Influence of bacteria on phytoplankton cell mortality with phosphorus or nitrogen as the algal-growth-limiting nutrient

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ABSTRACT: The effects of bacteria on phytoplankton mortality were studied with phosphorus or nitrogen as the algal-growth-limiting nutrients. Experiments were performed with the diatom *Ditylum brightwellii* using batch cultures, steady state continuous cultures and batch-mode cultures which were starved for the limiting nutrient after being preconditioned in a chemostat. With phosphorus limiting algal growth, the specific death rates of *D. brightwellii* generally increased upon bacterial inoculation. Bacteria enhanced algal mortality very likely due to competition with *D. brightwellii* for the limiting phosphate. With nitrogen as the algal-growth-limiting nutrient, however, the presence of bacteria had either no pronounced effect on or led to a reduction of the specific death rates of *D. brightwellii*. Remineralized ammonium was probably partly utilized by N-starved cells of *D. brightwellii*, leading to reduced death rates of the algal cells. Bacteria thus indirectly prolonged survival of *D. brightwellii* populations under N starvation. This study shows that bacteria can affect phytoplankton survival, which in turn may influence algal species succession. The degree of bacterial influence on algal death kinetics depends largely on the type of nutrient limiting algal growth and on the culture conditions.

KEY WORDS: Algal cell mortality · *Ditylum brightwellii* · Bacteria · N and P deficiency · Starvation

INTRODUCTION

In the field, phytoplankton populations experience loss of cells due to grazing, sinking and cell lysis. Under axenic culture conditions, algal cell lysis was found to be directly affected by the degree of nutrient deficiency (Brussaard et al. 1997). However, phytoplankton under natural growth conditions will have to compete for the growth-limiting nutrient with bacteria. Laboratory work by Currie & Kalff (1984) and Bratbak (1987) suggested that bacteria are better competitors for inorganic phosphorus than algae. Bratbak (1987) found that the diatom *Skeletonema costatum* is negatively affected by bacterial activity under phosphorus depletion. Small bacteria, with a higher surface:volume ratio, have been reported to have the highest

affinity for the limiting dissolved orthophosphate (Thingstad et al. 1993). Accordingly, bacteria would be also more efficient competitors for ammonium, but whether bacteria also promote extra algal mortality when ammonium is the algal-growth-limiting substrate is still unknown.

Under ammonium or phosphate limitation, bacteria will only be superior to phytoplankton when organic carbon is present in sufficiently high amounts to prevent C limitation (Bratbak & Thingstad 1985, Rothhaupt 1992, Kirchman 1994, Ietswaart & Flynn 1995). Bacterial growth rates in marine ecosystems are often considered to be carbon rather than mineral nutrient limited (Bratbak & Thingstad 1985, Kirchman et al. 1990, Brussaard et al. 1995). Besides exudation of organic components, interpreted as overflow of photosynthetically fixed carbon (photosynthetic extracellular release, PER; Mague et al. 1980, Bjørnsen 1988, Myklestad et al. 1989, Obernosterer & Herndl 1995),

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algal cellular components released through cell lysis may be an important C source for bacteria. Several field studies suggested that phytoplankton cell lysis products are potential C sources for bacteria, allowing balancing of their C budgets (Lancelot & Billen 1985, Riemann & Søndergaard 1986, Christoffersen et al. 1990, Brussaard et al. 1996). Laboratory studies on microbial decomposition of phytoplankton cells showed rapid mineralization rates (Fukami et al. 1981, Newell et al. 1981, Garber 1984, Hansen et al. 1986, Biddanda 1988). Bacterial mineralization of the relatively N- and P-rich lysed algal cellular components (Garber 1984, Caron et al. 1988, Jürgens & Güde 1990, Goldman & Dennett 1991) might also be an important process to supply algae with ammonium or phosphate, potentially influencing algal survival and species succession under nutrient deficiency.

This report presents data on the bacterial influence on phytoplankton cell mortality with either phosphorus (P) or nitrogen (N) as the algal-growth-limiting substrate. The experiments were performed with algal populations in batch cultures, in steady state continuous cultures, and with algal populations precultured in continuous cultures before being starved by switching off the medium supply. Batch cultures start with high concentrations of inorganic nutrients, which may resemble early spring bloom situations typical for temperate waters as argued by Bratbak (1987). In field situations, algae may also encounter prolonged periods of nutrient limitation and exhaustion (e.g. summer periods in coastal areas or oligotrophic waters). Such conditions are not well represented by ordinary batch cultures in which the physiological state of the algal cells constantly changes, but are better mimicked by nutrient-limited continuous cultures and by nutrient-starvation experiments with algal cultures preconditioned under nutrient limitation in continuous cultures.

MATERIAL AND METHODS

Batch culture systems. Batch culture experiments were performed with the axenic diatom *Ditylum brightwellii* (West) Grunow (#358 Bigelow culture collection). During earlier experiments with this axenic *D. brightwellii* strain, no virus-like particles were observed extra- and intracellularly using transmission electron microscopy. To create xenic cultures of *D. brightwellii* (unialgal cultures containing bacteria), axenic cultures (75 ml each) were inoculated directly at the start of the experiments with a mixed community of marine bacteria. The latter was obtained from xenic cultures of *D. brightwellii* (originally isolated from Dutch coastal waters) at the end of the exponential phase by filtration through a sterile glass fiber filter

(GF/C Whatman, approximately 50 % of total bacterial cells passed through the filter). The filtrate (5 ml) was used as bacterial inoculum for the axenic culture of *D. brightwellii*. The mixed bacterial community (dominated by rods) thus consisted of a consortium of bacterial species presumably adapted to the specific culture conditions. The concentrations of bacteria at the start of the experiments were between 0.25 and 0.4×10^6 ml^{-1} ($\approx$ 20 to 30 ng C ml^{-1}, using a conversion factor of 0.22×10^{-12} g C μm^{-3} biovolume). The axenic cultures received 5 ml of 0.2 µm filtrate (sterile cellulose acetate disposable filters, Schleicher & Schuell) containing no bacteria. No heterotrophic nanoflagellates grew in the cultures.

