## BBP

VOLUME IV

APPENDIX G

1987 - 1988 NUTRIENT SPIKE EXPERIMENTS

EXPERIMENTAL ASSESSMENT OF PHYTOPLANKTON COMPOSITION, GROWTH AND PRIMARY PRODUCTION IN NEW BEDFORD OUTER HARBOR IN RESPONSE TO NUTRIENT AND SEWAGE EFFLUENT ENRICHMENTS

submitted to

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MCA Report No. 89-2

15 June 1989

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### CONCLUSIONS

Based on nine nutrient enrichment experiments carried out monthly between December 1987 and August 1988 using natural phytoplankton populations collected at the present (Station 2) and proposed sewage outfall site (Station 1), and their responses to nine different enrichment treatments, including 5% and 1% dilutions of treated sewage effluent composited over 48-hr periods at the New Bedford Primary Treatment Facility:

- 1. The total ( $\Sigma$ ) and < 10  $\mu$ m (= nannophytoplankton) size groups at the same station frequently differed in their primary production, biomass and growth rate responses to enrichment.
- During December 1987, diatom growth was not nutrient-limited, unlike the nannophytoplankton component, which was markedly stimulated by Si and sewage effluent addition.
- 3. All January enrichments repressed primary production (relative to Control) of the total (∑) and < 10 µm size classes at Station 1 and total community at Station 2, where nannophytoplankton production was markedly stimulated by enrichment.
- 4. The January experiments demonstrated that exposure to elevated nutrient levels can induce various phytoplankton responses: immediate stimulation; immediate repression; an initial lag effect, followed by stimulation.
- Effluent enrichment selectively stimulated growth rates of individual species.
- 6. Primary production was nutrient-limited during March; N-limitation being more significant than Si-limitation.
- 7. The non-toxic red tide dinoflagellate <u>Heterocapsa triguetra</u> present during May was not stimulated to a bloom event in any of the nine enrichment treatments. Growth rates of 1.0 to 1.3 d<sup>-1</sup> were recorded in the N+P+Si and N+P+Si/2 treatments at Station 1. <u>Heterocapsa</u> persisted in June; its modest growth rates in response to enrichment suggest that it was not nutrient-limited and that other factors regulated its failure to bloom.
- 8. Inter-specific and regional differences (Station 1 vs. Station 2) in response to nutrient enrichment level and composition characterize New Bedford Harbor; a vivid demonstration of this was provided by the July experiments.
- 9. Seasonal differences in the effects of the different nutrient combinations used characterized the experiments.
- 10. Observed inter-specific, regional and seasonal differences in nutrient effects make predictions as to the responses of given species to given nutrient loadings problematic. Outbreaks of unusual species, notably "nuisance" species, were not stimulated during the 48 hr experimental incubation periods.

- 11. The July August experiments revealed phytoplankton growth at the proposed outfall site (Station 1) to be nutrient-limited. In contrast, nutrient addition at the present outfall site generally either repressed summer primary production, or failed to stimulate it above Control levels.
- 12. Inorganic nutrient levels in the sewage effluent composited at the New Bedford Treatment Facility varied seasonally: about 2-fold for NH<sub>4</sub> (349 to 639  $\mu$ M), 2.5-fold for PO<sub>4</sub> (54 to 137  $\mu$ M) and 1.5-fold for SiO<sub>4</sub> (87 to 125  $\mu$ M).
- 13. Dissolved organic nitrogen levels in the sewage effluent varied about 3-fold, from 106 to 309  $\mu$ M; dissolved organic phosphorus 2.5-fold, from 1.4 to 49  $\mu$ M. Total nitrogen and total phosphorus varied from 364 to 805  $\mu$ M and 46 to 156  $\mu$ M, respectively.
- 14. The inorganic N:P and N:Si nutrient ratios (by atoms) in the sewage effluent varied 2-fold over the annual cycle, the P:Si ratio by 3-fold, and the DON:DOP ratio by about 33-fold.
- 15. Sewage effluent discharged into New Bedford Harbor between August 1987 - August 1988 was characterized by variable concentrations and compositional ratios of the inorganic and organic nutrients, and exhibited seasonal maxima and minima in these properties.
- 16. High temperatures, high nutrient levels and the nutrient ratios during the summer potentially favor blooms of non-diatomaceous species. The mixing cnaracteristics, however, facilitate summer diatom growth in response to N and P loadings and Si availability. These conditions diminish somewhat the prospects of extensive non-diatom blooms as a recurrent, annual summer problem.
- 17. Primary production assimilation numbers in the sewage effluent enrichment treatments were repressed in one-third of the experiments at the current outfall site (Station 2) and in only 14% of the experiments at the proposed outfall site (Station 1). Sewage enrichment stimulated production twice as frequently (64%) at Station 1 than at Station 2 (33%).
- 18. Seasonal responses to effluent addition differed between sites. During June - August, Station 1 communities were enhanced to a greater extent than at Station 2; the reverse response occurred during December -March; April - May responses were similar.
- 19. A notable feature of processed effluent from the New Bedford Sewage Treatment Facility was the considerable variation between monthly samples in color, Schlieren and Tyndall effects, and apparent viscosity of the filtrate.
- 20. Effluent enrichment did not alter phytoplankton community composition and individual species' growth rates from that in the inorganic enrichment experiments. Nuisance species blooms were not triggered in the 48 hr incubation periods, nor was the red-tide species <u>Heterocapsa</u> <u>triquetra</u> stimulated during its May - June presence.

- 21. Evidence was obtained for apparent utilization of the dissolved organic nitrogen fraction in the sewage effluent by the phytoplankton community and its associated microflora.
- 22. The organic components of sewage effluent mixtures must be factored into engineering assessments of potential nutrient loading levels and associated increases in phytoplankton biomass and oxygen demand of the latter during respiration and decomposition.
- 23. A strong correlation occurred between chlorophyll biomass growth rate and NH<sub>4</sub> uptake in the 5% and 1% effluent enrichments during the nine monthly experiments. For Station 1,  $r^2 = 0.67$ ; at Station 2  $r^2 = 0.81$ .
- 24. The experimental data consistently identified N as the principal nutrient regulating phytoplankton growth in New Bedford Harbor, although increased Si availability further enhanced NH<sub>4</sub> uptake.
- 25. The experimental data indicate that increased phytoplankton production, biomass and growth accompanied exposure to treated sewage effluent, particularly at the proposed relocated outfall site at Station 1. However, the phytoplankton dynamics, responses and composition did not differ substantially upon enrichment from those characterizing natural conditions.
- 26. The seasonal phytoplankton dynamics, species composition, succession and (generally) enrichment responses were similar at both stations. The primary difference was in the elevated phytoplankton abundance, primary production, nutrient levels and less frequent stimulation upon effluent enrichment at Station 2.
- 27. Based on the present study, the proposed relocation of the outfall site to the region of Station 1 would not cause serious alterations or negatively impact the indigenous phytoplankton community and its dynamics.
- 28. The experimental results also suggest that continuance of the outfall site at its present location (Station 2) would not significantly modify, or negatively impact local phytoplankton dynamics already established in response to the present effluent discharge.

This report presents the results of nine nutrient-spike experiments carried out between December 1987 and August 1988 to assess the response of the indigenous phytoplankton communities at the current and proposed outfall sites in New Bedford Harbor when exposed to various combinations and a range of nutrient loadings of NH<sub>4</sub>-N, PO<sub>4</sub>, SiO<sub>2</sub> and two dilutions of waste water effluent composited over 48-hr periods at the New Beaford Waste Treatment Facility. This report constitutes a companion report to that previously submitted to CAMP DRESSER & MCKEE by MACKEREL COVE ASSOCIATES (MCA) as MCA Report No. 89-1 and entitled: ASSESSMENT OF PRIMARY PRODUCTIVITY AND EUTROPHICATION POTENTIAL IN NEW BEDFORD OUTER HARBOR IN RESPONSE TO NUTRIENT REGIME AND POTENTIAL INPUTS. That report presented the results of 14 field surveys carried out between August, 1987 to August, 1988 which evaluated the seasonal cycles of phytoplankton species composition, biomass and primary production in relationship to the physical environment and nutrient levels.

The present report deals with an experimental study initiated as Amendment No. 1 to the originally scheduled field surveys, the substance of MCA Report No. 89-1. This expanded effort was formulated based on discussions during a meeting held on 9 October 1987 with representatives of DEQE/DWPC, CDM and MCA, and formalized in an Amended Scope of Work prepared and submitted to CDM by MCA on 27 October 1987. The primary objective of the nutrient enrichment experiments was to provide an experimental perspective in the evaluation of the potential impact of waste water discharge on primary productivity in New Bedford Harbor should the current outfall be relocated to a site designated as Station 1 in both this and MCA Report No. 89-1. The latter report will be cited frequently to establish the accompanying physical and nutrient fields and phytoplankton dynamics present at the current and proposed outfall sites during the nine surveys when the natural phytoplankton assemblages were collected and processed for use in the nutrient-spike experiments.

### ME THODS

Nutrient spike experiments were carried out using natural phytoplankton populations composited from samples collected at Stations 1 and 2 (see Fig. 1 in MCA Rpt. No. 89-1) between December 1987 and August 1988. Nine nutrient and effluent enrichment treatments were tested. An approximately 12 L sample was composited at each station in the field by mixing equal sample volumes collected at the 100%, 60%, 25%, 10% and 3% isolume depths. Samples were collected by Dr. Brian Howes and his group from the Woods Hole Oceanographic Institution, who carried out the field sampling program under contract to CAMP DRESSER & MCKEE (CDM). The samples were stored in insulated Gott containers for shipment to MACKEREL COVE ASSOCIATES (MCA) on the collection dates.

For experimental use, beginning the morning following the collection date, 3.5 L composited samples from Stations 1 and 2 were gently screened through a < 10  $\mu$ m mesh-screening, and this size class (= Nannophytoplankton) retained for experimental use. The remaining 7.5 L of the composited sample was not filtered and used for experimental determination of the total community ( $\Sigma$ ) response. The primary production rates, chlorophyll biomass growth, and nutrient uptake of both the total ( $\Sigma$ ) and < 10  $\mu$ m phytoplankton size-classes were determined.

Appropriate aliquots of the total ( $\sum$ ) and < 10 µm size-classes were dispensed into 1-L Erlenmeyer flasks and enriched with NH<sub>4</sub>-N, PO<sub>4</sub>-P, SiO<sub>2</sub>-Si and sewage effluent dilutions. The inorganic enrichments, made using reagent grade chemicals, included CONTROL; 100% N+P (T1); 50% N+P (T2); 20% N+P (T3); 100% N+P+Si (T4); 50% N+P+Si (T5); 20% N+P+Si (T6). The added concentrations of each nutrient in the enrichment series are given in Table 1.

These inorganic enrichment additions were identical to those previously used by MCA to assess experimentally potential effects of Deer Island secondary effluent on phytoplankton response in Boston Harbor (see p. 7 in MCA Rept. No. 87-10). In that study, a 50:1 initial dilution of sewage effluent upon discharge into the ZID was projected, with mean effluent concentrations prior to dilution being:  $NH_4 = N$ , 17.0 mg L<sup>-1</sup>;  $PO_4 = P$ , 2.31 mg L<sup>-1</sup> and  $SiO_2 = Si$ , 13.0 mg L<sup>-1</sup>. For New Bedford Harbor, the corresponding secondary effluent concentrations were stated to be: 18.0 mg L<sup>-1</sup> for NH<sub>A</sub>-N and 7.0 mg L<sup>-1</sup> for PO<sub>A</sub>-P; projected SiO2-Si levels were not provided MCA (ANNEX 1). A 20:1 ZID dilution for full-strength effluent was to be assumed for New Bedford Harbor (Howard Yamaguchi, CDM, personal communication on 27 July 1987). In consultation with Mr. Howard Yamaguchi of CDM and Mr. Russ Isaac of DEQE/DWPC, MCA was directed to supplement the above inorganic enrichment series (T1 - T6) with treated effluent enrichment experiments carried out at two doses: a 5% enrichment (20:1) designated as T7, and a 1% enrichment (100:1), designated as T8.

