Vol. 319: 103–110, 2006 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published August 18

Photosynthesis-induced phosphate precipitation in seawater: ecological implications for phytoplankton

Lasse M. Olsen*, Murat Öztürk, Egil Sakshaug, Geir Johnsen

Department of Biology, Norwegian University of Science and Technology, Trondheim Biological Station, 7491 Trondheim, Norway

ABSTRACT: A relationship between nutrient concentration, cell density and pH increase due to photosynthesis was established for 3 phytoplankton species, *Prorocentrum minimum* (Dinophyceae), *Phaeodactylum tricornutum* (Bacillariophyceae) and *Tetraselmis* sp. (Prasinophyceae), using batch cultures. Experiments were carried out to find out whether changes in pH induced by photosynthesis can cause precipitation of phosphate (PO_4) in seawater. Nutrient concentrations similar to those of North Atlantic water induced phytoplankton growth, which in turn caused a pH increase of 0.75 U. At pH ≈ 9, up to 20% of the phosphate in our nutrient solutions precipitated. The precipitation was positively correlated with the carbonate concentration. At pH > 9.7, another precipitation reaction, not correlated with carbonate concentration, caused flocculation of particles and a highly efficient PO_4 removal, presumably involving $Mg(OH)_2$. In contrast to the other 2 species that grew at pH ≤ 10.2, *P. minimum* only grew at pH ≤ 9.7, thus surviving high pH without itself causing catastrophic precipitation of phosphate. This could be one reason why *P. minimum* is able to bloom in eutrophic estuarine waters.

KEY WORDS: Phosphate precipitation · *Prorocentrum minimum* · *Phaeodactylum tricornutum* · *Tetraselmis* sp. · Total inorganic carbon · Photosynthesis · pH · Seawater

INTRODUCTION

The average pH of the global surface ocean is 8.2 and the total inorganic carbon (TIC) concentration at a salinity of 34 is 2 to 2.5 mM (Sverdrup et al. 1942). TIC fills the gap that would otherwise exist between the positive and negative charges in seawater. Through a series of dissociation reactions, TIC constitutes a buffer system:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad (1)$$

This system is the most essential determinant for seawater pH under normal conditions. At pH 8.2, approximately 95% of TIC exists as bicarbonate (HCO_3^-). Addition of acid or base skews the system towards CO_2 or carbonate (CO_3^{2-}), respectively (Stumm & Morgan 1996). Photosynthesis (in gross terms, $CO_2 + H_2O \rightarrow CH_2O + O_2$) removes CO_2 from the TIC system, yet many algal species also utilise HCO_3^- as a source of CO_2 (Nimer et al. 1997). Because biological carbon fixation can be faster than the association-dissociation reactions between CO_2 and carbonic acid (H_2CO_3) and between CO_2 and HCO_3^-, photosynthesis disturbs the TIC buffer system, resulting in lower TIC concentration, higher O_2 concentration, elevated pH and a skewing of the buffer system towards CO_3^{2-} (Falkowski & Raven 1997).

Algal photosynthesis creates microenvironments around cells and aggregates of cells, with high pH and O_2 concentration (Richardson & Stoltzenbach 1995). Alkaline and oxidising conditions will initiate or accelerate many precipitation reactions in seawater, such as the oxidation of Mn(II) and Fe(II) (Richardson et al. 1988, Gunnars et al. 2002) and the production of $CaCO_3$ and $Mg(OH)_2$ (Chave & Suess 1970, Neville & Morizot 2002). Surfaces of these precipitates are suited for adsorption of PO_4 and will therefore mediate pre-

*Email: lasse.olsen@nt.ntnu.no

cipitation of PO_4 (Karl & Tien 1992, Slomp & van Raaphorst 1993). The extent to which these processes affect bulk water properties depends on the size of the algal biomass, which in turn is a function of nutrient availability. In addition, phytoplankton creates other surfaces, like the cells themselves or extracellular organic material, where adsorption of PO_4 can take place (Chin et al. 1998, Sanudo-Wilhelmy et al. 2004).

Hansen (2002) showed that the pH range for algal growth varies widely among marine species and that pH therefore can affect the succession of species in natural waters. However, very little is known about why algae differ so widely in terms of pH tolerance. Different explanations are possible, such as being related to the particular CO_2 acquisition mechanism (Nimer et al. 1997) or disturbance of transport across membranes and alteration of intracellular chemistry (Taraldsvik & Myklestad 2000). In the present study we propose that the pH tolerance range might also be related to the ability to take up nutrients in an environment in which the formation of precipitates increases as pH and O_2 concentration increase.

Photosynthesis-induced coprecipitation of PO_4 with carbonates has been observed in freshwater (Murphy et al. 1983, Hartley et al. 1997) but has to our knowledge not been studied explicitly in seawater. We established an empirical relationship between nutrient concentration, cell density and pH increase for 3 phytoplankton species in batch cultures. Phosphate (PO_4) removal experiments measured PO_4 precipitation as a function of pH and CO_3^{2-} concentration in seawater where the chemistry had been modified by phytoplankton metabolism in batch cultures. We combined these results to elucidate whether algal photosynthesis can cause precipitation of PO_4 in natural seawater, and discuss possible ecological implications regarding phytoplankton growth.

