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Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession

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ABSTRACT: Ten years of pH measurements (1990 to 1999) in the surface waters of the eutrophic Mariager Fjord, Denmark, revealed profound seasonal variation. Typically, pH was relatively constant around 8 from January to March, increased during spring, reached maximum levels in July to August (9 to 9.7), and declined during autumn to about 8 in October. The influence of pH on the growth rate of phytoplankton was tested on 3 species (*Ceratium lineatum*, *Heterocapsa triquetra* and *Prorocentrum minimum*) in laboratory experiments. The growth rate was highest at pH 7.5 to 8.0 in all species. The growth rate of *C. lineatum* declined by ~20% at pH 8.3 to 8.5, while a similar reduction in the growth rate in *H. triquetra* and *P. minimum* was observed at pH 8.8 to 8.9. *C. lineatum* stopped growing above pH 8.8, while growth ceased at about pH 9.45 in *H. triquetra* and 9.6 in *P. minimum*. Compilation of literature data on pH and phytoplankton growth suggested that while some species cannot grow at pH 8.4, others are able to grow up to pH 10. However, none of the species studied can attain their maximum growth rate above pH 9. Competition experiments using a mixture of *C. lineatum*, *H. triquetra* and *P. minimum* always resulted in the species with the highest pH tolerance (*P. minimum*) outcompeting the other species, irrespective of the initial pH value. The role of high pH in the succession of marine phytoplankton in nature is discussed.

KEY WORDS: pH · Species succession · Competition · Marine · Phytoplankton · Growth · Inorganic carbon · DIC

INTRODUCTION

Uptake of inorganic carbon by phytoplankton during photosynthesis has the potential to increase pH in the surrounding water. However, while pH can rise above 10 in freshwater (e.g. Talling 1976), it is usually quite constant around 8.2 in surface seawaters. This is because seawater contains high concentrations (2 mM) of inorganic carbon, which buffers pH. Apart from some reports from natural environments (e.g. Hinga 1992, Macedo et al. 2001), high pH has usually been observed in marine enclosures after the addition of nutrients or in laboratory cultures of phytoplankton (Goldman 1982a,b, Hinga 1992). Although the occurrence of high pH in marine waters may not be uncommon, pH has generally not been considered an important determinant of pelagic processes, and papers dealing with the possible effect of pH on the growth and succession of marine phytoplankton are sparse. However, a few studies that have been carried out indicate that pH indeed may have an effect on phytoplankton growth and that this effect drives species succession (e.g. Goldman et al. 1982a,b, Schmidt & Hansen 2001).

The present study was initiated in response to the observation of very high pH (up to 9.75) in the surface waters of Mariager Fjord, Denmark, during a summer

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bloom of the dinoflagellate *Prorocentrum minimum* in 1997 and to some recent results which have pointed to the fact that pH may be responsible for species succession (Schmidt & Hansen 2001).

The first phase of this study was to collect data on the seasonal change in pH in the highly productive Mariager Fjord in order to describe the natural range of pH in a marine environment. The second phase aimed to study (1) the role of pH in limiting growth in laboratory cultures of marine phytoplankton and (2) the role of pH in the succession of species in mixed phytoplankton cultures in the laboratory.

MATERIALS AND METHODS

Seasonal and inter-annual variation in pH in a eutrophic fjord in Denmark. pH has been measured regularly during the past 20 yr at a fixed station in Mariager Fjord, Denmark, by the counties of Aarhus and North Jutland as part of their monitoring program. The station is located at 56° 39′ 80″ N, 9° 58′ 69″ E close to the center of the deep basin, which has a water depth of 28 m. The salinity in the surface water at this station varies from 16 to 17 ppt. For the past 10 yr measurements have been carried out weekly to bi-weekly, and these data were kindly provided by the counties. Data on pH are from the top part of the water column. Water, subsequently used for primary production, was gently poured into polyethylene bottles (2.5 l) to capacity to avoid aeration of the samples. The bottles were stored in the dark in a thermo-container until the measurement of pH shortly after, using a standard Radiometer® glass pH electrode (sensitivity 0.01). The pH sensor was calibrated (2 point) using buffers of pH 7 and 10.

Isolation and culture of phytoplankton. Information on isolation date, isolation place and clone designation of the 3 species of dinoflagellates used in the present study is listed in Table 1. The Scandinavian Culture Collection of Algae and Protozoa, Botanical Institute, University of Copenhagen, provided *Heterocapsa triquetra* and *Prorocentrum minimum*, while the Marine Biological Laboratory, University of Copenhagen, provided *Ceratium lineatum*. These species were chosen based on previous experience concerning their different tolerance to pH in batch cultures. According to Schmidt & Hansen (2001), *C. lineatum* should have a pH limit for growth at pH 8.79, while the pH limits for growth of *H. triquetra* and *P. minimum* should be 9.43 and 9.62, respectively. The algae were grown as non-axenic cultures in f/2 medium (Guillard 1983) based on seawater (30 psu) at 15 ± 1°C following a light:dark cycle of 16:8 h. Illumination was provided by cool white fluorescent lamps, and cultures were kept at an irradiance of 25 µmol photons m^{-2} s^{-1}. Irradiance was measured using a LI-COR LI-1000 radiation sensor equipped with a spherical probe.