Sewage effluent composited over 48-hr periods at the New Bedford Sewage Facility was used in treatments T7 and T8. The sampler was set up at the influent pipe at primary No. 1, 2 and 4 depending, <u>inter</u> <u>alia</u>, on the facility maintenance schedule. Details of each monthly

## TABLE 1. NUTRIENT AND SEWAGE ENRICHMENT TREATMENTS AND CONCENTRATIONS USED IN EXPERIMENTS

	NH4 <sup>-</sup> N	PO4-P	SiO <sub>2</sub> -Si
CONTROL	0	0	0
Tl	25.0 µM	5.0	0
т2	12.5	2.5	0
Т3	5.0	1.0	0
Т4	25.0	5.0	10.0
т5	12.5	2.5	5.0
Т6	5.0	1.0	2.0
T7 = 5% Efflue	ent		

T8 = 1% Effluent

effluent collection can be provided by Mr. J. Mello of CDM who procured and delivered approximately 1-L aliquots of the composited effluent sample to MCA. Upon receipt, the effluent sample was deep-frozen until required for use in the enrichment experiment. On the experimental date, the composited sample was gently thawed, settled for several hours, a 250 ml aliquot removed, filtered three times through HA 0.43 (um membrane filters, and the filtered effluent then autoclaved (to kill bacteria) and stored (3°C) for use later that day. The color of the filtrate varied considerably between collection dates. Unfiltered, frozen aliquots of the initial composited samples have been archived by MCA, and will be retained through December 1989 should CDM or its designee wish additional chemical analysis on these.

Following enrichments (Table 1) of the total ( $\Sigma$ ) and < 10  $\mu$ m size-classes, the treated, composited samples collected from Stations 1 and 2 were dispensed into the following experimental bottles. 30 ml aliquots were dispensed into primary productivity bottles and incubated for 6 hrs at the 60% irradiance level in the outdoor productivity incubator, following the procedures described for the standard productivity measurements during the New Bedford study (MCA Report No. 89-1). 100 ml aliquots of the total ( $\Sigma$ ) and < 10  $\mu$ m size-class enrichment treatments were dispensed into 250 ml polycarbonate bottles and incubated outdoors for 24 hrs at 60% incident irradiance. Seawater circulated through this growth chamber, having a 30 min. replacement time. After 24 hrs, aliquots of each treatment were filtered for chlorophyll analyses using the procedures described in MCA Rept. No. In addition, 20 ml aliquots from each experimental flask were 89-1. filtered, dispensed into plastic vials, deep frozen and delivered to Dr. Ted Loder (University of New Hampshire) for analyses of NHA,

NO<sub>2</sub>+NO<sub>3</sub>, PO<sub>4</sub> and SiO<sub>4</sub>. Samples for nutrient and chlorophyll analyses were also collected after enrichment and prior to incubation to establish initial levels. This experimental series sought to establish chlorophyll growth rates and associated nutrient uptake during the initial 24 hrs following enrichment.

After spiking the total community ( $\geq$ ) from the composited samples with the nine different nutrient treatments, 300 ml aliquots were dispensed into Falcon Tissue Flasks and incubated outdoors at the 60% irradiance level. After 24 and 48 hrs, aliquots were removed from each treatment to determine the chlorophyll, NH<sub>4</sub>, NO<sub>2</sub>+NO<sub>3</sub>, PO<sub>4</sub> and SiO<sub>2</sub> concentrations. This procedure sought to determine the amount of nutrient taken up in each spike and its relationship to biomass (= chlorophyll) production, i.e., the yield-dose relationship. After 48 hrs incubation, the phytoplankton community structure and abundance were determined. This procedure examined the influence of the different nutrient enrichments on phytoplankton community composition, abundance and growth rate. The procedures followed in the phytoplankton census were as described in MCA Report No. 89-1.

In summary, the experiments sought to establish the influence of various inorganic enrichment levels and combinations of N, P and Si and 20:1 and 100:1 dilutions of New Bedford sewage effluent on the species composition, abundance. growth rates, biomass and primary production of natural phytoplankton communities present at the current and proposed sewage outfall sites in New Bedford harbor. The seasonal variations in chemical composition of treated effluent, specifically inorganic N, P and Si and dissolved organic N and dissolved organic P were also assessed.

### RESULTS

1. DECEMBER, 1987, ENRICHMENT EXPERIMENT

This experiment was carried out on 9-10 December. Experimental temperatures ranged from 1.0 to 7.5°C. Total daily incident irradiance was similar during both experimental days, 142 and 146 ly d-1, respectively. These corresponded to 85 and 88 ly d-1 at the 60% irradiance level used.

The field survey (MCA Report No. 89-1) revealed that the maximal annual production rates found in New Bedford harbor during the 1-year survey occurred during this survey (Figure 1): ca. 6.0 g C m-2 at the current outfall site (Station 2) and 2.2 g C m-2 at the proposed outfall site (Station 1). A diatom bloom occurred at Station 2, dominated by <u>Chaetoceros compressus</u>; at Station 1, <u>Chaetoceros <u>curvisetum</u> and several other chaetocerids dominated a sparser diatom community. Chlorophyll levels were high at both stations, a continuance of the late summer to early winter diatom bloom found during the surveys (Figure 2).</u>

Primary production was not stimulated by the nutrient and effluent additions (Figures 3, 4). At Station 1, total community ( $\Sigma$ ) responses in the N+P enrichment series (T1, T2, T3) were similar to the Control (170 mg C m<sup>-3</sup> d<sup>-1</sup>). A progressive inhibition accompanied the N+P+Si series (T4, T5, T6), with the response to the N+P+Si/5 treatment (106 mg C m<sup>-3</sup> d<sup>-1</sup>) about 40% lower than the Control. Exposure to the 5% and 1% sewage effluent levels decreased daily primary production 12 to 18% below the Controls. The associated daily assimilation number (mg C mg Chl<sup>-1</sup> d<sup>-1</sup>) was 7% to 10% below Control responses.

The nannophytoplankton (< 10  $\mu$ m) component responded differently

to enrichment. The N+P/2 and N+P/5 enrichments decreased the assimilation number  $(AN_0)$  by 35% relative to the Control. In contrast, the N+P+Si/2 (= T5) and 5% effluent enrichment (= T6) stimulated the  $AN_0$  by about 20%, and the N+P+Si/5 (= T6) and 1% effluent treatments (= T8) by 10%.

The nutrient amendments at the current outfall site (Station 2) suppressed the total community ( $\sum$ ) An<sub>0</sub> by 19% to 34% (Figure 4). In contrast, the nannophytoplankton AN<sub>0</sub> was stimulated in all enrichments, ranging from 16% in the N+P/2 (= T2) to 233% in the N+P+Si/5 treatment. The addition of 5% and 1% sewage effluent stimulated the AN<sub>0</sub> by about 70%.

Thus, nutrient and effluent enrichment during the December experiment did not stimulate diatom primary production (i.e., the larger size-class) above control levels. This suggests that their production was then not nutrient-limited. In fact, inhibition or suppression accompanied enrichment. The nannophytoplankton component, on the other hand, was generally stimulated, particularly by the N+P+Si and sewage erfluent enrichments. This suggests that this size class (< 10 µm) was then nutrient-limited.

The experimental results reveal that the different phytoplankton size functional groups present within a given community can differ in their response to nutrient enrichment.

Stimulation of the mannophytoplankton primary production was paralleled by high numerical growth rates. At Station 1, microflagellates exhibited a growth rate of 1.59 d<sup>-1</sup> in the Control; rates in the enrichment treatments ranged up to 2.28 d<sup>-1</sup> (N+P). At Station 2, a 25% decrease in the Control daily growth rates contrast to rates ranging from 0.39 to 1.0 d<sup>-1</sup> in the enrichments.

Among diatoms at Station 1, the addition of treated sewage effluent markedly stimulated growth of <u>Chaetoceros compressus</u> (1.61 d-1 (T7) and 1.03 d-1 (T8) above Control levels (0.72 d-1). However, Control responses were otherwise not stimulated by nutrient enrichment. In fact, there was some indication that inorganic nutrient addition repressed growth relative to effluent addition; for example:

	Control	N+P+Si	1% Effluent
<u>Asterionella</u> <u>glacialis</u>	1.32 d-1	1.05	1.40
<u>Chaetoceros</u> <u>debilis</u>	1.71	0.87	1.65

At Station 2, inorganic nutrient enrichment did not stimulate growth of the major diatoms, whereas effluent enrichment stimulated growth rates above Control levels: Leptocylindrus minimus (0.75 d<sup>-1</sup>); <u>Skeletonema costatum</u> (0.66 d<sup>-1</sup>) and <u>Chaetoceros diadema</u> (2.25 d<sup>-1</sup>). Enrichment conspicuously failed to stimulate growth of <u>Chaetoceros</u> <u>compressus</u> at Station 2, where it dominated the community. This result and the stationary level of the control population over the 2-day growth period indicate that growth of <u>Chaetoceros compressus</u> was then being regulated by non-nutritional factors which induced its senescense.

The general conclusion from this early December experiment is that diatom growth was then not nutrient-limited, unlike the nannophytoplankton component. The latter were stimulated by Si in the N+P treatments and by sewage effluent.

### 2. JANUARY, 1988 ENRICHMENT EXPERIMENTS

This experiment was carried out on 12-13 January. The field surveys revealed that in the four weeks since the previous experiment, phytoplankton numerical abundance. biomass and primary production plummeted (MCA Rept. No. 89-1). Primary production decreased to 350 mg C m<sup>-2</sup> d<sup>-1</sup> at the current and proposed outfall sites, a decrease from 6.0 and 2.2 g C m<sup>-2</sup> d<sup>-1</sup>, respectively, since December. Carbon generation times were 26 to 38 hrs for the entire ( $\sum$ ) community and six to nine days for the nannophytoplankton. These dynamics were accompanied by extremely low temperatures, which varied vertically within the water column at Station 1 from -0.1° to 0.5°C and as low as -0.4°C at Station 2. Temperature-limitation of phytoplankton growth was partly contributory to the precipitous decline since the December experiments (Figures 1, 2).

The January experiments were carried out at the extremely low temperatures (-0.5°C) then prevalent. Ice formation in many of the phytoplankton-speciation experimental flasks precluded assessment of species responses in most treatments. Irradiance levels were 157.4 ly  $d^{-1}$  on 12 January and 78.5 ly  $d^{-1}$  on 13 January, i.e., a cloudy, very cold experimental date. The primary production responses to the enrichment treatments are snown in Figures 5, 6. At Station 1, none of the enrichments stimulated production above the control level (72 mg C  $m^{-3} d^{-1}$ ) for the total ( $\sum$ ) community. Production was repressed by 10% in the N+P+Si/5 treatment and (maximally) by 44% in the 5% effluent enrichment. Curiously, the severity of the repression <u>decreased</u> with attenuation of the enrichment, i.e., it was most intense in the N+P treatment (T1) and least intense in N+P/5 (= T3) of the N+P enrichment series; corresponding responses occurred in treatments T4 and T6 of the N+P+Si enrichment series and T7 and T8 of the effluent enrichment series.