Because pH values varied between 8.0 and 8.7 in these batches, the experiments were repeated including cultures buffered with 20 mM HEPES at pH 8.0 ± 0.1. This pH range had no significant effect on algal growth and mortality under either phosphate, ammonium or nitrate depletion (for all limitations p > 0.1; data not shown). Time interval between the duplicate unbuffered experiments was 1 mo. A list of culture conditions is presented in Table 1.

Media were based on aged, nutrient-poor, 0.2 µm filtered, autoclaved seawater from the Central North Sea, acidified with HCl (pH < 3.5). The algal-growth-limiting factor was either NH_4^+ (concentration at the start of the experiments was 15 µM), NO_3^- (starting concentration 15 µM) or PO_4^{3-} (starting concentrations 10 and 2.5 µM). Concentrations of the other macronutrients in the sterile medium were 2.7 mM $NaHCO_3$, 150 µM $Na_2SiO_3 \cdot 9H_2O$, and either 300 µM N (150 µM NH_4Cl and 150 µM $NaNO_3$) or 25 µM $NaH_2PO_4^{3-}$, depending on the type of limiting nutrient. The basic medium was enriched with micronutrients and vitamins according to Brussaard et al. (1997). The pH of the medium was aseptically adjusted to 8.0 ± 0.1. Light was provided in a 16/8 h light/dark cycle at a density of 100 to 125 µmol photons $m^{-2} s^{-1}$. Temperature was kept at 13°C. Batch cultures were gently mixed twice a day by hand, which gave the most reliable results compared to the use of several other mixing devices. Axenic algal cultures were checked for bacterial contamination at least once a week by epifluorescence microscopy after staining with acridine orange.

Continuous culture systems. Continuous culture experiments were conducted with *Ditylum brightwellii* (Table 1). The 900 ml cultures were held at 13.0 ± 0.2°C under a 16/8 h light/dark regime, receiving 100 to 125 µmol photons $m^{-2} s^{-1}$ during the light period. Autoclaved, aged, nutrient-poor seawater was enriched with sterile $NaHCO_3$ (2.7 mM final concentration), $NaSiO_3$ (300 µM), trace metals and vitamins (Brussaard et al. 1997). Phosphorus-limited media contained 3 µM NaH_2PO_4 and 300 µM nitrogen ($NaNO_3$ and NH_4Cl

at 150 µM each). Nitrogen-limited media contained 50 µM N (NaNO₃ and NH₄Cl, 25 µM each) and 50 µM NaH₂PO₄. The pH of the medium was adjusted to 8.0 ± 0.1. Cultures were mixed at 30 rpm by a glass clapper hanging just above the bottom of the vessel. The cultures were allowed to run for at least 4 volume changes and were considered to be at steady state when algal cell concentrations (total and dead cells) showed no significant changes (<5% standard deviation) for at least 5 d. Dilution rate (D, d^{-1}) under N limitation was 0.22 d^{-1}. Because a strong effect of the physiological state of the algal cells on algal lysis rates had been found for axenic *D. brightwellii* populations cultured in P-limited chemostats at D between 0.28 and 0.42 d^{-1} (Brussaard et al. 1997), D under P limitation was chosen at the lower and the upper limit of this range (0.24 and 0.44 d^{-1}). Ambient concentrations of the limiting nutrients at steady state were <0.26 µM PO₄³⁻ in the P-limited chemostats and 0.43 µM NH₄⁺ and 0.99 µM NO₃⁻ in the N-limited chemostat. Average cell volumes in axenic *D. brightwellii* cultures at steady state were 6000 µm³ under P limitation and 25000 µm³ under N limitation. Axenic algal continuous cultures at steady state received 10 ml GF/C Whatman filtrate containing a mixed community of bacteria, generally selected in xenic batch cultures of *D. brightwellii*. Bacterial cell concentrations in the continuous cultures directly upon inoculation were around 0.1 × 10⁶ ml^{-1} (±10 ng C ml^{-1}).

After new steady states had been established (3 to 4 culture volume changes of the medium), the medium supply was stopped (by turning off the pump) to study the influence of bacteria on algal cell mortality under nutrient starvation (batch-mode cultures; Table 1). The results from these batch-mode starvation experiments were compared with the data on cell lysis kinetics of axenic cultures of *Ditylum brightwellii* (Brussaard et al. 1997).

Sampling and analyses. Algal cells were preferably counted every day, but at least once every 2 d. Samples were taken 2 to 3 h into the light period, fixed with acid Lugol/glutaraldehyde solution (v/v, final concentration 15 to 20%), and counted by inverse light microscopy (Zeiss) after settling on the bottom of multiwell plates. At least 300 algal cells were counted. Cells with empty frustules were counted as dead. Since dead cells of *Ditylum brightwellii* often broke into parts; 2 end parts were counted as 1 dead cell. Batch experiments with *D. brightwellii* were ended after 1 mo or as soon as the percentage of dead cells had reached >35% of total cell concentrations.