MATERIALS AND METHODS

Dilution experiments. Three marine phytoplankton species were used: *Prorocentrum minimum* (Dinophyceae), *Phaeodactylum tricornutum* (Bacillariophyceae) and *Tetraselmis* sp. (Prasinophyceae). A gradient of decreasing nutrient concentration was obtained by successively diluting Guillard's f-recipe (Guillard 1975) from half concentration (f/2) to f/128. Nutrient medium was prepared using pasteurized seawater prefiltered on 0.45 µm glass fibre filters (Whatman) with a salinity of 34. Non-axenic batch cultures were grown in triplicate in the different nutrient concentrations in 50 ml Nunclon tissue culture vessels with airtight caps. The temperature was 15 ± 1°C and the irradiance at the outside of the vessels was 80 µmol photons m^{-2} s^{-1} and continous. The bottles were stirred once per day and pH was measured approximately every other day after stirring using the pH electrode described below. The cultures were terminated early in the stationary phase. Subsamples of 60 ml were filtered through precombusted GF/F filters and the filtrates stored at –20°C for subsequent measurement of dissolved organic carbon (DOC) in a Dorman DC 190 High Temp TOC analyzer. For algal cell counts 5 ml samples were preserved with Lugol's solution to a final concentration of 1%. The cells were counted in a CASY1 Model TTC (Schärfe System) cell counter or in sedimentation chambers in an inverted microscope (Sournia 1981).

Total alkalinity (A_T), TIC and pH. A combined glass electrode with an Ag/AgCl reference electrode (Mettler Toledo) was used for the determination of pH to an accuracy of 0.01 U. The electrode was calibrated using NBS buffer solutions at laboratory temperature (15°C). The apparent activity coefficient of H^+ (f_{H^+}) was determined by 4 point titration of 50 ml seawater by 15 ml standard acid with normalities in the range of 0.008 to 0.014 N HCl. Using Eq. (2), f_{H^+} was found to be 0.8 from the slope of the plot of the acid normalities (N) against $10^{-pH_{(NBS)}}$:

$$N = \frac{A_T \times V_S}{V_A} + \left(\frac{(V_S + V_A)}{V_A} \times \frac{10^{-pH_{NBS}}}{f_{H^+}} \right) \quad (2)$$

where V_S is seawater volume and V_A added standard acid volume. A_T was calculated from Eq. (2) after titration with 0.01 N HCl. TIC was calculated after conversion of the measured pH (pH_{NBS}) to $pH_{total} = -\log([H^+]+[HSO_4^-]) = f_{H^+} \times pH_{NBS}$ (Grasshoff et al. 1999) and using:

$$TIC = \frac{\left(A_T + 10^{-pH_T} - \frac{K_w}{10^{-pH_T}} - \frac{K_B B_T}{K_B + 10^{-pH_T}} \right) \times \left(\frac{(10^{-pH_T})^2}{K_1 + 10^{-pH_T} + K_2} \right)}{10^{-pH_T} + 2K_2} \quad (3)$$

where K_W is the ionic product of water, K_B the dissociation constant of boric acid, B_T the total borate and K_1 and K_2 are the dissociation constants for H_2CO_3 (Grasshoff et al. 1999). Total non-carbonate alkalinity except for the borate contribution (PO_4, silicate, NH_4^+ and HS^-) was <1% of TIC and therefore ignored. The maximum SDs of pH and A_T measurements are ~0.5%, causing a maximum error of approximately 10% in the TIC calculations. The concentrations of CO_2, HCO_3^- and CO_3^{2-} were calculated using equations from Grasshoff et al. (1999).

pH drift experiment with a dense culture of *Phaeodactylum tricornutum*. *P. tricornutum* was precultured in a 600 ml chemostat with f/2 medium under the same irradiance and temperature conditions as for the experimental batch cultures, at a dilution rate of 0.7 d^{-1}.

The chl *a* concentration, measured spectrophotometrically after methanol extraction (Mackinney 1941), had stabilised at approximately 2 mg l^{-1}. Five samples of 50 ml each were transferred to 50 ml Nunclon culture bottles for the pH drift experiment. These bottles were closed with airtight caps. One bottle was used immediately for measurement of pH and total alkalinity by acid titration, as described above. The same variables were also measured after 2, 4, 6 and 8 h, using one of the remaining bottles each time.

Phosphate removal experiments. Fig. 1 depicts the procedure of the PO_4 removal experiments. Three batch cultures of 3.5 l volume were established in 4 l Nalgene polyethylene culture flasks with f/10 medium (Guillard 1975). One culture each of *Tetraselmis* sp. and *Prorocentrum minimum* were stagnant without aeration. One culture of *P. minimum* was bubbled with sterile air to maintain the TIC concentration and pH. The batch cultures were grown until the algae reached stationary phase. Particulate matter, mainly algal cells, was then separated from dissolved fractions by centrifugation at 13 000 × *g* for 5 min. The supernatant was removed and metabolic activity in the particulate matter was stopped by autoclaving it. The particulate matter was then rediluted with filtered and pasteurized seawater to approximately the same volume and concentration as in the batch culture (Fig. 1). The particle suspension from the *P. minimum* cultures contained a mixture of particles from the stagnant and the bubbled cultures. Then A_T, pH and PO_4 concentration were measured in the supernatant and particle suspension, before 10 µM PO_4 was added. In addition, f/10 mineral media were prepared with 10 µM PO_4, one during the experiment with *Tetraselmis* sp. and one during the experiment with *P. minimum*. These solutions were treated exactly like those from the batch cultures, and served as control solutions where seawater chemistry had not been modified by photosynthesis. The solutions were distributed to 30 ml glass bottles and the pH was adjusted by adding 1 M NaOH or 0.45 M HCl. The solutions from the *Tetraselmis* sp. culture were exposed to 4 pH levels (7, 8, 9, 10) and those from the *P. minimum* cultures to 5 levels (8, 9, 9.5, 10, 10.5), the latter in duplicate. The samples were incubated at 15 ± 1°C for 22 h. In the *Tetraselmis* sp. solutions, pH and A_T were measured again after 22 h to enable calculation of the speciation of inorganic carbon in the pH gradient. For the *P. minimum* solutions, the speciation was calculated using the TIC concentration (Table 1) and the percentage distribution obtained from the pH drift experiment with *Phaeodactylum tricornutum* (Fig. 2). All samples were filtered through acid-washed 0.45 µm Whatman GF/F filters before PO_4 was measured according to the method in Koroleff (1976).