In marked contrast, the nannophytoplankton component was markedly stimulated by all nutrient amendments, particularly in the N+P+Si enrichment series (Figure 5). The  $AN_O$  in these treatments relative to the Control response are shown in Table 2.

Nannophytoplankton production was also stimulated at Station 2 (Figure 6); the AN<sub>0</sub> increased by about 3- to 4-fold above Control levels (Table 2). In contrast, the total community ( $\Sigma$ ) did not respond to the nutrient enrichments, the AN<sub>0</sub> were similar to Control levels (Figure 6), unlike the inhibition noted at Station 1. Thus, similar to the December experiments, the nannophytoplankton component during the January experiment was markedly stimulated by the various enrichments.

Individual species responses could be determined only in the Control, N+P/5, N+P+Si/2 and 5% effluent experiments for Station 1 because of the aforementioned icing problems. The highest growth rates in excess of Control responses occurred in the 5% effluent treatment: <u>Chaetoceros compressus</u> (1.18 d-1), <u>Asterionella glacialis</u> (1.90 d-1), <u>Leptocylindrus minimus</u> (0.73 d-1) and <u>Rhizosolenia delicatula</u> (0.69 d-1).

### 3. FEBRUARY, 1988 ENRICHMENT EXPERIMENTS

This experiment was carried out on 2-3 February at a temperature ranging from 2.2°C to 3.9°C. During the first experimental day, incident irradiance was only 46 ly d-1, increasing to 141 ly d-1 the following day. CDM did not collect a January sewage effluent sample during January at the New Bedford Sewage Treatment Facility. The TABLE 2. ASSIMILATION NUMBER (mg C mg Chl-l d-l) RATIO OF NANNO-PHYTOPLANKTON (< 10  $\mu$ m) RESPONSE IN ENRICHMENT TREATMENTS RELATIVE TO CONTROL DURING JANUARY EXPERIMENT.

	<u>Stn 1</u>	<u>Stn_2</u>
N+P	1.93	3.28
N+P/2	2.02	3.72
N+P/5	1.83	3.07
N+P+Si	2.64	3.09
N+P+Si/2	2.72	2.98
N+P+Si/5	3.22	3.72
5% Effluent	2.17	3.19
1% Effluent	1.83	2.99

. .. .....

February experiment was completed before delivery (4 February) of the February sample. Therefore, the December effluent sample was used to prepare the 5% (T7) and 1% (T8) enrichment treatments during this experiment.

The field survey revealed that the very low primary production and chlorophyll biomass found during mid-January persisted (MCA Report No. 89-1; Figures 1, 2). The diatom population increased to about 1,500 cells ml-1, representing a 2- to 4-fold increase over January levels. <u>Chaetoceros compressus</u> persisted as the dominant species, with <u>Asterionella glacialis</u> also being important at Station 1.

Unlike the December and January experiments, total ( $\overline{\Sigma}$ ) community primary production was markedly stimulated at both stations by all nutrient and effluent additions (Figures 7, 8). Expressed as the AN<sub>O</sub>, enrichment stimulated production by 1.8- to 2.4-fold at Station 1 and generally by about 2-fold at Station 2 (Table 3). The N+P+Si (T4, T5, T6) and effluent enrichment series (T7, T8) were more stimulatory than the N+P series at (T1, T2, T3) Station 1. Nannophytoplankton production at Station 1 was also markedly stimulated by all enrichments, particularly in the N+P+Si series (T4 - T6) and 5% effluent addition (Figure 7, Table 2). At Station 2, however, all enrichments inhibited nannophytoplankton production relative to the Control, by 28% to 41%.

Enrichment generally stimulated chlorophyll biomass above Control levels. Total community chlorophyll growth rates after two days' incubation ranged from 0.09 to 0.50 d<sup>-1</sup> at Station 1 and from 0.17 to 0.32 d<sup>-1</sup> at Station 2, being maximal in the N+P+Si/2 enrichment (Table 4).

Several noteworthy trends characterized the chlorophyll responses.

## TABLE 3. ASSIMILATION NUMBER (mg C mg Chl-l d-l) RATIOS RELATIVE TO CONTROL DURING FEBRUARY ENRICHMENT EXPERIMENTS.

	STN 1		STN 2	
	(Σ)	< 10 µm	(٢)	< 10 µm
N+P	1.85	1.45	1.74	0.69
N+P/2	1.75	1.30	1.92	0.62
N+P/5	1.75	1.63	2.02	0.62
N+P+Si	2.20	1.62	1.86	0.68
N+P+Si/2	2.17	2.32	2.14	0.75
N+P+Si/5	2.43	1.78	1.96	0.5 <b>9</b>
5% Effluent	2.04	2.40	1.91	0.67
1% Effluent	2.25	1.54	2.04	0.72

TABLE 4. MAIN DAILY TOTAL COMMUNITY CHLOROPHYLL GROWTH RATES AFTER 48 HR INCUBATION DURING THE FEBRUARY ENRICHMENT EXPERIMENTS.

	STN 1	STN 2
CONTROL	0.09	0.17
N+P	0.19	0.26
N+P/2	0.23	0.18
N+P/5	0.25	0.20
N+P+Si	0.20	0.23
N+P+Si/2	0.50	0.32
N+P+Si/5	0.37	0.27
5% Effluent	0.09	0.17
1% Effluent	0.41	0.18

Inhibition of nannophytoplankton primary production at Station 2 in the 6 hr incubation period by all enrichments was pointed out (Table 3). This is paralleled by the generally lower chlorophyll growth rates in the 24 hour incubations relative to control levels:

Control, 1.24 d-1

N <b>+</b> P	0.65	N+P+Si	0.51	5% Effluent	0
N+P/2	0.15	N+P+Si/2	0.95	l% Effluent	1.08
N+P/5	0.32	N+P+Si/5	0.19		

At Station 1, 24 hr chlorophyll growth rates exceeded Control levels only in the T3 - T5 enrichments, reaching 0.92 d-1.

There is some indication that the total ( $\sum$ ) community at Station 1 required a 24 hr adaptation period to the enrichments prior to commencing cellular (= biomass) growth. The 24 hr and 48 hr chiorophyll growth rates are presented in Table 6.

At Station 1, a net increase in chlorophyll biomass generally did not occur during the first 24 hrs, but increased rapidly the following day. This also characterized the Station 2 responses, excluding the responses in the N+P, N+P/2 and 1% effluent enrichments. First day rates in these were about twice those during the following day. The very low growth rates during the first 24 hr experimental period were accompanied by the lowest 24 hr irradiance levels (46 ly d-1) encountered during the annual enrichment study, and probably reflect light-limitation of growth. During the second experimental day (3 February), incident irradiance trebled to 141 ly d-1, accompanied by generally higher growth rates.

These limited data reveal that exposure to elevated nutrient

TABLE 6. TOTAL COMMUNITY CHLOROPHYLL GROWTH RATES AFTER 24 AND 48 HRS DURING FEBRUARY EXPERIMENT; ( $\Sigma$ ) INDICATES NEGATIVE GROWTH RATE.

	<u>STN 1</u>		STN	2
	24 hr	48 hr	24 hr	<u>48 hr</u>
Control	(0.15) d-1	0.09	(0.30)	0.17
N+P	0.08	0.19	0.57	0.26
N+P/2	0.01	0.23	0.37	0.18
N+P/5	(0.05)	0.25	0.05	0.20
N+P+Si	0	0.20	0.13	0.23
N+P+Si/2	0.01	0.50	0.17	0.32
N+P+Si/5	(0.05)	0.37	0.05	0.2/
5% Eftluent	0	0.09	(0.14)	0.17
1% Eftluent	0.01	0.41	0.43	0.18

levels may induce the following phytoplankton responses: immediate stimulation; immediate repression; an initial lag effect, followed by stimulation.

Significant stimulation of individual species and their growth rates also occurred during the February experiment. The microflagellate component of the nannophytoplankton (< 10 µm) group grew very rapidly; the 24 hr responses are presented in Table 7. Maximal rates occurred in the N+P series and exceeded 3.0 d-1, significantly above the 1.8 d-1 rate characterizing Control populations. The 5% effluent enrichment was also quite stimulatory to the microflageilates.

Among the diatoms, the dominant species <u>Chaetoceros compressus</u> grew sluggishly in the Control at both stations, 0.06 to 0.11 d<sup>-1</sup>. At Station 1, the maximal stimulated growth rate of 0.64 d<sup>-1</sup> occurred in the 1% effluent enrichment; at Station 2, in the N+P+Si enrichment (0.69 d<sup>-1</sup>), with the next highest rate (0.42 d<sup>-1</sup>) also occurring in the 1% effluent addition. The 5% effluent treatment was particularly stimulatory to <u>Rhizosolenia delicatula</u>, with rates of 0.45 and 1.53 d<sup>-1</sup> occurring at Stations 1 and 2, respectively, where Control rates were 0.13 and 1.21 d<sup>-1</sup>. In contrast to all other enrichments, the 5% and 1% effluent enrichments were stimulatory to <u>Thalassionema nitzschioides</u> at both stations:

5% Effluent		5% Effluent	<u>l% Effluent</u>
Stn	1	0.67 d-1	0.24
Stn	2	1.12	0.69

Thalassiosira nordenskioeldii, at both stations, was stimulated only by

# TABLE 7.DAILY MICROFLAGELLATE GROWTH RATES DURING 24 HRINCUBATIONS IN FEBRUARY ENRICHMENT EXPERIMENTS.

	STN 1	STN 2
Control	1.76 d-1	1.82
N+P	1.76	2.97
N+P/2	2.64	3.88
N+P/5	3.21	1.85
N+P+Si	1.65	2.32
N+P+Si/2	1.86	1.84
N+P+Si/5	1.13	1.98
5% Effluent	2.12	2.57
l% Eftluent	0.83	1.81

the N+P+Si and 1% effluent enrichments:

<u>N+P+Si</u>		<u>1% Effluent</u>
Stn 1	0.48 d-1	0.50
Stn 2	0.62	0.63

These experiments clearly establish that effluent discharge into New Bedford Harbor, under certain conditions, can selectively stimulate the growth rates of individual species.

4. MARCH, 1988 ENRICHMENT EXPERIMENT

This experiment was carried out between 7-8 March at about 4°C; daily total incident radiance levels were similar at about 390 ly d-1. The field survey revealed that nutrient-poor waters persisted in the well-mixed water column, and that both production and biomass at Station 2 increased somewhat at Station 2, but remained at very low levels at Station 1 (MCA Report No. 89-1). <u>Chaetoceros compressus</u> persisted in its dominance of the taxonomically-diverse phytoplankton communities.

Primary production of the total community ( $\Sigma$ ) was stimulated by all enrichments at Stations 1 and 2, exclusive of the 5% and 1% effluent enrichments and the N+P/2 (= T2) amendment (Figures 9, 10). The responses in the latter three treatments were similar to that of the Control. The AN<sub>0</sub> relative to the Control is given in Table 8. At Station 2, total community production (expressed as AN<sub>0</sub>) was stimulated by about 1.4 to 1.7-fold, with the sewage effluent additions being least stimulatory. This also characterized enrichment responses of the < 10  $\mu$ m size-class at Station 2, which responded much more

## TABLE 8. ASSIMILATION NUMBER (mg C mg Chl-l d-l) RATIOS RELATIVE TO CONTROL DURING MARCH ENRICHMENT EXPERIMENTS.

	STN 1		STN 2	
	(2)	mu < 10 ×	(∑)	< 10 µm
N+P	1.37	1.41	1.51	2.28
N+P/2	1.05	1.13	1.61	2.85
N+P/5	1.25	1.06	1.56	2.42
N+P+Si	1.28	1.23	1.73	2.49
N+P+Si/2	1.18	0.91	1.65	2.85
N+P+Si/5	1.21	1.36	1.53	2.49
5% Effluent	1.09	1.11	1.36	1.30
l% Effluent	0.94	0.86	1.38	1.70

vigorously to enrichment.

