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Large-volume (20-liter) bioassays were carried out in order to assess the effects of major nutrients and micro-nutrients on natural phytoplankton populations in water collected from a site close to the mouth of Yaquina Bay, Oregon. Seven long-term experiments were conducted during the years 1975-1976. A stripping technique employing activated carbon was used to reduce the trace metal and dissolved organic load of a portion of the water. Various combinations of major nutrients (nitrate, phosphate, and silicate) and micro-nutrients (trace metals and vitamins) were added to both the carbon-stripped and the non-carbon-stripped water in order to assess the effects of various nutrient substances on the growth rate, biomass yield, and species composition of the phytoplankton populations which developed from small natural inocula.

The major nutrients and the micro-nutrients appear to have fundamentally different effects on the species composition and population growth characteristics of the phytoplankton. The

micro-nutrients had marked systematic effects on the species composition of the phytoplankton populations which developed in the experimental vessels, while the major nutrients had minimal effects on species composition. The micro-nutrients appeared essential for the achievement of maximal population growth rates and minimal lags, while the major nutrients had no effect on growth rates or lags. Both the major nutrients and micro-nutrients had significant effects on the final biomass yields. There were seasonal variations in the control of final biomass yields by major nutrients and micro-nutrients.

Effects of Micro-Nutrients and Major Nutrients on the Growth and Species Composition of Natural Phytoplankton Populations

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INTRODUCTION

Primary production in the oceans occurs within certain bounds defined by the availability of resources necessary for growth. Light, for example, is limited to the upper strata of the oceans (the euphotic zone), thereby confining primary production to a small portion of the total ocean volume. Primary production is further constrained by the availability of chemical substances needed for growth. Some (water and carbon, for example) do not typically limit growth in the oceans, but other substances are commonly depleted to the point of limiting growth. These latter substances are referred to as limiting nutrients. Ultimately it is the rate at which these nutrients become available in the euphotic zone, through recycling or mixing and diffusion from below the euphotic zone, which determines the distribution and magnitude of primary production in the oceans.

Relatively large amounts of nitrogen and phosphorus (and silica in the case of the diatoms and silicoflagellates) are needed for the building of tissue in the phytoplankton. Lesser quantities of other substances, especially trace metals and vitamins, are often required for the functioning of cellular enzyme systems. Trace metals and vitamins, which are referred to here collectively as micro-nutrients, generally function as enzyme activators and co-enzymes (White et al., 1964).

Growth of cells is a function of the uptake and utilization

of nutrients from the environment. The velocity of substrate nutrient uptake is a function of the rate of formation of a substrate-enzyme complex (White et al., 1964) as shown by:

$$\text{Enzyme} + \text{substrate} \rightarrow \text{enzyme-substrate complex} \downarrow \text{enzyme} + \text{product}.$$

Exactly where in the cell the enzyme-nutrient complex is formed is not known, and it may differ for different nutrients. Falkowski (1975) has postulated an ATPase-nitrate complex associated with outer cell membrances, and it is likely most complexes involved with uptake are formed at sites in, on, or near the outer cell membranes. When the concentration of substrate is far greater than the enzyme concentration, the rate of formation of the enzyme-substrate complex is a function of enzyme activity. When the substrate concentration is less than that required to saturate the enzyme active sites, then substrate concentration limits the rate of formation of the complex. This rate then becomes a function of the substrate concentration, and can be described by a Michaelis-Menton-Monod (Monod, 1942) hyperbolic relationship.

A nutrient which is abundant in the environment will not limit the rate of growth. In a closed or partially closed system, where nutrient concentration is decreasing through biological uptake, the growth rate will become limited by nutrient concentration only when the concentration is reduced to the point where it can no longer saturate the enzyme active sites. At that point a short period characterized by a constantly diminishing growth rate will ensue, as the limiting nutrient approaches zero concentration. The

decrease in growth rate is abrupt and often difficult to detect (O'Brien, 1972). Logarithmic growth is terminated at that point, and a limit to further growth is imposed in the classical sense of Liebig's "Law of the Minimum" (1840).

My experiments were conducted with closed (batch) cultures, and as such can be considered analogues of natural phytoplankton systems under bloom conditions (Platt and Rao, 1970). The growth of phytoplankton in closed culture is typically described in three phases: lag phase, logarithmic (log) phase, and senescent phase (Fogg, 1966). Natural phytoplankton blooms behave in a similar manner (Platt and Rao, 1970).

The lag phase is an initial period of little measurable growth, and typically occurs in batch culture when a senescent (described below) inoculum is used or when growth conditions are altered (Fogg, 1966). Lag phase may or may not take place. During lag, some cells may be dying while others are growing, giving little measurable change in cell numbers over time. Also, cells may be adjusting enzyme systems for altered nutrient conditions, or a selective process for cells with adaptive genotypes may be going on.

Logarithmic phase is the period of active cell division when cells are dividing at some relatively steady rate (giving an exponential increase in cell numbers) and nutrients are sufficient to maintain the cells in a state of health. A sustained growth rate can be regulated only by factors affecting the cellular metabolic rates, or by flux into the system of some limiting factor (light, for example). Log phase ceases when the rate of cell

division is markedly reduced or becomes zero. This reduction or cessation of growth is typically caused by the exhaustion of some essential nutrient, accumulation of a toxic metabolite, or self-shading in dense cultures.

Senescence is a state of low activity, dormancy, or dying. It may be a time of rapid death of most or all cells, or may be a period of low metabolic activity, with little or no net growth, from which cells may recover when conditions improve (Frey, 1974).

Much of the work involving nutrient-enrichment algal assays has ignored the various phases of growth of micro-organisms. This is unfortunate, because the various characteristics of the growth curve can be controlled or influenced by different factors. The total yield, or biomass reached under a given set of conditions, for example, may be controlled by factors different from the ones which influence the rate of growth before the final state is reached. To simply measure the yield after X number of hours or days, as many studies have done, may miss or lump these factors, resulting in a considerable loss of information.

Goldman (1972) outlined four general approaches for assessing the nutrient-limiting factors in natural waters. The first method entails direct measurement of nutrients in the water to infer limitation by nutrients in short supply. For the major nutrients this can provide useful information, although some problems exist. One such problem is that fluxes of nutrients into the environment and cycling of nutrients are difficult to quantify and can be of major importance (Pomeroy, 1970). Another inherent problem is

deciding which forms of an essential element are available to the phytoplankton. Available nitrogen, for example, occurs as nitrate, nitrite, ammonia, urea, and amino acids. Accurate measurement of all the variations can be difficult, and even then, the relative availability of these forms to the phytoplankton may be questionable. With the micro-nutrients these problems are compounded. Measurement of trace substances is difficult and the accuracy of results is often questionable, due to low concentrations, analytical detection limits, and problems of contamination. Little is known about the relative availability of the many physical and chemical forms which trace nutrients can assume.

A second approach is to collect the organisms and measure the elements and their ratios inside the cell. The ratio of carbon to nitrogen in naturally-occurring phytoplankton has been used effectively as an indicator of nitrogen depletion (Small and Ramberg, 1971; Eppley et al., 1971; Banse, 1974). Gerloff and Skoog (1954) used this technique with nitrogen and phosphorus. Interpretations are complicated by the ability of algae to store excesses of some nutrients, and by contamination of samples with non-living organic material. With trace substances this is further complicated by problems of detection limits.

A third approach is to add substances to natural unfiltered water and measure the growth responses of natural phytoplankton in that water. This bioassay method measures the actual effect of a substrance on the organisms of direct interest. This approach can be quite sensitive and avoids most of the problems inherent in the

previous two approaches. It is the general approach which I have found effective in work on Yaquina Bay.

The fourth approach is similar to the third, except that pure or unialgal cultures are used rather than the natural assemblage of phytoplankton. This is often used as a standard bioassay technique (Specht and Miller, 1973; Smayda, 1970, 1971, 1974; Carlucci, 1966, 1967).

There are significant advantages in using the natural assemblage of endemic phytoplankton to assay the effects of nutrients in natural waters. A single species, originally isolated from waters remote from the area of testing and maintained for years in highly artificial (unialgal, high-nutrient) laboratory cultures, cannot be expected to behave the same as diverse, endemic, natural phytoplankton assemblages. Further, species sufficiently tolerant to be easily maintained in laboratory culture are likely the ones to be least sensitive to environmental perturbations. Experimental results derived with natural populations can be more reasonably extrapolated to the actual ecosystem.