Specific algal growth (μ, d^{-1}) and algal cell death rates (δ, d^{-1}) at steady state in the chemostats were calculated according to Brussaard et al. (1997):

$$\delta = \frac{D \cdot y^{\bullet}}{x^{\bullet}} \cong \frac{D \cdot (x+y)^{\bullet}}{x^{\bullet}} - D \qquad (1)$$

$$\mu = D + \delta \qquad (2)$$

where D is the dilution rate, $x^{\bullet}$ and $y^{\bullet}$ are the concentrations of living and dead cells (l^{-1}) at steady state, and $(x + y)^{\bullet}$ is the total algal cell concentration (l^{-1}).

Table 1. List of experiments performed in the present study. All experiments were performed without (axenic algal culture) and with (xenic cultures) addition of bacteria. The concentrations of the nutrients limiting algal growth presented here are at the start of the experiments (batch and batch-mode cultures) or at steady state (continuous cultures)

Type of experiment	Type of nutrient limiting algal growth	Nutrient concentration (µM)	Buffered with 20 mM HEPES	pH during experiment	Replicates
Batch ('nutrient depletion')	Phosphate	2.5	No	8.0–8.7	Duplicate
	Phosphate	10	No	8.0–8.7	Duplicate
	Ammonium	15	No	8.0–8.7	Duplicate
	Nitrate	15	No	8.0–8.7	Duplicate
Batch ('nutrient depletion')	Phosphate	2.5	Yes	8.0±0.1	Single
	Phosphate	10	Yes	8.0±0.1	Single
	Ammonium	15	Yes	8.0±0.1	Single
	Nitrate	15	Yes	8.0±0.1	Single
Continuous culture ('nutrient limitation')	Phosphate	<0.3 (3) [a]	No	8.0–8.7	Single
	Nitrogen (ammonium and nitrate)	<1.5 (50) [a]	No	8.0–8.7	Single
Batch mode ('nutrient starvation')	Phosphate	<0.3	No	8.0–8.7	Single
	Nitrogen (ammonium and nitrate)	<1.5	No	8.0–8.7	Single

[a] Concentrations of limiting nutrients in the medium vessel given in parentheses

Aquat Microb Ecol 14: 271–280, 1998

For the batch experiments, specific algal growth rates (μ_b, d^{-1}) and cell death rates (δ_b, d^{-1}) were calculated according to Brussaard et al. (1997) using the formula:

$$\delta_b = \frac{\ln x_t - \ln x_0}{t \cdot \left(\dfrac{(x+y)_t - (x+y)_0}{y_t - y_0} - 1 \right)} \quad (3)$$

$$\mu_b = \delta_b \frac{(x+y)_t - (x+y)_0}{y_t - y_0} \quad (4)$$

where x is the concentration of living cells (l^{-1}), $(x + y)$ is the total cell concentration (l^{-1}), and y is the concentration of dead cells (l^{-1}). The total concentration of cells at time t is represented by $(x + y)_t$ and the concentration of dead cells at time t by y_t. Time intervals of 24 h were used to calculate μ_b and δ_b.

Bacteria were counted on 0.2 μm polycarbonate membrane filters (Poretics) by the acridine orange direct count method of Hobbie et al. (1977). After being filtered at 20×10^3 Pa over 0.2 μm polysulfone filters (Acrodisc) and stored at −50°C or 4°C (in the case of silicate), the ambient concentrations of dissolved inorganic nitrogen, phosphate and silicate were analyzed within 1 mo after collection on a TrAAcs 800 autoanalyzer (Technicon).
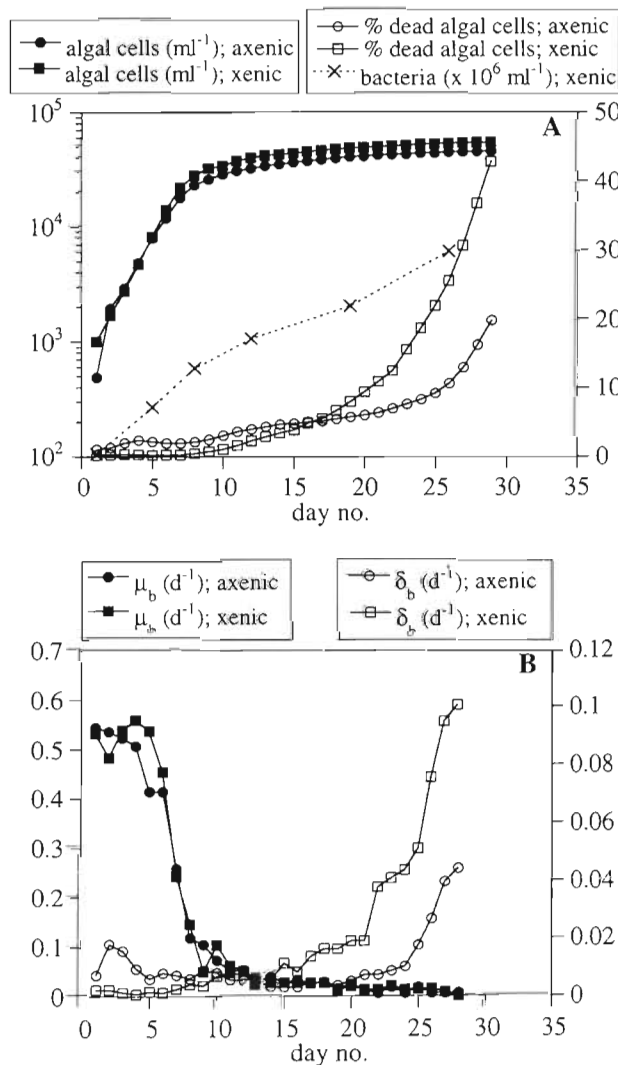