Experimental conditions. All experiments were carried out at an irradiance of 60 µmol photons m^{-2} s^{-1}. The dinoflagellates were adapted to this irradiance for at least 14 d prior to each experiment. Only cells from exponentially growing cultures were used for inoculation. For enumeration of cells, subsamples were fixed in acidic Lugol's iodine (2.5% final concentration). Cells were counted in a Sedgewick-Rafter chamber or a multidish well (Nunclon®). Growth rates (μ) were measured as increase in cell number and were calculated assuming exponential growth:

$$\mu\ (\mathrm{d}^{-1}) = \frac{(\ln N_1 - \ln N_0)}{t}$$

N_0 and N_1 are number of cells at time t_0 and t_1, and t is the difference in time (d) between t_0 and t_1 samples. All experiments were carried out in triplicate, and data from each triplicate were the mean of at least 3 growth rates (= 4 sampling dates). pH was measured using a Sentron® pH-meter (model 2001) equipped with Redline probe, which is an ISFET® sensor (Semi-conductor Ion Field Effect Transistor) with a detection limit of 0.01. The pH sensor was calibrated (2 point) using Sentron buffers of pH 7 and 10.

Effect of pH on phytoplankton growth rate. The growth rates of the 3 dinoflagellates were measured at different pH within the range of 7.5 to 10. The pH was adjusted by the addition of 0.1 M NaOH or HCl. All experiments were carried out in Nunclon® tissue culture flasks (250 ml), which were mounted on a plankton wheel (1 rpm) in order to keep the phytoplankton in suspension. Each experiment was initiated by the inoculation of between 20 and 100 cells ml^{-1} and allowed to run for 7 d. Daily, pH of the culture media was measured, and subsamples (6 ml) were taken for enumeration of phytoplankton cells. After subsampling, the bottles were refilled to capacity with f/2 growth medium, which had the same pH as the respective experimental bottle. If the pH differed more than 0.03 from the

Table 1. List of dinoflagellate species used in the experiments, their clone designation and isolation place and time

Species	Clone	Isolation place and time
Ceratium lineatum		Øresund, Denmark, 1995
Heterocapsa triquetra	K-0481	Øresund, Denmark, 1988
Prorocentrum minimum	K-0295	Kattegat, Denmark, 1989

set point, it was adjusted by addition of small amounts of 0.1 M NaOH or HCl. The first 2 d of the experiments were considered an acclimation period; therefore cell counts from these samplings were not included in the calculations of growth rates. Also, the daily dilution (of 6 ml) was adjusted for in the calculations of algal growth rates.

Growth experiments with mixed phytoplankton cultures. Two types of succession experiments were carried out using the 3 dinoflagellates in mixture. In the first type of experiments, mixed cultures were initiated at pH levels of 8, 8.5, and 9.0. The pH was adjusted by the addition of 0.1 M NaOH or HCl. All experiments were carried out in Nunclon® tissue culture flasks (250 ml), which were mounted on a plankton wheel (1 rpm) in order to keep the phytoplankton in suspension. The species were inoculated together at an initial concentration of 100 cells ml^{-1} of each species and allowed to grow. Every 2 to 3 d, pH of the culture media was measured, and subsamples (6 ml) were retrieved for enumeration of phytoplankton cells. Immediately after sampling, the bottles were refilled to capacity with f/2 growth medium, which had the same pH as the sampled medium.

Because the species *Heterocapsa triquetra* and *Prorocentrum minimum* have an almost similar tolerance to high pH, a second type of experiment was carried out in order to exclude other possible effects, such as nutrient/vitamin limitation or effects of allelochemicals (toxins). This experiment was carried out in a 1 l Pyrex flask containing 500 ml of f/2 medium in triplicate. Atmospheric air was applied by bubbling in order to supply CO_2 to the medium and thereby reduce the elevation of pH, when cultures become dense. The species were inoculated together at an initial concentration of 100 cells ml^{-1} of each species and allowed to grow. Every 2 to 3 d, pH of the culture media was measured, and subsamples (6 ml) were retrieved for enumeration of phytoplankton cells.

