Limnology & Oceanography 22: 132-139 (1977)

Bioassay of lower Narragansett Bay waters during the 1972–1973 winter–spring bloom using the diatom *Skeletonema costatum*[1]

Gary L. Hitchcock and Theodore J. Smayda
Graduate School of Oceanography, University of Rhode Island, Kingston 02881

Abstract

Samples collected biweekly from lower Narragansett Bay from December 1972 through March 1973 were bioassayed with *Skeletonema costatum* at nine different enrichments with nutrients equivalent to f/25-Tris medium. In early December growth rates were low in all treatments relative to complete enrichment; thereafter there was no evidence of nutrient limitation until late February. During this period of the winter–spring bloom growth was limited by the omission of NO_3, SiO_3, and PO_4; deletion of vitamins and trace metals was less important. The addition of EDTA alone increasingly suppressed growth as the bloom developed; lysis was observed during late March.

Hitchcock and Smayda (1977) evaluated the role of light intensity in causing the delayed winter–spring phytoplankton bloom during 1972–1973 in Narragansett Bay; here we examine the potential role of various nutrients in regulating growth during the course of this bloom. Pratt (1965) concluded on the basis of field data that Si and N regulate maximum diatom abundance and the termination of the winter–spring bloom in this bay. Experimenting with natural populations incubated in situ during the 1971 bloom, Smayda (1973) observed that the addition of NO_3 and SiO_3 to natural samples stimulated growth of *Skeletonema* during the bloom's termination, supporting Pratt's view. PO_4 enrichment had less of an effect, and trace metal additions during late March stimulated growth. The response of *Skeletonema costatum* was emphasized, since it is numerically the dominant species in Narragansett Bay throughout the winter–spring bloom and usually initiates it (Smayda 1957, 1973; Pratt 1959, 1965). For these reasons, the potential role of nutrients during the course of the delayed 1972–1973 winter–spring bloom was examined in a series of bioassay experiments conducted with an axenic Narragansett Bay clone of *S. costatum*.

We thank C. Tomas for carrying out the urea and ammonia analyses, and G. Vargo for the NO_3, PO_4, and SiO_3 analyses. B. Coyne typed the manuscript and drafted the figures.

Methods

Water samples were collected with Niskin bottles at station 2 (Pratt 1959) from December 1972–March 1973 (Hitchcock and Smayda 1977). Equal volumes of the surface, middepth (4 m) and bottom (8 m) water samples were mixed. The concentrations of SiO_3, PO_4, and NO_3 in this pooled sample were measured by Auto-Analyzer techniques (Strickland and Parsons 1972); NH_3 (Solórzano 1969) and urea (McCarthy 1970, as modified by McCarthy and Kamykowski 1972), were also determined. Phytoplankton was counted in a Sedgwick-Rafter counting chamber. Other observations are as given by Hitchcock and Smayda (1977).

At bimonthly intervals, a portion of the pooled sample was filtered through a prerinsed, precombusted Gelman glass fiber filter and frozen in a polyethylene container. Within 4 weeks, this filtered sample was thawed and 50-ml aliquots added to sterile 125-ml Erlenmeyer flasks (capped with 50-ml beakers) and enriched in nine different ways: 1—complete (ALL) medium (Table 1); 2—ALL-P; 3—ALL-N; 4—ALL-Si; 5—ALL-trace metals (TM); 6—Na_2EDTA (1 mg liter^{-1}); 7—ALL-vitamins; 8—vitamins

[1] This study was supported by National Science Foundation grant GA 31319X.

Limnology & Oceanography 22: 132-139 (1977)

(Table 1); 9—no nutrients added (NONE). The concentrations (f/25) used equalled those at 1/25 of full strength Guillard's (Guillard and Ryther 1962) f-1 medium. The nutrient stock solutions were prepared in distilled, deionized water, placed in polycarbonate tubes, autoclaved, frozen, and then thawed for each bioassay. The total volume of stock solutions added to the 50-ml samples to yield the various f/25 enrichments was 0.5 ml.