Fig. 1. Variations of (A) total algal cell concentrations, percentages of dead algal cells, bacterial cell concentrations and (B) specific algal growth (μ_b) and cell death rates (δ_b) in axenic and xenic batch cultures of *Ditylum brightwellii* with phosphate as the algal-growth-limiting nutrient. The ambient concentration of phosphate at the start of the experiments was 10 μM. (○, ●) Axenic cultures; (□, ■) xenic cultures
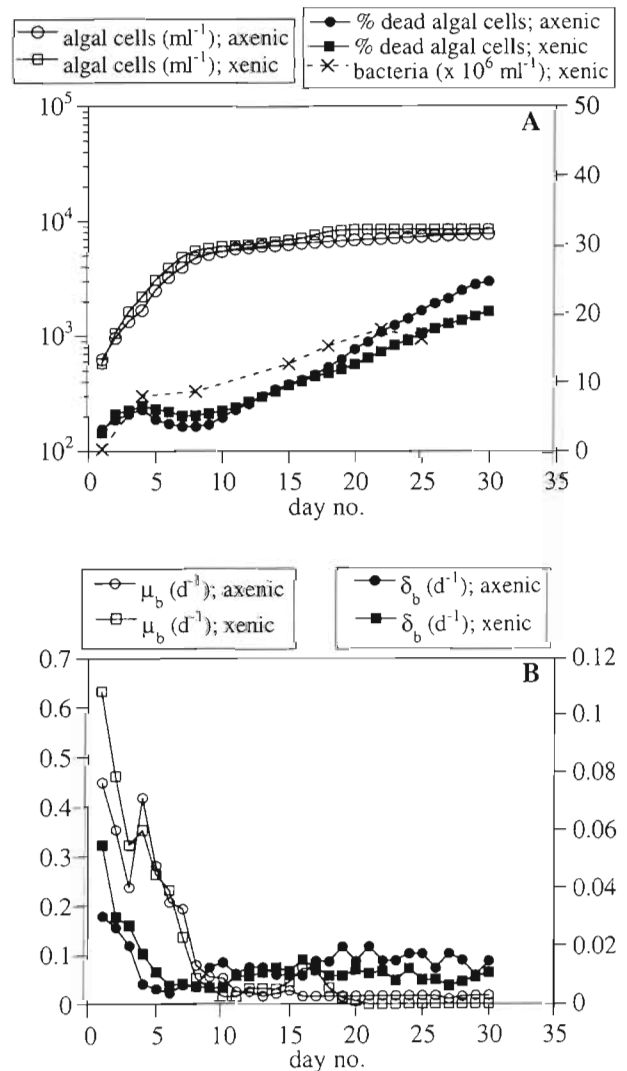
Fig. 2. Variations of (A) total algal cell concentrations, percentages of dead algal cells, bacterial cell concentrations and (B) specific algal growth (μ_b) and cell death rates (δ_b) in axenic and xenic batch cultures of *Ditylum brightwellii* with ammonium as the algal-growth-limiting nutrient. The ambient concentration of ammonium at the start of the experiments was 15 μM. (○, ●) Axenic cultures; (□, ■) xenic cultures

RESULTS

Batch cultures

Figs. 1 & 2 show representative examples of the variations in concentrations of total algal cells, percentages of dead algal cells, bacterial abundances, δ_b and μ_b during batch culture experiments with phosphate or ammonium as the algal-growth-limiting nutrient (phosphate depletion and ammonium depletion, respectively). Bacterial cell concentrations were generally lower in the cultures with N limiting algal growth compared to P limiting algal growth. Maximum bacterial biomass was 1.1 and 2.7 µg C ml^{-1} under ammonium and phosphate depletion, respectively.

Because the batch cultures were performed in duplicate, we were able to test the statistical significance of the different treatments on the algal cell death and growth kinetics. For each batch culture the specific algal cell death rates (δ_b, d^{-1}) of the last 10 d of the experiment were averaged. Maximum growth rates and gradients of the decline in algal growth rates were used to test the significance of bacterial presence on algal growth kinetics. Both the treatment using different types of nutrients limiting algal growth (phosphate, ammonium and nitrate) and the treatment without (axenic) and with (xenic) bacteria had significant effects on the average algal cell death rates (Table 2). No significant variance was found for the algal growth kinetics among the different treatments (data not shown). Bacterial addition to axenic batch cultures of *Ditylum brightwellii* led to increased δ_b when P was limiting algal growth, independent of the concentration of phosphate at the start of the experiments (Fig. 3). Under ammonium depletion, the presence of bacteria had no significant effect on δ_b, whereas under nitrate depletion a significant decline in δ_b was recorded in the xenic compared to the axenic cultures (Fig. 3).
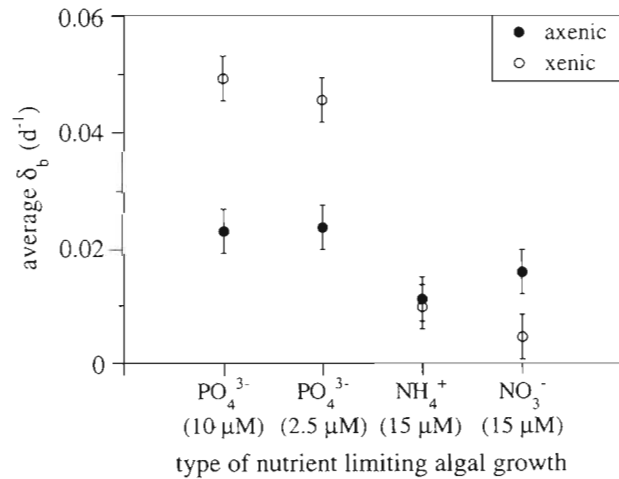

Fig. 3. Least squares means of specific algal death rates (δ_b, calculated according to Eq. 3) from duplicate batch culture experiments with phosphate, ammonium or nitrate as the algal-growth-limiting nutrient. Concentrations of limiting nutrients at the start of the experiments were 10 and 2.5 µM phosphate, and 15 µM ammonium and 15 µM nitrate, respectively. For each batch culture, the algal death rates were averaged over the last 10 d of the experiments to allow statistical analysis. (●) Axenic algal cultures; (○) xenic (containing algae and bacteria) cultures. Overall standard errors are based on the residual mean square (error) presented in Table 2

Continuous cultures

In contrast to batch cultures, chemostats represent growth conditions under nutrient limitation with a restricted but continuous supply of the limiting nutrient. Organisms cultured in chemostats adapt to nutrient limitation, resulting in balanced growth (steady state). Addition of bacteria to algal continuous cultures can be expected to disturb algal steady state because bacteria will compete with algae for the limiting nutrient.