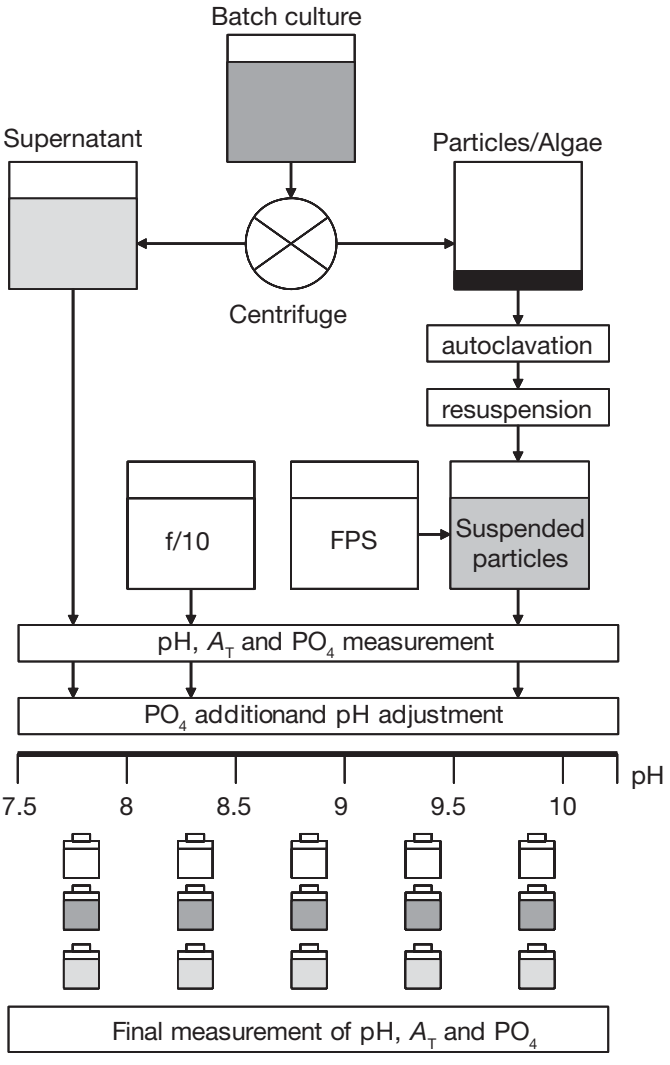

Fig. 1. Schematic overview of the procedure of the phosphate removal experiments. (See 'Materials and methods' for details). A_T: total alkalinity; white bottles: f/10 medium; dark grey bottles: suspended particles; light grey bottles: supernatant; FPS: filtered and pasteurized seawater

RESULTS

Dilution and pH drift experiments

Maximum pH obtained in the batch cultures of *Tetraselmis* sp. was 10.2 (Fig. 2E). The f/32 strength medium (2.25 µM P) was sufficient to reach the upper pH limit for growth. The f/128 medium (0.56 µM P) induced an increase in pH of approximately 0.75 U (Fig. 2E). From earlier experiments we know that the cell density reached at pH limitation is approximately 1×10^5 cells ml^{-1}. A culture with 0.25 µM P (≈f/256) yielded 1×10^4 cells ml^{-1}. At early stationary phase, the DOC concentration in a pH-

Table 1. Total alkalinity (A_T), pH, total inorganic carbon (TIC), carbonate (CO_3^{2-}), bicarbonate (HCO_3^-) and phosphate (PO_4) concentrations in the solutions obtained after centrifugation of the batch cultures of *Tetraselmis* sp. and *Prorocentrum minimum* in the phosphate removal experiments, and in the f/10 mineral nutrient media used as control solutions. nd: no data

	pH	A_T (mM)	[TIC] (mM)	[CO_3^{2-}] (mM)	[HCO_3^-] (mM)	[PO_4] (µM)
f/10 from *Tetraselmis* experiment	7.4	2.1	2.1	0.04	2.0	nd
f/10 from *P. minimum* experiment	7.7	2.1	2.1	0.07	2.0	nd
P. minimum stagnant supernatant	9.6	2.3	1.2	0.85	0.35	2.7
P. minimum bubbled supernatant	8.8	2.5	1.9	0.56	1.3	0.7
Tetraselmis stagnant supernatant	9.7 (10.2)[a]	0.4	0.02	0.016	0.004	0
Particle suspensions *P. minimum*	7.8	1.8	1.7	0.08	1.6	nd
Particle suspensions *Tetraselmis*	7.9	2.5	2.3	0.13	2.2	nd