RESULTS

pH in Mariager Fjord

Median, minimum, and maximum pH values from 10 yr of pH measurements (1990 to 1999) of surface water from the deep basin are shown in Fig. 1. The seasonal variation in pH was bell-shaped. From October to March, pH varied around 7.7 to 8.2. In April and May, pH increased rapidly to 8.7. The highest pH was found in July and August (pH 9). By August pH decreased again to reach pH 8 in late September. The data also show that pH above 9 (max. 9.75) could be found at any time from May to August.

pH limits for phytoplankton growth/survival in batch cultures

The effect of pH on the growth rate was very profound in the 3 phytoplankton species *Ceratium lineatum*, *Heterocapsa triquetra* and *Prorocentrum minimum* (Fig. 2). The growth rate was highest at pH 7.5 to 8.0 in all species. The growth rate of *C. lineatum* declined by ~20% at pH 8.3 to 8.5, while a similar reduction in the growth rate in *H. triquetra* and *P. minimum* was achieved at pH 8.8 to 8.9. *C. lineatum* stopped growing above pH 8.8, while growth ceased at about pH 9.45 and 9.6 in *H. triquetra* and *P. minimum*, respectively

Succession experiments in mixed phytoplankton batch cultures

The importance of pH in the succession of phytoplankton species was studied using mixed cultures of the 3 species at 3 different initial pH levels: 8, 8.5, and 9 (Fig. 3). The experiments initiated at pH 8 and 8.5 were quite similar. In both cases all species grew until pH reached 8.70 to 9 on Day 7. At Day 10 the pH had increased to above pH 9.4, and the entire population of *Ceratium lineatum* had died out. At Day 10 the growth of *Heterocapsa triquetra* also stopped, and during the following 2 wk the concentration of *H. triquetra* declined. At Day 24 the entire population of *H. triquetra* had died out. The degradation of the *H. triquetra* population caused pH to decrease to 9 at Day 24. *Prorocentrum minimum* was able to keep a positive growth rate until Day 17. During the period from

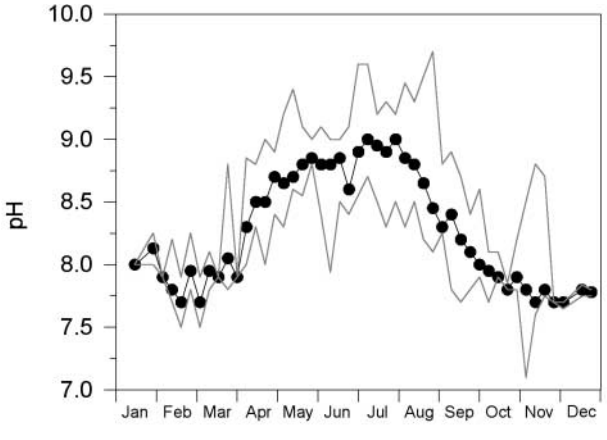

Fig. 1. Seasonal distribution of median pH (•) in surface waters of Mariager Fjord, Denmark, for a 10 yr period (1990–1999). Maximum and minimum values are indicated with a solid grey line

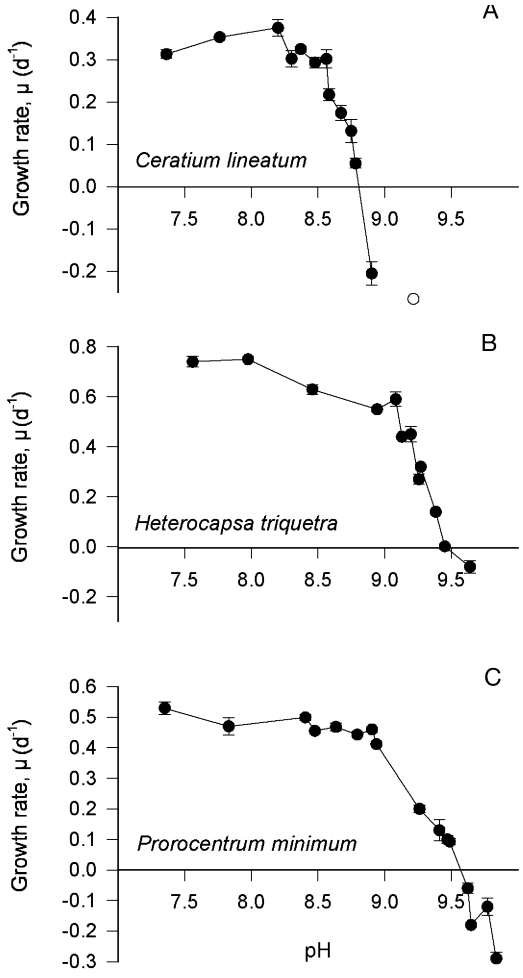

Fig. 2. Growth rate as a function of pH in laboratory cultures of (A) *Ceratium lineatum*, (B) *Heterocapsa triquetra*, and (C) *Prorocentrum minimum*. Data points refer to treatment means ± SE (n = 3)

Days 17 to 21, the *P. minimum* population just maintained itself, but after Day 21 it regained positive growth, resulting in an increase in pH.

In the experiment initiated at pH 9, the *Ceratium lineatum* population died within the first 3 d of incubation, while *Heterocapsa triquetra* and *Prorocentrum minimum* grew until Days 14 and 19, respectively, when pH had increased to above 9.4 (Fig. 3E,F). The *H. triquetra* population declined from Day 16, and after 31 d of incubation had completely died out. In that period, pH decreased to below 9. The *P. minimum* population maintained itself from Day 19 to Day 31, whereupon it increased again, resulting in an increase in pH.

