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Effect of acidification on an Arctic phytoplankton community from Disko Bay, West Greenland

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Abstract:

Long-term measurements (i.e. months) of *in situ* pH have not previously been reported from the Arctic; this study shows fluctuations between pH 7.5 and 8.3 during the spring bloom 2012 in a coastal area of Disko Bay, West Greenland. The effect of acidification on phytoplankton from this area was studied at both the community and species level in experimental pH treatments within (pH 8.0, 7.7 and 7.4) and outside (pH 7.1) *in situ* pH. The growth rate of the phytoplankton community decreased during the experimental acidification from 0.50 ± 0.01 d^{-1} (SD) at pH 8.0 to 0.22 ± 0.01 d^{-1} at pH 7.1. Nevertheless, the response to acidification was species-specific and divided into 4 categories: I, least affected; II, affected only at pH 7.1; III, gradually affected and IV, highly affected. In addition, the colony size and chain length of selected species were affected by the acidification. Our findings show that coastal phytoplankton from Disko Bay is naturally exposed to pH fluctuations exceeding the experimental pH range used in most ocean acidification studies. We emphasize that studies on ocean acidification should include *in situ* pH before assumptions on the effect of acidification on marine organisms can be made.

Keywords: Ocean acidification ; Coastal ; Arctic phytoplankton ; Growth rate ; pH ; CO_2 ; DIC

1. Introduction

The impacts of ongoing ocean acidification on marine organisms are a highly debatable topic within the scientific community. Anthropogenic emissions are expected to increase the level of atmospheric CO_2 (carbon dioxide) from ~280 parts per million (ppm) in the mid-18th century to ~700 ppm by the end of the 21st century (Riebesell et al. 2009). Approximately 25% of the emitted atmospheric CO_2 is absorbed into the oceans (Riebesell et al. 2010) where chemical reactions alter the composition of dissolved inorganic carbon (DIC) while lowering pH (acidification). The average pH of ocean surface waters is expected to decrease from ~8.2 in the mid-18th century to ~7.8 by the end of the 21st century (Feely et al. 2009). When pH of seawater decreases, the concentration of CO_3^{2-} (carbonate ion) also decreases while HCO_3^- (bicarbonate ion) remains in vast concentrations (for details on seawater carbonate chemistry see(Zeebe & Wolf-Gladrow 2003).

The majority of phytoplankton examined in the literature utilize both HCO_3^- and CO_2 for photosynthesis, while some use either HCO_3^- or CO_2 (e.g. Giordano et al. 2005). Increased CO_2 could potentially increase primary production, especially for species relying on diffuse uptake of CO_2 since the energy usage for the uptake of inorganic carbon through carbon concentrating mechanisms (CCM's) is likely to be reduced (Rost et al. 2008). However, extracellular pH (pHe) may influence the intracellular pH (pH_i) of unicellular organisms and affect physiological processes

such as ion transport, enzyme activity, protein function and nutrient uptake (Gattuso & Hansson 2011 and references therein, Nimer et al. 1994). Thus, increased CO_2 may not result in enhanced primary production if other physiological processes are affected negatively by acidification.

The formation of chains and colonies in marine phytoplankton is well-known. For diatoms, it is presumed that this reduces sinking velocity and predation, and increases sexual reproduction (Bergkvist et al. 2012, Kooistra et al. 2007, Smayada & Bolelyn 1966, Takabayashi et al. 2006). Likewise, the prymnesiophyte *Phaeocystis globosa* forms different sized colonies depending on the type of grazer present (Jakobsen & Tang 2002). The effects of acidification on the formation of chains and colonies have, to our knowledge, not been studied until now. Considering that acidification affects physiological processes, it is likely that these formations are affected.

Ocean pH is considered stabile on seasonal and diurnal scales due to the buffering capacity of seawater with an average open ocean surface pH of ~8.2 (Feely et al. 2009). This stability in pH is particularly true for open oceans where algal biomass is usually low. Contrary, pH in coastal ecosystems can exhibit large seasonal and diurnal fluctuations shaped by factors such as photosynthesis, respiration, upwelling, CO_2 venting, trophic state and water residence time (e.g. Duarte et al. 2013, Feely et al. 2008, Hofmann et al. 2011, Wootton et al. 2008). Seasonal variations in pH levels in temperate coastal ecosystems correlate with productivity, and the fluctuation decreases with distance from the shore (Provoost et al. 2010). In shallow and temperate coastal areas, the pH can vary from 7.4 to 9.2 on a diurnal basis (Middelboe & Hansen 2007), and in the Danish eutrophic Mariager Fjord, the minimum and maximum pH during a 10 year period was ~7.1 and ~9.7, respectively (Hansen 2002). Smaller fluctuations in the range of 0.1 to ~0.6 pH units were measured in inner Danish waters during 6 decades (Duarte et al. 2013) and yearly fluctuations of pH in the western Gulf of Finland regularly spanned 1 pH unit (Brutemark et al. 2011). Studies on natural fluctuations of pH have focused on temperate coastal waters, and studies on the tolerance of phytoplankton to acidification have primarily dealt with temperate phytoplankton (e.g., Berge et al. 2010, Kim & Lee 2006, Lohbeck et al. 2012, Lundholm & Hansen 2004, McCarthy et al. 2012). Unfortunately, the majority of studies on the effect of acidification has been conducted in the pH range 7.7 to 8.2, despite the fact that natural fluctuations of pH are much greater in coastal areas and often decrease <7.7 (e.g., Hofmann et al. 2011, Hansen 2002). Measurements of natural fluctuations in pH in a given area are needed in order to expose organisms to pH values beyond today's naturally occurring levels. This will provide the conditions to obtain valid results of the effects from ocean acidification.

The Arctic Ocean is presumed to be very sensitive to ocean acidification due to the high solubility of CO_2 in cold water and the decreasing sea ice cover (Slagstad et al. 2011). However, only short-term measurements (over days) of pH levels in the polar open ocean and in the brine of sea ice have been conducted (e.g. Gleitz et al. 1995, Hofmann et al. 2011). Short-term measurements do not provide thorough insight on the pH fluctuations that organisms are exposed to over a longer time scale. It is hereby crucial to collect *in situ* pH data in the Arctic region during long-term measurements (over months). This will allow for a more qualified study on the effects of acidification on Arctic organisms, and contribute to a better estimate of the impact of ocean acidification in the Arctic region.

The aim of the present study was to monitor the long-term fluctuations of *in situ* pH levels in a coastal water column during the Arctic phytoplankton spring bloom while examining the effect of acidification on phytoplankton growth via incubations with natural phytoplankton assemblages exposed to different pH levels.

Materials and methods

Study site: The experiment was conducted in spring 2012 at Arctic Station (University of Copenhagen, Denmark) in Qeqertarsuaq on Disko Island, West Greenland (Fig. 1). Few days after the breakup of sea ice (April 14th) the plankton community in Disko Bay was collected from the research vessel Porsild at a 300 m deep monitoring station (69°13'N, 53°22'W) outside of Qeqertarsuaq. Depth distribution of salinity and temperature was recorded with a Seabird CTD every 3rd day and samples for pH measurements were collected at depths of 1, 40, 50, 75, 100, 150, 200 and 250 m with a Niskin bottle.

The plankton community was collected using a 10 L Niskin bottle from the depth of the chlorophyll maximum (5 m depth) and the sample was reverse filtered into a 25 L container via a silicon tube through a cylinder with a 250 µm nylon mesh to remove mesozooplankton. Immediately after filtration, the plankton assemblage was carefully mixed and transferred to 12 incubation bottles via a silicon tube.