[a]pH down from the maximum of 10.2

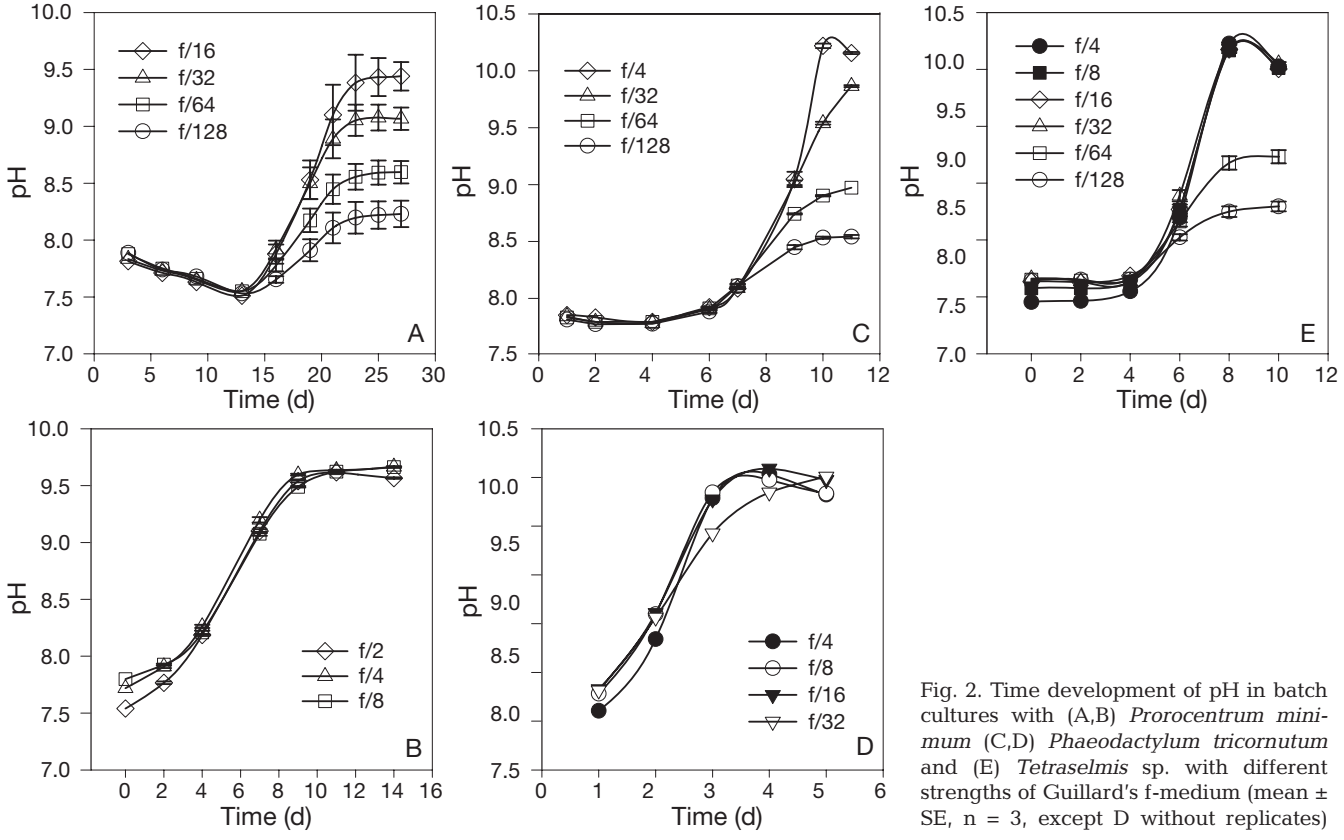

Fig. 2. Time development of pH in batch cultures with (A,B) *Prorocentrum minimum* (C,D) *Phaeodactylum tricornutum* and (E) *Tetraselmis* sp. with different strengths of Guillard's f-medium (mean ± SE, n = 3, except D without replicates)

limited culture was approximately 83 µmol C l^{-1}, in contrast to 16 µmol C l^{-1} in a culture with 0.25 µM P (Olsen et al. 2002), implying that net production of DOC during active growth was 0.83 to 1.67 pmol C $cell^{-1}$.

In the *Prorocentrum minimum* batch cultures, pH never exceeded 9.7 (Fig. 2B). Nutrient enrichment higher than f/16 (4.5 µM P) was required for the cultures to reach the growth-limiting pH. The f/128 medium allowed an increase in pH by approximately

0.6 U (Fig. 2A), with a final cell yield of 2.4×10^4. The cell density at pH limitation of growth was approximately 8×10^4 cells ml^{-1} while the DOC concentrations at early stationary phase ranged from 233 to 742 µmol C l^{-1} in the f/128 to f-medium gradient. Average net DOC produced per algal cell was 5.25 pmol C $cell^{-1}$.

Cell carbon content was 8.3 pmol for *Tetraselmis* sp. (authors' unpubl. results). Because they are in the same size range, this factor was used both for *Tetraselmis* sp. and *Prorocentrum minimum* to calculate carbon biomass. The sum of carbon biomass and DOC in the cultures at the onset of pH limitation was 1 and 1.1 mmol C l^{-1} for *Tetraselmis* sp. and *P. minimum*, respectively.