A control experiment was carried out in which bubbling with atmospheric air was applied to reduce the elevation of pH in a mixed culture of *Heterocapsa triquetra* and *Prorocentrum minimum* (Fig. 4). The pH in this experiment stayed fairly constant around 7.75 to 8 for the first 12 d, whereupon it increased to reach about 9 on Day 17 and stayed at this level until the experiment was terminated. At this time the cell concentrations of *H. triquetra* and *P. minimum* had reached 185 000 and 18 500 cells ml^{-1}, respectively. In terms of total cell concentration, this was about 10 times higher than the cell concentration found in the experiment without bubbling initiated at pH 8, and 20 times the cell concentration reached at the experiment initiated at pH 9.

DISCUSSION

High pH in marine waters

A number of biological and physical processes may influence the pH of marine surface waters. Uptake of inorganic carbon by phytoplankton during photosynthesis may increase pH, while release of CO_2 through respiration processes will decrease pH. Excursions of pH in marine waters are counteracted by the physical exchange of CO_2 between the atmosphere and the surface water. Thus, the exchange of CO_2 at the air-water transition is highly dependent on the vertical mixing of the water column, and thereby very much dependent on the strength of the wind stress and the stability of the water column.

In Mariager Fjord, the data from the past 10 yr show a median pH of close to 9 during the summer (May to August). The maximum pH recorded within the past 10 yr was 9.75, which is close to the maximum pH that can be achieved in seawater. The development of such high pH values in Mariager Fjord is largely due to its high primary production and to its morphology and hydrography. The fjord is highly productive, and summer chlorophyll *a* concentrations may reach >50 μg l^{-1} (Fenchel et al. 1995, Fallesen et al. 2000, Olesen 2001). The high productivity of the fjord is supported by inorganic nutrients draining from the surrounding farmland (Fallesen et al. 2000). The fjord has a sill and is permanently stratified with a salinity of 16 to 17 psu in the top 10 m. The water exchange of Mariager Fjord is relatively small, and the average residence time of water in the central part (above the 16 m) is about 8 mo (Fenchel et al. 1995, Fallesen et al. 2000). Thus, the combination of a stable water column, a low residence time of surface waters, high solar radiation during summer, and sufficient supply of nutrients form the physical basis for the development of phytoplankton blooms and high pH levels in Mariager Fjord.

Although the pH can become extremely high in Mariager Fjord, the fjord is not unique. In the