Upon inoculating continuous cultures of *Ditylum brightwellii* with bacteria, new steady states established after several volume changes. Specific algal cell death rates at the newly established situations of balanced growth (δ, d^{-1}) were comparable to δ at axenic steady states (Fig. 4). However, algal cell mortality in the P-limited cultures firstly increased 2-fold directly upon inoculation with bacteria (max. $\delta = 0.108$ and 0.013 d^{-1} at $D = 0.24$ and 0.44 d^{-1}, respectively). For $D = 0.24$ d^{-1} this resulted finally in a 2-fold decrease in algal cell concentration (Fig. 4). Algal cell concentration under N limitation had increased slightly (Fig. 4, $D = 0.22$ d^{-1}), whereas the diameter of the algal cells had become smaller (up to 2-fold) during the establishment of the new equilibrium (Fig. 4). Bacter-

Table 2. *Ditylum brightwellii*. Statistical analysis of variance for specific cell death rates in (unbuffered) batch cultures (n = 16). Experiments were performed in duplicate. Specific algal death rates were averaged over the last 10 d of the experiments. Treat A: treatment with different types of nutrients limiting algal growth (phosphate, ammonium and nitrate); Treat B: treatment without (axenic) and with (xenic) bacteria present in the algal cultures. Error: residual mean square giving an estimation of the error variance

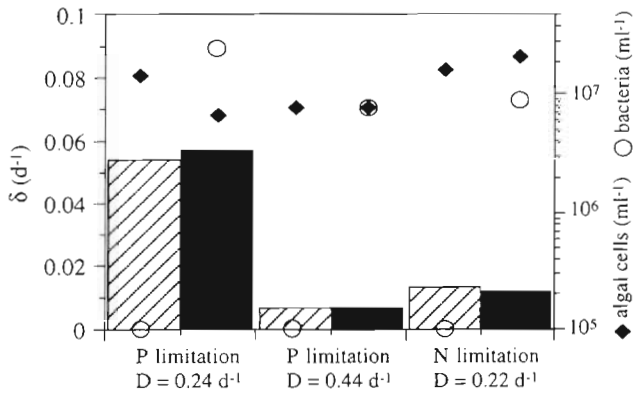| Source	SS	df	MS	F	p
Treat A	0.00251	3	0.00084	28.352	0.00013
Treat B	0.00032	1	0.00032	10.863	0.01093
Treat A × Treat B	0.00099	3	0.00033	11.177	0.00312
Error	0.00024	8	0.00003		

Fig. 4. Total algal cell concentrations of *Ditylum brightwellii*, bacterial cell concentrations and specific algal cell death rates (δ) during steady state in P- and N-limited continuous cultures. Algal cell death rates were calculated according to Eq. (1). Steady state growth rates ($\mu = \delta + D$) were 0.30 and 0.45 d^{-1} under P limitation and 0.23 d^{-1} under N limitation. Striped bars represent the axenic cultures and black bars the xenic cultures

ial abundance reached highest levels under P limitation at D = 0.24 d^{-1} (Fig. 4). Upon addition of bacteria, the ambient concentration of ammonium in the N-limited chemostat decreased from 0.45 to 0.15 µM, after which it increased again to 0.3 µM. Under P limitation, no such strong fluctuations were observed. Ambient concentrations of phosphate were 0.15 and 0.27 µM at D = 0.24 and 0.44 d^{-1}, respectively.

Because specific algal growth rate (μ) is the sum of D + δ (Brussaard et al. 1997), we will refer to μ instead of D below. Under P limitation, μ was 0.30 and 0.45 d^{-1}, and under N limitation it was 0.23 d^{-1}.