Nutrient enrichment experiments with natural phytoplankton populations have been in fairly common use for some time (Ryther and Guillard, 1959; Eppley et al., 1971; Thomas et al., 1974; Glooschenko and Curl, 1971). One technique has commonly been to "spike" a small (less than 250 ml), untreated sample of water with some combination of nutrients along with carbon-14, incubate the sample for several hours, and measure the amount of carbon-14 fixed. The results from this type of short-term experiment are

supposed to show any stimulation of growth caused by addition of a nutrient, and thereby indicate which nutrients are limiting. An interesting comparison of this technique to longer-term monitoring of large volume experiments was performed by Gerhart and Likens (1975). They found that nutrient additions which proved highly stimulatory over a period of several days in a large-volume container, gave confusing and inconsistent results in short-term C-14 uptake experiments. Small-volume C-14 uptake experiments lasting over 24 hours were found to have a great deal of scatter between replicates.

My research examines the seasonal dynamics and nutrient interactions of the marine phytoplankton in Yaquina Bay, Oregon. The experiments were long-term algal assays designed to assess the effects of various nutrient combinations on the phytoplankton in the bay, from the summer of 1975 through the summer of 1976. These experiments are unique in a number of respects: 1) they involve using the natural phytoplankton assemblages which occur through the various seasons of the year; 2) they employ large volumes of water (20 liters) and very small initial concentrations of phytoplankton, allowing the developing populations to be studied over many generations without greatly depleting the total volume by sampling; 3) treatment effects on the speciation of the phytoplankton assemblage are determined, in addition to growth rates and final yields; and 4) in addition to the usual technique of adding substances to the water in order to study their effects, techniques are employed to strip naturally occurring trace substances from the water, in order to gain a better understanding of the role of these substances in the dynamics of the natural

phytoplankton community.

Trace substances of biological concern in sea water consist of trace metals and dissolved organic matter (DOM). DOM accumulates in sea water through the breakdown of dead tissue, excretion from live organisms, or from land run-off.

Dissolved organic matter may interact with the phytoplankton in a number of ways. It may act directly upon the phytoplankton cells or it may act indirectly by influencing the chemical environment. Saunders (1957), in an excellent review, has characterized four basic categories of dissolved organic substances which may have an effect on phytoplankton: 1) nutritional substances which may be utilized directly as energy sources or as structural elements in the building of protoplasm; 2) accessory growth factors which are required for growth or which stimulate growth; 3) toxic substances which inhibit metabolic activities or cause death; and 4) chelators which may complex with trace metals, to the benefit or detriment of the organisms involved.

In the marine environment, DOM may at times be important to some phytoplankton as a nutritional source. This may be particularly true in post-bloom periods, when inorganic nutrients are low. It is not uncommon for certain phytoplankton to utilize nitrogen-containing DOM as a nitrogen source, especially urea and amino acids (Provasoli and McLaughlin, 1963; Hayward, 1965; Bruce, 1969; McCarthy, 1971). Dinoflagellates and other microflagellates seem to be particularly successful heterotrophs, although heterotrophy is not limited to these groups.

Accessory growth factors include substances such as vitamin co-enzymes which may be required for growth or which stimulate growth. These substances are required in only trace amounts, and requirements have been shown in many species of phytoplankton (Saunders, 1957; Provasoli, 1963). Vitamins have been shown to be produced in the planktonic community by certain species of phytoplankton (Carlucci and Bowes, 1970), as well as by bacteria (Strickland, 1965).

There is an accumulation of evidence that some organic excretion products (in addition to vitamins) can be stimulatory to the growth of phytoplankton (Kroes, 1971; Monahan and Trainor, 1970; Huntsman and Barber, 1975). Bacterial excretion products have also been shown to enhance the growth of several marine phytoplankton species (Ukeles and Bishop, 1975).

Algal production of metabolic products which have inhibitory or toxic effects on phytoplankton has been well documented (Proctor, 1957; Harris, 1970; Sze and Kingsbury, 1974). Lefevre et al. (1948) suggested the possibility that this may be of ecological significance among competing phytoplankton species.

Dissolved organic matter may be an important regulator of trace metals in the marine environment. Organic chelators can form relatively stable complexes with the metal ions. The specificity of the chelator and the stability of the metal-chelator complex are characteristics of the particular chelator. Trace metals are essential components of many enzyme systems, and thus are required in some relatively low concentration. In somewhat higher

concentrations, trace metals can be toxic to the phytoplankton.

Chelation of trace metals may have several effects on the phytoplankton. If the organo-metal complex is stable and the complexed form is not usable by the phytoplankton, then the chelator competes with the cells for the metal ions and makes them less available to the phytoplankton. If the metal ions in normal sea water tend to precipitate into a particulate form unavailable to the cells, a chelator may help to maintain the metal in solution and thus make it more available to the phytoplankton. If toxic levels of a trace metal are in solution, a chelator may reduce the toxicity of the ions. Since various phytoplankton species react differently to the presence of specific chelators (Shapiro, 1966), chelators may affect the species composition of the phytoplankton assemblage.

Barber et al. (1971) have suggested that natural chelators may play an important role in the primary production of oceanic ecosystems. Their data suggest that growth in newly upwelled sea water is poor unless the water has been "conditioned" with natural organic chelators. By "conditioning", they refer to the accumulation of dissolved organic matter in sea water through the natural life and death processes of organisms in the water. The need for "conditioning" may reflect the need of the phytoplankton for chelators to make trace nutrients more available, or may indicate a need for the detoxification of toxic levels of a trace metal such as copper.

The overall objective of my experiments was to compare the growth of endemic phytoplankton populations in natural sea water to

growth in sea water from which dissolved organic material and trace metals had been partially removed. The experiments were designed to determine if logarithmic growth rates, final yields, or species composition of the developing populations were affected. Known substances were added to see if any combination could restore the phytoplankton growth characteristics to the original state. Restoration of growth characteristics by addition of known substances indicates a number of things: 1) that similarly-acting substances occur naturally in sea water and are removed by my removal techniques; 2) that such substances are biologically significant; and 3) that these substances exert the specific sorts of control over growth rates, final yields, or species composition indicated by the experimental populations.

MATERIALS AND METHODS

General Experimental Design

Sea water was collected from a point close to the mouth of Yaquina Bay (44°37' north latitude, 124°03' west longitude), near Newport, Oregon (see below for details). This water was coarsely filtered (3 μm nominal pore size) and divided into two parts. One part was treated with activated carbon to remove trace substances from the water, while the other part served as a control. All water was then re-filtered (0.45 μm nominal pore size) into 20-liter growth containers. Treatment additions (three replicates) consisted of various combinations of the major nutrients, vitamins, trace metals, and certain chelators (see Table I for specific treatment additions). The growth containers were then inoculated with 20 ml of unfiltered bay water, and were suspended in open, outdoor concrete tanks of water circulating from the bay.

Growth of phytoplankton was monitored for several weeks by a variety of measurements, including *in vivo* fluorescence, carbon-14 uptake, disappearance of the major nutrients, and microscopic cell counts. Monitoring was continued until algal populations were well into senescence. The effects of treatments on community growth rate, community biomass yield, and taxonomic composition were examined.

Collection and Treatment of Sea Water

Sea water was collected at high tide from the Oregon State University Marine Science Center docks, 2.4 km from the mouth of the Yaquina Bay Estuary, at nine times during the years 1975-76 (see Table 2 for dates). This included two preliminary experiments, the results of which are not included here. The water was pumped at high tide from a depth of 0.5 meter by a Little Giant® submersible pump. The water was passed through a spun polyethylene Framweb® pre-filter, and a Pall DE® 3 μm cartridge filter. Filter housings were of polypropylene with ethylene propylene "O" rings. All tubing used in these experiments was clean Tygon®.

Water thus filtered was pumped into several 50-liter polyethylene carboys, previously rinsed with this same water. Some of the carboys were designed for treatment with activated carbon. To each of these was added 50 grams of Darco G-60® activated carbon. The carboys were stored for two days, during which period they were vigorously shaken several times.

Not all trace substances are completely removed from water by activated carbon, but most trace substances are at least substantially reduced. Jeffery and Hood (1958) reported 100% removal of measurable DOM from sea water, while Kerr and Quinn (1975) report a 55% - 86% reduction of DOM by activated carbon adsorption. Copper, manganese, and molybdenum were 100% removed and iron was about half removed from growth media in experiments reported by Donald et al. (1952). Frey (1974) found that activated carbon removed about half the

available iron from sea water.