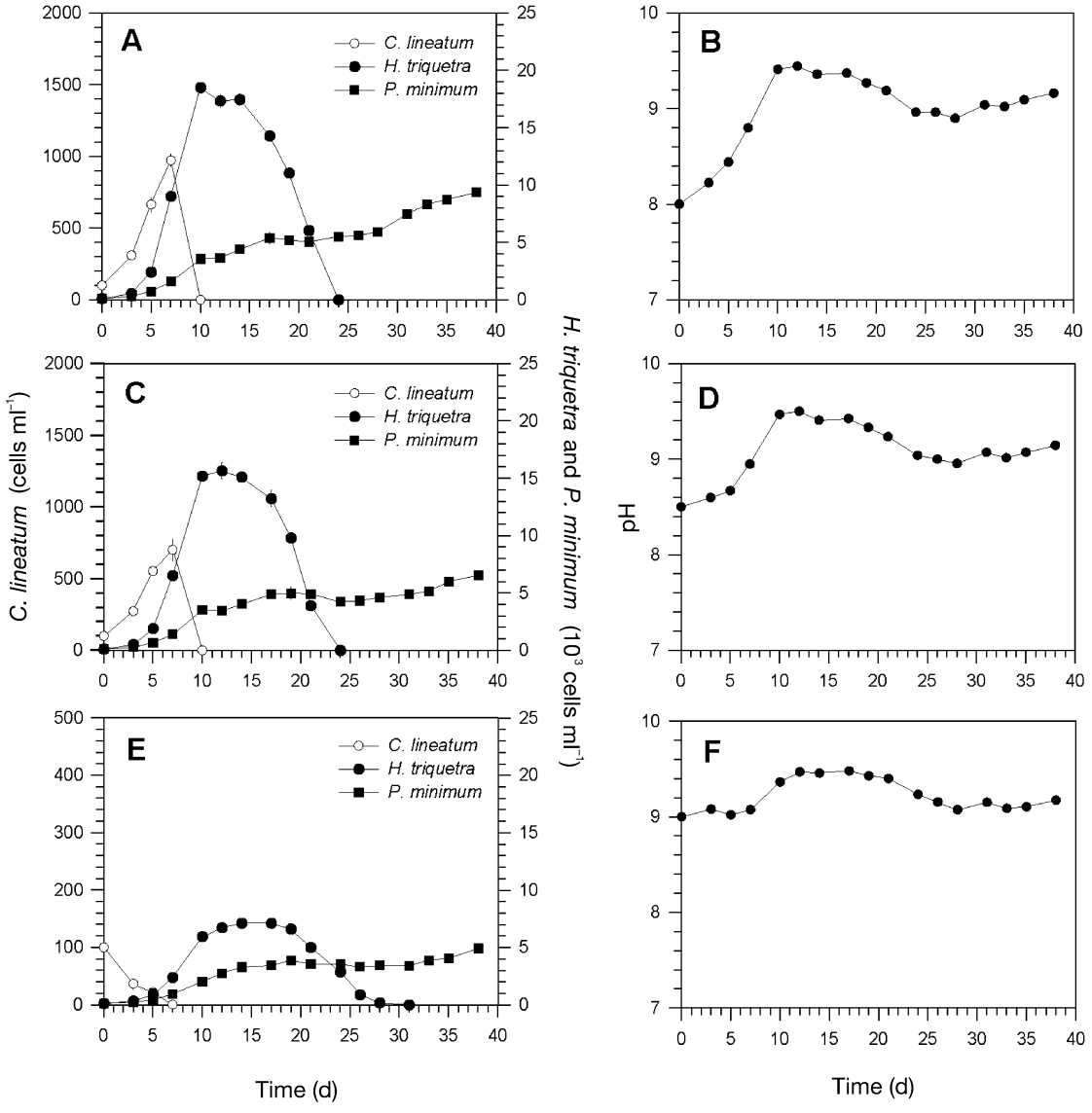

Fig. 3. Succession experiment. (A,C,E) Changes in cell concentration of the species *Ceratium lineatum*, *Heterocapsa triquetra* and *Prorocentrum minimum* as a function of time (d) from inoculation at 3 initial pH levels. (B,D,F) pH as a function of time from inoculation. Please note that the cell concentration of *C. lineatum* is shown on the left *y*-axes, while the cell concentration of the 2 other species is shown on the right *y*-axes

German Bight and in the Chesapeake Bay system, maximum pH of between 8.7 and 9.25 has been observed during the summer (e.g. Pegler & Kempe 1988, Hinga 1992 and references therein). In enclosed lagoons that are only temporarily flooded, pH can reach values as high as 9.5 to 9.6 (Macedo et al. 2001). Thus, elevated pH may be a common phenomenon in the pelagial of many highly productive coastal waters and is a parameter that we should consider when dealing with phytoplankton production and species succession.

Importance of pH for the growth of marine phytoplankton

Chen & Durbin (1994) studied the growth of 2 diatoms (*Thalassiosira* spp.) as a function of pH in the culture medium. Both diatoms were able to maintain their maximum growth rate up to pH 8.6. Above this pH, the growth rate of the diatoms decreased to only ca. 10% of the maximum growth rate at pH 9.4, resembling the pH dependency of the dinoflagellates *Heterocapsa triquetra* and *Prorocentrum minimum* in

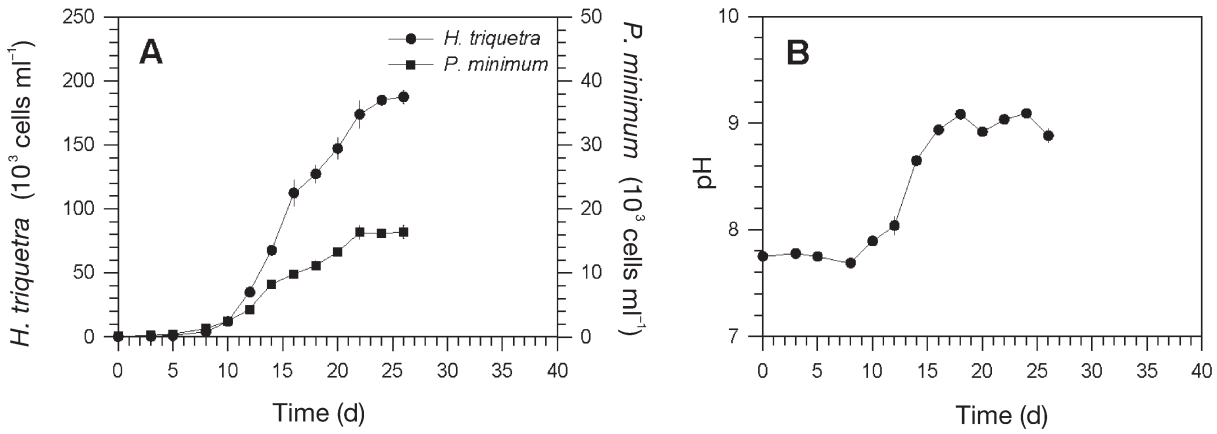

Fig. 4. Succession experiment — control experiment. (A) Changes in cell concentration of *Heterocapsa triquetra* and *Prorocentrum minimum* as a function of time from inoculation in mixed cultures, which were bubbled with atmospheric air to buffer pH. (B) pH as a function of time from inoculation. Please note that the cell concentration of *H. triquetra* is shown on the left *y*-axes, while the cell concentration of *P. minimum* is shown on the right *y*-axes

the present study. Apart from the work by Chen & Durbin (1994) and the present study, information on pH limits for the growth of marine phytoplankton comes from studies of batch cultures in which pH has not been kept constant.