Phaeodactylum tricornutum exhibited approximately the same upper pH limit for growth as *Tetraselmis* sp. (10.2), which was obtained when the nutrient concentration equalled that of the f/32 medium (Fig. 2C,D). When the very dense chemostat cultures of *P. tricornutum* were transferred to batch-culture bottles without CO_2 supply, pH increased from 8.3 to 10.1 in 8 h. During this time nearly 1 mM of TIC was fixed by photosynthesis, and at pH 10.1 more than 95% of the remaining inorganic carbon was CO_3^{2-} (Fig. 3).

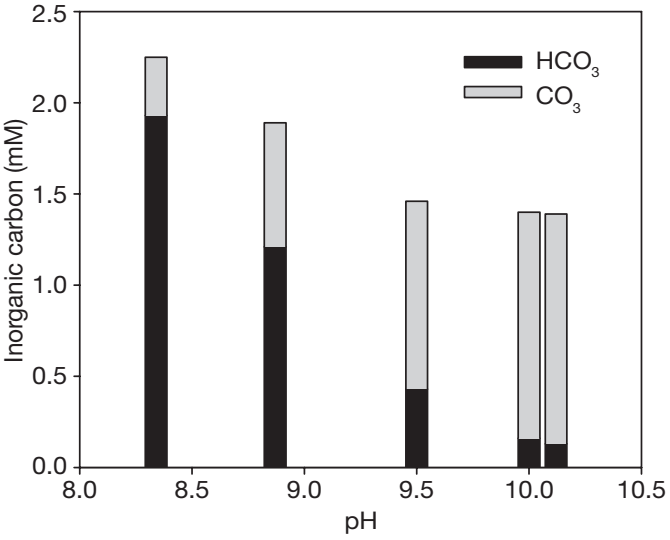

Fig. 3. Changes in the inorganic carbon system due to changes in pH caused by photosynthetic carbon fixation over 8 h in a dense culture (2 mg chl *a* l^{-1}) with *Phaeodactylum tricornutum*

Phosphate removal experiments

The solutions obtained after centrifugation of the batch cultures in the PO_4 removal experiments differed with respect to pH and A_T, thus also with respect to TIC and the equilibrium between CO_3^{2-} and HCO_3^-. In the supernatant from the stationary *Tetraselmis* sp. culture, TIC was only 0.02 mM (Table 1), and no PO_4 was left in the water after centrifugation. This indicates that all remaining CO_3^{2-} and PO_4 had precipitated when the culture reached the maximal pH of 10.2. The pH had decreased from the maximum level of 10.2 to 9.7 in the batch culture before the removal experiment was started. In the *Prorocentrum minimum* cultures, alkalinity changed negligibly in the supernatants, indicating little precipitation of CO_3^{2-}, and most excess PO_4 stayed in solution. The particle suspensions and the f/10 medium had TIC concentrations around 2 mM, typical for natural seawater (Table 1).

Fig. 4 shows PO_4 removal as a function of pH in the different fractions from the batch cultures with the 2 algal species and the f/10 control solutions. After 22 h of incubation, pH had drifted slightly away from the preadjusted values in some of the bottles. Generally, the curves for mineral nutrient medium and particle suspensions were very similar and showed the most efficient removal with increasing pH, but apparently the particle surfaces did not enhance the precipitation (Fig. 4). In the supernatants, a gradient of removal efficiency was observed, highest for the bubbled *Prorocentrum minimum* culture, medium for the stagnant *P. minimum* culture and the lowest for the stagnant *Tetraselmis* sp. culture (Fig. 4). Table 1 shows that there was a gradient in TIC with the least in the *Tetraselmis* sp. supernatant, and the most in the mineral media and the particle suspensions. The *Tetraselmis* sp. supernatant was almost stripped of inorganic carbon, and no PO_4 was removed at any pH up to 10.2 (Fig. 4).

In Fig. 5, data from all PO_4 removal experiments are compiled. Fig. 5A shows that PO_4 removal was initiated at pH 9, and at pH between 9 and 10, 0 to 40% of the PO_4 had disappeared from solution. Data with high removal (70 to 100%) clustered within a narrow pH range of 9.9 to 10.2 (Fig. 5A). Fig. 5B shows that the high removal data were scattered over a wide range of CO_3^{2-} ion concentrations.

DISCUSSION

Photosynthesis-induced pH increase and phosphate precipitation

The results from the dilution experiments and the pH drift experiment demonstrate that when approximately 1 mM of TIC was fixed by photosynthesis in a closed or semi-closed system with full salinity seawater, pH increased to 9.5–10 and the inorganic carbon left is mainly CO_3^{2-} (Fig. 3). Respiration in the algae and other organisms, however, counteract the increase in pH. In a mesocosm with a natural plankton community, 2.4 µM PO_4 (≈f/32) was added gradually over 18 d, leading to an increase in pH from 8.2 to 8.75 (Öztürk et