At the end of the two-day period all water (both carbon-stripped and non-stripped) was filtered directly into the 20-liter growth containers. The water was pumped with a Teel® centrifugal pump through a polyethylene Framweb® pre-filter and a Pall Ultipore® AX 0.45 μm cartridge filter. Completely separate filtration systems were used for the carbon-treated and non-carbon-treated sea water in order to avoid any possibility of cross-contamination.

Growth Containers

Growth containers were 20-liter Reliance Products Fold-A-Carriers.® To avoid contamination, rubber gaskets were removed from the caps and threads were wrapped with Teflon® tape. A short length of Tygon® tubing with a plastic Duraclamp® was attached to each valve to provide a double closure and to facilitate sampling. The container walls attenuated 20% of the incident light.

Treatment Additions and Inoculation

Treatment additions were made after the growth containers were rinsed and filled with the filtered sea water. Additions consisted of various combinations of the major nutrients (N, P, Si), trace metals (Fe, Cu, Zn, Co, Mn, Mo), vitamins (thiamin, biotin, B_{12}), EDTA, and humic acid. The levels of addition are shown in Table 1. Treatments performed for each experiment are shown in Table 2. Humic acid was prepared as described by Frey (1974).

Following treatment additions, the containers were placed

Table 1. Treatment Additions

	Concentration per liter	
Major Nutrients		
$NaNO_3$	12.8 mg	150 µg-at N
$NaH_2PO_4 \cdot H_2O$	1.0 mg	7.3 µg-at P
$Na_2SiO_3 \cdot 9H_2O$	6.0 mg	21.4 µg-at Si
Trace Metals		
$CuSO_4 \cdot 5H_2O$	2.0 µg	0.0078 µg-at Cu
$ZnSO_4 \cdot 7H_2O$	4.4 µg	0.0015 µg-at Zn
$CoCl_2 \cdot 6H_2O$	2.0 µg	0.0084 µg-at Co
$MnCl_2 \cdot 4H_2O$	0.036 µg	0.182 µg-at Mn
$Na_2MoO_4 \cdot 2H_2O$	0.0013 µg	0.0053 µg-at Mo
Fe sequestrene	1 mg	0.232 µg-at Fe
Vitamins		
Thiamin · HCl	20 µg	
Biotin	0.1 µg	
B_{12}	0.1 µg	
EDTA	0.147 mg	0.387 µ-mole
Humic Acid	0.3 mg	
Iron		
Fe sequestrene	1 mg	0.232 µg-at Fe

Table 2. Treatments Performed in Each Experiment

Treatment indicated by XX. Blank indicates no treatment performed.

		TREATMENTS											
		Non-carbon		Carbon Treated									
Exp. no.	Date begun	None	N,P,Si Vit., T.M.	None	N,P,Si Vit., T.M.	N,P,Si Vit.	N,P,Si	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug. 10, 1975	XX		XX		XX		XX		XX			XX
IV	Sept. 8, 1975	XX	XX	XX		XX		XX		XX			XX
V	Feb. 9, 1976	XX	XX	XX	XX		XX	XX	XX	XX			XX
VI	April 11, 1976	XX	XX	XX	XX		XX	XX	XX	XX		XX	XX
VII	May 21, 1976	XX	XX	XX	XX		XX	XX	XX	XX	XX	XX	
VIII	July 7, 1976	XX	XX	XX	XX		XX	XX	XX	XX	XX	XX	
IX	Aug. 20, 1976	XX	XX	XX	XX		XX	XX	XX	XX	XX	XX	

in outdoor concrete tanks filled with circulating water from the bay. After the water temperature inside the growth containers had equilibrated to that of the circulating bay water (about two hours), inoculation with phytoplankton was carried out.

Water for the inoculum was collected from the O.S.U. Marine Science Center docks from a depth of 0.5 meter, during the high tide preceding inoculation. This water was stored for up to three hours in a closed thermos at bay temperature until it was used. Twenty milliliters were added to each growth container, giving a ratio of inoculum to treated sea water of 1:1000.

Measurement of Cultures

Growth of phytoplankton within the containers was measured in a variety of ways. Small samples (50 ml) were removed daily from the containers during times of active measurable growth, and less frequently during both senescence and the initial period when cell density was so low that detection was not possible. Containers were routinely shaken before drawing samples, and sample bottles were rinsed twice with sample water. Total experimental time was determined by the length of time required for the phytoplankton populations to achieve maximum yield. This time was generally about two weeks. Never was more than 25% of the total culture volume cumulatively removed for sampling purposes.

Fluorescence

Fluorescence was measured *in vivo* for all cultures whenever

samples were taken, using a Turner model 111® fluorometer. Fluorescence measurement was a rapid and dependable technique for following the growth of the experimental populations, and required relatively little sample removal. It gave results very similar to the much more cumbersome carbon-14 uptake technique, and exhibited less variability. Thomas et al. (1974) also found in vivo fluorescence to be a reliable measure of growth.

Carbon-14 Uptake

Carbon-14 uptake was measured frequently during the first two experiments. Two replicate 50 ml samples were taken from each growth container. To each of these was added 4 microCuries of buffered carbon-14 solution prepared according to Strickland and Parsons (1972). Incubation was for approximately 2 hours, beginning at 11 A.M. standard time. Incubation was in 55 ml polyethylene bottles with Teflon® lined caps. The bottle walls caused 20% light attenuation (the same as that of the growth containers). The bottles were incubated at the surface in the same outdoor tanks used to incubate the growth containers.

At the end of the incubation period, the samples were vacuum-filtered (5 p.s.i.) onto 0.8 μm pore-size Millipore HA membrane filters. Each filter was placed directly into a glass scintillation vial filled with 10 ml of Aquasol® liquid scintillation "cocktail". Radioactivity of the samples was determined on a Packard Tri-Carb® liquid scintillation spectrometer. Counts per minute were converted to disintegrations per minute by the internal spike method of Neame

and Homewood (1974). Disintegrations per minute were converted to mg carbon according to the equation of Strickland and Parson (1972).

Nutrients

Nutrient samples were stored frozen and in the dark in 55 ml polyethylene bottles until they were analyzed on a Technicon Auto-analyzer®. The analyses measured nitrate plus nitrite, reactive silicate, and reactive phosphate. Analyses were performed according to the methods described by Atlas et al., (1971). Ammonia determinations were not made because it was not possible to run samples fresh. Because of the volatility of ammonia, ammonia measurements are not reliable after a period of storage.

Microscopic Counts

Samples were preserved for microscopic examination by addition of Lugol's solution. They were concentrated prior to counting by settling 24 hours in a graduated cylinder and subsequent aspiration of the supernatent. Counting was done with a Palmer® counting cell, using a Nikon phase microscope. At least 100 cells were generally counted for each sample. In order to facilitate the processing of large numbers of samples, certain taxa were identified only to genus.

Light and Temperature

Temperature in the concrete water tanks was continuously monitored during experiments with a Taylor recording thermometer.

Incident light was recorded continuously with an Epply pyrheliometer shielded to record wavelengths between 400 and 700 nm.

RESULTS

There are, in these experiments, two levels of response that are of interest. One is the group of responses to treatment shown by the composite phytoplankton community through the seasons. This group consists of those responses which are measured with fluorescence, carbon-14 uptake, and nutrient uptake, and which give growth rate and total yield information. The other level of response is that related to community structure; that is, the gross taxonomic composition of the phytoplankton community which develops as a response to each treatment.

Physical Conditions

Incident light between 400-700 nm was recorded continuously during the experimental period. The numbers of gram-calories/cm^2 (langleys) recorded per day were averaged for each week and these values are plotted in Figure 1. Values were highest in late spring and early summer (about 250 langleys/day), and lowest in winter (about 30 langleys/day). Fog created by strong upwelling caused lower incident light levels in late summer than in early summer.

Temperature in the circulating water baths was recorded continuously during each experiment. The range of temperatures recorded during the course of each experiment is given in Table 3. Average temperatures ranged from about 8.5° C in February 1976, to 15° C in August 1976. Temperatures in each experiment fluctuated as much as ± 3° C. This temperature fluctuation reflects tidal and longer-term temperature fluctuations in the bay.