Seawater for dilution: Seawater used for diluting the treatments during the experiment was collected from the sampling station below the pycnocline (150-200 m) to ensure nutrient-rich water. The water was filtered through a 0.45 µm Whatman® polycap filter and stored dark and cold (3±2 °C) in 25 L containers. Average pH, temperature and salinity of the seawater were 7.9 ±0.1, 3.3

±0.1 °C and 34.3 ±0.0, respectively.

Experimental setup and CO_2 manipulation: The experiment was run in triplicated 1 L Nalgene® polycarbonate bottles and pH was adjusted by applying gaseous CO_2 (Air Liquide Denmark A/S. UN 1013 Carbon dioxide, Class 2, 2A, ADR.). The incubation bottles were filled to capacity (1.23 L), allowing no headspace in order to avoid fluctuations of seawater chemistry as well as avoiding negative effects of air bubbles on protists, followed by sealing the top with parafilm before applying the lid. Triplicate bottles of four treatments (pH 8.0, 7.7, 7.4 and 7.1) were placed at 3 ±2 °C on a flat turning plankton wheel (1 rpm) in front of a light source (100 µE m^{-2} s^{-1}, 12 h light: 12 h dark cycle). The phytoplankton community exposed to pH 7.7, 7.4 and 7.1 was lowered in steps of 0.5 pH units per 12 h by adding strongly acidified seawater with a pH of 4.9 ±0.1 (Fig. 2; Day 0). After 24 hours, all incubation bottles had reached their respective pH set points. To aquire acidic seawater, 2 L of 0.45 µm filtered seawater were bubbled strongly with CO_2 (ca 5 min) and afterwards O_2 (Hede Nielsen A/S) (ca 5 s) to increase the concentration of O_2 >100%, which was measured with a WTW Oxi 3210 oxygen meter with a WTW DurOx oxygen probe. During the remaining part of the experiment, the acidic seawater was added to bottles of 2 L 0.45 µm filtered seawater to obtain seawater for dilution with the specific experimental pH values; 7.7, 7.4 and 7.1 (Fig. 2; Day 1 to end of experiment). Before each sampling, the acidic seawater and the pH-specific seawater for dilution were produced as described. The phytoplankton community at pH 8.0 was used as a control, resembling the pH in Disko Bay at the time of sampling, and diluted with 0.45 µm filtered seawater without adjustment of pH. The treatment at pH 8.0 was terminated on day 11, while pH 7.7 and 7.4 were terminated on day 17, and pH 7.1 on day 16.

Sampling and dilution: The incubation bottles were removed in triplicates from the plankton wheel and kept cold in a cooling box filled with snow during sampling (~20 min). The phytoplankton community was diluted throughout sampling and additional dilutions were done to avoid high biomasses that would cause large fluctuations in pH and nutrient limitation. First, pH was measured and samples were withdrawn. Then, the incubation bottles were refilled with pH-specific seawater (dilution). If dilution was insufficient to obtain the experimental pH, a few drops of acidic seawater were added. The phytoplankton community was always diluted to a concentration of ~5 µg Chl *a* L^{-1}. The intensity of sampling depended on the growth rate of the phytoplankton community (using Chl *a* as a proxy) and due to the extraction time of Chl *a* the

dilution to ~5 µg Chl *a* L^{-1} was an approximate estimate. The control treatment at pH 8.0 was sampled nearly every day, while pH 7.7, 7.4 and 7.1 were sampled every 2nd or 3rd day. On day 5, the desired concentration of 5 µg Chl *a* L^{-1} was reached in all pH treatments and day 0 to 5 was set as the period of acclimation (not included in the results).

pH: pH was measured with a WTW pH 3210 pH meter equipped with a WTW SenTix 41 electrode. The electrode was calibrated using a 2-point calibration and regularly checked for correct measurements in buffers of pH 7 and 10 (WTW Technical Buffer, NIST scale). Prior to use (≥10 min), the pH electrode was immersed in filtrated seawater to stabilize.

DIC and nutrients: Samples for DIC were fixed with 100 µL Hg_2Cl_2 (mercury(I)chloride) in airtight glass vials (12 ml) without headspace to avoid CO_2 leaking out of the water phase. Samples were stored in dark and cold conditions until measurements one month later at Marine Biological Laboratorium in Helsingør, Denmark. Triplicate measurements were conducted on an IRGA (infrared gas analyzer) by comparing with a HCO_3^- (bicarbonate) standard of 2.0 mM (for procedure see Nielsen et al. 2007). The carbon speciation (HCO_3^-, CO_3^{2-} and CO_2*) was calculated using the program CO2SYS developed for CO_2 system calculations (Lewis & Wallace 1998).

Samples for measurements of the inorganic nutrients phosphate (PO_4^{3-}), nitrate (NO_3^-) and silicate ($Si(OH)_4$) were transferred into plastic bottles (35 ml) and frozen immediately. The samples were analysed at the Institute for Bioscience, University of Aarhus, following the procedures of Grasshoff (1976) and Valderrama (1981).

Chl *a*: 50-100 ml sample of the phytoplankton community was filtrated through a 0.7 µm GF/F filter and a 10 µm Nitex® plankton gauze, respectively. Immediately afterwards, the filters were extracted in 5 ml 96% ethanol in glass vials and stored in the dark at room temperature. The next day, Chl *a* was measured on a TD-700 Fluorometer (Turner Designs) following Jespersen & Christoffersen (1987). The growth rate of the phytoplankton community (Chl *a*) was calculated from the cumulative growth due to the dilution technique.

Enumeration and identification of phytoplankton species: Samples for enumeration of phytoplankton taxa (120-250 ml) were transferred to brown glass bottles (250-300 ml) containing acidic Lugol's iodine (2% final conc.) and stored in the dark at room temperature. Cells of

dominating species were counted on an inverted microscope (Olympus CK40) using 25 or 50 ml sedimentation chambers (Hydro-Bios Kiel) with 24 hrs settling time. A minimum of 400 cells or 6 transects were enumerated. Cells in colonies or chains, single cells, or both were enumerated (Table 1). The growth rate was calculated from the cumulative growth due to the dilution technique. Species were identified based on light microscopic characters using Tomas (1997), except for *Navicula* cf. *granii* which was identified using a JEOL-1010 transmission electron microscope (TEM) (Jeol, Tokyo, Japan). Prior to identification by TEM, a sample of the phytoplankton community at pH 8.0 was rinsed following Lundholm et al. (2002).

Colony, chain and cell size: The diameter of 25 random colonies of the prymnesiophyte *Phaeocystis pouchetii* was measured on an inverted microscope (Olympus CK40). The number of cells in the chains of the diatom species *Thalassiosira* spp., *Navicula vanhoeffenii*, *Chaetoceros* spp. and *Navicula* cf. *granii* were enumerated in 25 random chains. Single cells and chains ≥2 cells were included for *Thalassiosira* spp., *Navicula vanhoeffenii* and *Chaetoceros* spp. Only chains ≥2 cells were included for *Navicula* cf. *granii* due to difficulty identifying single cells on the inverted microscope.

Calculations

DIC speciation (HCO_3^-, CO_3^{2-} and CO_2*) was calculated with the program CO2SYS and the following available inputs: *Set of constants*: K1, K2 from Mehrbach et al. 1973 refit by Dickson and Millero, 1987; *KHSO4*: Dickson; *pH scale*: Seawater scale (mol/kg-SW). For further information about CO2SYS see Lewis & Wallace (1998).

Dilution of the phytoplankton community (Chl *a*):

$$\mathrm{vol_r} = \left(1 - \frac{\mathrm{C_2}}{\mathrm{C_1}}\right) * \mathrm{vol_t}$$

where vol_r and vol_t is the volume replaced with pH-specific seawater and total volume of the incubation bottle, respectively, and C is the Chl *a* concentration before (C_1) and after (C_2) dilution.