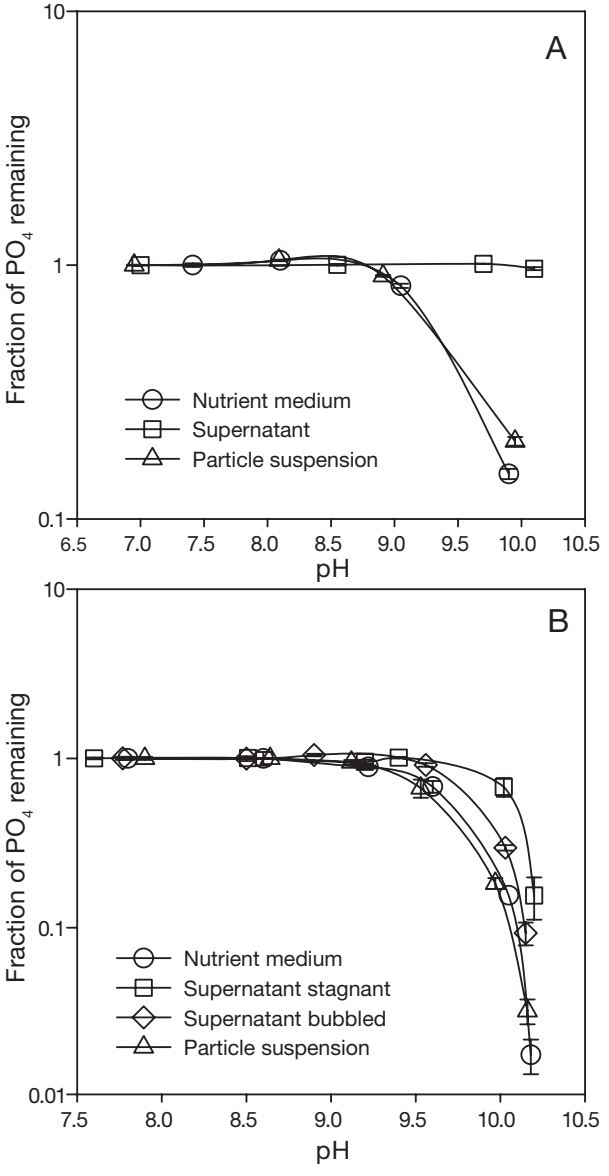

Fig. 4. Results from the phosphate removal experiments with the different fractions from batch cultures with (A) *Tetraselmis* sp. (mean ± SE, n = 2 replicate samples and (B) *Prorocentrum minimum*. All data were normalized to the value at the lowest pH (mean ± SE, n = 2 replicate bottles)

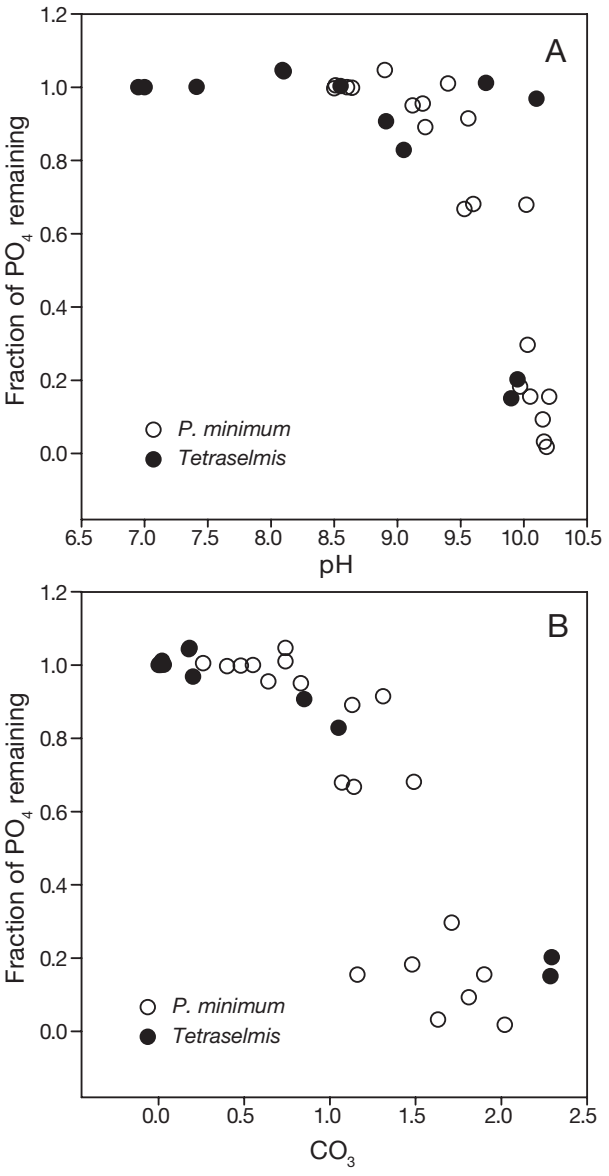

Fig. 5. Compilation of data from all phosphate removal experiments. (A) The fraction of phosphate removed as a function of pH in all solutions and (B) carbonate concentration. All data were normalized to the value at the lowest pH

al. 2003). In batch cultures of algae and associated bacteria, nutrient concentration of this magnitude induced a rise in pH of approximately 2 U (Fig. 2).