The various inorganic enrichments stimulated the AN<sub>O</sub> by about 2.3to 2.9-fold; sewage effluent by 1.3- to 1.7-fold. This marked stimulation of the nannophytoplankton contrasts with their production inhibition during the February experiments (Table 3). At Station 1, the total community and nannophytoplankton responded similarly to enrichment. These responses indicate that nutrient-limitation of primary production occurred during March, and that N was probably a more significant limiting nutrient than Si. This is suggested by the responses in the appropriate nutrient pairings, for example:

	STN 1		STN 2	
	(Σ)	m <b>ير 1</b> 0 >	( <u>Z</u> )	mبر 10 <
N+P	1.37	1.41	1.51	2.28
N+P+Si	1.28	1.23	1.73	2.49
N+P/5	1.25	1.06	1.56	2.42
N+P+Si/5	1.21	1.36	1.53	2.49

Unlike February, significant chlorophyll growth rates were not found in the 24 hr and 48 hr incubations. Maximal total community growth rates occurred at Station 2 in the sewage effluent enrichments: 0.13 to 0.18 d<sup>-1</sup>.

Several diatom species present in the Station 2 community were markedly stimulated. The Control growth rates and maximal growth rate and associated enrichment for some of these species are:

	Control	Enric	chmen	t	
Chaetoceros compressus	0.65 d-1	0.94	(5%	Effluent)	1
		0.87	(N+P	+Si)	
<u>Leptocylindrus</u> minimus	0.52	1.09	(N+P	+Si/2)	
<u>Rhizosolenia</u> <u>delicatula</u>	0.32	0.79	(5%	Effluent)	ł
<u>Asterionella glacialis</u>	0	1.64	(1%	<b>n</b> )	ļ
<u>Thalassiosira nordenskioeldii</u>	0.17	0.69	(5%	<b>"</b> )	į

<u>Skeletonema costatum</u>, which was not found in the Control and in most enrichments, was detected in the 5% Effluent (73 cells  $ml^{-1}$ ) and N+P/5 (41  $ml^{-1}$ ) enrichments.

The nannophytoplankton component exhibited maximal growth  $(1.13 d^{-1})$  in the N+P+Si/5 treatment, with Control rates of 0.85 d<sup>-1</sup>, corresponding to generation times of 21 and 28 hrs, respectively, a difference of 33%. Nannophytoplankton at Station 1 grew at rates of 1.7 and 1.35 d<sup>-1</sup> in the N+P/5 and N+P+Si/5 treatments, respectively; considerably faster than the Control population  $(0.28 d^{-1})$ . In contrast, the dominant diatom at Station 1 (<u>Chaetoceros compressus</u>) grew at the Control rate  $(0.48 d^{-1})$  in the various enrichments. Leptocylindrus minimus and Rhizosolenia delicatula did not exhibit net growth in the Control. Leptocylindrus minimus grew maximally at 1.0 to 1.2 d<sup>-1</sup> in the N+P+Si enrichment series and effluent additions. Rhizosolenia delicatula grow maximally in the N+P/5 enrichment  $(0.72 d^{-1})$ .

### 5. APRIL, 1988 ENRICHMENT EXPERIMENTS

This experiment carried out between 12-13 April assessed the influence of the 5% and 1% sewage enrichments at 60% and 10% of the

total incident irradiance which was 445 ly d-l. This experiment evaluated potential effects of irradiance on the response to effluent enrichment. Experimental temperature was 8°C.

The low primary production rates and phytoplankton biomass present since January persisted (Figures 1, 2). <u>Chaetoceros compressus</u> which dominated since January was replaced by <u>Chaetoceros socialis</u> at Station 1, whereas both species co-dominated at Station 2 (MCA Report No. 89-1).

There was no significant consistent difference in AN<sub>0</sub> between the various treatment pairs, other than the considerably higher production rates of the Station 2 community relative to Station 1 in both the Control and treatments (Figure 11). Neither irradiance level nor sewage erfluent strength induced different responses in the total community ( $\Sigma$ ) and nannophytoplankton (< 10 µm). The AN<sub>0</sub> rates are presented in Table 9 and Figure 11). Chlorophyll biomass growth rates and changes in the treatments were similar to Control responses. Maximal Control growth rates of 0.65 to 0.69 d-1 occurred at Station 2, where growth rates in the 1% treatment ranged from 0.72 to 0.76 d-1.

Maximal micro-flagellate growth rates occurred in the 5% treatment at 60% irradiance at both stations: 0.6 (Station 1) and 1.70 d-1 (Station 2). Diatom growth was stimulated at Station 1, with the following maximal growth rates recorded:

<u>Chaetoceros socialis</u>	0.83	(5% H	ffluer	it)
Chaetoceros compressus	0.79	(1%		)
<u>Thalassiossira</u> <u>nordenskioeldij</u>	1.12	(1%	•	)

### TABLE 9. ASSIMILATION NUMBER (mg C mg Chl-l d-l) RATIOS RELATIVE TO CONTROL DURING APRIL SEWAGE EFFLUENT ENRICHMENT EXPERIMENTS.

		STN 1		STI	STN 2		
		<u>60% Io</u>	10% I <sub>0</sub>	<u>60% Io</u>	<u>10% I</u> o		
5%		1.04	2.13	1.31	1.00		
	< 10 µm	1.67	1.48	5.47	?		
1%		1.49	2.27	1.33	1.22		
	mu 10 <	2.06	?	2.82	?		

### 6. MAY, 1988 ENRICHMENT EXPERIMENTS

This experiment was carried out on 10-11 May at 12°C; total irradiance during the first day was 214 ly d-1 and 228 during the second day. The field survey revealed the lowest phytoplankton biomass and primary production rates recorded to date (Figures 1, 2). The other significant characteristic was the abundance of the non-toxic, red tide species <u>Heterocapsa triquetra</u> at Station 2, where the maximal dinoflagellate abundance recorded to date (73 ml-1) was found (MCA Report No. 89-1). Dinoflagellates were not abundant at Station 1.

At both stations N+P (= T1) enrichment repressed production of the total community ( $\overline{\geq}$ ), the AN<sub>0</sub> being about 15% below Control levels (Figures 12, 13). The nannophytoplankton, in contrast, were markedly stimulated by this enrichment (Table 10). Otherwise, all enrichments were stimulatory to both the total ( $\overline{\geq}$ ) community and nannophytoplankton. At Station 1, the 1% (= T8) sewage effluent treatment was most stimulatory, and the nannophytoplankton were generally stimulated to a greater extent than the entire community ( $\overline{\geq}$ ). At Station 2, the < 10  $\mu$ m size-class was also stimulated to a greater extent than the total ( $\overline{\geq}$ ) community.

Chlorophyll growth rates were not substantially different from Control responses. The maximal chlorophyll growth rates of about 0.6  $d^{-1}$  occurred in all treatments, exclusive of N+P/5 and the 1% effluent at Station 1. Despite the abundance of <u>Heterocapsa triquetra</u> at Station 2, net growth was not observed. However, at Station 1 where this non-toxic, red tide dinoflagellate was initially sparse, growth rates of 1.0 and 1.3  $d^{-1}$  occurred in the N+P+Si and N+P+Si/2 treatments, respectively. The <u>Chaetoceros socialis</u> + <u>furcellatus</u> TABLE 10. ASSIMILATION NUMBER (mg C mg Chl-l d-l) RATIOS RELATIVE TO CONTROL DURING MAY ENRICHMENT EXPERIMENTS.

	STN 1		STN 2	
	<u>(</u> 2)	< 10 µm	<u>(</u> <b>Z</b> )	< 10 µm
N+P	0.86	1.74	0.88	2.25
N+P/2	1.36	1.42	1.35	1.96
N+P/5	1.16	1.68?	1.36	1.88
N+P+Si	1.10	1.54	1.42	1.63
N+P+Si/2	1.12	1.48	1.38	2.40
N+P+Si/5	1.30	1.73	1.31	1.99
5% Effluent	1.21	1.61	1.16	1.58
1% Effluent	1.40	1.92	1.31	1.90

complex grew at rates of 1.8 d<sup>-1</sup> in N+P, 1.93 d<sup>-1</sup> in N+P+Si/2 and 1.76 d<sup>-1</sup> in the 5% effluent treatment, as opposed to a Control rate of 0.85 d<sup>-1</sup>. At Station 2, it grew maximally (1.21 d<sup>-1</sup>) in 1% sewage effluent.

### 7. JUNE, 1988 ENRICHMENT EXPERIMENTS

This experiment was carried out from 7-9 June at 15° - 16°C, total irradiance levels were 626 and 583 ly d<sup>-1</sup>, respectively. The field survey showed that both production and chlorophyll biomass increased above the very low May levels (Figures 1, 2). Dinoflagellates dominated by <u>Heterocapsa triquetra</u> exceeded diatoms at both stations, although the dinoflagellate population levels (36 to 72 cells ml-1) did not reach red-tide proportions.

Primary production of the total community and nannophytoplankton size class was markedly stimulated in all enrichments, excluding T8 at Station 2 (Figures 14, 15, Table 11). At Station 1, effluent stimulated about a 2.5-fold increase in the AN<sub>0</sub>, where the responses were generally about 2-fold, or greater, than the Control. At Station 2, maximal stimulation of about 1.5-fold occurred in the sewage effluent enrichments of the < 10  $\mu$ m phytoplankton size-class. The nannophytoplankton at this station generally were stimulated to a greater extent than the total community. Chlorophyll growth rates were greatest, about 0.3 d<sup>-1</sup>, in the effluent treatments.

The red-tide dinoflagellate <u>Heterocapsa triquetra</u> exhibited maximal growth rates in the Control of 0.55 d<sup>-1</sup> and 0.23 d<sup>-1</sup> at Stations 1 and 2, respectively. At Station 1, growth rates in the other enrichments ranged from 0.17 to 0.59 (N+P+Si). This suggests that <u>Heterocapsa</u> was not nutrient-limited. At Station 2, excluding a growth rate of 0.35 d<sup>-1</sup> in the N+P/5 treatment, all other enrichments

## TABLE 11. ASSIMILATION NUMBER (mg C mg Chl-l d-l) RATIOS RELATIVE TO CONTROL DURING JUNE ENRICHMENT EXPERIMENTS.

	STN 1		STN 2	
	( <u>E</u> )	< 10 µm	(∑)	m < 10 د
N+P	2.45	2.02	1.22	1.37
N+P/2	2.61	1.44	1.17	1.43
N+P/5	2.47	2.23	1.34	1.45
N+P+Si	2.28	1.78	1.20	1.27
N+P+Si/2	2.22	1.91	1.23	1.31
N+P+Si/5	2.23	2.31	1.30	1.29
5% Effluent	1.95	2.55	1.34	1.42
1% Effluent	2.52	2.35	0.77	1.52

were either inhibitory or without effect. As during the May experiment, neither the unenriched Control nor eight enrichments over the two-day experimental period revealed a population surge of <u>Heterocapsa triquetra</u> indicative of a red-tide bloom event.