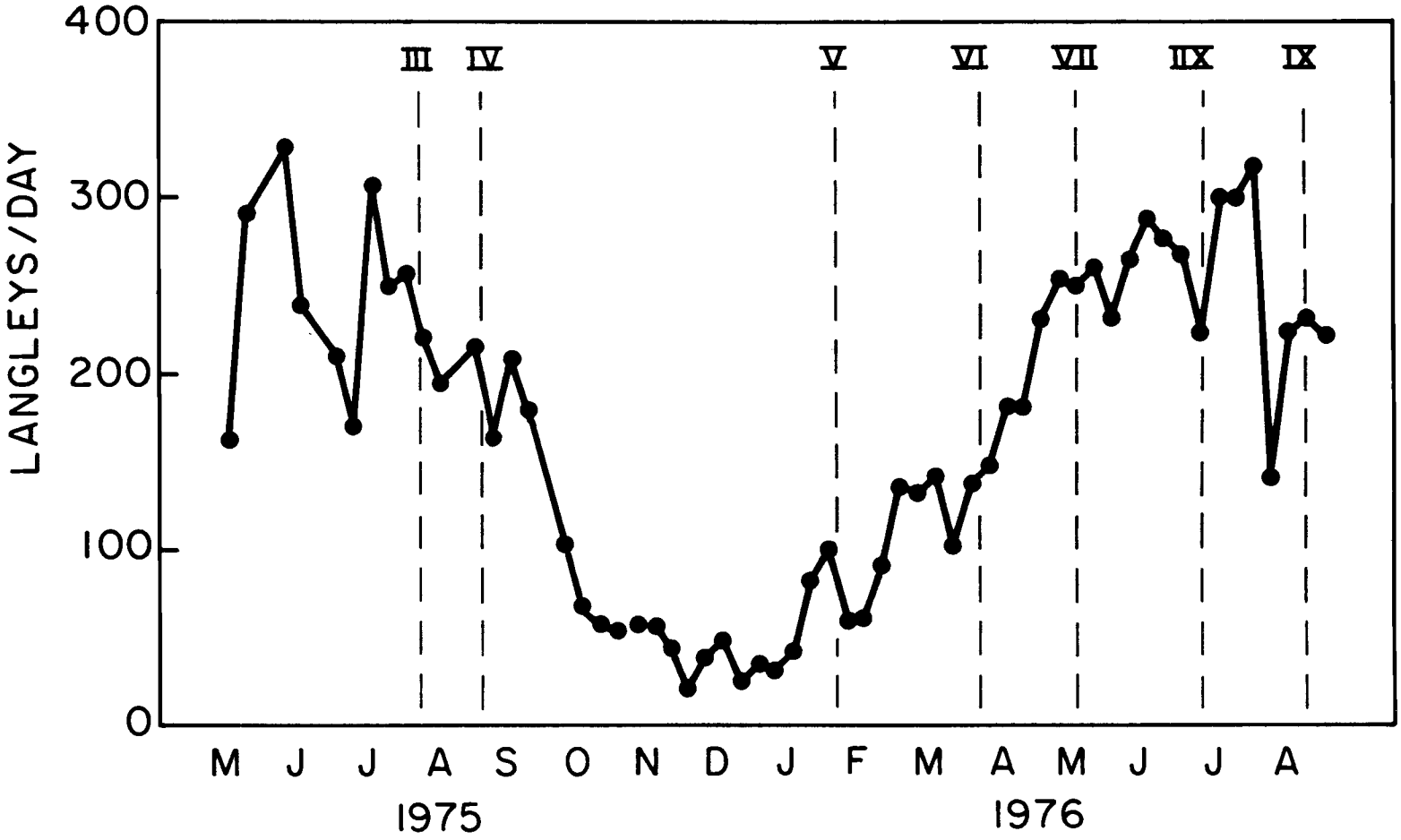

Figure 1. Incident light, 1975-1976; 400-700 nm. Gram-calories/cm^2 (langleys) per day. Dashed lines indicate starting date of each experiment.

Table 3. Temperature Ranges During Incubation

III	August 1975	9.0 - 15.0 °C
IV	September	11.0 - 14.0
V	February 1976	7.5 - 9.5
VI	April	10.2 - 10.8
VII	May	10.5 - 13.5
VIII	July	11.0 - 16.0
IX	August	13.0 - 17.0

Major Nutrients

Nitrate plus nitrite, reactive phosphate, and reactive silicate were measured through the course of each experiment. The initial major nutrient concentrations for each experiment are shown in Table 4. Figure 2 shows the typical correspondence between the disappearance of nutrients and the increase in in vivo fluorescence. Treatment of water with activated carbon had no effect on the concentrations of major nutrients.

Nitrate plus nitrite values ranged from high values of 29 and 21 μg-at/L in August 1975 and February 1976, respectively, to negligible amounts in July and August 1976. Phosphate values varied from 2.3 μg at/L in August 1975 to 0.8 μg at/L in July 1976. Silicate varied from 45 and 60 μg at/L in August 1975 and February 1976 to 9.0 in July 1976. The high August 1975 values probably reflect a pulse of recently upwelled water, while the high February values reflect winter mixing and minimal stratification. The low values from the summer of 1976 reflect a stable water column and no recent upwelling influence. These interpretations are in agreement with the Bakun upwelling indices for the time periods in question (Bakun, 1976).

Dissolved Organic Carbon

Samples for dissolved organic carbon analysis were taken from the filtered sea water used in each experiment. Samples of the non-carbon-treated (NC) and carbon-treated (C) water were analyzed. The mean values and standard deviations from three sub-samples are given

Table 4. Initial Water Characteristics.

Initial pre-addition nutrient concentrations are given (I), as well as final concentrations in the NC, No-Addition Control (F).

Experiment Number	Date	Nitrate & Nitrite (µg-at/L)		Phosphate (µg-at/L)		Silicate (µg-at/L)		Dixxolved Organic Carbon (mg C/L)	
		I	F	I	F	I	F	non-carbon treated	carbon-treated
III	August 10 1975	25	0	2.3	0.4	45	0	0.89 ± .04	0.42 ± .03
IV	September 8 1975	6	0	1.4	0.4	20	0	0.94 ± .04	0.54 ± .03
V	February 9 1976	21	0	1.3	0.4	60	40	0.75 ± .04	0.31 ± .01
VI	April 11 1976	12	0	0.9	0.4	40	25	0.78 ± .02	0.44 ± .02
VII	May 21 1976	14	0	1.3	0.2	21	2	0.90 ± .01	0.62 ± .02
VIII	July 7 1976	0	0	0.8	0.4	9	4	4.44 ± .06	0.63 ± .08
IX	August 20 1976	1	0	1.0	0.5	12	3	sample lost	0.29 ± .09