Initial TIC concentration (Table 1) could explain the observed differences in PO_4 removal at increased pH in the different seawater solutions used in our experiments, with the highest removal efficiency for highest TIC concentration (Fig. 4). This suggests an important role for CO_3^{2-} in the PO_4 precipitation reactions. The gap between the data showing low-to-moderate PO_4 removal and data showing large removal (Fig. 5) suggests that at least 2 different precipitation reactions took place. Fig. 5B shows that the first reaction caused an increase in PO_4 precipitation with increasing CO_3^{2-} concentration. The second reaction took place only in high-CO_3^{2-} environment, but the CO_3^{2-} concentration itself did not affect the extent of PO_4 precipitation. According to calculations using activity coefficients, precipitation of $CaCO_3$ has a pH of saturation around 7.5 in seawater at the temperature used in our experiments, whereas for $Mg(OH)_2$ it is approximately 9.5 (Neville & Morizot 2002). Thermodynamically, precipitation is possible above this pH.

Since surface seawater, however, is supersaturated with $CaCO_3$ by a factor of 7 because other ions and dissolved organic material inhibit precipitation (Chave & Suess 1970, Neville & Morizot 2002), only high pH (high CO_3^{2-} concentration) can initiate $CaCO_3$ precipitation. The initial PO_4 precipitation reaction in our experiments was at least partly associated with precipitation of $CaCO_3$. However, precipitation of Mn and Fe oxides probably also contributed (Schoemann et al. 2001, Gunnars et al. 2002). The second precipitation reaction was probably caused by the formation of brucite ($Mg(OH)_2$). Brucite particles are positively charged at the pH in question and therefore attract particles that are negatively charged, such as $CaCO_3$ and algal cells. This type of flocculation can effectively remove both particulate and dissolved material, as well as ions such as PO_4 (Semerjian & Ayoub 2003). We observed that the water became almost totally clear in some of the batch cultures when pH was around 10. The rapid decrease in pH after the maximum pH of 10.2 was reached in the *Tetraselmis* sp. and *Phaeodactylum tricornutum* cultures (Fig. 2C–E) could be an indication that $Mg(OH)_2$ precipitated, thus removing some OH^- from the solution.

In typical Norwegian coastal water the PO_4 concentration during winter is approximately 0.5 µM (Öztürk et al. 2003), which corresponds to the f/128 medium that caused a pH increase of 0.6 to 0.75 U in batch culture (Fig. 2). In surface seawater with an average pH of 8.2, this can potentially result in a rise of pH to 8.8–8.95 during an algal bloom, given that grazing is negligible. At pH 9, up to 20% of the PO_4 precipitated in our bottles. If the extent of PO_4 adsorption is dependent on the number of available binding sites, a much higher percent would precipitate in a PO_4-poor environment. During a *Phaeocystis* sp. bloom in the North Sea, a pH increase from 7.9 to 8.7 was registered (Brussaard et al. 1996). It has been shown that Mn precipitates in the high pH/high O_2 environment of *Phaeocystis* colonies (Schoemann et al. 2001) and that PO_4 can accumulate in the matrix of these colonies (Veldhuis et al. 1991). *Phaeocystis* colonies admittedly represent a special case, but it is possible that polymeric material released by other algae during a bloom could form a similar but looser matrix with embedded $CaCO_3$, Mn and Fe-oxides onto which PO_4 could adsorb (Chin et al. 1998). We chose to use seawater that had been modified by metabolic activities in phytoplankton batch cultures in order to approach the properties of natural seawater, while still being able to manipulate the inorganic carbon system. One natural next step would be to elucidate how extracellular organic material may modify the precipitation reactions induced by photosynthesis. A recent study showed that a substantial amount of PO_4 may adsorb to living phytoplankton cells (Sanudo-Wilhelmy et al. 2004). We used autoclaved algal cells to avoid further metabolic activity during the PO_4 removal experiments. These particle surfaces did not enhance the precipitation, which suggests that adsorption to phytoplankton cells is enhanced by the photosynthetic activity of the cells.

Ecological implications for phytoplankton

In eutrophic areas, such as the shallow and brackish Mariager fjord in Denmark, a pH of 9 or higher was prevalent from May to August, with a maximum of 9.75 (Hansen 2002). In such waters, the precipitation of PO_4 can presumably become substantial (Fig. 5). Lower salinity, and thus also lower alkalinity and TIC concentration, may modify the conclusions from our experiments with full salinity seawater. Hansen (2002) argued that the ability to tolerate pH increase could affect algal species succession, thus explaining why *Prorocentrum minimum* frequently form blooms in Mariager fjord. In agreement with Hansen (2002), our results indicated that *P. minimum* has an upper pH limit for growth of 9.6 to 9.7, higher than many other algal species yet just below the pH at which fatal precipitation through flocculation starts (Fig. 5A). It appears, thus, that these dinoflagellates can sustain themselves at a pH that is lethal to other species yet avoid ultimate death by phosphorus starvation through self-induced precipitation.

Phaeodactylum tricornutum is often found in inshore waters and rock pools (Droop 1953). In enclosed tidal pools the pH can often reach 10 or higher due to photosynthesis in macro- and microalgae (Schmidt-Nielsen 1991). With a pH limit for growth at 10.2, *P. tricornutum* seems to be well adapted to such environments.

Tetraselmis sp. is common in coastal and inshore waters (Massana et al. 2004), but as far as we know no bloom of these algae has ever been reported. Its high pH tolerance enables survival in tide pools and ponds. But such high pH limits for growth are probably of no particular advantage in open seawater, since pH around 10 will only induce precipitation and possibly make PO_4 and some other essential minerals inaccessible.