Each enrichment treatment (in duplicate) was inoculated with axenic *S. costatum* (clone SK6C) in exponential growth phase to yield ca. 500 cells ml^{-1} and incubated at 2°C (except for the 5 January assay at 5°C) and 0.022 ly min^{-1} ("cool-white" fluorescent light) on a 9L:15D photoperiod cycle. This photoperiod approximates that at Narragansett Bay from December through February (Smayda 1973). The parent culture was preconditioned for 6 days in filtered, *unenriched* Narragansett Bay water under the experimental conditions of temperature and light. The *unenriched* water used in preconditioning was a portion of the water to be assayed; hence, all inocula were preconditioned to the sample to be assayed.

Growth in NONE and ALL cultures for each bioassay series was determined from cell counts made every 48 h. Live samples were counted with a Palmer-Maloney chamber at 100×. The duration of each bioassay experiment varied depending on when the population in NONE reached stationary phase. After *Skeletonema* ceased to divide in NONE, all bioassay samples were preserved with Lugol's preservative and the cells counted. The average daily growth rate for each treatment was then determined from

$$K = \ln \frac{C_t}{C_0}\left(\frac{1}{t \ln 2}\right),$$

where C_t is the cell concentration at time t in days and C_0 the cell concentration at the beginning of the logarithmic phase. Values for C_0 were selected from NONE at day 2 (December, January, and February bioassays) and at day 4 in the March bioassay. Values for these days were selected to overcome any effect of the lag period (generally 2 days) in computing cell division rates. Cell densities (C_0) in NONE were within 40% of those in ALL on the same day. Use of C_0 values from ALL changes the growth rates by 10% at most. Thus, using C_0 from NONE to compute C_0 does not have a major effect on either the results or the conclusions. Ideally, of course, each culture should have been counted throughout the entire bioassay; unfortunately, this was impractical.

Table 1. Complete medium (f/25) used in nutrient enrichment experiments.

NO_3	70 μg-atoms $liter^{-1}$
SiO_3	8
PO_4	3
Trace metals:	
Fe EDTA	1
Mo	2
Cu	3 ng-atoms $liter^{-1}$
Zn	6
Co	4
Mn	72
Vitamins:	
Biotin	40 ng $liter^{-1}$
B_{12}	40
Thiamin	8 μg $liter^{-1}$

For comparison of the effect of nutrients throughout the period studied, the average daily growth rate ($\langle K \rangle$) for each treatment is expressed as a percent of that for the complete enrichment (ALL). These data are plotted against time of collection to provide an indication of the potential effect of the different nutrients on growth of *Skeletonema* throughout the 1972–1973 winter–spring bloom.

Results

Nutrient concentrations were highest in December and gradually decreased through January; after a precipitous increase in early February, N, P, and Si exhibited marked, continuous declines to reach very low concentrations during March (Fig. 1). Maximum and minimum concentrations in the pooled sample were: 21.54 and 0.3 μg-atoms $liter^{-1}$ for N (NH_3

Limnology & Oceanography 22: 132-139 (1977)

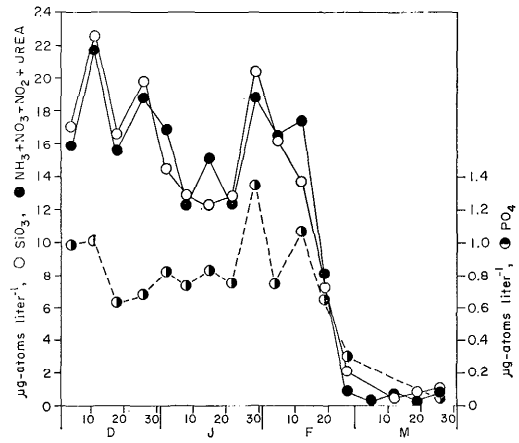

Fig. 1. Concentrations of nutrients in pooled sample from 3 December 1972–26 March 1973.