The work of Schmidt & Hansen (2001) suggested that marine phytoplankton cultures grown in a standard phytoplankton growth medium (like the f/2 growth medium) are limited by high pH rather than inorganic nutrients such as nitrogen and phosphorus. My data support this conclusion.

First, a simple calculation using the maximum concentrations of *Ceratium lineatum*, *Heterocapsa triquetra* and *Prorocentrum minimum* reached in f/2 medium (Schmidt & Hansen 2001) and their cellular carbon content clearly suggests that such cultures are not nutrient-limited. In fact, the estimated uptake of nitrogen and phosphorus only accounted for about 5 to 11 and 7 to 17% of the available nitrogen and phosphorus, respectively (Table 2).

Table 2. Estimated uptake of C, N and P in *Ceratium lineatum*, *Heterocapsa triquetra* and *Prorocentrum minimum* cultures that have reached maximum cell concentrations in f/2 medium. Addition of N and P to the seawater in the f/2 medium was 883 and 36 µM, respectively. Estimates were based on a red field ratio of 106 C:16 N: 1 P (by mol). The carbon contents of the species were calculated from the carbon to cell volume equation published by Strathmann (1967)

Species	Maximum cell concentration (cells ml^{-1})	C uptake (µM)	N uptake (µM)	P uptake (µM)
Ceratium lineatum	4.0×10^3	280	42.3	2.64
Heterocapsa triquetra	4.0×10^4	639	96.6	6.03
Prorocentrum minimum	5.5×10^4	652	98.3	6.15

Second, the pH at which the 3 selected dinoflagellates just maintained themselves in the constant pH experiment at low cell concentrations in the present study was similar to the pH obtained in the stationary growth phase of the same species in enriched batch cultures (cf. Table 3 with Fig. 2). Likewise, the pH causing a reduction in growth rates of the 3 studied species by 20% in the present study was similar to the pH causing a ca. 20% reduction of the growth rate in ordinary batch cultures (cf. Table 3 with Fig. 2).

Thus, in the absence of data from experiments carried out at constant pH levels, data on pH limits for maximum growth rate (reduction ca. 20%) as well as the pH limits for growth for marine phytoplankton can be obtained from ordinary batch culture experiments (Table 3). Comparison of literature data on 35 species of marine phytoplankton suggested a great variation in their tolerance to high pH. Some species (*Ceratium tripos*, *C. furca*) stopped growing at a pH above 8.3 to 8.4, while others were able to grow at a pH close to 10 (e.g. *Phaeodactylum tricornutum*, *Rhodomonas salina*). It is notable that within the same family some species were sensitive while others were insensitive to high pH (e.g. dinoflagellates and diatoms). When the number of phytoplankton species capable of growing at a specific pH was plotted as a function of pH, the data fitted a 1-tailed normal distribution (Fig. 5). Only half of the species investigated could sustain growth at pH above 9.2.

For 17 species, data were also available on the pH at which maximum growth was reduced by 20%. While

the growth of some species was affected at pH below 8.4, other species could maintain their maximum growth at pH 9. However, none of the tested species could grow at their maximum growth rate much above pH 9. Thus, although *Rhodomonas salina* or *Prorocentrum minimum* could grow at pH close to 10, their growth rates were already affected at a pH of 9 to 9.1. It is also noteworthy that only half of the species investigated reached maximum growth rates at pH between 8.6 and 8.8. Therefore pH should be taken into account when dealing with phytoplankton growth in the laboratory and in natural environments.

Several reasons for the effects of high pH on the growth of phytoplankton may be suggested. Changes of pH in seawater influence the inter-speciation of inorganic carbon (as CO_2 aq., HCO_3^-, CO_3^{2-}). In seawater at pH 8, ca. 1% of DIC is present as CO_2, while at pH 9 only ca. 0.1 % of the DIC is available in this form. Limitation in the supply of CO_2 caused by elevated pH may therefore potentially restrict photosynthesis and growth in marine phytoplankton. However, at least some phytoplankton species have active transport systems by which they can take up HCO_3^- in order to avoid DIC limitation at elevated pH (e.g. Colman & Gehl 1983, Dixon & Merrett 1988, Colman & Rotatore 1995, Korb et al. 1997, Huertas et al. 2000). Thus, elevated pH should favour species which can utilize HCO_3^- as an inorganic carbon source. Alternatively, high extracellular pH may cause gross alterations in the membrane transport processes and metabolic

Table 3. pH limits for exponential growth and for positive growth ($\mu = 0$) in highly enriched batch cultures for phytoplankton belonging to a variety of taxa. Salinity was in all cases between 30 and 34 psu. Taxa abbreviations refer to eumastigohytes (Eumast.), cryptophytes (Cryp.), dinoflagellates (Dino.), prymnesiophytes (Prym.), raphidophytes (Raphid.), silicoflagellates (Silico.), prasinophytes (Prasino.), chlorophytes (Chloro.). *Criterion: growth is affected when growth rate is reduced by >20%