At both stations, the microflagellate component was markedly stimulated in certain enrichments, most notably in the effluent treatments. At Station 1, the growth rate of 0.71 d<sup>-1</sup> in the 5% effluent compares with the Control rate of 0.30 d<sup>-1</sup>. At Station 2, the Control rate was 0.70 d<sup>-1</sup> versus 2.57 and 1.55 d<sup>-1</sup> in the 5% and 1% effluent treatments, respectively.

### 8. JULY, 1988 ENRICHMENT EXPERIMENTS

This experiment was carried out between 19-20 July at 21.5° to 22°C; it rained during both days, the total daily irradiance being 126 and 76 ly d<sup>-1</sup>, respectively. The field survey indicated that a significant increase in diatom abundance occurred since June. This primarily reflected a new bloom of <u>Chaetoceros compressus</u>, accompanied by a secondary predominance of <u>Skeletonema costatum</u> and <u>Chaetoceros</u> <u>affinis</u> (MCA Report No. 89-1). Chlorophyll biomass doubled since June (Figure 2) whereas primary production rates were similar to those in June (Figure 1).

The responses to added nutrients differed markedly between stations (Figures 16, 17). At the proposed outfall site (Station 1), all nutrient additions stimulated the  $AN_0$  of the total community by 2to 2.6-fold above Control levels (Table 12). The nannophytoplankton were also stimulated, but to a lesser extent (1.4- to 1.7-fold). The responses to the N+P series (T1 - T3) were similar to the effluent enrichment effects (T7, T8). The addition of silica (T4 - T6) to the
# TABLE 12. ASSIMILATION NUMBER (mg C mg Chl-l d-l) RATIOS RELATIVE TO CONTROL DURING JULY ENRICHMENT EXPERIMENTS.

	STN	STN 1		2
	<u>(Σ)</u>	< 10 µm	<u>(Σ)</u>	(mu <u>10 (</u> m
N+P	2.66	1.71	0.92	0.82
N+P/2	2.39	1.69	0.78	0.80
N+P/5	2.12	1.56	0.68	0.80
N+P+Si	2.13	1.52	0.74	0.75
N+P+Si/2	2.04	1.40	0.63	0.58
N+P+Si/5	2.32	1.68	0.61	0.73
5% Effluent	2.36	1.73	0.58	0.70
l% Effluent	2.15	1.56	0.60	0.70

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N+P levels did not further stimulate production, suggesting that silica was not then limiting.

The responses at the present outfall site (Station 2) were the opposite: all enrichments repressed production, with maximal repression occurring in the effluent treatments (T7, T8). For the entire community, the AN<sub>0</sub> was 40% below Control levels; for the nannophytoplankton, 30%. The response curve (Figure 17) suggests a progressively increasing inhibition of the total community along the T1 - T8 enrichment gradient. These responses indicate that ambient nutrient levels at the current outfall site were not limiting to phytoplankton production, unlike at Station 1. Rather, increased nutrient enrichment or effluent loading repressed production.

Mean total community ( $\Sigma$ ) chlorophyll growth rates during the 48 hr incubations are presented in Table 13. Excluding the N+P+Si/5 treatment, all enrichments at Station 1 stimulated biomass growth above Control levels, being maximal in the effluent treatments, 0.64 to 0.77 d<sup>-1</sup>. These rates correspond to generation times of about 38 and 31 hrs, respectively; about twice as fast as Control generation time (70 hrs). At Station 2, growth rates were slightly higher than Control levels (0.33 d<sup>-1</sup>) only in the N+P/2, N+P/5 and 5% effluent additions. These differences between stations are generally consistent with the primary production responses in the various enrichments.

Individual species responded differently to the enrichments, within and between stations. At Station 1, <u>Chaetoceros compressus</u> exhibited a Control rate of 0.20 d<sup>-1</sup>, which was exceeded by all enrichments (excluding N+P (= T1)), ranging from 0.31 (N+P+Si/5) to 0.91 d<sup>-1</sup> (1% effluent). <u>Skeletonema costatum</u> grew rapidly in the Control (1.21 d<sup>-1</sup>), slightly exceeded in the N+P/2 and N+P/5 TABLE 13. TOTAL COMMUNITY CHLOROPHYLL GROWTH RATES OVER 48-HR PERIOD DURING JULY EXPERIMENT

	STN 1	STN 2
CONTROL	0.34 d-1	0.33
N+P	0.54	0.31
N+P/2	0.53	0.45
N+P/5	0.56	0.41
N+P+Si	0.40	0.19
N+P+Si/2	0.56	0.31
N+P+Si/5	0.34	0.22
5% Effluent	0.77	0.54
1% Effluent	0.64	0.32

enrichments (1.32 d<sup>-1</sup>). <u>Nitzschia seriata</u> grew rapidly (1.33 d<sup>-1</sup>) in the N+P+Si treatment, but otherwise exhibited rates equal to (0.83 d<sup>-1</sup>), or below Control levels. <u>Cerataulina pelagica</u> grew (0.82 d<sup>-1</sup>) only in the 5% effluent enrichment, whereas <u>Chaetoceros affinis</u> was stimulated only at:

N+P/2	0.25 d-1	N+P+Si/2	0.50
N+P/5	0.56	N+P+Si/5	0.64

This response suggests that both silica and, probably, N were limiting to <u>Chaetoceros affinis</u>. In the case of <u>Skeletonema costatum</u> inclusion of Si in the N+P series dampened its growth rate:

N+P	1.32 d-1	N+P+Si	1.12
N+P/2	1.33	N+P+Si/2	0.78
N+P/5	1.13	N+P+Si/5	0.93

Inter-specific differences in response to sewage effluent also occurred at Station 1:

	5% Effluent	1% Effluent
<u>Chaetoceros compressus</u>	0.60 d-1	0.91
<u>Skeletonema costatum</u>	1.27	1.11
<u>Cerataulina pelagica</u>	0.82	0
<u>Nitzsch</u> ia <u>se</u> riata	0	0.83

Thus, the 1% effluent enrichment was more favorable to growth than the 5% enrichment for two of these four species, and <u>vice versa</u> for the

other two species.

At Station 2, <u>Chaetoceros compressus</u> was also stimulated above Control responses (0.38 d<sup>-1</sup>) at all treatments excluding N+P+Si/2 and N+P+Si/5. Growth rates, otherwise, ranged from 0.59 d<sup>-1</sup> (N+P/5) to 0.99 d<sup>-1</sup> (5% effluent). Unlike at Station 1, <u>Skeletonema costatum</u> generally was stimulated above Control rates (0.47 d<sup>-1</sup>), with its maximal growth (1.17 d<sup>-1</sup>) occurring in the 5% effluent treatment (T7). <u>Nitzschia seriata</u>, likewise, was not stimulated at Station 1, whereas at Station 2 it was markedly stimulated by all N+P (T1 - T3) and N+P+Si (T4 - T6) amendments; maximal growth (1.95 d<sup>-1</sup>) occurred in the N+P+Si treatment. The response to effluent enrichment varied between species:

	5% Effluent	<u>l% Effluent</u>
<u>Chaetoceros compressus</u>	0.99 d-1	0.78
<u>Skeletonema costatum</u>	1.17	0.77
<u>Cerataulina pelagica</u>	0.13	0
<u>Nitzschia</u> <u>seriata</u>	0	0

Clearly, inter-specific and regional differences (Station 1 vs. 2) in response to nutrient enrichment level and composition are characteristic of New Bedford Harbor. Moreover, seasonal differences in effects of the different nutrient combinations also characterize the experiments. These characteristics make predictions as to the responses of given species to given nutrient loadings at any given time problematic. However, outbreaks of unusual species, notably "nuisance" species, were not observed, at least over the 48 hr incubation periods.

#### 9. AUGUST, 1988 ENRICHMENT EXPERIMENTS

The last nutrient enrichment experiment was carried out between 16-18 August 1988 at a temperature of 22° - 23°C; total irradiance levels were 391 and 464 ly d<sup>-1</sup>, respectively. The field survey indicated a strong summer bloom of the diatoms <u>Skeletonema costatum</u> and the large species <u>Eucampia cornuta</u> (MCA Report No. 89-1). Both primary production rates (Figure 1) and chlorophyll biomass (Figure 2) increased significantly since July.

At Station 1, all nutrient additions stimulated primary production (Figure 18); at Station 2, in contrast, production of the entire community ( $\Sigma$ ) was generally similar to, or slightly below Control levels, but generally stimulated in the nannophytoplankton fraction (Figure 19). The AN<sub>0</sub> at Station 1 generally increased along the enrichment series gradient (from Tl to T8), being maximal in the effluent treatments (Table 14). The < 10  $\mu$ um size-class was stimulated to a much greater extent (2- to 2.7-fold) than the total community (1.2- to 1.9-fold).

Repression of production at Station 2 occurred during July (Table 13). During August, for the entire  $(\sum)$  community, repression persisted in the effluent treatments (10% to 30% below Control levels); the other nutrient treatments did not appreciably modify the responses from those in the Control. The AN<sub>0</sub> nannophytoplankton fraction was generally stimulated (by 1.1- to 1.2-fold), excluding the effluent treatments which were similar to Control levels.

The interesting feature of these results is that whereas nutrient enrichment of the Station 1 phytoplankton community generally stimulated production during July and August, at the present outfall site (Station 2) community production was either repressed generally or

# TABLE 14. ASSIMILATION NUMBER (mg C mg Chl-1 d-1) RATIOS RELATIVE TO CONTROL DURING AUGUST ENRICHMENT EXPERIMENTS.

	STN 1		STN	2
	<u>(Σ)</u>	< 10 µm	<u>(</u> <b>Σ</b> )	< 10 µm
N+P	1.24	2.08	1.04	1.12
N+P/2	1.46	2.04	1.08	0.97
N+P/5	1.28	2.01	1.06	1.42
N+P+Si	1.31	2.00	0.85	1.26
N+P+Si/2	1.39	1.97	0.97	1.24
N+P+Si/5	1.58	2.61	0.99	1.24
5% Effluent	1.93	2.74	0.89	1.11
l% Effluent	1.60	2.49	0.72	1.01

not stimulated above Control levels. This reveals that in the outer reaches of New Bedford Harbor summer phytoplankton growth is nutrient-limited. However, this limitation is eliminated through outfall loading (as at Station 2). Furthermore, under certain conditions stimulation of primary production through accreted nutrients in the sewage discharge is inhibited. For the N+P and N+P+Si enrichment series, this may reflect excessive loadings of these nutrients (although this interpretation is problematic). In the case of the sewage erfluent dilutions used, perhaps one or more substances may be phyto-inhibitory in these effluent mixtures. A conspicuous feature of the effluent mixture after filtration was the considerable difference in color and a variable Tyndall effect between sample collections. This suggests that industrial contributions to the chemical composition of the effluent are variable.

Chlorophyll biomass growth rates are presented in Table 15. The 24 hr rates at Station 1 ranged from 1.2 to 1.9 d<sup>-1</sup>, considerably above Control rates (0.35 d<sup>-1</sup>). At Station 2, 24 hr rates were similar to, or below Control levels (0.67 d<sup>-1</sup>) in the N+P+Si series (T4 - T6), but nearly equally stimulated in the N+P, N+P/2 and effluent enrichments. The 48 hr rates were generally lower.