Batch-mode starvation cultures

Starvation represents a situation with insignificant levels of the limiting nutrient to start with and during which there is no supply of nutrient. Brussaard et al. (1997) reported for axenic populations of *Ditylum brightwellii* that the growth rates prior to P starvation affected algal cell death kinetics. The starvation experiments were therefore conducted with cultures preconditioned under well-defined growth conditions in N- or P-limited continuous cultures. Fig. 5 shows that the impact of bacteria on algal death response under P starvation was influenced by the growth rate of the P-limited continuous culture to which the algal population was preconditioned prior to starvation. Bacteria were found to reduce the survival of the P-starved algal population precultured at μ = 0.45 d^{-1} compared to the axenic control, as clearly represented by the cumulative representation of δ$_b$ (Fig. 5A). Average δ$_b$
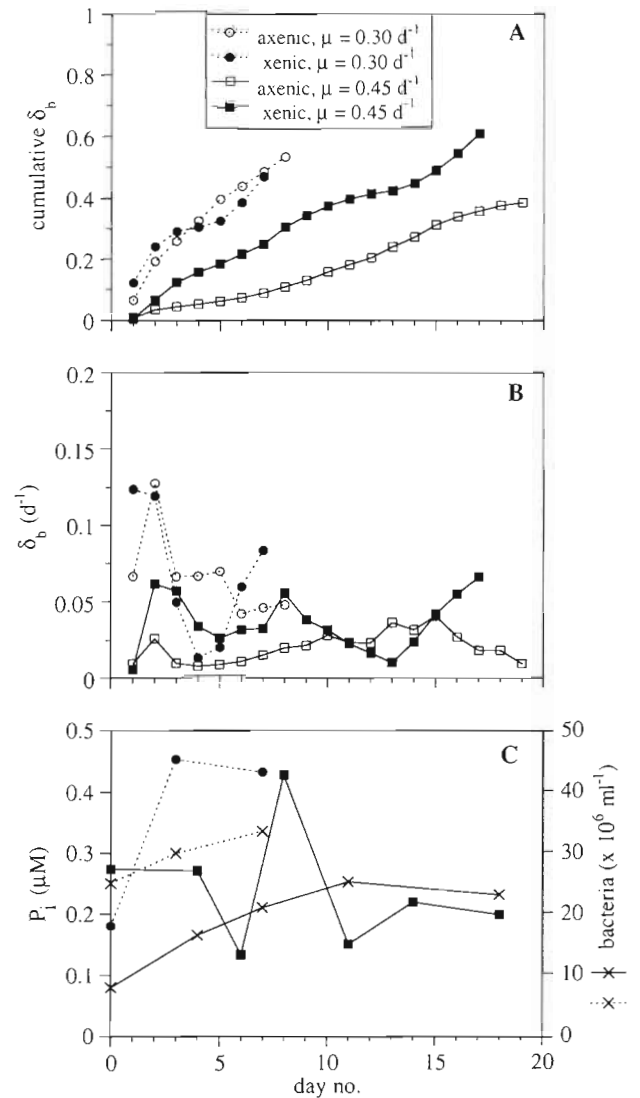

Fig. 5. *Ditylum brightwellii*. Influence of bacteria on cell death kinetics during P starvation. Cultures had been preconditioned in axenic and bacteria-containing (xenic) P-limited continuous cultures at steady state growth rates of 0.30 and 0.45 d^{-1} prior to starvation. (A) Cumulative representation of algal cell death; (B) specific algal cell death rates (δ$_b$). (C) Bacterial abundance (broken and solid lines represent preconditioning at μ = 0.30 and 0.45 d^{-1}, respectively) and ambient phosphate concentrations (µM) in the xenic cultures

over the whole experimental time period was 0.020 ± 0.010 for the axenic and 0.036 ± 0.018 d^{-1} for the xenic cultures. No differences in survival of the algal population between axenic and xenic cultures under P starvation were recorded when preconditioned at μ = 0.30 d^{-1} (Fig. 5A). For both treatments average δ$_b$ over the whole experimental time period was 0.067 d^{-1}. Oscillating patterns in δ$_b$ were observed for both growth rates (Fig. 5B). Preconditioned at μ = 0.30 d^{-1}, ambient concentration of the limiting nutrient phosphate

increased directly after the start of the experiment to 0.45 µM on Day 3 (Fig. 5C). At $\mu = 0.45$ d^{-1} an increase in ambient concentration of phosphate was recorded 5 d later on Day 8 (Fig. 5C). Although bacterial cell concentrations reached highest levels in the cultures preconditioned at $\mu = 0.30$ d^{-1} (Fig. 5C), maximum bacterial biomasses were comparable for both growth rates (3.5 and 3.0 µg C ml^{-1} for $\mu = 0.30$ and 0.45 d^{-1}, respectively).

Survival of N-starved *Ditylum brightwellii* populations preconditioned at $\mu = 0.23$ d^{-1} was, after 10 d, positively affected by bacteria (Fig. 6A). Average δ_b of the xenic culture (0.011 ± 0.006 d^{-1}) was more than 2-fold smaller than of the axenic cultures. Bacterial presence resulted in reduced δ_b after the first week of N starvation compared to axenic culture conditions (Fig. 6B). Ambient concentration of ammonium increased strongly after Day 7 to 1.8 µM on Day 10 (Fig. 6C). Bacterial cell concentrations were relatively low (Fig. 6C) and had already reached their maximum after 3 d (≈ 1.5 µg C ml^{-1}).

DISCUSSION

Phosphorus as algal-growth-limiting nutrient

Our results on phytoplankton-bacteria interactions in batch cultures and continuous cultures with phosphate as the algal-growth-limiting nutrient are consistent with the literature (Rhee 1972, Currie & Kalff 1984, Bratbak & Thingstad 1985, Bratbak 1987). In the P-limited continuous cultures, lower dilution rates led to less algae and more bacteria, due to competition for the limiting phosphate. The present study, however, is the first report actually recording algal cell death rates during such competition experiments. Resulting from competition for phosphate, specific cell death rates of *Ditylum brightwellii* increased upon inoculation with bacteria. Algal cell numbers declined as a result until bacteria became limited by a substrate other than phosphate, very likely organic carbon (Rhee 1972, Bratbak 1987, Caron et al. 1988).