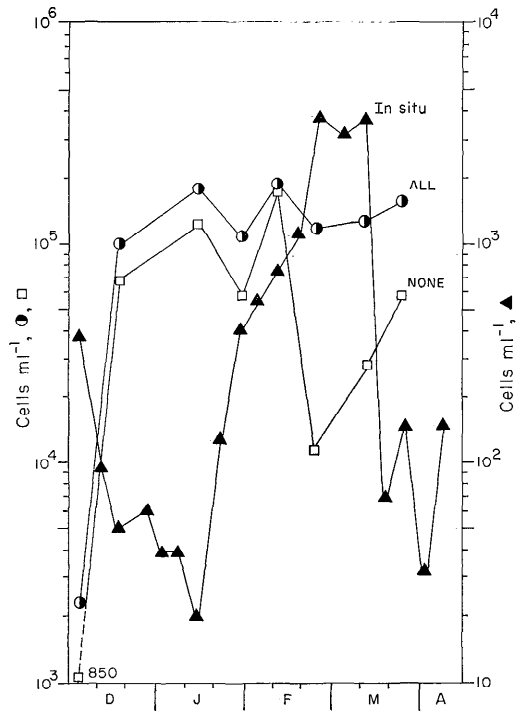

Fig. 2. Abundance of *Skeletonema costatum* in surface waters (in situ) at station 2, and yield in unenriched (NONE) and complete (ALL) enrichment bioassay treatments.

+ NO_3 + NO_2 + urea); 22.6 and 0.6 μg-atoms liter^{-1} for SiO_3, and 1.01 and 0.45 μg-atoms liter^{-1} for PO_4. These cycles reflect in situ uptake by phytoplankton. Figure 2 illustrates the changes in abundance of *S. costatum*, which initially dominated the community. Active growth began after mid-January; a peak population of about 3,500 cells ml^{-1} persisted from late February through mid-March (when *Detonula confervacea* at 5,000 cells ml^{-1} was more abundant). Subsequently, the populations of *Skeletonema* and all diatoms decreased significantly with the decrease in available nutrients.

December bioassay series—Nutrient concentrations were maximal during December, which was characterized by the progressive decrease in *Skeletonema*'s abundance to its winter minimum (Figs. 1, 2). In the first bioassay experiment, initiated on 5 December, the maximum yield of *Skeletonema* in the unenriched treatment (NONE) was 850 cells ml^{-1}, as compared to 2,300 cells ml^{-1} produced in the f/25 enrichment (ALL) (Fig. 2). The response in ALL corresponded to a mean growth rate of 0.42 division per day. The omission of the trace metal (TM) mixture from the complete enrichment (ALL-TM) prevented growth, a limitation which the addition of EDTA alone significantly overcame (Fig. 3C). The mean growth rate in this latter treatment was 67% of that in ALL. Of the other treatments, the omission of N, Si, and P singly from ALL (Figs. 3B, D, F) increased the mean generation time by 40–100% (3.2 and 4.7 days); omission of vitamins caused a more substantial decrease in $\langle K \rangle$ (Fig. 3E).

In the next bioassay experiment (18 December), the final yield in NONE was 68,000 cells ml^{-1} ($\langle K \rangle = 0.72$) and 100,000 cells ml^{-1} in ALL, which corresponds to a $\langle K \rangle$ of 0.78 division per day. These in vitro growth rates of *Skeletonema* were considerably faster than those found 2 weeks earlier (Fig. 2). The omission of the individual nutrients from ALL had little effect on yield and growth rate (Fig. 3). The results suggest that the nutrient water quality supporting growth of *Skeletonema*

Limnology & Oceanography 22: 132-139 (1977)