Acknowledgements. We thank S. Kirkvold for DOC analyses. This work was supported by the Norwegian Research Council (Contract: 143511/213) via Promar AS, Oslo, Norway.

LITERATURE CITED

Brussaard CPD, Gast GJ, van Duyl FC, Riegman R (1996) Impact of phytoplankton bloom magnitude on a pelagic microbial food web. Mar Ecol Prog Ser 144:211–221

Chave KE, Suess E (1970) Calcium carbonate saturation in seawater: effects of dissolved organic matter. Limnol Oceanogr 15:633–637

Chin WC, Orellana MV, Verdugo P (1998) Spontaneous assembly of marine dissolved organic matter into polymer gels. Nature 391:568–572

Droop MR (1953) On the ecology of flagellates from some brackish and freshwater rockpools of Finland. Acta Bot Fenn 51:1–52

Falkowski P, Raven JA (1997) Aquatic photosynthesis. Blackwell, Oxford

Grasshoff K, Kremling K, Ehrhardt M (1999) Methods of seawater analysis. Wiley-VCH, Weinheim

Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum, New York, p 29–60

Gunnars A, Blomquist S, Johansson P, Andersson C (2002) Formation of Fe(III) oxyhydroxide colloids in freshwater and brackish seawater, with incorporation of phosphate and calcium. Geochim Cosmochim Acta 66:745–758

Hansen PJ (2002) Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. Aquat Microb Ecol 28:279–288

Hartley AM, House WA, Callow ME, Leadbeater BSC (1997) Coprecipitation of phosphate with calcite in the presence of photosynthesizing green algae. Water Res 31:2261–2268

Karl DM, Tien G (1992) MAGIC: a sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnol Oceanogr 37(1):105–116

Koroleff F (1976) Determination of phosphorus. In: Grasshoff K (ed) Methods in seawater analysis. Verlag Chemie, Weinheim, p 117–125

Mackinney G (1941) Absorption of light by chlorophyll solutions. J Biol Chem 140:315–322

Massana R, Balague V, Guillou L, Pedros-Alio C (2004) Picoeukaryotic diversity in an oligotrophic coastal site studied by molecular and culturing approaches. FEMS Microbiol Ecol 50:231–243

Murphy TP, Hall KJ, Yesaki I (1983) Coprecipitation of phosphate with calcite in a naturally eutrophic lake. Limnol Oceanogr 18(1):58–69

Neville A, Morizot AP (2002) Calcareous scales formed by cathodic protection — an assessment of characteristics and kinetics. J Crystal Growth 243:490–502

Nimer NA, Iglesias-Rodriguez MD, Merret MJ (1997) Bicarbonate utilization by marine phytoplankton species. J Phycol 33:625–631

Olsen LM, Reinertsen H, Vadstein O (2002) Can phosphorus limitation inhibit dissolved organic carbon consumption in aquatic microbial food webs? A study of three food web structures in microcosms. Microb Ecol 43:353–366

Öztürk M, Vadstein O, Sakshaug E (2003) The effects of enhanced phytoplankton production on iron speciation and removal in mesocosm experiments in a landlocked basin of Hopavågen, Norway. Mar Chem 84:3–17

Richardson LL, Stolzenbach KD (1995) Phytoplankton cell size and the development of microenvironments. FEMS Microbiol Ecol 16:185–192

Richardson LL, Aguilar C, Nealson KH (1988) Manganese oxidation in pH and O_2 microenvironments produced by phytoplankton. Limnol Oceanogr 33(3):352–363

Sanudo-Wilhelmy SA, Tovar-Sanchez A, Fu FX, Capone DG, Carpenter EJ, Hutchins DA (2004) The impact of surface-adsorbed phosphorus on phytoplankton Redfield stochiometry. Nature 432:897–901

Schmidt-Nielsen K (1991) Animal physiology. Cambridge University Press, Cambridge

Schoemann V, Wollast R, Chou L, Lancelot C (2001) Effects of photosynthesis on the accumulation of Mn and Fe by *Phaeocystis* colonies. Limnol Oceanogr 46(5):1065–1076

Semerjian L, Ayoub GM (2003) High-pH magnesium coagulation-flocculation in waste water treatment. Adv Environ Res 7:389–403

Slomp CP, van Raaphorst W (1993) Phosphate adsorption in oxidized marine sediments. Chem Geol 107:477–480

Sournia A (1978) Phytoplankton manual. UNESCO monographs on oceanographic methodology No. 6, Paris

Stumm W, Morgan JJ (1996) Aquatic chemistry, 3rd edn. Wiley, New York

Sverdrup HU, Johnson MW, Fleming RH (1942) The Oceans: their physics, chemistry, and general biology. Prentice Hall, NJ

Taraldsvik M, Myklestad SM (2000) The effect of pH on growth rate, biochemical composition and extracellular carbohydrate production of the marine diatom *Skeletonema costatum*. Eur J Phycol 35:189–194

Veldhuis MJV, Colijn F, Admiraal W (1991) Phosphate utilization in *Phaeocystis pouchetii* (Haptophyceae). PSZN I: Mar Ecol 12:53–62

Editorial responsibility: Victor N. de Jonge (Contributing Editor), Haren, The Netherlands

Submitted: April 25, 2004; Accepted: April 9, 2006
Proofs received from author(s): July 14, 2006