Species	Taxon	pH limits for exponential growth*	pH limits for growth ($\mu = 0$)	Source
Phaeodactylum tricornutum	Diatom		>10; 10.3;10.4	1,2,3
Amphidinium carterae	Dino.		>10	1
Nanochloropsis sp.	Eumast.		10.08	4
Rhodomonas marina	Crypt.	8.74	9.93	5
Prorocentrum micans	Dino.	8.75	9.92	5
Thalassiosira pseudonana	Diatom		9.77	4
Prorocentrum minimum	Dino	9.20	9.62	5
Dunaliella tertiolecta	Chloro.		8.69; 9.5	4,1
Nitzschia closterium	Diatom		9.5	1
Nitzschia sp.	Diatom		9.5	1
Heterocapsa triquetra	Dino	8.80; 8.90	9.43	5
Synechococcus sp.	Cyano.		9.40	4
Chroomonas sp.	Crypto.		9.3	1
Emiliania huxleyi (heavy coccoliths)	Prym.		9.29	4
Chrysochromulina simplex	Prym.	9.20	9.25	5
Eutrieptiella gymnastica	Eugl.	9.00	9.22	5
Skeletonema costatum	Diatom	8.49	9.21	5
Chrysochromulina polylepis	Prym.	8.70	9.20	5
Heterosigma akashiwo	Raphid.	8.52	9.15	5
Phaeocystis globosa	Prym.		9.14	4
Biddulphia aurita	Diatom		9	1
Chaetoceros didymus	Diatom		9	1
Gymnodinium splendens	Dino.		9	1
Monochrysis lutheri	Prym.		9	1
Gymnodinium mikimotoi	Dino.	8.72	9.00	5
Alexandrium ostenfeldii (2 clones)	Dino.	8.66	8.90	5
Thalassiosira punctigera	Diatom		8.90	4
Alexandrium tamarense	Dino.	8.66	8.85	5
Dictyocha speculum	Silicofl.	8.30	8.81	5
Emiliana huxleyi (weak coccoliths)	Prym.		8.80	4
Pyramimonas propulsa	Prasino.	8.55	8.80	5
Ceratium lineatum	Dino.	8.30	8.79	5
Cylindrotheca closterium	Diatom		8.5	1
Ceratium furca	Dino.	8.29	8.40	5
Ceratium tripos	Dino.	8.24	8.30	5

1: Humphrey (1975), 2: Goldmann et al. (1982), 3: Nimer et al. (1997), 4: Elzenga et al. (2000), 5: Schmidt & Hansen (2001)

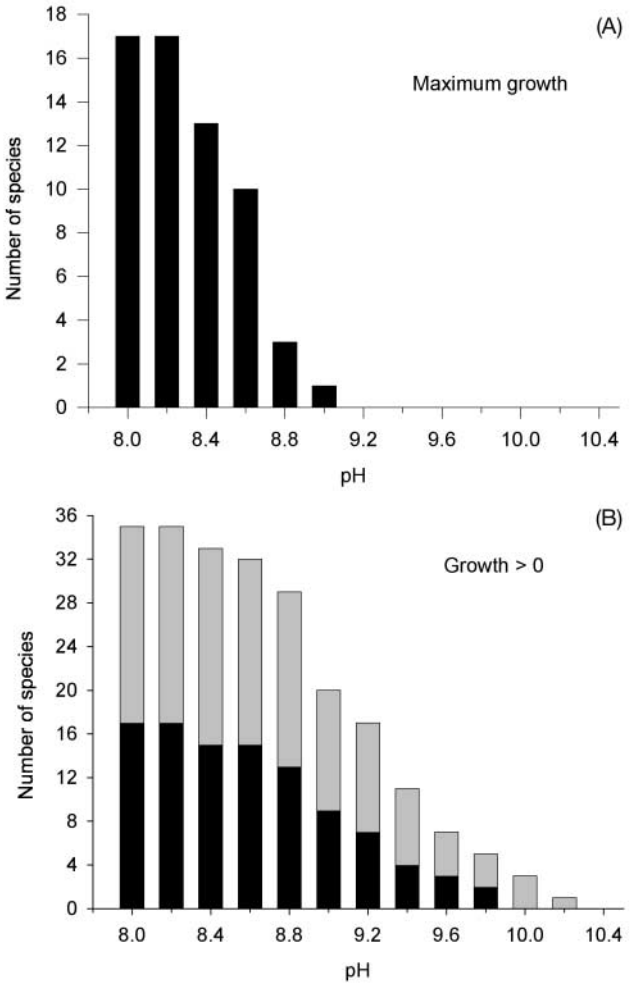

Fig. 5. pH limits of marine phytoplankton obtained in batch cultures. (A) pH at which the growth rate is reduced by >20%. (B) pH obtained in stationary growth phase. Data collected from the literature (Table 3). The black bars refer to data from Schmidt & Hansen (2001)

functions involved in internal pH regulation (Smith & Raven 1979, Raven 1980, 1993) or cause changes in cellular content of amino acids and their relative composition, possibly affecting cellular growth (e.g. Taraldsvik & Myklestad 2000).