Growth rate of the phytoplankton community (Chl *a*) and species was calculated assuming exponential growth:

$$\mu = \frac{\ln\left(\frac{N_{t2}}{N_{t1}}\right)}{t_2 - t_1}$$

where μ is the growth rate and N is Chl *a* or cell concentration at time t_2 and t_1. The cumulative concentration (μ') of Chl *a* and cells were calculated using μ due to the dilution technique:

$$\mu' = N_{t_1} * e^{\mu_{t2} * (t_2 - t_1)}$$

where N is Chl *a* or cell concentration and μ is the growth rate at time t_2.

The growth rate at a pH treatment was calculated as the average of the triplicate samples' linear regressions of ln(cumulative Chl *a* conc.) or ln(cumulative cell conc.) after acclimation with the slopes equalling to the growth rates.

Statistics: Significant differences were analyzed using ANOVA (one-way) followed by pairwise multiple comparisons with the Holm-Sidak method. The null-hypothesis (H_0) was that there was no difference between pH treatments and H_0 was rejected when $p < 0.05$. The significance level was set to 0.05 in all analyses.

Results

Hydrography of sampling station in Disko Bay: On the day of sampling (April 14th 2012), the temperature and salinity of the surface water was -1.6 °C and 33.2, respectively. At a depth of 300 m, this increased to 3.6 °C and 34.4, respectively. A pycnocline at 160 m depth kept the spring bloom in the upper water column with a maximum Chl *a* concentration of 1.4 µg L^{-1} at 5 m depth (data not shown). The phytoplankton community was sampled from the maximum Chl *a* where temperature, salinity and pH was -1.4 °C, 33.2 and 7.9, respectively. The vertical distribution of *in situ* pH at a water column depth of 250 m was measured during the spring of 2011 and 2012 (Fig. 3). In 2011, pH was not measured throughout spring bloom but varied between 7.9 and 8.5 from the end of April to the end of May. In 2012, pH varied between 7.5 and 8.3 from the end of March to the end of May. In March 2012, pH in the water column was 7.8 for approximately two weeks and increased during April. However, there was a decrease to pH 7.5 in early April and mid-May. In the end of May, pH decreased for a longer period to 7.8 with a small increase to pH 8.0 in the upper part of the water column.

Experimental pH, DIC and nutrients: pH in the experimental treatments fluctuated minimally from the designated pH levels of 8.0, 7.7, 7.4 and 7.1 throughout the experimental period (Table 2). The concentration of DIC in the triplicate of each pH treatment was not significantly different midway and at the end of the experiment (Holm-Sidak, $p > 0.05$) (data not shown). DIC increased

from 2222.2 ±2.3 µmol kg^{-1} at pH 8.0 to 2522.6 ±35.9 µmol kg^{-1} at pH 7.1 (Table 2). The DIC concentration at pH 7.1 was significantly different from the other pH treatments (Holm-Sidak, p <0.05). The carbon speciation of DIC in the pH treatments was dominated by bicarbonate ion (HCO_3^-) with >90%, while the concentration of carbonate ion (CO_3^{2-}) decreased and carbon dioxide (CO_2*) increased with lowered pH. The concentration of CO_3^{2-} and CO_2* was significantly different between all pH treatments (Holm-Sidak, p <0.05).

In situ concentration of the nutrients NO_3^-, PO_4^{3-} and $Si(OH)_4$ in the depth of the chlorophyll maximum was 9.27 ±0.1 µM, 0.77 ±0.01 µM and 7.51 ±0.12 µM, respectively, and fitted the Redfield ratios of 16N:1P and 15Si:16N. The nutrient concentrations in the pH treatments were not significantly different from the *in situ* concentrations throughout the experimental period (ANOVA, p >0.05).

Growth of the phytoplankton community: The growth rate of the total phytoplankton community (i.e. Chl *a* >0.7 µm) was 0.50 ±0.01 d^{-1} at pH 8.0 and decreased to 0.22 ±0.01 d^{-1} at pH 7.1 (Fig. 4). The growth rate of the phytoplankton community >10 µm (i.e. Chl *a* >10 µm) was affected to the same degree but had a significantly higher growth rate at pH 8.0 (0.58 ±0.02 d^{-1}), 7.7 and 7.4. There was no significant difference between the growth rates of the total phytoplankton community and the phytoplankton community >10 µm at pH 7.1 (Holm-Sidak, p <0.05).

The phytoplankton community >10 µm was the dominating size fraction after day 10 at pH 8.0, 7.7 and 7.4 (Fig. 5). To the contrary, at pH 7.1 the size fraction >10 µm did not exceed 40% during the experiment.

Growth of the phytoplankton species: In general, the highest growth rates of the phytoplankton species were obtained at pH 8.0 and the lowest at pH 7.1, but the growth rates differed among species (Fig. 6). At pH 8.0, the growth rates of diatoms were the highest among the species (~0.4 to ~0.6 d^{-1}) whereas the prymnesiophyte *Phaeocystis pouchetii* and the prasinophyte *Pyramimonas* sp. had lower growth rates (~0.3 d^{-1}). Depending on the species, the growth rates at pH 7.1 were significantly lower (>50% compared to pH 8.0) and a few species did not grow at pH 7.1.

The effect of acidification on the growth rate was divided into four categories (Fig. 6): **I)** The species least affected which was the prasinophyte *Pyramimonas* sp. Although there was a significant difference in growth rate between some of the pH treatments (Holm-Sidak, p <0.05) a 32% reduction in growth rate from pH 8.0 to 7.1 was the smallest compared to the other species. **II)**

Species not affected by acidification in the pH range 8.0 to 7.4 comprised the diatom *Navicula* spp. and the prymnesiophyte *P. pouchetii*. At pH 7.1 neither species could sustain growth (Holm-Sidak, $p < 0.05$). **III)** Species gradually affected by acidification were the chain-forming diatoms *Thalassiosira* spp., *Navicula vanhoeffenii* and *Chaetoceros* spp. with similar growth rates and an similar reduction in growth rate reaching ~0.2 d^{-1} at pH 7.1. The growth rate for each species was not significantly different between pH 7.7 and 7.4 (Holm-Sidak, $p > 0.05$). **IV)** The species highly affected by acidification was the chain-forming diatom *Navivula* cf. *granii* with a significantly different growth rate among every pH treatment (Holm-Sidak, $p < 0.05$). This species had one of the highest growth rates at pH 8.0 but was also the most sensitive species and could not sustain growth at pH 7.1.

Effect of acidification on colony size and chain length: The colony size of *Phaeocystis pouchetii* was reduced during acidification (Fig. 7). On day 5, the frequency of *P. pouchetii* colonies with a diameter of up to 280 µm were similar at pH 8.0, 7.7 and 7.4 with a slight decrease in size with increasing acidification (only data for pH 8.0 is shown). However, at pH 7.1 a decrease in colony size was observed on day 5 with the majority of colonies measuring up to 80 µm. Microscopical observations of *P. pouchetii* colonies at pH 7.1 revealed that the cells were unorganized and clustered, making it impossible to enumerate the cells in the colonies after day 5. After 10 days no *P. pouchetii* colonies were present at pH 7.1 (Fig. 6, marked +).

The chain length (i.e. no. of cells) of *Thalassiosira* spp. decreased with acidification (Fig. 8). At pH 8.0, the majority of chains contained up to 8 cells on day 11 but was reduced to 4 cells at pH 7.1. A similar effect on chain length was observed for *N. vanhoeffenii* and *Chaetoceros* spp. while the chain length of *N.* cf. *granii* did not appear to be affected (data not shown).

Discussion

Fluctuations of pH in Disko Bay during spring: Few data are available on fluctuations of pH in Arctic marine ecosystems and these mostly report the fluctuations in open surface waters. Data on pH from coastal regions of the Arctic are completely lacking but here we document prominent fluctuations from pH 7.5 to 8.3 in a 250 m water column in Disko Bay during the spring bloom 2012. These fluctuations resemble observations in other coastal areas, e.g. pH in 2 meters depth at the upwelling area of Point Ano Nuevo, USA, varied from 7.7 to 8.1 during 30 days from mid-May

(Hofmann et al. 2011) and pH in Narragansett Bay, USA, fluctuated from ~7.6 to ~8.5 during 1 year in 5 m depth (Hinga 1992).