Marked stimulation of species growth rates occurred. Most species grew rapidly in the Controls, with extremely high growth rates often accompanying enrichment. Some examples of these, using Control and maximal rates, are:

	ç	ontrol	<u>Maximal</u>	
<u>Chaetoceros</u>	compressus			
Stn 1		1.22 d-1	2.58	(N+P+Si)
Stn 2		3.11	4.53	(5% Effluent)
<u>Skeletonema</u>	costatum			
Stn 1		0.71	2.00	(5% Effluent)
Stn 2		0.97	3.19	(N+P+Si)
Asterionella	<u>glacialis</u>			
Stn 1		1.65	2.39	(1% Effluent)
Stn 2		2.18	2.71	(N+P+Si)

More species were stimulated at Station 1 than at Station 2:

	<u>Control</u>	<u>Maximal</u>	
<u>Nitzschi</u> a <u>seriata</u>	0.31	1.48 (5%	Effluent)
<u>Nitzschia delicatissima</u>	2.72	2.90 (5%	• )
<u>Nitzschia closterium</u>	1.47	2.61 (1%	• )
<u>Thalassionema</u> <u>nitzschioides</u>	0	1.62 (5%	<b>•</b> )

These results, together with the July experiments, reveal that summer nutrient enrichment in New Bedford Harbor markedly stimulates species biomass and individual species responses over a 24 to 48 hr period. In contrast, the six-hr production experiments during these months revealed marked stimulation of Station 1, but repression and/or neutral effects of added nutrients relative to the Control responses at the present outfall site.

## TABLE 15. TOTAL COMMUNITY CHLOROPHYLL GROWTH RATES OVER 24 AND 48 HR PERIODS DURING THE AUGUST EXPERIMENT.

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	STN 1		STN 2		
	24 HR	48 HR	24 HR	48 HR	
CONTROL	0.35	0	0.67	0.21	
N+P	1.85	0.95	0.95	0.57	
N+P/2	1.88	0.78	0.86	0.51	
N+P/5	1.17	0.17	0.65	0.88	
N+P+Si	1.73	1.04	0.71	0.80	
N+P+Si/2	1.64	0.61	0.38	0.13	
N+P+Si/5	1.22	0.23	0.33	0.32	
5% Effluent	1.73	1.52	0.85	1.06	
1% Effluent	?	0.79	1.04	0.62	

#### DISCUSSION

The discussion will focus on the experimental responses of the phytoplankton community to the sewage effluent enrichments provided at 1:20 (5%) and 1:100 (1%) dilutions. The 1:20 dilution simulates the expected full strength effluent dilution in the zone of initial dilution (ZID) (H. Yamaguchi, CDM, personal communication). The 1:100 dilution simulates a loading further downstream in the effluent plume. The responses at Station 1, the proposed outfall-site, particularly are of interest. Should outfall-discharge be relocated to this site, the phytoplankton communities would be subjected to significantly increased nutrient loading and water quality amendment. Effluent additions in the Station 2 experiments, in contrast, are in addition to background levels of effluent to which the phytoplankton communities are presently exposed to at this outfall site. Thus, the experimental results at Station 1 represent potential phytoplankton responses in that region following environmental modification due to effluent enrichment and altered chemical water quality. The experimental results at Station 2, in contrast, represent responses to increased modification of an environment already perturbed by effluent discharge. The question in focus becomes: will the Station 1 phytoplankton community dynamics and responses become similar to those which presently characterize Station 2?

Two distinct aspects of effluent chemical composition must be recognized which directly influence phytoplankton dynamics: its nutrient content and the presence of phyto-stimulants/phytoinhibitors/phyto-toxicants. The major nutrient sources, N, P, Si in the effluent mixtures used experimentally were measured; but micro-nutrients (Fe, Mn, Zn, Co. Cu, vitamins) were not. Of these two nutrient categories, macro-nutrients are more involved in regulating biomass and productivity than the micro-nutrients. which may be important in regulating species composition and succession. Since biomass overloading in excess of grazing may result in anoxia, the focus on macro-nutrients (which determine biomass yield) over micro-nutrients is appropriate to assessment of potential New Bedford Harbor phytoplankton responses to effluent discharge.

Phytoplankton communities are also influenced by altered water quality, including chemical factors which may be phyto-stimulatory, phyto-inhibitory or toxic. The chemical nature, presence, abundance and seasonal aspects of these properties have not been determined in the effluent mixtures, nor stipulated contractually. This precludes evaluation of their potential effects on New Bedford Harbor phytoplankton dynamics and their effects during the experiments. In reality, contemporary experimental techniques and our current state of knowledge preclude meaningful assessments of such "water-quality" affects from being made. Thus, the experimental phytoplankton responses in relation to the major nutrient component (N, P, Si) of the sewage effluent mixtures used will be the focus of the ensuing discussion.

## Chemical Composition of Sewage Effluent

Macro-nutrient levels in the sewage effluent composited at the New Bedford Treatment Facility varied seasonally (Table 16). NH<sub>4</sub> levels varied about 2-fold, from 349 to 639  $\mu$ M; NO<sub>2</sub>+NO<sub>3</sub> levels 94-fold, from 1.2 to 113  $\mu$ M; PO<sub>4</sub> levels 2.5-fold, from 54 to 137  $\mu$ M, and SiO<sub>4</sub> about

COLLECTION DATES	NH4	NO3+NO2	PO4	SiO4	DON	TN	DOP	TP
<u>1987</u>								
15-17 Sept	370	4.0	99	87	309	683	17	116
1-3 Oct	527	3.7	78	113	27 4	80 5	44	122
10-12 Dec	485	1.7	82	125	264	750	15	97
<u>1988</u>								
2-4 Feb	4/2	51.0	75	96	106	629	1.4	76
28 Feb - 1 Mar	443	91.2	76	8 <b>9</b>	158	692	9	85
29-31 Mar	286	113.0	61	105	27 <del>9</del>	678	16	77
25-2/ Apr	503	7.3	137	95	161	671	19	156
25-2/ May	536	2.5	114	88	141	680	3	117
27-29 June	639	1.2	147	99	120	761	9	155
29-31 July	464	1.2	125	105	116	582	49	124
29-31 Aug	349	6.1	54	112	?	364	?	46

TABLE 16. NUTRIENT CONCENTRATIONS IN SEWAGE EFFLUENT COMPOSITED AT NEW BEDFORD SEWAGE TREATMENT FACILITY (µM).

1.5-fold, from 87 to 125 µM. Dissolved organic nitrogen (DON) levels varied about 3-fold, from 106 to 309 µM, and dissolved organic phosphorus 2.5-fold, from 1.4 to 49 µM. Total nitrogen (TN) varied about 2.2-fold, from 364 to 805 µM. and total phosphorus (TP) 3.4-fold, from 46 to 156 µM. This clearly indicates that macro-nutrient levels within sewage effluent are not constant. There was a quasi-seasonal aspect to macro-nutrient concentrations in the effluent. NHA levels were highest (> 500 µM) from April - June; lowest during August -September (Table 16). NO2+NO3 levels were maximal during February -March; in fact, a 10- to 100-fold surge in levels occurred during this period. POA levels were maximal (> 110 AuM) during April - July. Levels then increased about 2- to 3-fold above March and August levels. In contrast, there was no clear seasonality in SiO<sub>2</sub> concentrations. Thus, the sewage effluent was characterized not only by variable concentrations of macro-nutrients, but also by seasonality in maximal levels which differed between the N and P fractions in terms of seasonal phasing.

DON levels were generally lowest (< 165 µM) during April - July, the period when NH<sub>4</sub> levels were at their annual maximum. DOP levels, in contrast. were more variable in the monthly collections and without clear seasonality.

Another feature of the sewage effluent mixture is the variable ratios between nutrients (Table 17). Nutrients are assimilated stoichiometrically by phytoplankton, rather than as individual moreties, in a classical stoichiometric association known as the Redfield Ratio:

$$O:C:N:P = 276:106:16:1$$
 (by atoms)

	N:P	N:Si	P:Si	DON:DOP
<u>1987</u>				
15-1/ Sept	3.8	4.3	1.1	18.2
1-3 Oct	6.8	4.7	0.7	6.2
10-12 Dec	5.9	3.9	0.7	17.6
<u>1988</u>				
2-4 Feb	7.0	5.4	0.8	80.0
28 Feb - 1 Mar	7.0	6.0	0.9	17.6
29-31 Mar	6.5	3.8	0.6	17.4
25-2/ Apr	3.7	5.4	1.4	8.5
25-27 May	4.7	6.1	1.3	47.0
27-29 June	4.4	6.5	1.5	13.3
29-31 July	3.7	4.4	1.2	2.4
29-31 Aug	6.6	3.2	0.5	?

TABLE 17. NUTRIENT RATIOS IN TREATED SEWAGE EFFLUENT COMPOSITED AT NEW BEDFORD SEWAGE TREATMENT FACILITY (atom:atom basis).

Moreover, competition between species and selection of phytoplankton functional groups are influenced by nutrient ratios. The N:P and N:Si ratios (by atoms) varied about 2-fold in the effluent mixtures, from 3.7 to 7.0:1 and 3.2 to 7.5:1, respectively. The P:Si ratio varied by about 3-fold, from 0.5 to 1.4:1, and the DON:DOP ratio about 33-fold, from 2.4 to 80:1.  $(NH_4+NO_2+NO_3)$  levels were summed to obtain N.) The DON:DOP ratio was very variable seasonally, whereas the N:P ratio was lowest (< 5:1) from April - July. During this latter period, the P:Si ratio increased indicating an excess of inorganic P relative to Si. N:Si ratios also tended to be higher then. Such ratios tend to favor the summer predominance of flagellates and non-motile, non-siliceous requiring phytoplankters in waters which are stratified. The well-mixed waters of New Bedford Harbor allow the faster sinking diatoms to thrive during the summer. However, the reduced availability of Si relative to N and P results in a shared dominance with < 10  $\mu$ m nannophytoplanktonic species. Thus, the high temperatures, high nutrient levels and nutrient ratios during the summer in New Bedford Harbor potentially favor non-diatom blooms during that season. However, the mixing characteristics also facilitate diatom growth then in response to N and P loadings. This aspect diminishes somewhat the prospects of extensive non-diatom blooms as a recurrent, annual problem.