Both organic components released through algal cell lysis and organic carbon supplied by photosynthetic extracellular release (PER) may serve as C sources for bacteria (Ignatiades & Fogg 1973, Myklestad 1977, Larsson & Hagström 1979, Wolter 1982, Bratbak & Thingstad 1985, Riemann & Søndergaard 1986, Obernosterer & Herndl 1995, Brussaard et al. 1996). Assuming a bacterial growth efficiency of 0.33, and the algal cellular C under P limitation to be 240 pg C cell^{-1} (Strathmann 1967), the organic carbon released through algal cell lysis at steady state in the P-limited continuous cultures was highest (7.7 times) at the
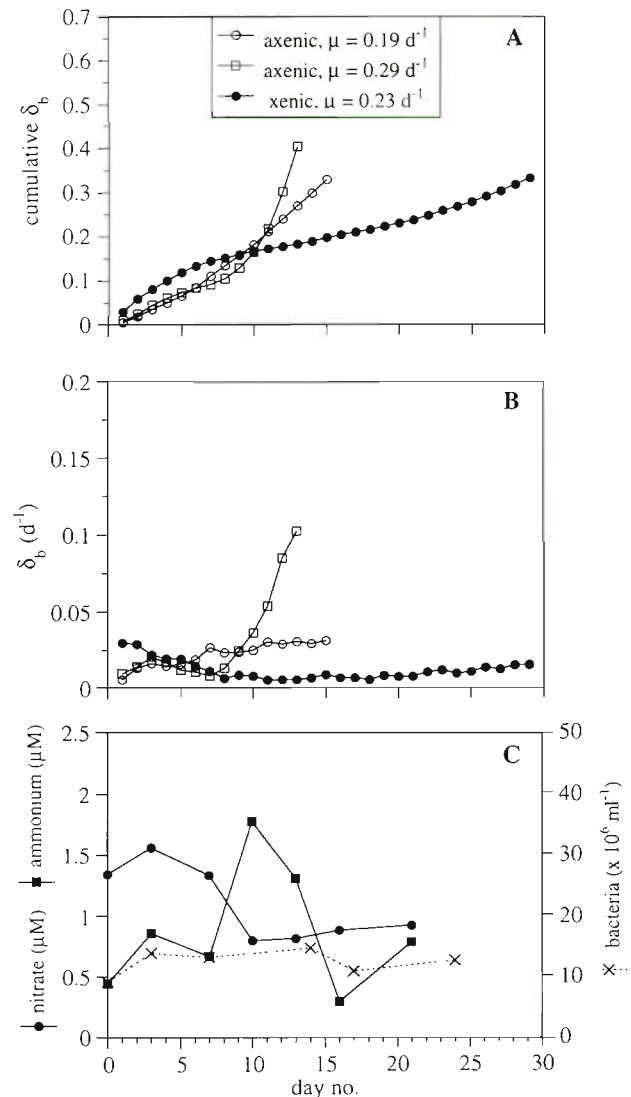

Fig. 6. *Ditylum brightwellii*. Influence of bacteria on cell death kinetics during N starvation. Cultures had been preconditioned in axenic and bacteria-containing (xenic) N-limited continuous cultures at $\mu = 0.23$ d^{-1} prior to starvation. (A) Cumulative representation of algal cell death; (B) specific algal cell death rates (δ_b). (C) Bacterial abundance and ambient concentrations of ammonium and nitrate in the xenic cultures. Because the xenic cultures had been preconditioned at slightly different growth rates in the N-limited chemostat than the axenic cultures, data were compared with 2 axenic cultures precultured at somewhat lower ($\mu = 0.19$ d^{-1}) and higher ($\mu = 0.29$ d^{-1}) steady state growth rates

lower growth rate ($\mu = 0.30$ d^{-1}). However, algal cell lysis as a source of organic carbon for bacteria was insuffient to account for the total bacterial C demand. Organic carbon supply by PER may have been another C source for bacteria.

By turning off the medium supply to the continuous cultures, the cultures became exhausted in phosphate

(P starvation). As argued above, bacteria were probably already C limited prior to starvation. Bacterial utilization of relatively P rich lysed algal cellular components during P starvation led therefore to remineralization of phosphate (Bratbak 1987, Jürgens & Güde 1990). Above a certain concentration, it can be expected that remineralized phosphate will be readily taken up by the P-deficient algal cells, which may lead to reduced algal cell death. As soon as the concentration of phosphate becomes too low, P deficiency in algal cells and subsequently algal cell death will increase again. The oscillations in δ_b of *Ditylum brightwellii* seem to reflect variations in the concentration of remineralized phosphate and thus reflect alternations in the degree of bacterial P and C limitation. The reduced survival of the *D. brightwellii* population in the cultures preconditioned at the higher growth rate ($\mu = 0.45$ d^{-1}) suggests that the increases in δ_b due to competition for phosphate with bacteria were more important than the reductions in δ_b due to algal utilization of periodically bacterial regenerated phosphate. Preconditioned at the lower growth rate ($\mu = 0.30$ d^{-1}), however, fluctuations in δ_b compensated each other for no differences in survival abilities were recorded for the axenic and xenic algal populations. It has to be noted that maximum values of δ_b in the xenic cultures adapted to $\mu = 0.45$ d^{-1} were still lower than those in cultures held at $\mu = 0.30$ d^{-1}. Enhanced δ_b due to the presence of bacteria was also observed in the batch culture experiments with P as the algal-growth-limiting nutrient. In batch cultures, algal growth rates were relatively high prior to exhaustion of the limiting nutrient (exponential growth versus stationary phase). Apparently, *D. brightwellii* cells which had been growing at higher rates were not so well equipped to compete with bacteria for phosphate as the algal populations preconditioned at lower growth rates.

Nitrogen as algal-growth-limiting nutrient

Upon inoculation with bacteria in N-limited continuous culture at $\mu = 0.23$ d^{-1}, algal cell concentration did not decline as expected (Bratbak & Thingstad 1985). Instead, algal cell size reduced (cells had become thinner). This increased their surface:volume ratios (up to 1.3) and most likely lowered their cellular N demand, allowing the algal cells to grow at $\mu = 0.23$ d^{-1} without a decline in population size. Models on interactions between phytoplankton and bacteria under N limitation (Ietswaart & Flynn 1995) should include such adaptations of algal cells to increased N deficiency.