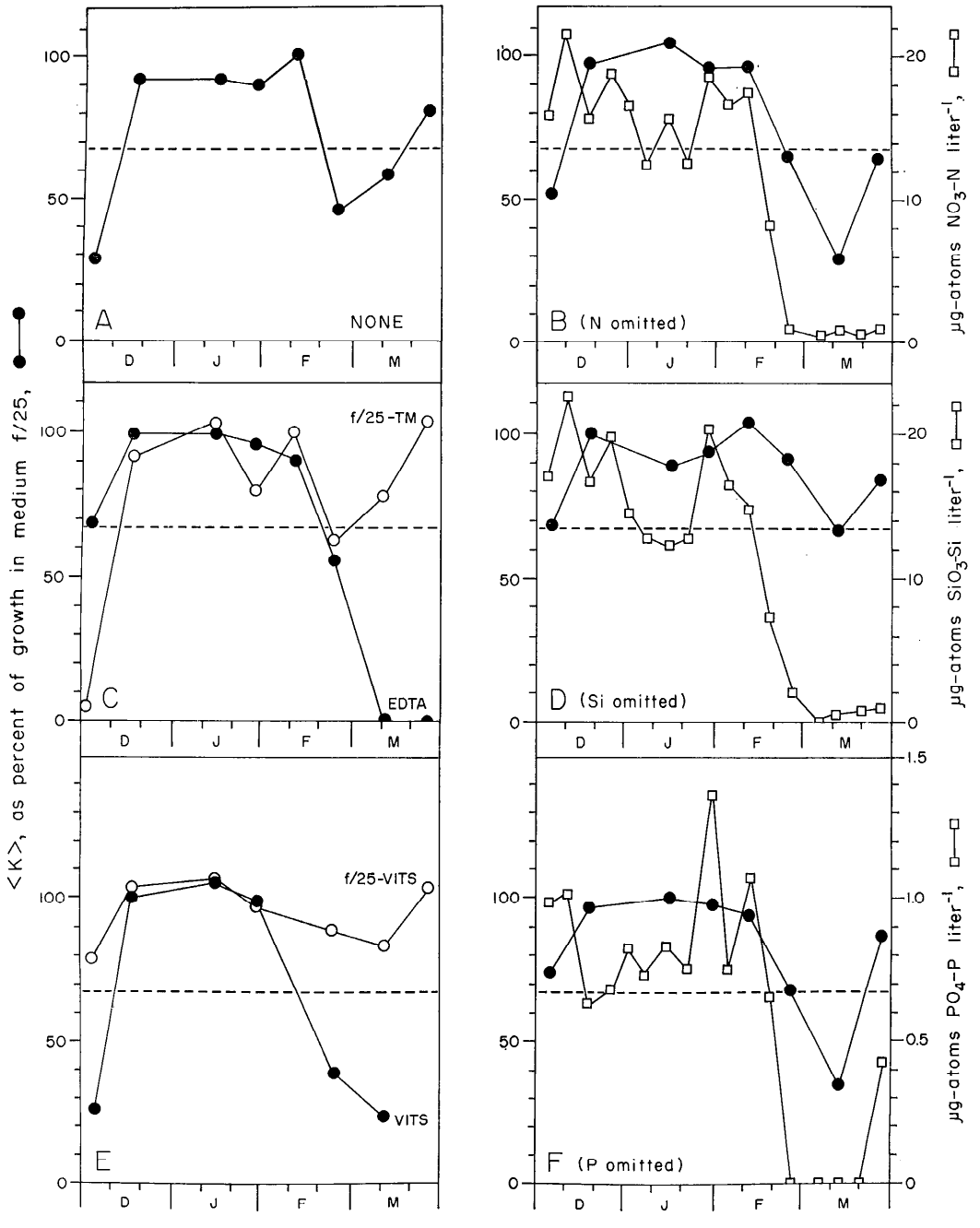

Fig. 3. Mean growth rates ($\langle K \rangle$) of *Skeletonema costatum* in various enrichment treatments during bioassay study, expressed as percent of that occurring in complete enrichment (ALL)—●; appropriate in situ nutrient concentrations—□; NONE refers to unenriched treatment. Broken line at 66% of $\langle K \rangle$ represents level at which generation time is increased by 50%.

Limnology & Oceanography 22: 132-139 (1977)

improved considerably in 2 weeks since 5 December, and that the response of the sparse, declining population in situ (Fig. 2) is not attributable to nutrient availability.