## Sewage Effluent Effects on Phytoplankton

The seasonal changes in the primary production assimilation number in response to the 1:20 (5%) and 1:100 (1%) effluent enrichments are presented in Table 18. To facilitate analyses, the assimilation

TABLE 18.	SEASONAL CHANGES IN	PRIMARY PRODUCTION	ASSIMILATION NUMBER
	(mg C·mg Chl-l·d-l)	RATIOS RELATIVE TO	CONTROL IN RESPONSE
	TO 5% AND 1% SEWAGE	EFFLUENT ADDITIONS	AND MAXIMAL RESPONSE
	(MR) IN INORGANIC EN	NRICHMENT TREATMENT	SERIES (code in ( )
	identifies treatment		

STATION	11			2			
EFFLUENT	5%	1%	MR	5%	1%	MR	
December ∑ < 10	0.88 1.20	0.82 1.09	1.04(C) 1.20(T5)	0.56 1.74	0.79 1.69	0.81(C) 2.33(T6)	
January 乏 く10	0.62 2.17	0.87 1.83	0.90(T6) 3.22(T6)	1.00 3.19	1.02 2.99	1.15(T3) 3.72(T6)	
February Z < 10	2.04 2.40	2.25 1.54	2.43(T6) 2.32(T5)	1.91 0.67	2.04 0.72	2.14(T5) 0.75(T5)	
March Z < 10	1.09 1.11	0.94 0.86	1.28(T4) 1.36(T6)	1.36 1.30	1.38 1.70	1.73(T4) 2.85(T5)	
April Z < 10	1.54 1.67	1.49 2.06	? ?	1.31 5.47	1.33 2.82	? ?	
May Z < 10	1.21 1.61	1.40 1.92	1.30(T6) 1.73(T6)	1.16 1.58	1.31 1.90	1.42(T4) 2.40(T5)	
June Z < 10	1.95 2.55	2.52 2.35	2.61(T2) 2.31(T6)	1.34 1.42	0.77 1.52	1.34(T3) 1.45(T3)	
July × < 10	2.36 1.73	2.15 1.56	2.66(T1) 1.68(T6)	0.58 0.70	0.60 0.70	0.92(T1) 0.82(T1)	
August < 10	1.93 2.74	1.60 2.49	1.58(T6) 2.61(T6)	0.89 1.11	0.72	1.08(T3) 1.42(T3)	

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numbers  $(AN_0)$  relative to the Control responses have been sub-grouped into various categories: < 0.9 (repression); 0.91 - 1.10 (without effect); 10% to 50% stimulation (1.11 - 1.5); 50% to 100% stimulation (1.51 - 2.0); 100% - 200% stimulation (2.01 - 3.0); > 200% stimulation (> 3.0). Pooling the responses of the total ( $\Sigma$ ) and < 10 µm size classes exposed to the 5% and 1% effluent enrichments yields 36 treatments at each station for the nine monthly experiments. The percentages of these treatments exhibiting the response patterns established were:

	STN 1	STN 2
Repression	14	31%
No Effect	8	8
10 to 50% Stimulation	14	28
51 to 100%	31	19
101 to 200% "	33	8
> 200%	-	6

Collectively, relative to the Control the production rate per unit chlorophyll biomass (Assimilation No.) at Station 2 was repressed once in every three experiments in contrast to once in every seven experiments at Station 1. With regard to stimulation, a > 50% enhancement occurred twice as frequently at Station 1 (64%) than at Station 2 (33%). That is, considerable enhancement occurred in two-thirds of the experiments at Station 1. These results clearly suggest that effluent discharge at Station 1 would generally stimulate production.

At Station 1, both the total ( $\Sigma$ ) and < 10  $\mu$ m size classes

responded similarly to effluent enrichment. For example, the total  $(\Sigma)$  community was repressed (< 0.90) in ll% of the experiments; the < 10  $\mu$ m (= nannophytoplankton) in 17%. The corresponding percentages for > 50% enhancement of AN<sub>0</sub> were 66% and 61%, respectively. For Station 2, the > 50% enhancement frequency was 17% ( $\Sigma$ ) and 39% (< 10  $\mu$ m), suggesting the nannophytoplankton community at the current outfall site was frequently stimulated by effluent addition.

The seasonal responses to effluent addition differed between stations (Table 18). During June - August, the Station 1 communities were enhanced to a greater extent than at Station 2. The reverse situation occurred during December - March, whereas the April - May responses were similar. A conspicuous feature of the phytoplankton at both stations was the similarity in species composition and dominance. Thus, the observed differences do not reflect differences in taxonomic structure, but most likely differences in the chemical milieu, such as water quality and macro-nutrient availability.

Although the effluent series was generally stimulatory, notably at Station 1, the persistent evidence of occasional repression, particularly at Station 2, prompted further evaluation of the latter. Table 18 presents the maximal AN<sub>0</sub>, relative to the Control found in the inorganic enrichment treatment series T1 - T6 (Table 1). The primary production AN<sub>0</sub> was usually enhanced, notably by the N+P+Si enrichment series (T4, T5, T6) above the effluent enrichment responses. Effluent enrichment AN<sub>0</sub> relative to the Control (in those experiments where it was  $\geq$  1.0) was exceeded by 10% or more in 18 of 22 experiments at Station 2, and 12 of 28 experiments at Station 1. For example, the < 10 µm fraction at Station 2 in December was enhanced over the Control by 1.74 and 1.69 in the 5% and 1% treatments. respectively. Both

responses were secondary to the maximal response (2.33) in this experiment obtained with the N+P+Si/5 (= T6) enrichment. At Station 2, from December - May, the relative AN<sub>0</sub> in one of the N+P+Si treatments exceeded those in the effluent treatments, unlike June - August. This enhancement does not appear to be due to additional Si levels in the T4 - T6 series relative to those in the 5% and 1% effluent mixtures. The following lists the initial Si levels in the effluent treatments and in the more stimulatory N+P+Si treatment (= NPS):

	58	18	NPS	<u>Series No</u> .
December				
< 10	6.63 µм	2.52	3.13	Т6
January				
< 10	7.56	5.61	3.65	Т6
February				
	13.26	8.19	5.91	Т5
March				
Σ	5.79	1.80	5.84	Т4
< 10	5.96	2.75	3.37	Т5
May				
Σ	12.56	7.22	11.96	Т4
< 10	16.80	7.52	8.13	Т5

Similar demonstrations can be made for the Station 1 responses. Clearly, the Si levels present in the N+P+Si levels are often lower than effluent levels. Hence, enhancement above effluent responses can not be attributed to increased Si availability, and reduced responses to effluent enrichment can not be attributed to toxic levels of Si

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(where they exceed T4, T5, T6 Si levels). These elevated concentrations are well within normal Si levels to which diatoms are exposed to and utilize. Neither can limiting effects due to N or P availability be detected in the nutrient data when analyzed in a fashion similar to that for Si, and illustrated above.

These data suggest therefore that New Bedford sewage effluent additions have a dual effect. The major effect: considerable stimulation of primary production, expressed as the ANo relative to the Control, frequently occurs. On occasion, a more subtle, accompanying effect appears to be a mild repression of primary production preventing even greater production rates and utilization of the elevated This possibly reflects the presence of chemical moieties nutrients. which influence water quality and modify physiological processes. A notable feature observed during the processing of the New Bedford Sewage Treatment Facility's composited effluent sample was the considerable variability in color, Schlieren and Tyndall effects and apparent viscosity between monthly samples. Presumably, this reflected a variable chemical composition, moieties of which may have had the presumed inhibitory impact on the primary production ANo suggested by the data.

#### Effect of Sewage Effluent on Chlorophyll Biomass Growth Rates

Frequently, neither the total ( $\sum$ ) community nor the nannophytoplankton (< 10 µm) size fraction exhibited net chlorophyll growth rates during the first 24 hrs. This occurred despite active primary production during the 6 hr incubations. Chlorophyll levels often decreased initially. Significant exceptions to this generalization occurred during the February, July and August experiments at both

stations; during May and June at Station 1, and during March and April at Station 2. This response may partly reflect active grazing by microzooplankton which passed through the 153 um mesh screen used to filter out larger zooplankton during transfer of the samples into the experimental containers. Table 19 presents the annual cycles of tintinnids and ciliates revealing an abundant year-round population of these herbivorous microzooplankters. Heterotrophic phytoplankton were also abundant, including a <u>Dinobryon</u> sp. which grew at daily rates of 1.0 to 1.5 divisions, and various holozoic dinoflagellates. The contractually defined experiments were not designed to evaluate grazing rates, preventing quantification of the importance of grazing in regulating observed growth responses to enrichment. Grazing, however, did not significantly modify the final outcome of the responses. The frequent first day reduction in and/or lag in chlorophyll synthesis and growth probably represented a period of adaptation to the sudden exposure of the natural phytoplankton communities to elevated nutrient levels. This temporary stasis, despite the measureable nutrient uptake during the first 24 hrs of incubation, together with any micrograzing activity, most likely contributed to the declining or unchanged chlorophyll biomass where it occurred. In those experiments, marked adjustment to the elevated nutrient led to a recovery and active chlorophyll growth. Table 20 presents the chlorophyll growth rates of the total ( $\sum$ ) community between experimental days 1 and 2 in the effluent enrichment treatments. Growth rates in the effluent treatments generally were highly stimulated over Control rates. However, it is also clear that seasonal and station-to-station differences in biomass responses to effluent enrichment characterize New Bedford Harbor.

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+ CILIATES DU	KING ANNUAL SURVEY.	
STATION	1	2
18 August, 1987	4,000	3,000
2 September	7,000	6,000
15 September	3,000	6,000

TABLE 19.	RECORDED ABUNDANCE	(ANIMALS L-1)	OF HERBIVOROUS	TINTINNIDS
	+ CILIATES DURING	ANNUAL SURVEY.		

2 September	7,000	6,000
15 September	3,000	6,000
30 September	8,000	3,000
13 October	3,000	4,000
8 December	2,000	2,000
ll January, 1988	2,000	2,000
l February	2,000	2,000
3 March	2,000	2,000
ll April	0	1,000
9 May	2,000	4,000
6 June	6,000	8,000
18 July	4,200	5,000
15 August	6,000	9,000

#### Effect of Effluent Enrichment on Species Composition and Growth Rates:

Effluent enrichment did not alter the community composition and individual species' growth rates from that observed in the inorganic nutrient enrichments (Tl - T6) over the 48 hr experimental period. Moreover, nuisance species were not stimulated to bloom proportions. The non-toxic, red-tide dinoflagellate <u>Heterocapsa triquetra</u> present in modest abundance during May was not triggered into a bloom event by any of the nutrient treatments.

#### Apparent Utilization of Dissolved Organic Nitrogen

The utilization of NH<sub>4</sub>,  $NO_2 + NO_3$ , PO<sub>4</sub> and SiO<sub>4</sub> during the enrichment experiments was evaluated from changes in levels from initial concentrations in the various treatments. During the July and August experiments, periods of especially intense phytoplankton growth, the changes in dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were assessed. (These measurements were not stipulated in the contractual agreement.) The intent was to evaluate whether these dissolved organic components in the effluent might be utilized by some component of the phytoplankton community and associated microflora. Table 21 presents the results.

During the July experiment, DON levels decreased during the first 24 hrs by > 5  $\mu$ M in four of the eight experiments; were relatively unchanged (-1.9 to 2.5  $\mu$ M) in four experiments. During the August experiment, DON levels also decreased by about 5.0 to 15  $\mu$ M in four experiments; were relatively unchanged in two experiments and decreased by about 5  $\mu$ M twice. The observed decreases in DON in eight of the 16 experiments were not accompanied by equivalent increases in NO<sub>2</sub>+NO<sub>3</sub> or

STATION		11				2	
	С	5%	18		с	5%	18
December	-	0.24	-		-	0.55	-
January				???			
February	0.33	0.19	0.40		0.63	0.47	-
March	-	0.49	-		-	0.38	0.06
April	0.07	-	0.19		0.61	0.61	0.80
Мау	1.03	0.90	0.23		1.37	-	-
June	0.43	0.50	0.43		-	0.39	0.80
July	0.44	0.81	0.74		0.27	0.56	0.16
August	-	1.30	3.24		-	1.26	0.20

TABLE 20. CHLOROPHYLL BIOMASS GROWTH RATES (d-1) BETWEEN EXPERIMENTAL DAYS 1-2 RELATIVE TO CONTROL (C) GROWTH RATES (- indicates no growth; underlined rates exceed Control rates). NH<sub>4</sub>. This suggests that actual utilization of DON was occurring in addition to any deamination and remineralization of DON into inorganic nitrogenous compounds. Both the total ( $\Sigma$ ) and nannophytoplankton (< 10 µm) experimental series exhibited apparent utilization of DON. During July, apparent utilization of DON in the 5% effluent enrichment at Station 1 was about 2- ( $\Sigma$ ) to 5-fold (< 10 µm) greater than NH<sub>4</sub> uptake, the predominant apparent N source at Station 2. During August at Station 1, the apparent NH<sub>4</sub> and DON utilization rates over the 1-day experimental period were similar. At Station 2, apparent NH<sub>4</sub> utilization tended to exceed apparent DON utilization rates.