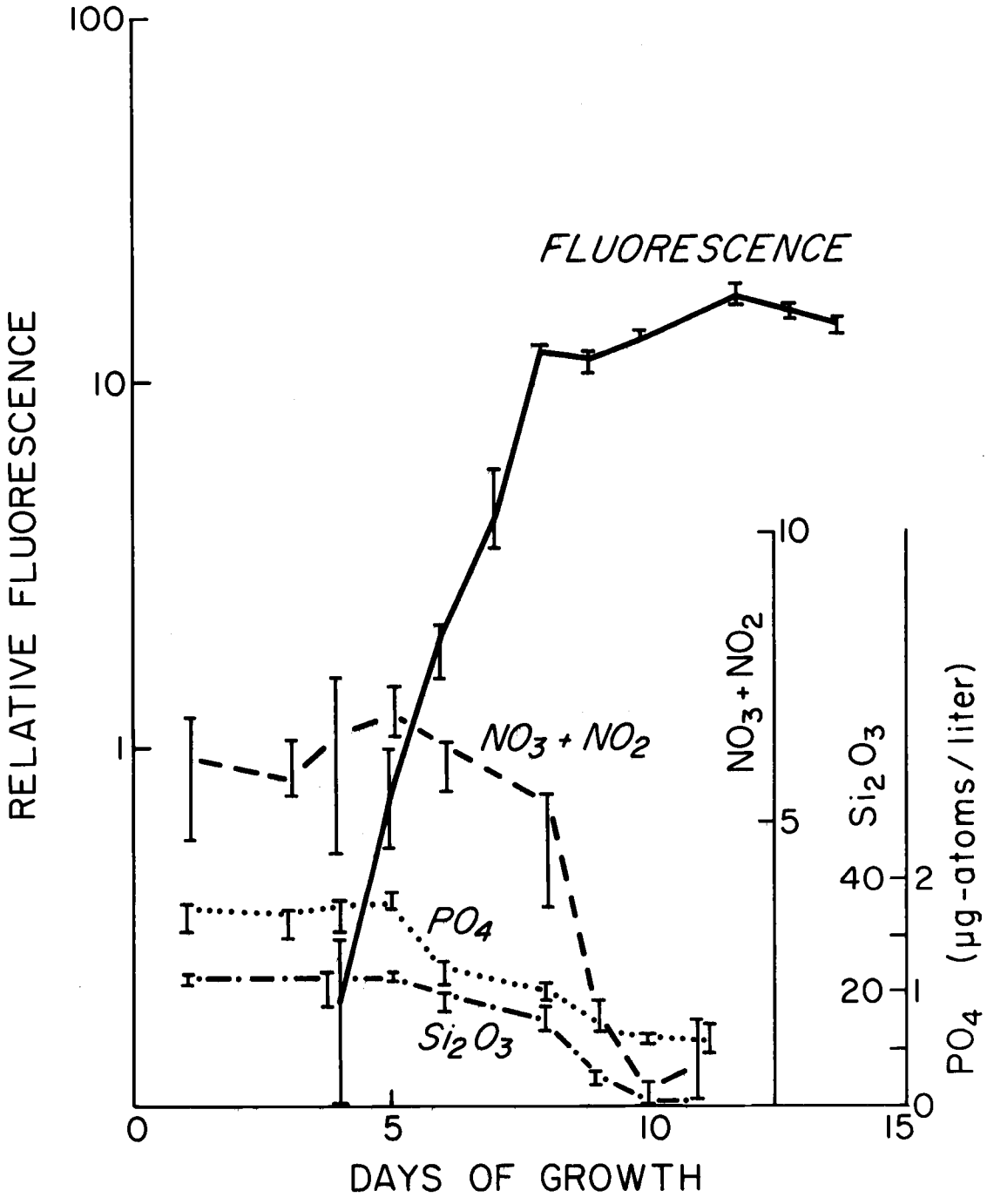

Figure 2. Comparison of fluorescence and nutrient data. Experiment IV, Carbon-treated, No-addition Control. All data points from each of three replicates are shown. Curves join median values.

in Table 4. Values for the non-carbon-treated water ranged from 0.75 mg C/L in February 1976 to 4.44 mg C/L in July 1976. Water treated with activated carbon had significantly lower values, ranging from 0.29 mg C/L in August 1976, to 0.63 in July 1976.

Overall Population Characteristics

Final Populations Yields

During the first two experiments (August and September, 1975), in addition to the in vivo fluorescence measurements made through all the experiments, phytoplankton growth and yield was also monitored by frequent carbon-14 uptake determinations performed on replicate subsamples from each growth container. Two representative figures (Figures 3 and 4, two different treatments in September 1975) are given which show the typical good correspondence between the carbon-14 data and the fluorescence data. After evaluating the first experiments, it was decided to drop the time-consuming and sample-consuming carbon-14 procedure in order to streamline the sampling and increase the number of treatments.

The highest fluorescence values attained by each treatment in each experiment are shown in Figure 5. The median values from each set of three replicates are displayed, along with the 95% confidence interval, computed on the basis of ranges, according to the method of Link and Wallace (1952). For small samples, ranges are only slightly more conservative measures of variance than are standard deviations (Snedecor and Cochran, 1969). Where values are off-scale, the bar

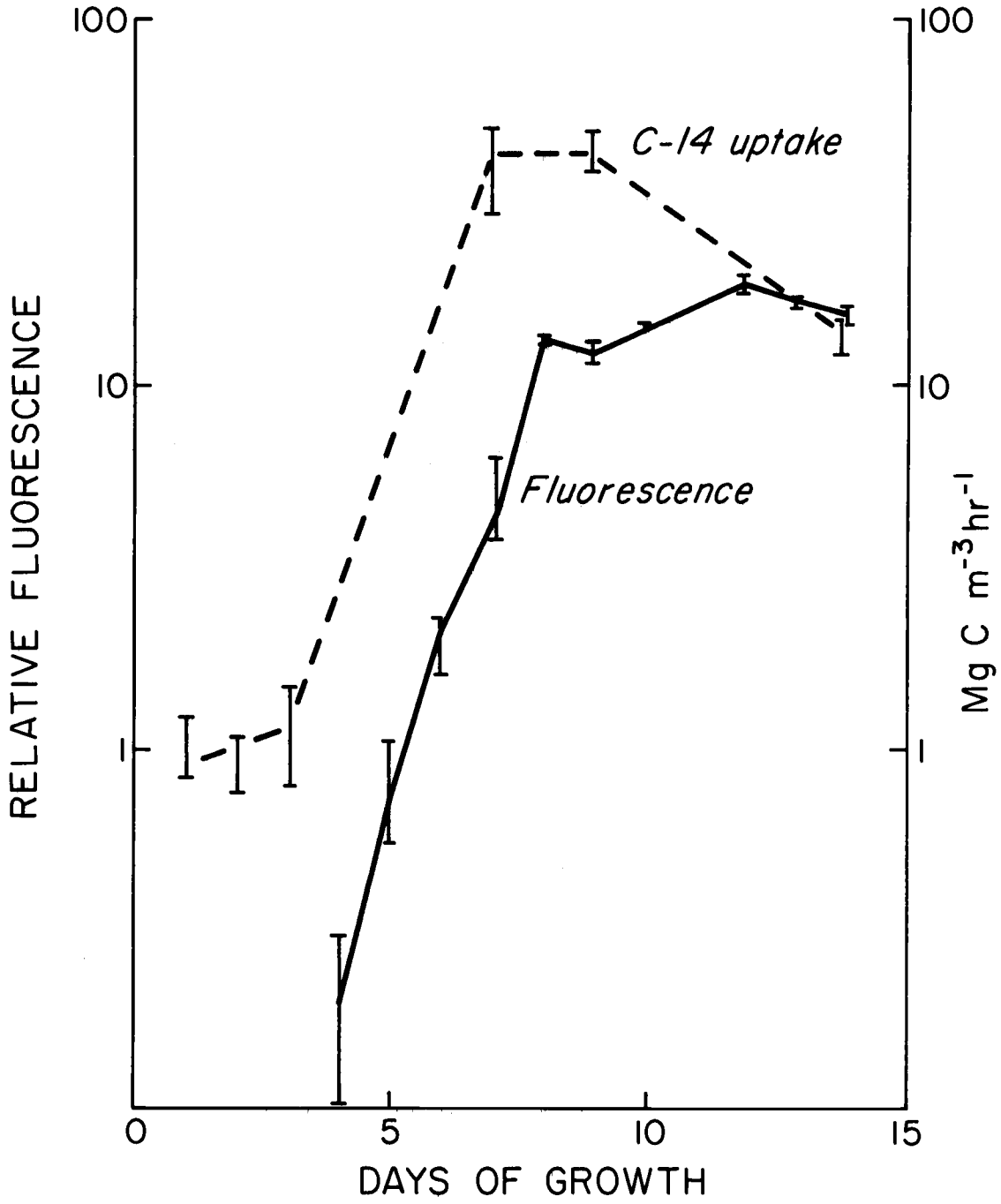

Figure 3. Comparison of fluorescence and carbon-14 uptake data. Experiment IV, Carbon-treated, No-addition Control. All data points from each of three replicates are shown. Curves join median values.