January bioassay series—Nutrient levels in situ were now reduced, but still high (Fig. 1); the *Skeletonema* population continued to decline to a very sparse mid-month minimum before the inception of growth (Fig. 2). The results of the experiments conducted on 15 and 29 January were similar to those in mid-December. Growth was excellent in the unenriched and ALL treatments and the omission of individual nutrients from the complete enrichment did not influence the response of *Skeletonema* (Fig. 3). The only noteworthy difference was a slight increase in generation time in the ALL-TM treatment on 29 January. These experiments also suggest that the continued low populations of *Skeletonema* in situ during January (Fig. 2) are not attributable to limitation by nutrients.

February bioassay series—All nutrients began to decrease rapidly during February (Fig. 1), while growth of *Skeletonema* began in situ and culminated in a maximum during late February (Fig. 2). The intense growth of *Skeletonema* in the NONE and ALL treatments, which had characterized the bioassay experiments since mid-December, also occurred in the 12 February sample (Figs. 2, 3). In the bioassay initiated on 26 February (when *Skeletonema* was at its maximum in situ), the final yield (11,000 cells ml^{-1}) in NONE was a tenth that attained 2 weeks earlier (Fig. 2), and $\langle K \rangle$ was 0.41 in ALL. The omission of N, P, and TM (Figs. 3B, F, C) now increased the mean generation time (3.3 days) by 50% above that in ALL, although growth in these treatments was better than in NONE. The omission of Si and vitamins was less effective in reducing growth (Figs. 3D, E). The poorer growth in NONE, ALL-N, and ALL-P during late February parallels the considerably reduced nutrient concentrations in situ during this month.

March bioassay series—The in situ nutrient concentrations were at their winter–spring minima (Fig. 1). *Skeletonema* continued at its maximum population levels through mid-March, and then decreased sharply in abundance (Fig. 2). In the 12 March bioassay, the response in NONE was better than in the previous equivalent experiment (Fig. 2), although the final yield of 28,000 cells ml^{-1} represents a mean growth rate ($\langle K \rangle = 0.22$) only 59% of that in ALL. The omission of N and P was particularly important; the mean generation time (relative to that in ALL) increased by threefold to 8 days (Figs. 3B, F). In ALL-Si the generation time increased to 4 days (Fig. 3D). These decreased growth rates (relative to ALL) accompanying single omissions of N, P, and Si in this experiment were the lowest observed during the study.

In the final bioassay, conducted on 26 March, poor growth again characterized the partial or complete omission of nutrients relative to the response in ALL (Fig. 3). However, the $\langle K \rangle$ responses as percent of ALL were generally higher than those in the previous bioassay. This modest improvement may partially reflect the slightly increased N and Si in situ (Fig. 1). The most conspicuous response of the various treatments was in the EDTA enrichment: as in the previous assay, enrichment with 1 mg $liter^{-1}$ of Na_2EDTA caused lysis of the inoculum (Fig. 3C).

Discussion

The sharp decrease in N, P, and Si concentrations in mid-February (Fig. 1) occurred 4–8 weeks later than the seasonal decreases during the previous winter–spring blooms at various locations in Narragansett Bay (Pratt 1965; Smayda 1973). This delay is not attributable to above-average nutrient concentrations during the 1972–1973 cycle. Nor can excretion of nutrients by zooplankton account for the observed cycles (Vargo 1976). Rather, this notable delay in nutrient reduction probably reflects the delayed diatom bloom

Limnology & Oceanography 22: 132-139 (1977)

during 1972–1973 (fig. 2: Hitchcock and Smayda 1977).

The bioassay experiments indicate that except for short term trace metal limitation during early December, nutrients did not limit the growth of *S. costatum* in the period immediately preceding the winter–spring bloom (Fig. 3) and, hence, were not responsible for the delayed bloom in 1973. Nutrients likewise did not regulate bloom inception during 1965–1966, according to bioassay of station 2 surface waters with *Thalassiosira pseudonana* (clone 13-1) (Smayda 1974). In both studies growth of the assay diatoms was nearly equal in unenriched and enriched water collected during the winter months before the bloom.