The observed fluctuations of pH in Disko Bay were caused by several factors. Before the spring bloom in the Arctic the polar night and sea ice cover, with continuous lack of solar irradiance, resulted in a low pH due to the dominance of respiration processes. As the solar irradiance increased, during the transition to polar day, factors such as Chl *a*, photosynthesis and pH increased in the water column. Advection of sea ice into the bay reduced the solar irradiance into the water column and resulted in a decrease in pH twice during 2012 (early and mid-May) due to decreased photosynthesis and increased respiration (Fig. 3). pH in the water column was generally higher in 2011 compared to 2012 due to an earlier breakup of sea ice allowing for a prolonged spring bloom (~6 weeks) with a longer period for photosynthesis to raise pH in the water column (T.G. Nielsen, personal observations). On the day of sampling (April 14th 2012), the salinity in the water column increased from the surface and downwards due to the melting of sea ice and a halocline was present at a depth of ~160 m maintaining the photosynthetic plankton above this depth. Nutrients (NO_3^-, PO_4^{3-} and $Si(OH)_4$) in the chlorophyll maximum (5 m) were plentiful at the time of sampling because the phytoplankton spring bloom had not yet peaked.

pH below 7.5 was not measured in Disko Bay during spring but it is not unlikely that pH may decrease to values near 7.1 during the next centuries with ongoing ocean acidification. For instance, Wootton et al. (2008) found a significant decrease of pH in coastal waters on the Washington Shelf during 8 years in association with increasing atmospheric CO_2 and a similar acidification could likely be occurring in Disko Bay at present time.

Nutrients, DIC and pH: The nutrient concentrations of $Si(OH)_4$, NO_3^- and PO_3^{4-} in the experimental treatments exceeded those shown to be limiting for phytoplankton, including cold water species (e.g. Egge & Aksnes 1992, Nelson & Tréguer 1992). This excluded nutrient limitation as a cause for the decreasing growth rates during the experiment.

The concentration of DIC and carbon speciation in the treatment at pH 8.0 correlate well with literature data on ocean surface waters (e.g. Feely et al. 2009). At increased experimental acidification the inorganic carbon availability, which could potentially benefit the autotrophic phytoplankton, was changed, theoretically, for the better with a vast concentration of HCO_3^- and increased CO_2. However, a benefit from this inorganic carbon availability was not reflected in the phytoplankton growth rate.

pH of the treatments fluctuated minimally (≤0.05 units) and were well separated throughout the experiment providing a valid response of the phytoplankton community to the experimental pH values.

The effect of acidification on the growth rate of the phytoplankton community: The growth rate of the phytoplankton community from Disko Bay was affected negatively by the experimental acidification (Fig. 4), even within the range of *in situ* pH measured in Disko Bay in 2012 (pH 7.5 to 8.3). The effect was observed as a gradual decrease in community growth rate with decreasing pH from 8.0 to 7.1.

Many studies have explored the effect of acidification on phytoplankton but tend to study the growth response within a narrow pH limit of 8.2 to 7.8 (e.g. Wang et al. 2010, Yang & Gao 2012), probably because this reduction has been predicted to occur for open oceans by the end of the 21st century. However, it is problematic to use such a narrow range of pH to study the tolerance of coastal phytoplankton to acidification because of pH in coastal waters fluctuating within a larger range as shown in this study. Furthermore, it is necessary to expose organisms to a pH lower than that measured *in situ* to evaluate the susceptibility of marine organisms to the ongoing ocean acidification.

The effect of acidification on the growth rate of phytoplankton species: The phytoplankton community in Disko Bay was dominated by diatoms, haptophytes and prasinophytes. The investigated species (Table 1) have previously been reported in Disko Bay (DATMAPD, Nielsen & Hansen 1999) except for *Navicula* cf. *granii*, in spite of it being a typical Arctic species (Guillard & Kilham 1977). The ciliate species *Strombidium* sp. and *Lohmaniella oviformis* were also present in the pH treatments but due to a very low cell concentration the grazing by these ciliates on the phytoplankton is assumed to have been insignificant.

The tolerance of marine protists to pH has previously been found to be species-specific (Berge et al. 2010, Kim & Lee 2006, Tortell et al. 2002) and the present study supports this as the phytoplankton species could be divided into four well-defined categories (Fig. 6). Studies show that prasinophytes are quite tolerant to acidification and actually increase in abundance possibly due to a higher exploitation of the elevated CO_2 levels (Meakin & Wyman 2011, Newbold et al. 2012). The prasinophyte *Pyramimonas* sp. in this study did not obtain a higher growth rate during acidification. However, it was the species least affected and our study supports the proposal that prasinophytes

may be rather tolerant to acidification. Also the diatom *Navicula* spp. and the haptophyte *Phaeocystis pouchetii* were quite tolerant to acidification and were only affected at pH 7.1. *Phaeocystis pouchetii* is very common in Arctic waters with colonies up to 2 mm in diameter (Schoemann et al. 2005) and the broad tolerance to acidification could be a contributing factor to the formation of the well-known large *Phaeocystis* blooms. However, the colony size of *P. pouchetii* was severely reduced at pH 7.1 (Fig. 7) and colonies disappeared after 10 days. Normally, the cells in *P. pouchetii* colonies are embedded in groups of 4 but at pH 7.1 the cells were clustered. It is possible that low pH hampered the uptake or utilization of certain cations necessary for the mucus gelling in these colonies (Boekel 1992). Only cells in colonies of *P. pouchetii* were enumerated so the present study did not clarify if single cells (4-7 µm; Schoemann et al. 2005) were either unable to tolerate a pH of 7.1 or if they were present but simply unable to form colonies.

Solitary species of the diatom genus *Navicula* are primarily benthic forms and common in sea ice wherefrom they are released into the water column when the sea ice melts (Tomas 1997). In contrast to our study, where *Navicula* spp. could not sustain growth at pH 7.1, another study found a tolerance to acidification of benthic diatoms as growth rates decreased merely ~20% from pH 8.0 to 7.0 (Wang et al. 1998). However, a comparison of the tolerance of species between different experiments is not optimal due to differences in experimental conditions and strains. Diatoms incorporate silicic acid ($Si(OH)_4$) into frustules during growth and this process is known to be negatively affected by acidification (Herve et al. 2012). This could be a cause for the decreasing growth rates and chain lengths found in the present study. However, the single-celled diatom *Navicula* spp. was more tolerant to acidification than the chain-forming diatoms (*Thalassiosira* spp., *Chaetoceros* spp., *Navicula vanhoeffenii* and *Navicula* cf. *granii*) which suggests that chain-forming diatoms possess similar physiological mechanisms affected by acidification (Fig. 8), especially regarding chain elongation. Contrary to this, the chain length of *N.* cf. *granii* did not differ between pH 8.0 and 7.1 although the growth rate was highly affected. The effect of acidification on the chain length of diatoms found in this study is unclear. The shorter chain lengths at low pH could simply be a result of lower growth rates of the diatoms obtained at pH 7.1 as shown by Takabayashi et al. (2006), where changes in temperature and nutrient availability caused a lower growth rate and shorter chain lengths of the diatom *Skeletonema costatum*. However, all diatoms in the present study could tolerate a pH range from 8.0 to 7.4 with either stable or reduced growth rates and similar results were obtained from natural enclosures at Rhode Island , USA, where the frequency of diatoms was highest at the pH range ~8.0 to ~7.4 and decreased with acidification

415 (Hinga 1992).