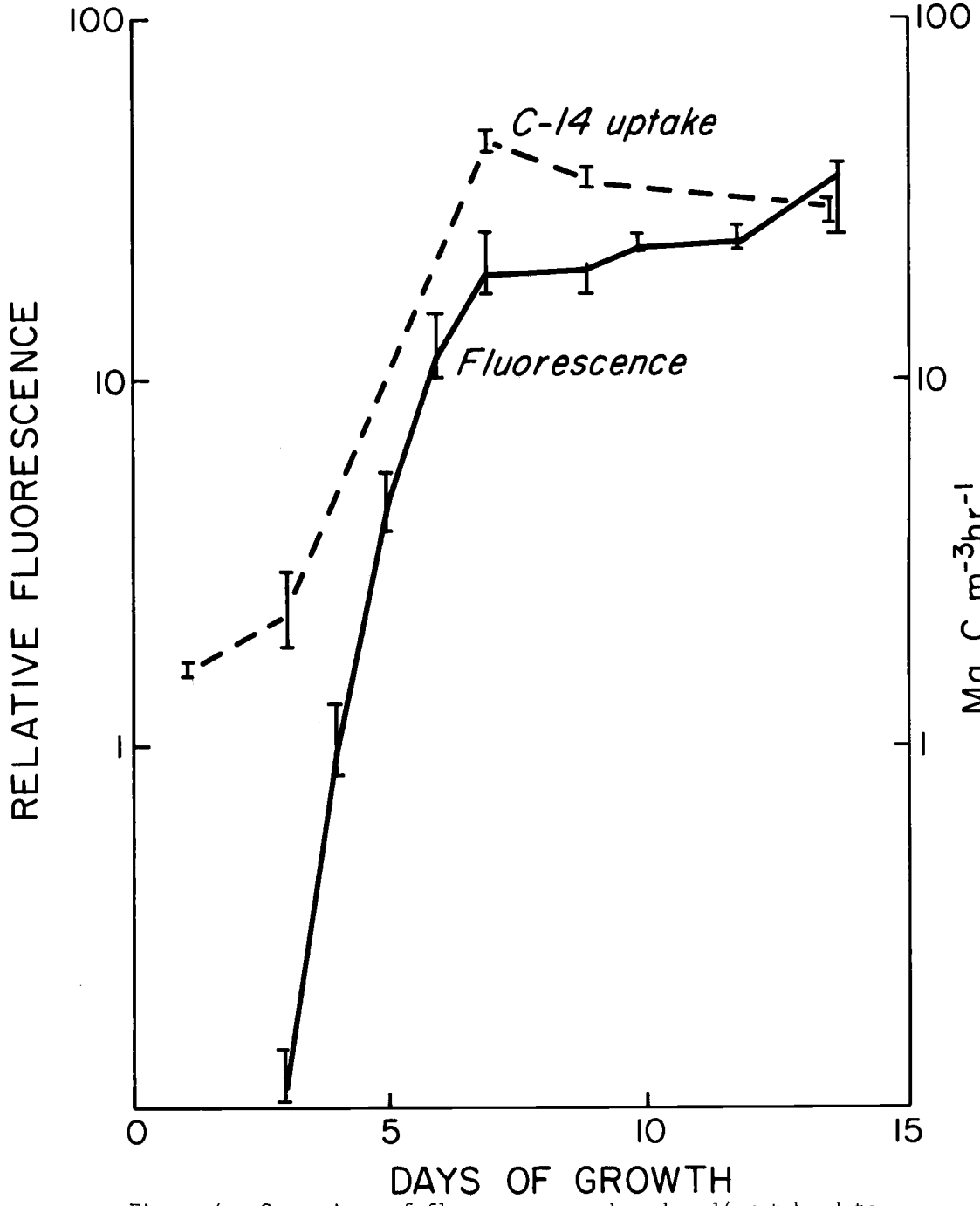

Figure 4. Comparison of fluorescence and carbon-14 uptake data. Experiment IV, Carbon-treated, major nutrient-plus-vitamin-addition treatment. All data points from each of three replicates are shown. Curves join median values.

Figure 5. Final fluorescence yield. NC = water not treated with activated carbon. C = water treated with activated carbon. Maj. = major nutrients (N, P, Si). Vit. = vitamins (see Table 1). T.M. = trace metals (see Table 1). H. A. = humic acids. Dotted line indicates NC, No-addition, Control value. △ indicates no treatment. Median values of three replicates are given. 95% confidence intervals for each experiment are indicated on the right. Where values are off-scale, the bar is broken and median and half-range values are given above the bar.
* Mean of two replicates.

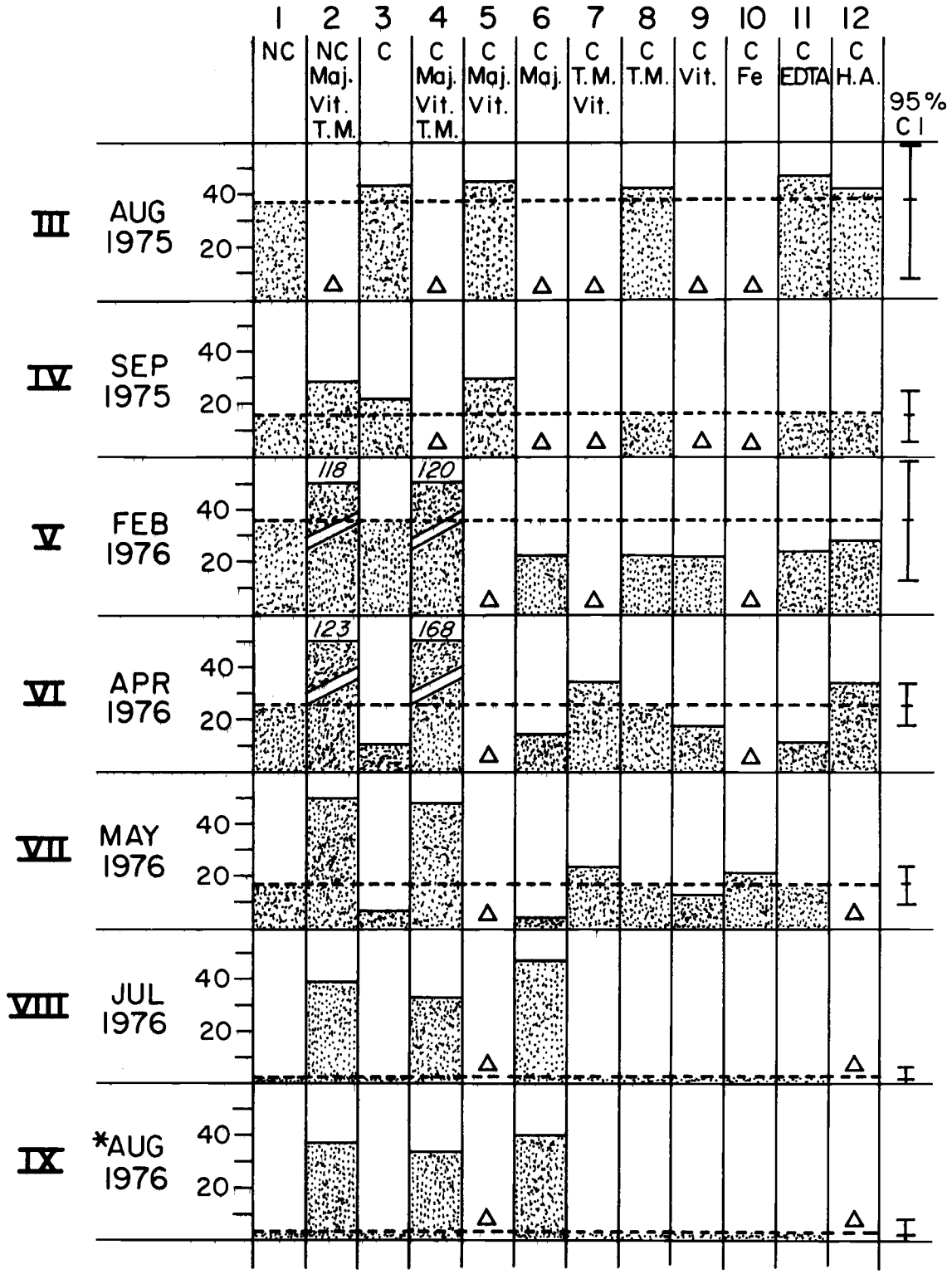
1 NC
2 NC Maj. Vit. T.M.
3 C
4 C Maj. Vit. T.M.
5 C Maj. Vit.
6 C Maj.
7 C T.M. Vit.
8 C T.M.
9 C Vit.
10 C Fe
11 C EDTA
12 C H.A.
95% CI
III AUG 1975
IV SEP 1975
V FEB 1976
VI APR 1976
VII MAY 1976
VIII JUL 1976
IX *AUG 1976
40
20
118
120
123
168

Figure 5.

is slashed and the value is given.

The final fluorescence yield was significantly reduced by carbon treatment in the experiments conducted during the spring (experiments VI, VII), and the addition of trace metals to the carbon-treated water restored the final yield to non-carbon-treated levels during these experiments. The yield in the summer and winter experiments was unaffected by carbon treatment alone. The addition of the major nutrients (N, P, and Si) without trace metals did not stimulate the final yield during the winter and spring experiments (V, VI, VII), but did stimulate the final yield in the summer (VIII, IX). These results indicate that trace metal limitation was induced by carbon stripping in the spring, but that carbon stripping did not induce trace metal limitation in the other experiments. The addition of iron sequestrene in the May 1976 experiment gave a result similar to the addition of the trace metal mix, suggesting that the yield limitation in the carbon-treated water, at least in May 1976, was specifically due to an iron limitation. In the experiments conducted during the summer of 1976 (VIII, IX), the stimulation resulting from the addition of N, P, and Si indicates that the final yield was limited by, and only by, the major nutrients. Nutrient analyses (Table 4) showed nitrate plus nitrite to be in short supply during these two experiments.