An apparent limitation during 1 week in December focuses attention on the possible role of water quality in causing the delayed bloom. Relatively low yields of *Skeletonema* occurred with and without enrichment in the 5 December series (Fig. 2). Although no specific nutrient limited growth then, the addition of EDTA alone increased the growth rate to 70% of that in the complete enrichment (Fig. 3C). Moreover, when the trace metal mixture was omitted (ALL-TM) growth was less than that in the NONE cultures. In the prebloom period assayed previously with *T. pseudonana*, growth did not depend on the addition of trace metals nor EDTA alone (Smayda 1974). However, during the 1964 prebloom period assayed with *Rhizosolenia fragilissima*, trace metals—especially cobalt—potentially limited its growth in the surface waters of Narragansett Bay (Ignatiades and Smayda 1970). Thus, during prebloom periods in Narragansett Bay the growth rate of some species may be potentially limited by short term trace metal unavailability. It may also be limited by high concentrations of certain trace metals, as demonstrated for the influence of copper on the growth of *T. pseudonana* (13-1) in another bioassay of local waters (Erickson 1972).

The extrapolation of bioassay results with trace metals and chelators to in situ conditions is problematical, given such methodological problems as reported by Lewin and Chen (1971) and other generally well known complications due to contaminants, chelation ratios, coprecipitates, etc. The fact that samples for the present bioassay experiments had to be deep-frozen initially is a further complication. Thus, the possibility has not been definitively excluded that our sample pretreatment and experimental procedure did not overcome in situ water quality effects which interfered with phytoplankton growth. Apart from this, the bioassay experiments consistently indicate that nutrients were not responsible for the delayed winter–spring bloom in 1972–1973 and strengthen the conclusion that inception of this bloom was light limited (Hitchcock and Smayda 1977).

At the time of the 1973 winter–spring bloom maximum the concentrations of N, P, and Si decreased and remained low (Figs. 1, 2, 3). Thereafter, growth of *Skeletonema* was markedly reduced in the unenriched treatment, as it was when these nutrients were omitted from the complete enrichment (Fig. 3). Thus, the lack of all three nutrients in situ and their effect on bioassay responses of *Skeletonema* generally became more pronounced as the bloom continued. Conventional methods of describing the nutrient regime by measuring ambient nutrient levels and in situ phytoplankton abundance could not have detected this increased limitation, as also noted in another bioassay study (Smayda 1974).

Silicon has been an important limiting nutrient in Narragansett Bay after the winter–spring bloom terminates (Pratt 1965; Smayda 1973). The present study suggests that in 1973 limitation by P and N was more severe than that by Si. The omission of Si from the complete enrichment did not suppress growth as much as deletion of N or P did (Fig. 3). This might be attributed to the leaching of Si from the culture vessels, although in this study the flasks were not autoclaved, a process which facilitates leaching. However, exclusive of leaching, enough Si was pres-

Limnology & Oceanography 22: 132-139 (1977)

ent when the lowest concentration was measured (12 March) to produce a minimum of 10^5 cells ml^{-1}, based on 1 μmole of Si supporting 17.4×10^6 cells of *Skeletonema* (Mitchell-Innes 1973). Since 3×10^4 cells ml^{-1} were then found in the ALL-Si treatment, other nutrients must have had more of an effect on growth in vitro.

Inorganic phosphorus was not detected in either the pooled or discrete depth samples for 4 consecutive weeks after the bloom reached its maximum. This contrasts with Pratt's observations (1965) that PO_4 was never as low as 0.3 μg-atom $liter^{-1}$ during the 1959 to 1963 winter–spring blooms. Smayda (1973) also observed that PO_4 was always detectable during the 1971–1972 winter–spring bloom and apparently never limited the growth of *Skeletonema*. Thus, the 1972–1973 winter–spring bloom was anomalous in that, aside from its late inception, P, in addition to N and Si, limited the growth of *Skeletonema* and reached undetectable levels.