416 It is well-known that voltage-gated H^+ channels in the plasma membrane of phytoplankton
417 are extremely sensitive to changes in external pH (pH_e) (e.g. Taylor et al. 2012). The predicted drop
418 in the average ocean surface water pH to ~7.8 by the end of this century (Feely et al. 2009) is
419 thought to reverse the proton motive force and thereby impairing the passive efflux of H^+ out of the
420 cell which regulates intracellular pH (pH_i). Changes in pH_i studied by Herve et al. (2012) with the
421 diatom *Thalassiosira weissflogii* found that a lowering of pH_e from 8.5 to 6.4 led to a drop in pH_i
422 from 7.6 to 6.7. This decrease in pH_i may have severe physiological consequences, e.g. ion
423 transport, enzyme activity and protein function, which will be reflected by reduced growth rates as
424 observed in the present study (Gattuso & Hansson 2011 and references therein, Nimer et al. 1994).

425 The phytoplankton species in the present study have been reported to be present also in the
426 brine channels of Arctic sea ice (Abelmann 1992, Ikävalko & Gradinger 1996, Niemi et al. 2011,
427 Werner et al. 2007) where pH can vary from the surrounding pH of seawater up to pH 9.9 (Gleitz et
428 al. 1995). Thus, the phytoplankton is subjected to a pH as high as 9.9 in the brine of sea ice and as
429 low as 7.5 in the water column (Fig. 3). This seasonal exposure to a broad pH range up to 2.4 pH
430 units may have led to some of the Arctic phytoplankton species currently operating at their
431 maximum physiological range. This could explain the inability of growth for some of the
432 investigated species at pH 7.1; a pH exceeding the natural fluctuations in Disko Bay during spring.

433 In the present study, the phytoplankton appears to be more or less adapted to the natural
434 fluctuations of pH, leading to a broad tolerance of the experimental acidification (pH 8.0 to 7.4).
435 The species-specific tolerances to pH may contribute to the dominance of different species
436 throughout the year as pH fluctuates. Three of seven phytoplankton species were not able to grow at
437 pH 7.1, and if pH in Disko Bay by the end of this century reaches pH 7.1, a shift in the
438 phytoplankton community structure may occur according to the obtained results. Such a shift in
439 species composition, colony size and chain length could affect the Arctic food web. *Phaeocystis* sp.
440 is an important component of the phytoplankton in Arctic marine ecosystems with a variety of
441 grazers grazing on different sizes of *Phaeocystis* colonies (Boekel 1992 and references therein,
442 Schoemann et al. 2005, Tang 2003). In addition, the success of *Phaeocystis* blooms has been
443 attributed to the ability to form large gelatinous colonies, and this study shows that the colonies
444 were severely affected at pH 7.1. Hence, if colonies of *P. pouchetii* are inhibited by the ongoing
445 acidification, they could become absent or lack as an important food source for certain grazers
446 should the pH decrease to values near pH 7.1.

Is pH tolerance size-dependent?: A size-dependent pH tolerance has been proposed for dinoflagellates and diatoms with small species typically being able to tolerate a higher pH than larger species (Lundholm & Hansen 2004, Søderberg & Hansen 2007). The large surface:volume area in small species is thought to provide a better regulation of pH_i during increased pH_e and the same could apply for the tolerance to low pH. However, considerable variations among species of similar sizes were also reported and a simulation study by Flynn et al. (2012) showed that large phytoplankton was better adapted to variable pH conditions compared to smaller sizes. In the present study, a size-dependent pH tolerance was found for the temporal composition of the phytoplankton community where large cells (>10µm) dominated at pH 8.0, 7.7 and 7.4 and small cells (≤10 µm) dominated at pH 7.1 (Fig. 5). Similar results were obtained by Nielsen et al. (2010) with a coastal plankton community from Øresund Strait, Denmark, where large cells (>15 µm) dominated at pH 8.0, 7.8 and 7.6 and small cells (10-15 µm) dominated at pH 6.0. Thus, small phytoplankton cells could be more tolerant to low pH (ca ≤7.1) compared to larger cells, but further research is needed to clarify the contrasting findings.

Population genetic adaption and experimental acidification: The present study was conducted over a short time span (17 days) which did not allow sufficient time for population genetic adaptation to the changing environment. The pH in treatments was adjusted from the *in situ* pH of Disko Bay (pH 7.9) to the experimental values of 7.7, 7.4 and 7.1 within 24 hours and does not reflect the rate of ocean acidification occurring gradually over centuries. However, the present study shows the response of phytoplankton to the natural variations of pH in Disko Bay (pH 7.5 to 8.3), including the tolerance when exposed to a low pH of 7.1.

Several strains of a species are present within a natural assemblage and population genetic adaption could potentially lead to the evolution of the more pH tolerant strains. Thus, the effect of ocean acidification on phytoplankton might not be as significant as could be expected. However, such predicaments are difficult to confirm, and studies of adaption are time consuming. Few studies on adaption have been conducted and studies on the coccolithophores *Emiliania huxleyi* and *Calcidiscus leptoporus* suggest that adaption to acidification may in fact occur over time (Langer et al. 2006, Lohbeck et al. 2012).

Studies on the effect of acidification, using natural phytoplankton assemblages, need to take the time of year into consideration because seasonal distribution and diversity of species change

over time due to variations of environmental conditions such as presence of sea ice, temperature, pH and salinity (Quillfeldt 2000). Thus, if the present study had been conducted later in the season, it is likely that species composition and pH tolerance would differ from that obtained by the present study conducted in spring.

Conclusion

In situ pH in Disko Bay varied from 7.5 to 8.3 during the spring bloom in 2012 and the present study shows that the growth rate of the phytoplankton community decreased with pH within the *in situ* pH range. The phytoplankton community, as well as the investigated phytoplankton species, did not benefit from acidification despite plenty of nutrients and inorganic carbon for growth and photosynthesis being available. A likely explanation could be that the phytoplankton growth is not limited by inorganic carbon at pH 8.0 and/or that negative effects of lowered pH on physiological processes exceed the potentially beneficial effect of increased CO_2. The study also reveals that a further decrease to pH 7.1 may have severe effects on the productivity of the phytoplankton community. The response to acidification was species-specific with some species being quite sensitive and others more tolerant. If ocean acidification in the future causes a decrease in pH in Disko Bay to ~7.1, a shift in the community structure and production may occur unless phytoplankton populations genetically adapt to the changing conditions over time.

Studies on the effect of ocean acidification should include the natural fluctuations of *in situ* pH in order to expose organisms from a specific site to an experimental pH range exceeding the natural fluctuations of pH. In addition, information on genetic diversity within species, and how this potentially affects the species' response to acidification, is required.

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Tables

Table 1. The dominating phytoplankton species from Disko Bay in this study. Enumeration of single cells, colonies or chains of the given species is indicated by *X*. The presence of single cells of species which were not enumerated is indicated by −. Blanks indicate that colonies or chains are not formed by the species.

	Species	Single cells	Colonies/chains
Diatoms	*Thalassiosira* spp.	X	X
	Chaetoceros spp. (incl. *C. socialis*)	X	X
	Navicula vanhoeffenii	X	X
	Navicula cf. *granii*	-	X
	Navicula spp. (incl. *N. transitans var. derasa f. delicatula*)	X	
Prymnesiophyte	*Phaeocystis pouchetii*	-	X
Prasinophyte	*Pyramimonas* sp.	X	

728 **Table 2.** Average pH in the experimental treatments from day 1 to the end of the experiment (±SD,
729 n = 3). The concentration of DIC (µmol kg^{-1}) is the average of two sampling times (midway and at
730 the end of the experiment). The carbon speciation of DIC is given in percentage.

pH	DIC (µmol kg^{-1})	HCO_3^- (%)	CO_3^{2-} (%)	CO_2* (%)
8.01 ±0.04	2222.2 ±2.3	94.3 ±0.2	4.7 ±0.3	1.0 ±0.1
7.71 ±0.04	2286.4 ±8.8	95.5 ±0.0	2.3 ±0.0	2.1 ±0.0
7.44 ±0.04	2341.7 ±40.0	95.1 ±0.1	1.4 ±0.1	3.5 ±0.2
7.15 ±0.05	2522.6 ±35.9	92.1 ±0.2	0.6 ±0.0	7.3 ±0.2

731

Figure legends

Fig. 1. Location of Qeqertarsuaq on Disko Island, West Greenland, where Arctic Station is situated.