In February, yield enhancement was induced only by the addition of both major nutrients and micro-nutrients together. In April and May 1976, although micro-nutrient additions enhanced the yield in the carbon-treated water, the addition of micro-nutrients and major

nutrients together produced a much higher final yield than did micro-nutrients alone. This data indicates that neither major nutrients nor micro-nutrients were present in excess concentrations, and that the addition of one would make the other the limiting factor. In February, the somewhat lower yields in the carbon-treated water with additions, as compared to the carbon-treated water without additions, are not statistically significant, and can be attributed to experimental variation.

EDTA restored the yield in May, but had no apparent effect in the other experiments. Vitamins by themselves had little effect toward restoring final yields in spring, and had no effect in summer and winter. Humic acid additions gave results similar to the addition of trace metals, probably indicating the availability of some trace metals in the humic acid addition.

Days to Reach Arbitrary Fluorescence Threshold (L)

In all experiments the inoculum was so small (and consequently the initial cell concentrations so dilute), that there was no practical way to measure growth in the container (using fluorescence values, C-14 uptake, or nutrient uptake) until the cell concentration had increased by two or three orders of magnitude. Therefore, it was difficult to determine if true population lags had occurred under different treatments. The response at low cell densities was quantified by measuring the time required to reach a low but measurable level of fluorescence (0.5 fluorescence units), arbitrarily selected. This interval reflects both growth rate and possible lag phase. I

refer to it as "$\underline{L}$".

Values shown in Figure 6 are the reciprocals of the number of days required to reach the 0.5 fluorescence threshold ($\underline{L}^{-1}$), relative to the No-carbon, No-addition treatment. Reciprocals were used in order to show increased time (inhibition) as a negative effect, and shorter time (enhancement) as a positive effect. The median values of $\underline{L}^{-1}$ from each set of three replicates are displayed along with the 95% confidence interval computed for each experiment. These values have been normalized for each experiment, so that all values are shown relative to the NC Control (no carbon stripping, no nutrient additions). Values of $\underline{L}^{-1}$ lower than those of the NC Control indicate a longer time to reach 0.5 fluorescence units while values of $\underline{L}^{-1}$ higher than those of the NC Control indicate a shorter time to reach 0.5.

The time required for phytoplankton populations to reach the fluorescence threshold was always increased by carbon stripping, although in each of the August experiments (III, IX) this was not significant. The addition of trace metals plus vitamins always restored or enhanced the $\underline{L}^{-1}$ value. The addition of the major nutrients by themselves did not in any case restore $\underline{L}^{-1}$ to the control value. Trace metals by themselves and vitamins by themselves sometimes showed a restorative effect over carbon-stripped samples with no additions, but it appeared to take the combination of vitamins and trace metals to greatly stimulate early growth. Only during May 1976 did nutrient additions (trace metals plus vitamins) enhance $\underline{L}^{-1}$ to values significantly above the NC Control, suggesting that micro-nutrients

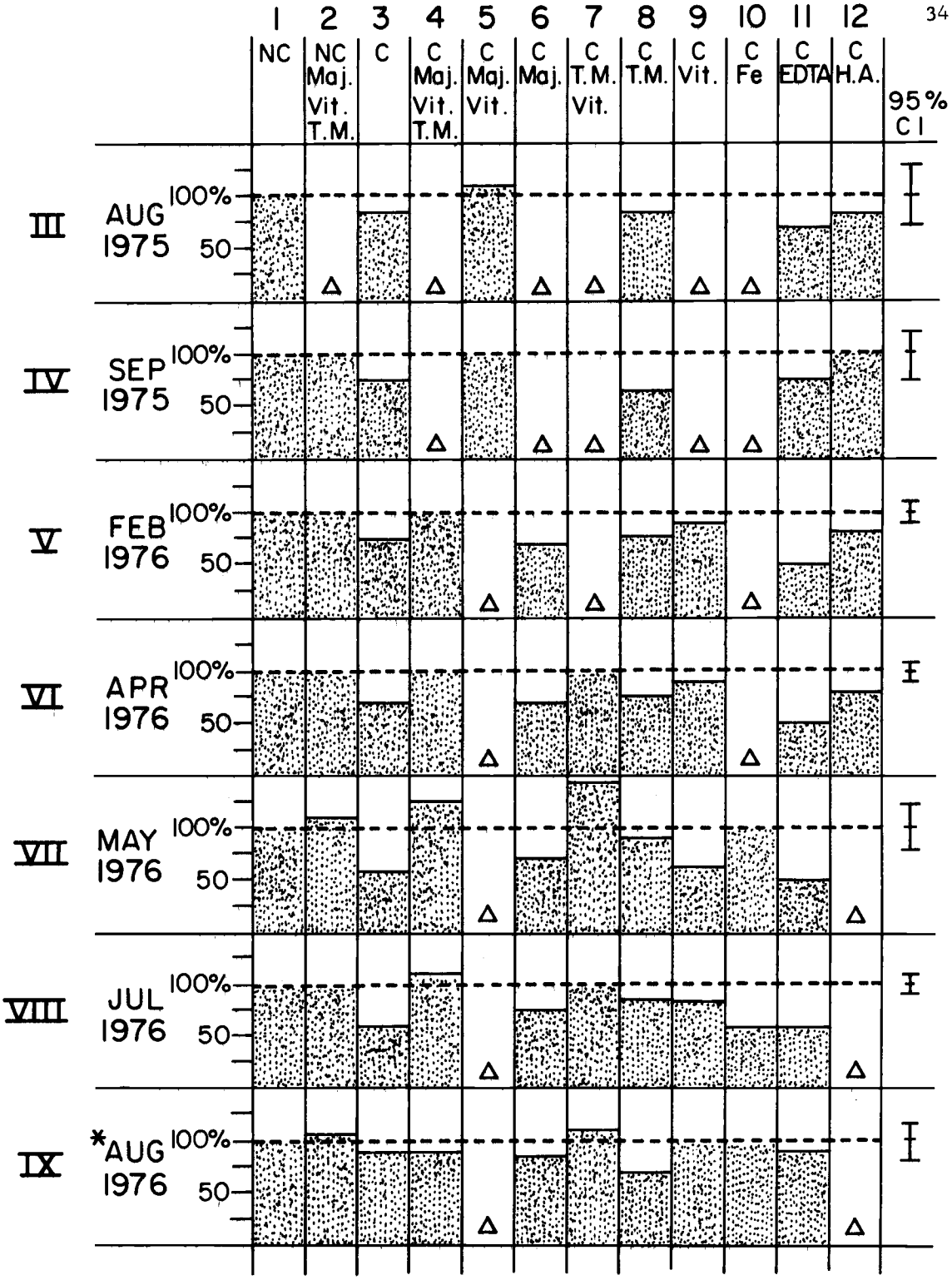

Figure 6. Days^{-1} for cultures to reach a fluorescence threshold of 0.5 units ($\underline{L}^{-1}$), relative to the NC Control. Symbols are the same as in Figure 5.

may have been limiting in May, or alternatively, that the untreated water contained some growth suppressor. The fact that iron re-established $\underline{L}^{-1}$ to the level of the NC Control in May suggests that iron was a major ingredient affecting early growth.

EDTA had a deleterious effect on $\underline{L}^{-1}$ during February and possibly April, but no apparent effect in the other experiments. Humic acid addition in February restored the carbon-treated water to the NC Control value of $\underline{L}^{-1}$, but showed no effect in the other experiments.

Specific Growth Rates

Specific growth rates (fluorescence increase/fluorescence present/day) were computed by a least squares fit to the equation $N_t = N_o e^{bt}$, where $\underline{t}$ is time (in days), $\underline{b}$ is the specific growth rate, and N_o and N_t are fluorescence values at time $\underline{t} = 0$ and time $\underline{t}$, respectively. Fluorescence values which visually appeared to lie along the logarithmic portion of each growth curve were used in the computation of the specific growth rate.