The addition of Na_2EDTA after the winter–spring maximum progressively decreased, and ultimately prevented, growth of *Skeletonema* (Fig. 3C), as reported for *T. pseudonana* (13-1) (Smayda 1974). A bioassay survey at the same location in Narragansett Bay with *R. fragilissima* (Ignatiades and Smayda 1970) revealed a similar negative effect during the winter–spring bloom. The addition of 1 mg $liter^{-1}$ of Na_2EDTA to surface waters then reduced the growth rate of *Rhizosolenia* from 0.72 division per day (no enrichment) to 0.45. On occasion, however, the addition of EDTA to Narragansett Bay water is beneficial to growth, as found for natural populations of *Skeletonema* incubated in situ during a bloom of *Phaeocystis pouchetii* (Smayda 1973). Moreover, the omission of EDTA from the complete bioassay enrichment for *T. pseudonana* during a winter–spring bloom usually prevented its growth, or increased the generation time by twofold to threefold (Smayda 1974). Thus, it is probable that growth of the natural population in situ *during* the winter–spring bloom in Narragansett Bay alters the water quality properties associated with trace metals and chelation, including potential toxicity by copper (Erickson 1972). But this effect is not usually detectable before the bloom, at least in those experiments conducted to date in local waters.

References

ERICKSON, S. J. 1972. Toxicity of copper to *Thalassiosira pseudonana* in unenriched nearshore water. J. Phycol. **8**: 318–323.

GUILLARD, R. R., AND J. H. RYTHER. 1962. Studies of marine planktonic diatoms. 1. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. **8**: 229–239.

HITCHOCK, G., AND T. J. SMAYDA. 1977. The importance of light in the initiation of the 1972–1973 winter–spring diatom bloom in Narragansett Bay. Limnol. Oceanogr. **22**: 126–131.

IGNATIADES, L., AND T. J. SMAYDA. 1970. Autecological studies on the marine diatom *Rhizosolenia fragilissima* Bergon. 2. Enrichment and dark viability experiments. J. Phycol. **6**: 357–364.

LEWIN, J., AND C. H. CHEN. 1971. Available iron: A limiting factor for marine phytoplankton. Limnol. Oceanogr. **16**: 670–675.

MCCARTHY, J. 1970. A urease method for urea in seawater. Limnol. Oceanogr. **15**: 309–313.

———, AND D. KAMYKOWSKI. 1972. Urea and other nitrogenous nutrients in La Jolla Bay during February, March, and April 1970. Fish. Bull. **70**: 1261–1274.

MITCHELL-INNES, B. 1973. Ecology of the phytoplankton of Narragansett Bay and the uptake of silica by natural populations and the diatoms *Skeletonema costatum* and *Detonula confervacea*. Ph.D. thesis, Univ. Rhode Island. 212 p.

PRATT, D. M. 1959. The phytoplankton of Narragansett Bay. Limnol. Oceanogr. **4**: 425–440.

———. 1965. The winter–spring diatom flowering in Narragansett Bay. Limnol. Oceanogr. **10**: 173–184.

SMAYDA, T. J. 1957. Phytoplankton studies in lower Narragansett Bay. Limnol. Oceanogr. **2**: 342–359.

———. 1973. The growth of *Skeletonema costatum* during a winter–spring bloom in Narragansett Bay, Rhode Island. Norw. J. Bot. **20**: 219–247.

———. 1974. Bioassay of the growth potential of the surface water of lower Narragansett Bay over an annual cycle using the diatom *Thalassiosira pseudonana* (oceanic clone 13-1). Limnol. Oceanogr. **19**: 889–901.

Limnology & Oceanography 22: 132-139 (1977)

SOLÓRZANO, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. **14**: 799–801.

STRICKLAND, J. D., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis, 2nd ed. Bull. Fish. Res. Bd. Can. 167.

VARGO, G. 1976. The influence of grazing and nutrient excretion by zooplankton on the growth and production of the marine diatom, *Skeletonema costatum* (Greville) Cleve, in Narragansett Bay. Ph.D. thesis, Univ. Rhode Island. 216 p.

Submitted: 28 January 1975
Accepted: 2 August 1976