Fig. 2. Experimental setup. Day 0: Acidic seawater was used to decrease the pH treatments of the phytoplankton community to the respective pH levels, except the control at pH 8.0. Day 1 to end of experiment: pH-specific seawater for dilution of the phytoplankton in the pH treatments was lowered to the respective pH levels with acidic seawater. The control treatment (pH 8.0) was diluted with filtered seawater without adjustment of pH.

Fig. 3. *In situ* pH of Disko Bay. Vertical distribution of pH at the coastal sampling station in a depth of 250 m during spring 2011 and 2012.

Fig. 4. Growth rate of the phytoplankton community at pH 8.0, 7.7, 7.4 and 7.1. Data points are means ± SD (n=3).

Fig. 5. The temporal size composition of the fraction of the phytoplankton community >10 µm at pH 8.0, 7.7, 7.4 and 7.1. Data points are means ±SD (n=3).

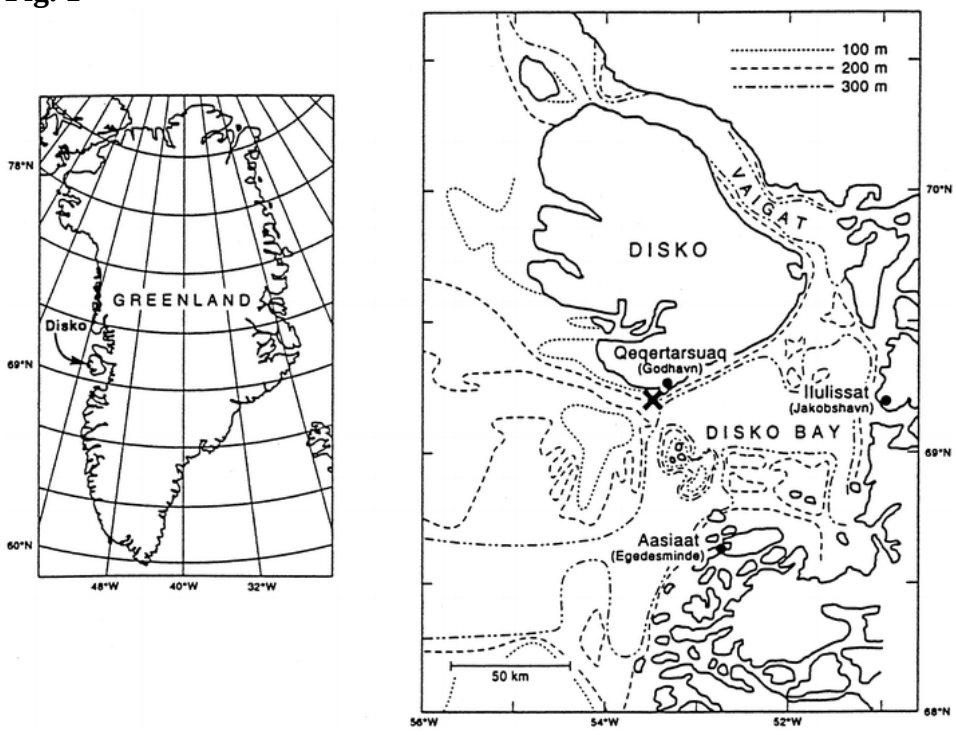
Fig. 6. Growth rates of phytoplankton species. The species were divided into categories based on their tolerance to acidification: least affected (I), affected only at pH 7.1 (II), gradually affected (III), and highly affected (IV). The symbol (+) indicates presence of *Phaeocystis pouchetii* colonies until day 10 at pH 7.1. Data points are means ±SD (n=3).

Fig. 7. Colony size. The frequency of the colony diameter of the prymnesiophyte *Phaeocystis pouchetii* at pH 8.0 and 7.1 on day 1 and 5.

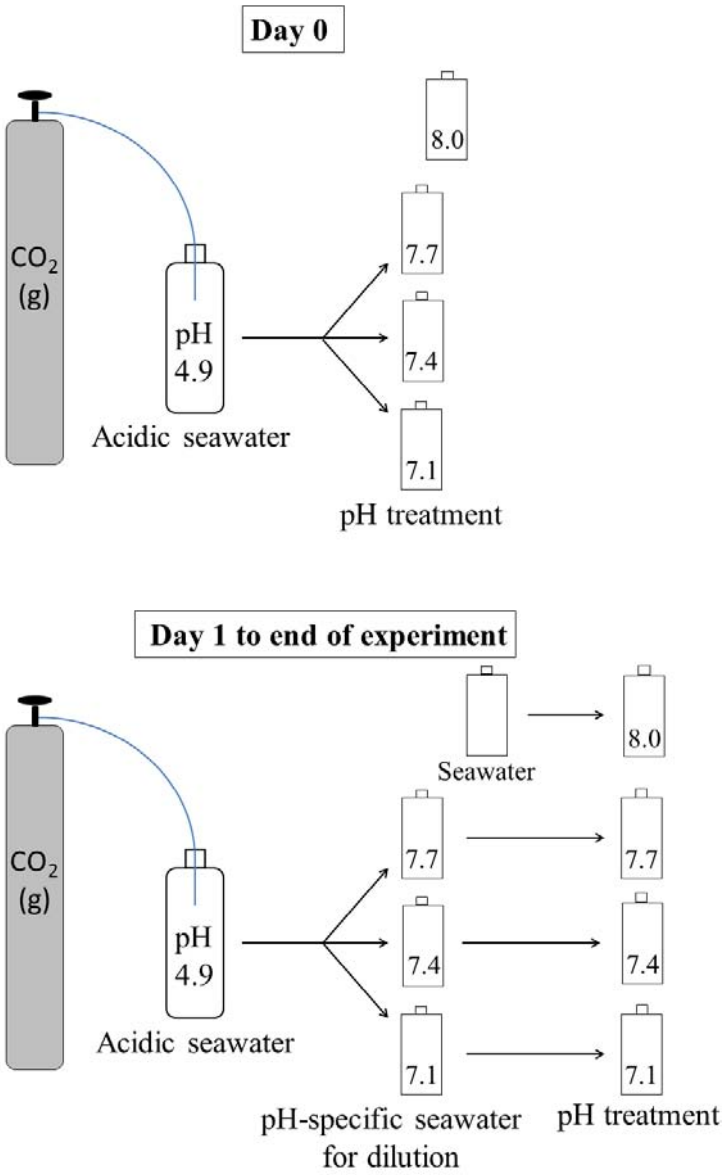
Fig. 8. Chain length. The frequency of the chain length (i.e. no. of cells) of the diatom *Thalassiosira* spp. at pH 8.0 and 7.1 on day 1 and 11.