The present study shows that, in contrast to xenic cultures with P as the algal-growth-limiting nutrient,

no reduced survival of *Ditylum brightwellii* populations was observed in xenic cultures with N as the algal-growth-limiting nutrient compared to axenic controls. In the ammonium-depleted batch cultures, there was no significant effect of bacteria on algal mortality kinetics. Under nitrate depletion, algal cell death rates actually declined in the presence of bacteria compared to the axenic control. Reduced algal mortality was also observed under N starvation. Accumulation of ammonium after the first week of N starvation indicates that bacteria were limited in their growth by carbon (Caron et al. 1988, Goldman & Dennett 1991). The lower bacterial biomass in cultures with N compared to cultures with P as the algal-growth-limiting nutrient (both batch and continuous cultures) indicates that the bacterial population was more C limited under N- than under P-deficient culture conditions. Degradation of relatively N rich algal lysis products by the C-limited bacteria probably resulted in the release of ammonium, which very likely was utilized by the N-deficient *D. brightwellii* cells.

Ecological relevance

The paradox that phytoplankton stressed by the lack of limiting nutrient stimulate growth of their competitors, as described by Bratbak & Thingstad (1985), did not apply to N deficiency. Under N deficiency, both algae and bacteria can actually benefit from each other. Axenic cultures of *Ditylum brightwellii* have been found to withstand N starvation better than P starvation, especially when preconditioned at a lower growth rate (Brussaard et al. 1997). The results in the present study show that these nutrient-type specific differences in algal death response to nutrient starvation are even more pronounced with bacteria present.

Since N starvation generally occurs in natural marine pelagic ecosystems, prolonged survival of N-starved populations of the neritic diatom *Ditylum brightwellii* due to bacterial mineralization of ammonium can give the algal species a competitive advantage. Besides algal mortality and PER responses to nutrient starvation, bacterial nutrient requirements also influence algal-bacterial interactions. The relatively higher bacterial requirements for P than for N (assuming C:N:P = 106:16:1 and 40:10:1 for algae and bacteria respectively; Kirchman 1994), compared to phytoplankton, imply higher remineralization rates for ammonium than for phosphate, which in turn may enhance phytoplankton survival.

Factors responsible for the different death responses of nutrient-depleted phytoplankton (e.g. differences in intrinsic rates of organic carbon supply through algal

cell lysis, in rates and molecular composition of exudation of photosynthetically fixed carbon, and in algal cell size) are very likely species-specific. Under phosphate depletion, the presence of bacteria enhanced δ_b of *Ditylum brightwellii* to a lesser extent than of *Skeletonema costatum* [maximum δ_b of 0.21 d^{-1}, calculated from data published by Bratbak (1987) according to Eq. (2)]. Under axenic growth conditions the cultures of *D. brightwellii* and *S. costatum* had comparable δ_b. A pilot study on the diatom *Lauderia borealis* showed that, in the presence of bacteria and compared to *D. brightwellii*, average algal death rates over a 3 wk experimental time period were about 2-fold higher in phosphate-depleted batch cultures (maximum δ_b of 0.28 d^{-1}) but 1.5 times lower under ammonium depletion (maximum δ_b of 0.04 d^{-1}; unpubl. data). These results are consistent with the lower death rates for *L. borealis* compared to *D. brightwellii* when cultured in the presence of bacteria in ammonium-limited chemostats (average $\delta = 0.001$ d^{-1} with μ ranging from 0.09 to 0.60 d^{-1}, calculated from data kindly provided by W. Stolte). The data mentioned above suggest that species-specific differences in the response of nutrient-deficient phytoplankton to the presence of bacteria do exist (although at this point it cannot be ruled out that inter-clonal variations may be as large as the variations found between species). Species-specific differences in algal cell mortality due to the presence of bacteria imply that bacteria may affect phytoplankton succession significantly, with potentially large differences in algal response for the various types of algal-growth-limiting nutrients.

Conclusions

The present experiments studying the influence of bacteria on cell death kinetics of *Ditylum brightwellii* under culture conditions with either N or P limiting algal growth showed that, compared to axenic cultures, enhanced mortality was only recorded for the P-deficient *D. brightwellii* populations (Table 3). Bacterial inoculation into cultures of *D. brightwellii* where N was the algal-growth-limiting nutrient either had no effect on algal mortality or indirectly reduced algal cell mortality and subsequently prolonged the survival of the algal population (Table 3). Bacterially regenerated ammonium was very likely utilized by N-starved cultures of *D. brightwellii*, thereby prolonging survival of the algal population. It is concluded from this study that bacteria do influence phytoplankton cell death kinetics, but that the degree of impact largely depends on the type of nutrient limiting algal growth and the culture history of the algal populations.

Table 3. *Ditylum brightwellii*. Summary of cell death responses in the presence of bacteria to various culture conditions under nitrogen (N) or phosphorus (P) deficiency. Symbols: increase (+), decrease (–), or no change (≡) in algal cell mortality compared to axenic controls. Starved batch-mode cultures were preconditioned in N- or P-limited continuous cultures. n.e.: no experiment was performed. *Algal death rates were comparable to axenic controls when ammonium limited algal growth, whereas death rates decreased when nitrate limited algal growth. **During establishment of new steady states upon bacterial inoculation, algal cell death rates first increased

Culture	Preconditioned at	N	P
Batch ('nutrient depletion')		= or –*	+
Chemostat ('nutrient limitation')		=	=**
Batch mode ('nutrient starvation')	$\mu = 0.45$ d^{-1}	n.e.	=
	$\mu \leq 0.30$ d^{-1}	–	+

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