Species succession

Although there are many studies on species succession in multi-species phytoplankton batch cultures, only a few have taken pH into account (see reviews by Maestrini & Bonin 1982a,b). The possible role of high pH in the succession of phytoplankton species was first addressed by Goldman et al. (1982a,b). They observed that the diatom *Phaeodactylum tricornutum* often successfully invaded other phytoplankton cultures in the laboratory. Through a set of competition experiments they demonstrated that *P. tricornutum* out-competed *Dunaliella tertiolecta* in continuous cultures when pH exceeded 9.2.

The role of high pH in species succession among marine phytoplankton was studied here by exposing 3 species in mixture to different initial pH values between 8 and 9 (Fig. 3). Irrespective of the initial pH, *Prorocentrum minimum* always outcompeted the 2 other species. *Ceratium lineatum* died out soon after the pH had exceeded 8.7, independently of the initial pH, which is in accordance with the results obtained for this species in the monoculture experiments at fixed pH levels (Fig. 2A). *Heterocapsa triquetra* grew in the mixture experiments until pH exceeded 9.4 (Fig. 3), which again is in accordance with the results obtained in the monoculture experiments at fixed pH levels (Fig. 2B).

Prorocentrum minimum has a pH limit for growth, which is only slightly higher than that of *Heterocapsa triquetra*, and thus other factors such as nutrient/vitamin limitation or production of allelochemicals (toxins) may have been involved in the interaction between these 2 species in the mixture experiment.

Earlier in this section (when discussing the importance of pH for growth of marine phytoplankton) a rough calculation suggested that nitrogen and phosphorus are not limiting the growth of either *Heterocapsa triquetra* or *Prorocentrum minimum* in f/2 growth medium. The results obtained in the aerated mixture experiment support that suggestion and also exclude vitamin limitation, because total cell yield in the aerated mixture experiment was at least 10 times higher than in the non-aerated mixture experiment (Figs. 3 & 4). Also, the maximum cell yield in the non-aerated mixture experiment decreased when the initial pH set point was elevated. Thus, any kind of nutrient/vitamin limitation can be ruled out as the cause of the species succession in these experiments.

A few studies have indicated that some isolates of *Prorocentrum minimum* can produce toxic substances (Trick et al. 1981, Grzebyk et al. 1997). In laboratory cultures, these toxins appear to be produced only during the stationary growth phase. In the present study no clear indications of negative effects due to *P. minimum* toxins on the other algae could be found. However, because *P. minimum* has a pH limit for growth which is only slightly higher than that of *Heterocapsa triquetra*, it cannot be completely ruled out that toxins exuded by *P. minimum* may have contributed to the decline of the *H. triquetra* population in the non-buffered mixture experiments (Fig. 3). Nevertheless, it seems justified to suggest that

elevated pH alone can drive a species succession among marine phytoplankton.

Very few data are available on the occurrence of the 3 studied species in relation to high pH in nature. However, in Mariager Fjord the dinoflagellates *Heterocapsa triquetra* and *Prorocentrum minimum* usually form almost mono-specific blooms during summer periods (Fenchel 1995, Fallesen et al. 2000, Olesen 2001), in which pH is extremely high (pH > 9.2, this data set). Similar observations have been made in the coastal Santo André Lagoon, SW Portugal (Macedo et al. 2001), where almost mono-specific blooms of *P. minimum* co-occur with pH of 9.5 to 9.6. It is noteworthy that the pH-sensitive *Ceratium* species are completely lacking in both Mariager Fjord and Santo André Lagoon (Fenchel et al. 1995, Macedo et al. 2001, Olesen 2001), although *Ceratium* spp. are common in waters just outside these areas (e.g. Taylor & Pollingher 1987).

It is evident from the compiled data on pH limits for marine phytoplankton growth that the ability to tolerate high pH is not related to any particular algal groups, but rather is species-specific (Table 3). A few studies have indicated that pH in nature in fact may be associated with certain groups of phytoplankton. Yoo et al. (1991) performed a correlation analysis between dinoflagellate abundance and environmental parameters in Masan Bay, Korea, which suggested that pH was the main factor influencing dinoflagellate abundance. Hinga (1992) found that high abundance of dinoflagellates was strongly correlated with high pH, whereas high abundance of diatoms was not. Similarly, in the Santo André Lagoon, blooms of dinoflagellates (*Prorocentrum*) have co-occurred with pH of 9.5 to 9.6 (Macedo et al. 2001). At slightly lower pH (<9.1 to 9.4), the phytoplankton community in this lagoon was more diverse, and other phytoplankton groups co-dominated. Thus, although a few reports suggest that mainly dinoflagellates are associated with extremely high pH in nature, our knowledge on the topic is still very limited, and other groups of algae, especially bloom-forming species, may in the future indeed be found to be associated with high pH.

CONCLUSIONS

pH can rise to very high levels in eutrophic estuaries, lagoons and embayments during summers with high insolation and calm weather. In such environments, pH changes may drive species succession of phytoplankton and impede primary production, because many species are quite sensitive to high pH. The reason for the growth reductions of phytoplankton at high pH needs yet to be assessed.

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