Figures

Fig. 1



762 **Fig. 2**
763



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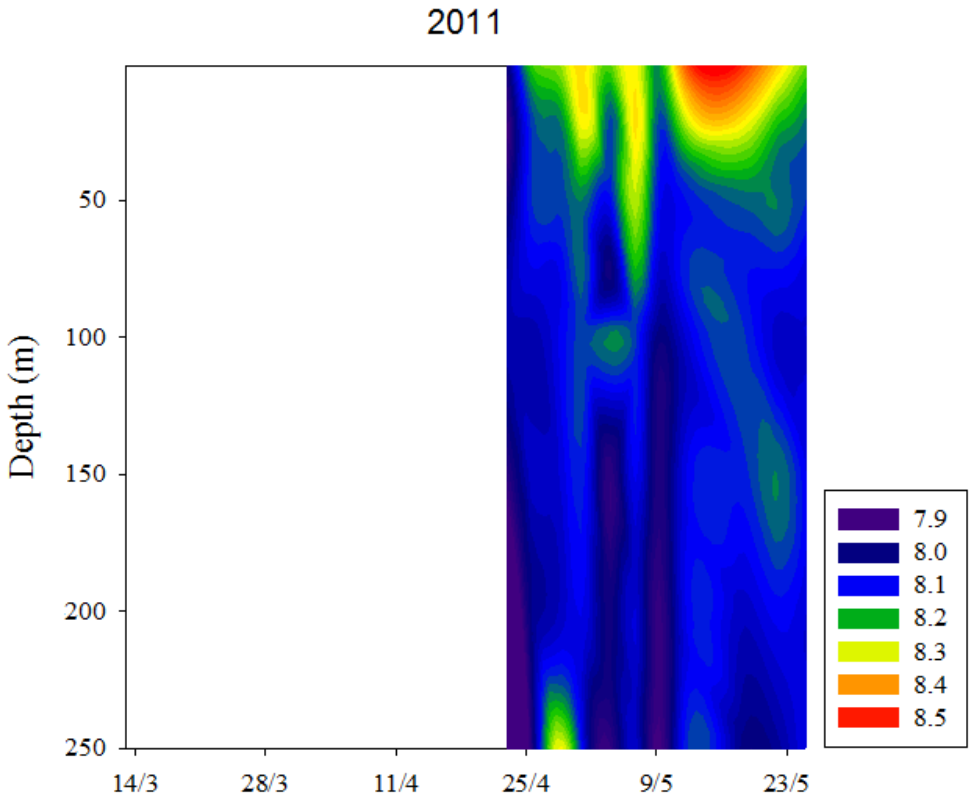
768 **Fig. 3**



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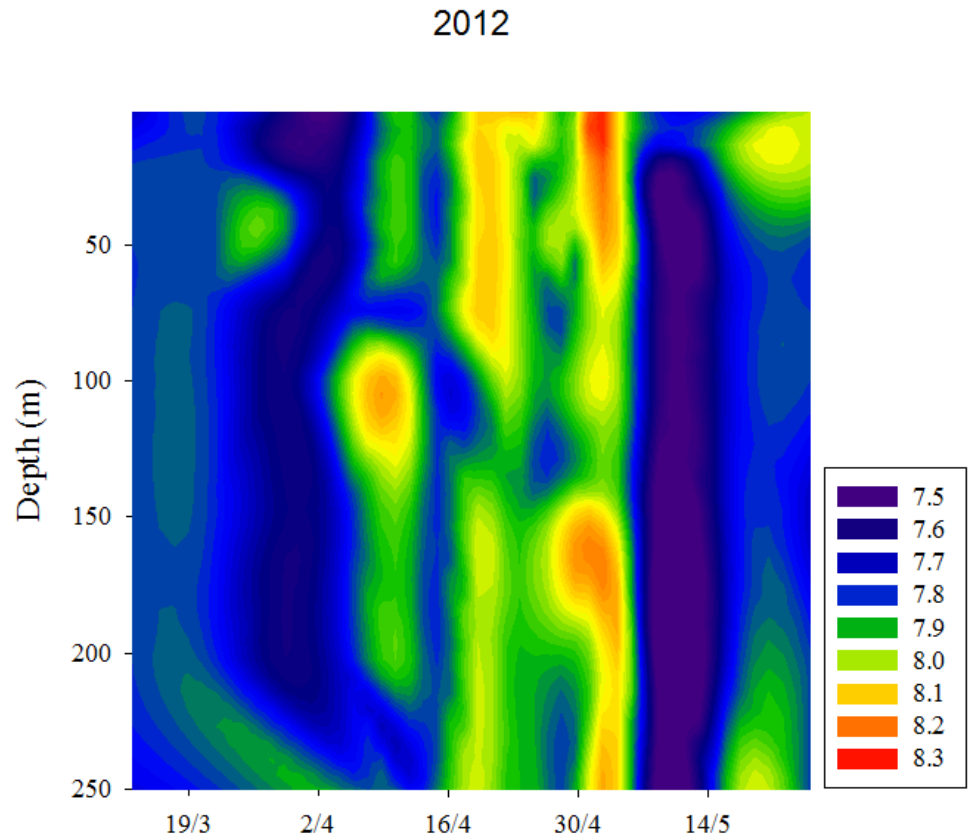

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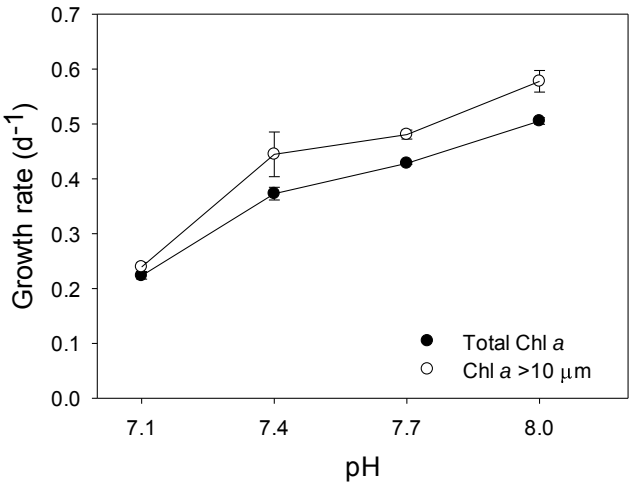
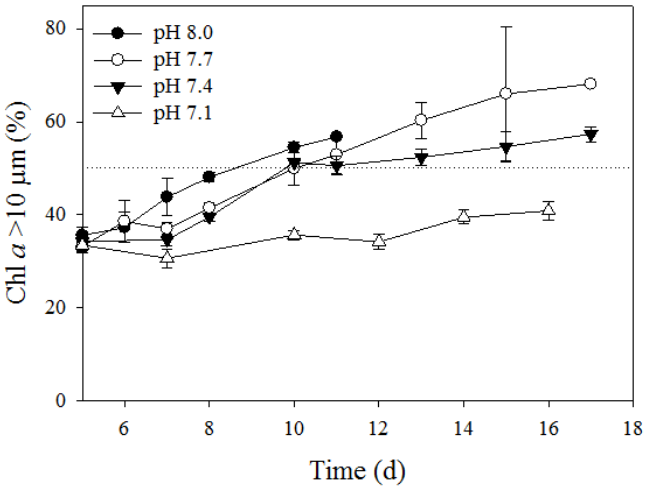
773 **Fig. 4**


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Growth rate (d-1)
7.1
7.4
7.7
8.0
pH
Total Chl a
Chl a >10 μm