Similar evidence for DOP utilization was not found; nor for apparent PO<sub>4</sub> utilization. In fact, both components frequently increased in the experimental treatments in erratic fashion.

Clearly, direct evidence for DON utilization requires the use of 15N techniques. The present, limited observations indicate that the DON fraction is not a refractory component in the effluent. Some component may be used directly by the phytoplankton community and its associated microflora, while another fraction may be remineralized into inorganic components more suitable for utilization by the phytoplankton. The organic components of the macronutrients must therefore be factored into engineering assessments of potential nutrient loading levels of proposed effluent discharge rates, associated increases in phytoplankton biomass and oxygen demand of the latter during decomposition and respiration.

### Phytoplankton Biomass Growth Rate and Nutrient Uptake

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From changes in  $NH_4$ ,  $PO_4$  and  $SiO_2$  concentrations during the incubation period, nutrient uptake rates were calculated.  $PO_4$  changes

TABLE 21. DAILY NH4, DISSOLVED ORGANIC NITROGEN (DON) AND DISSOLVED ORGANIC PHOSPHORUS (DOP) UPTAKE RATES (µM d-1) IN NEW BEDFORD 5% AND 1% SEWAGE EFFLUENT ENRICHMENT EXPERIMENTS DURING JULY AND AUGUST, () REPRESENTS INCREASE.

	<u> </u>	58	1	8		58	1	8
	NH4	DON	NH4	DON	PO4	DOP	PO4	DOP
July 18-19								
STN 1 Z	2.95	6.5	1.48	(1.9)	(1.25)	(0.44)	(0.83)	0.15
< 10	1.12	5.3	1.11	0.8	(0.87)	(0.31)	(0.69)	0.17
STN 2 Z	(5.83)	1.6	8.54	14.1	(3.08)	(2.92)	(0.85)	0.94
< 10	0	37.4	0	2.5	(1.02)	(0.03)	(0.10)	?
August 16-1	7							
STN 1 Z	20.01	14.9	4.38	3.0	0.33	0.73	0.68	(0.02)
< 10	16.87	14.9	5.36	4.8	0.49	0.14	0.73	(0.04)
STN 2 Z	7.61	(4.3)	5.25	(4.7)	(2.32)	(4.34)	(0.68)	(0.24)
< 10	13.96	9.7	(0.32)	2.9	0.79	(0.10)	(0.82)	(0.02)

. . . . . . . . . . . .

varied considerably, and no clear patterns of uptake were discernable. Both NH<sub>4</sub> and SiO<sub>2</sub> uptake, however, were commonly observed. NH<sub>4</sub> uptake will be focussed on since it is a major component of sewage effluent.

A strong correlation occurred between chlorophyll biomass growth rate and NH<sub>4</sub> uptake in the 5% and 1% sewage effluent treatments. For all nine monthly (December - August) enrichment experiments,  $r^2 = 0.67$ at Station 1, defined by the linear regression equation:

Y = 0.15 + 0.10X

where Y represents the daily chlorophyll growth rate and X represents NH4 uptake as uM.

For Station 2,  $r^2 = 0.81$ , defined by the linear regression equation:

Y = 0.05 + 0.05X

Combining both stations significantly reduced the correlation coefficient. Despite the strong linear correlations at both stations, the responses at each station were best described by treating the stations separately. The key finding, however, is that at both stations good correlations were obtained between NH<sub>4</sub> uptake and biomass growth, and clearly confirm the frequent stimulatory effect of sewage effluent on phytoplankton growth in response to NH<sub>4</sub> loading.

The relationship between NH4 uptake and chlorophyll growth rate in the nine enrichment treatments at each station during each month was also examined. Considerable monthly variations occurred in the strength of the correlations between NH4 uptake and biomass growth rate

and between stations. For example, for the July experiment at Stations 1 and 2,  $r^2 = 0.41$  and 0.35, respectively. During August,  $r^2$  increased to 0.89 and 0.60, respectively.  $r^2$  (0.60) for Station 2 was significantly influenced by the response in the N+P/5 (= T3) treatment. Eliminating this outlier in the correlation increased  $r^2$  to 0.87. Despite such monthly, station-to-station and individual treatment variability, the experimental data consistently identify nitrogen as a principal nutrient regulating phytoplankton growth in New Bedford Harbor.

This role of nitrogen is influenced by Si availability, as shown previously in the growth rate experiments. For example, the correlation between NH<sub>4</sub> uptake and chlorophyll growth rate in the N+P+Si (T4, T5, T6) enrichment series during the July and August experiments was  $r^2 = 0.50$ , whereas in the N+P series (T1, T2, T3)  $r^2$ was 0.33. For July alone, r<sup>2</sup> was 0.80 for the N+P+Si enrichment, but was uncorrelated  $(r^2 = 0.02)$  in the N+P enrichment series. The August r<sup>2</sup> values were 0.49 and 0.26, respectively. The data collectively indicate that increased NH<sub>4</sub> uptake accompanies increased Si availability. This facilitates increased diatom production in response to elevated nitrogen levels. A reduction in Si favors N uptake by non-diatomaceous species, with the potential for nuisance algal blooms. Therefore, it is desireable that high Si levels characterize the site of effluent discharge. Sewage effluent is not an effective source of Si favoring higher N:Si and P:Si ratios in recipient waters (Table 17); elemental ratios favorable to non-diatom groups. While such blooms were not evident during these experiments, the data point to the beneficial value of Si in routing N uptake to diatoms.

#### Summary of Sewage Effluent Effects on Phytoplankton:

The experimental data clearly established that increased phytoplankton primary production, biomass and growth rate accompanied exposure to treated sewage effluent. This effect was particularly evident at Station 1, the proposed relocation site of the effluent discharge. However, phytoplankton dynamics and composition did not differ substantially from those characterizing "natural" conditions. Within the framework of the experimental study, the proposed relocation of the discharge site to this region would not cause serious alterations or negatively impact the indigenous phytoplankton community and its dynamics. The experimental data also suggest that continuance of the outfall site at its current location, Station 2, would likewise not significantly modify, or negatively impact local phytoplankton dynamics already established in response to present effluent discharge. The seasonal phytoplankton dynamics, species composition and succession were similar at both stations. The primary difference was in the elevated phytoplankton abundance, primary production and nutrient levels at Station 2.

#### FIGURE LEGENDS

- Figure 1. Annual primary production cycles at Stations 1 and 2.
- Figure 2. Annual chlorophyll cycles at Stations 1 and 2.
- Figure 3. Primary production and assimilation number (mg C mg Chl-l d-l) responses to enrichment at Station 1 during December experiment.
- Figure 4. Primary production and assimilation number (mg C mg Chl-l d-l) responses to enrichment at Station 2 during December experiment.
- Figure 5. Primary production and assimilation number (mg C mg Chl-l d-l) responses to enrichment at Station l during January experiment.
- Figure 6. Primary production and assimilation number (mg C mg Chl<sup>-1</sup> d<sup>-1</sup>) responses to enrichment at Station 2 during January experiment.
- Figure 7. Primary production and assimilation number (mg C mg Chl-l d-l) responses to enrichment at Station l during February experiment.
- Figure 8. Primary production and assimilation number (mg C mg Chl-l d-l) responses to enrichment at Station 2 during February experiment.
- Figure 9. Primary production and assimilation number (mg C mg Chl-l d-l) responses to enrichment at Station l during March experiment.
- Figure 10. Primary production and assimilation number (mg C mg Chl-l d-l) responses to enrichment at Station 2 during March experiment.
- Figure 11. Primary production and assimilation number (mg C mg Chl-1 d-1) responses to 5% (Trt. 7) and 1% (Trt. 8) effluent enrichment and in Control at Stations 1 and 2 during April experiment following exposure to 60% and 10% of incident irradiance using

the total (∑) and < 10 µm nannophytoplankton size classes. Figure 12. Primary production and assimilation number (mg C mg Chl-1 d-1) responses to enrichment at Station 1 during May experiment. Figure 13. Primary production and assimilation number (mg C mg Chl-1 d-1) responses to enrichment at Station 2 during May experiment. Figure 14. Primary production and assimilation number (mg C mg Chl-1 d-1) responses to enrichment at Station 1 during June experiment. Figure 15. Primary production and assimilation number (mg C mg Chl-1 d-1) responses to enrichment at Station 2 during June experiment. Figure 16. Primary production and assimilation number (mg C mg Chl-1 d-1) responses to enrichment at Station 1 during June experiment. Figure 16. Primary production and assimilation number (mg C mg Chl-1 d-1) responses to enrichment at Station 1 during July experiment. Figure 17. Primary production and assimilation number (mg C mg Chl-1 d-1) responses to enrichment at Station 2 during July experiment. Figure 18. Primary production and assimilation number (mg C mg Chl-1 d-1)

Figure 19. Primary production and assimilation number (mg C mg Chl-l d-l) responses to enrichment at Station 2 during August experiment.

responses to enrichment at Station 1 during August experiment.

Integrated Production Aug. 18, 1987 - Aug. 16, 1988





# **STATION 1**



**STATION 2** 





## NUTRIENT SPIKE STATION 1 DEC. 8, 1987







## NUTRIENT SPIKE STATION 2 Dec. 8, 1987



TOTAL




# NUTRIENT SPIKE STATION 1 JAN 11, 1988







### NUTRIENT SPIKE STATION 2 JAN. 11, 1988



**PRODUCTION NUMBERS** 





mg C/m3/day

#### NUTRIENT SPIKE STATION 1 FEB. 2, 1988







#### NUTRIENT SPIKE STATION 2 FEB. 2, 1988







# NUTRIENT SPIKE STATION 1 MARCH 7, 1988





Figure 9

## NUTRIENT SPIKE STATION 2 MARCH 7, 1988







mg C/m3/day

NUTRIENT SPIKE APR. 11, 1988



#### NUTRIENT SPIKE STATION 1 MAY 9, 1988





## **NUTRIENT SPIKE STATION 2** MAY 9, 1988



C/m3/day

ßш

10 0 2 3 7 control 6 8 1 4 5

TREATMENT

### NUTRIENT SPIKE STATION 1 JUNE 6, 1988



**PRODUCTION NUMBERS** 450 400 TOTAL NANNOPLANKTON  $\boldsymbol{Z}$ 350 300 250 200 150 100 50 0 3 <sup>4</sup>TREATMENT <sup>5</sup> 8 2 7 control 1

mg C/m3/day

mg C/mg Chl a/day

NUTRIENT SPIKE STATION 2 JUNE 6, 1988





Figure 15

#### NUTRIENT SPIKE STATION 1 JULY 18, 1988





Figure 16

NUTRIENT SPIKE STATION 2 JULY 18, 1988

#### **CARBON PRODUCTION**







#### NUTRIENT SPIKE STATION 1 AUG. 16, 1988



**CARBON PRODUCTION** 

Figure 18

TREATMENT

2

5

6

7

8

0

control

1

### NUTRIENT SPIKE STATION 2 AUG 16, 1988



ANNEX 1

#### CAMP DRESSER & McKEE INC.

#### MEMORANDUM

TO: H. Yamaguchi

~ FROM: J. W. Small

- SUBJECT: New Bedford, MA Secondary Effluent Concentrations
- DATE: December 31, 1987

Based on discussions with Al Firmin, assume the following secondary effluent concentrations:

Ammonia – N	18 mg/1
Nitrate - N	None
Phosphorus - P	7 .
Sio <sub>2</sub>	Don't Know

Above data is also shown in red in attached memo.

JWS:gc Attachment

Ted-Ted-last!! Advard