the succession towards less tolerant species, including heterotrophic protists and non-diatom phytoplankton species such as *Phaeocystis pouchetii*.

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