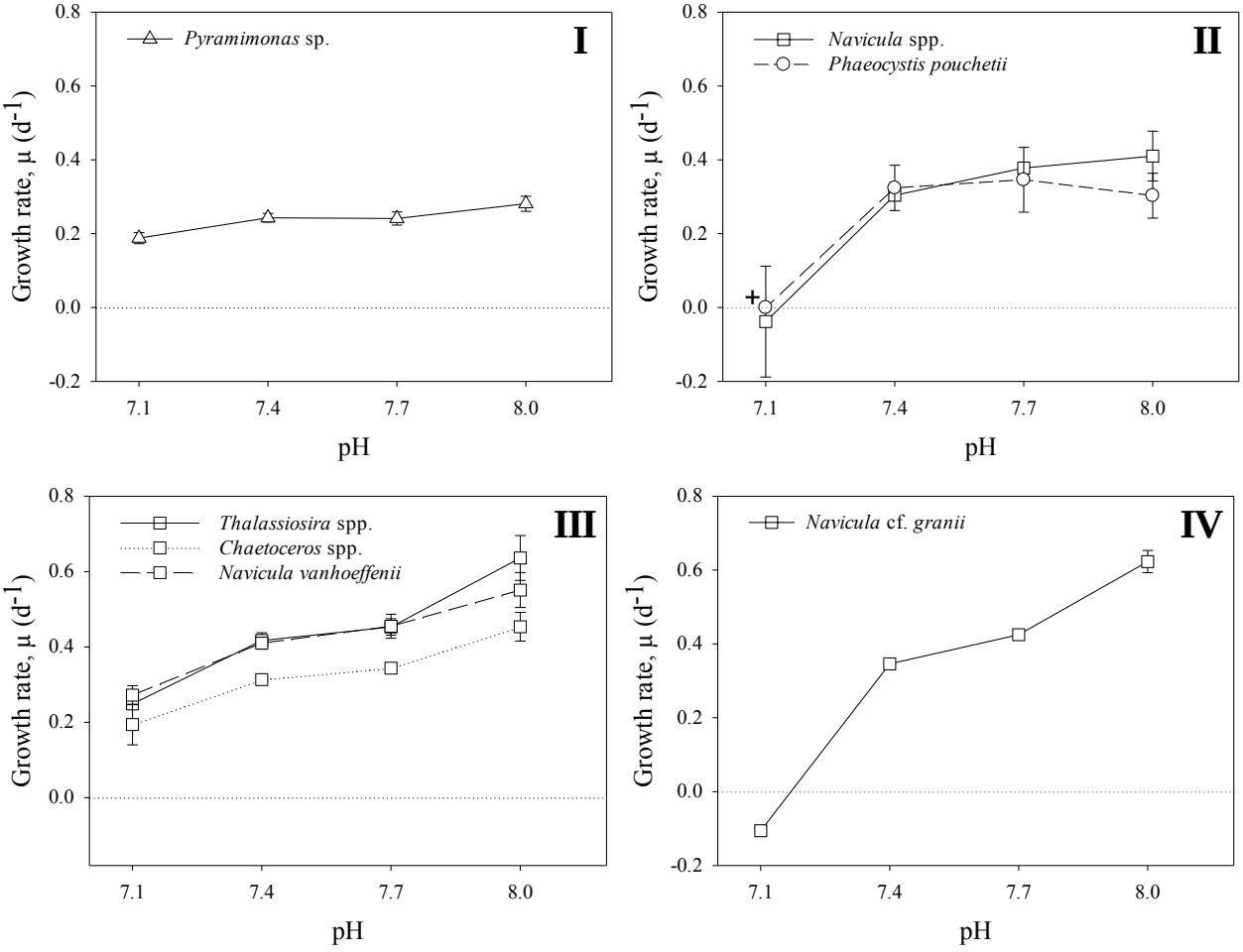
774

775 **Fig. 5**
776

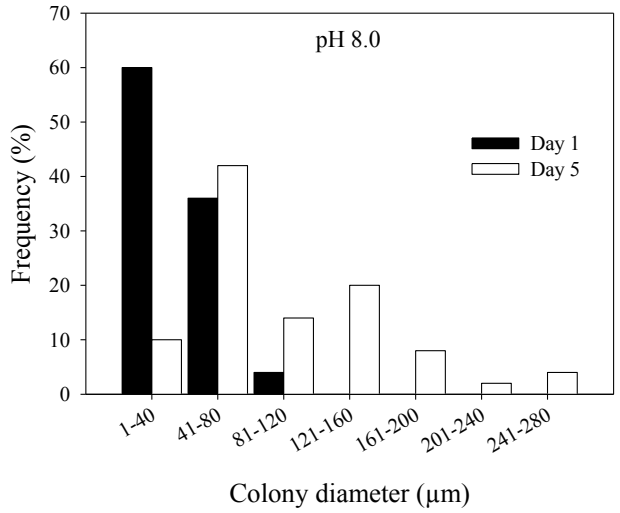


777

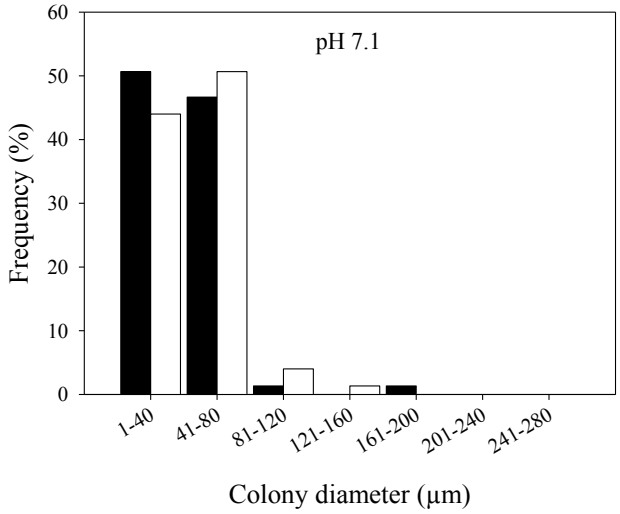
Fig. 6


I
Pyramimonas sp.
II
Navicula spp.
Phaeocystis pouchetii
III
Thalassiosira spp.
Chaetoceros spp.
Navicula vanhoeffenii
IV
Navicula cf. granii
Growth rate, μ (d-1)
pH
0.8
0.6
0.4
0.2
0.0
-0.2
7.1
7.4
7.7
8.0

782 **Fig. 7**

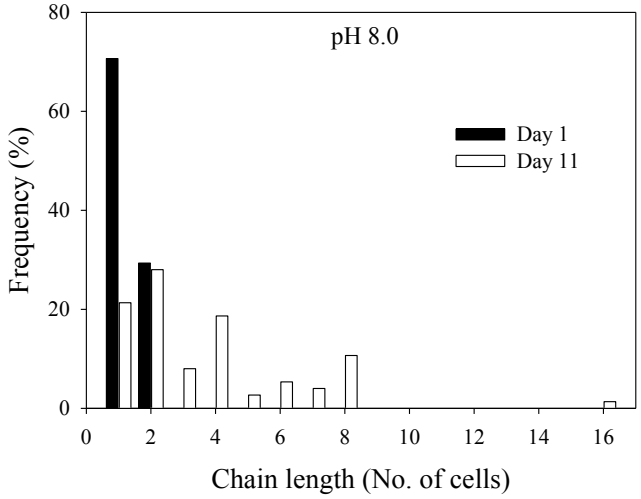


783
784

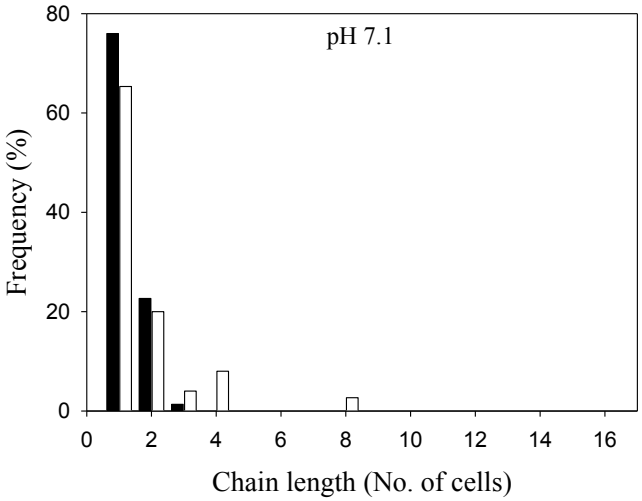


785

786 **Fig. 8**



787



788