The relative specific growth rates from each experiment are shown in Figure 7. The median values from each set of replicates are displayed along with the 95% confidence interval computed for each experiment. Values have been normalized for each experiment, so that all values are shown relative to the NC Control, except during July and August 1976. In these months, growth rates could not be accurately measured in most containers, due to a very short period of active growth. Addition of major nutrients increased the period of logarithmic growth in these experiments, so that specific growth rates under

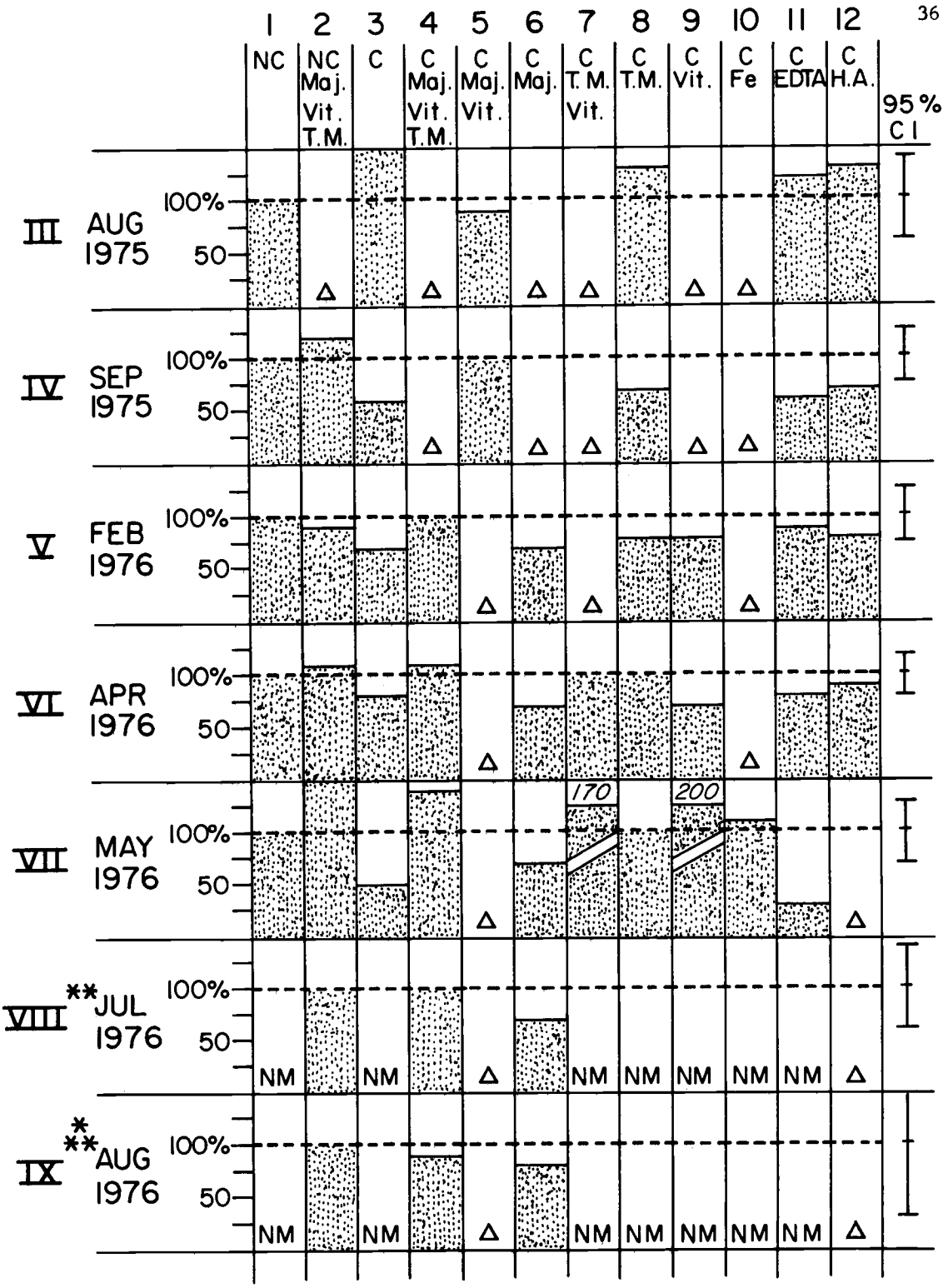

Figure 7. Specific growth rates, relative to the NC Control. NM = not measured. Symbols are otherwise the same as in Figure 5.
** Values relative to the NC, full-addition treatment.

various treatments are shown relative to the No-Carbon, Full-Addition Treatment (NC, N, P, Si, Vit, TM).

Carbon stripping significantly reduced the growth rate during all experiments except August 1975. In the anomalous experiment of August 1975, possibly some inhibitory organic or trace metal was removed by carbon stripping. Addition of a full range of nutrients always restored the growth rate to at least that of the control value. Addition of the major nutrients by themselves did not accomplish this. Addition of trace metals alone restored the growth rate to the control value in April and May, but not in September and February. Addition of vitamins alone greatly increased the growth rate in May, but in February and April they had no effect. EDTA had no significant effect on the growth rates. Humic acid additions showed no significant effect on growth rates.

Population Composition

The major genera which made up the final populations in the experimental vessels were Thalassiosira, Chaetoceros, Asterionella, Rhizosolenia, and Skeletonema. Several species of Thalassiosira and Chaetoceros were significant in the experimental populations, while the genera Asterionella, Rhizosolenia, and Skeletonema were each represented by a single species. In order to examine many samples, cells of the genera Thalassiosira and Chaetoceros were lumped at the genus level. Any cells of the genus Coscinodiscus would also have been lumped with Thalassiosira, but this genus did not appear to represent a significant portion of the experimental

populations. The major species making up each genus are discussed below. Although some information was lost by lumping at the level of genus, this procedure was necessary for the examination of a relatively large number of samples. Much of the significant taxonomic response to treatments was at the genus level, although some additional responses within the genera Thalassiosira and Chaetoceros may have been overlooked.

Flagellates and pennate diatoms (other than Asterionella and Rhizosolenia) were sometimes observed in the containers, but their numbers were never great during the experimental period. When the experimental populations were well into senescence, the pennates and flagellates became more prevalent. In the summer experiments, flagellates were consistently more abundant in the non-carbon-treated containers than in the carbon-treated ones, but this was not quanitifed.

The cell numbers/ml of each of the major genera at the peak of growth (as indicated by in vivo fluorescence) are summarized in Figures 8 through 12. The median values from each treatment (three replicates except as noted) are given as unbiased estimates of the population means. This value is represented by the height of each bar. Where values are off-scale, the bar is slashed and the value is given x 10^{-3}. The half-range values from each treatment are given as estimates of the population standard deviations. The half-range value is represented by the length of the vertical line extending above each bar on the figures. Where values are off-scale, the half-range value is indicated by * and is given x 10^{-3}. For

small samples, half-ranges are slightly more conservative estimates of population variance than are sample standard deviations (Snedecor and Cochran, 1969). It is the overall patterns of the species results which are of interest, rather than fine statistical differences; therefore the data is presented without detailed statistical analysis. The wide range of responses to treatments, coupled with the fact that ranges tended to be proportional to medians, made the development of overall 95% confidence intervals for each experiment impractical.

Thalassiosira

The genus Thalassiosira was present in all experiments (Figure 8). The important species of this genus included T. aestivalis, decipiens, and nordenskioldii.

The addition of a full range of nutrients (major plus micro-nutrients) to either carbon-treated or non-carbon-treated sea water always (with the exception of the anomalous August 1975 experiment) increased the final yield of Thalassiosira cells. Treatment of sea water with activated carbon always (with the exception of August 1975) reduced the yield of Thalassiosira cells. The addition of major nutrients alone to carbon-treated water had no effect on the Thalassiosira yield. Vitamins by themselves likewise had no effect, or else further reduced the yield (May 1976). The reduced Thalassiosira yield in May 1976 coincides with an enhanced yield of Asterionella and Rhizosolenia (Figures 10 and 11), strongly suggesting that this decrease in Thalassiosira yield is due to competition

Figure 8. Thalassiosira yield. Cells/ml at fluorescence asymptote. Median values of three replicates are indicated by height of bars. Half-range values are indicated by vertical lines atop bars. Where values are off-scale, the bar is broken and median and half-range values, in thousands, are given above the bar. O indicates this genus was not observed, and R indicates that cells of this genus were rare but were observed. Symbols are otherwise the same as in Figure 5.

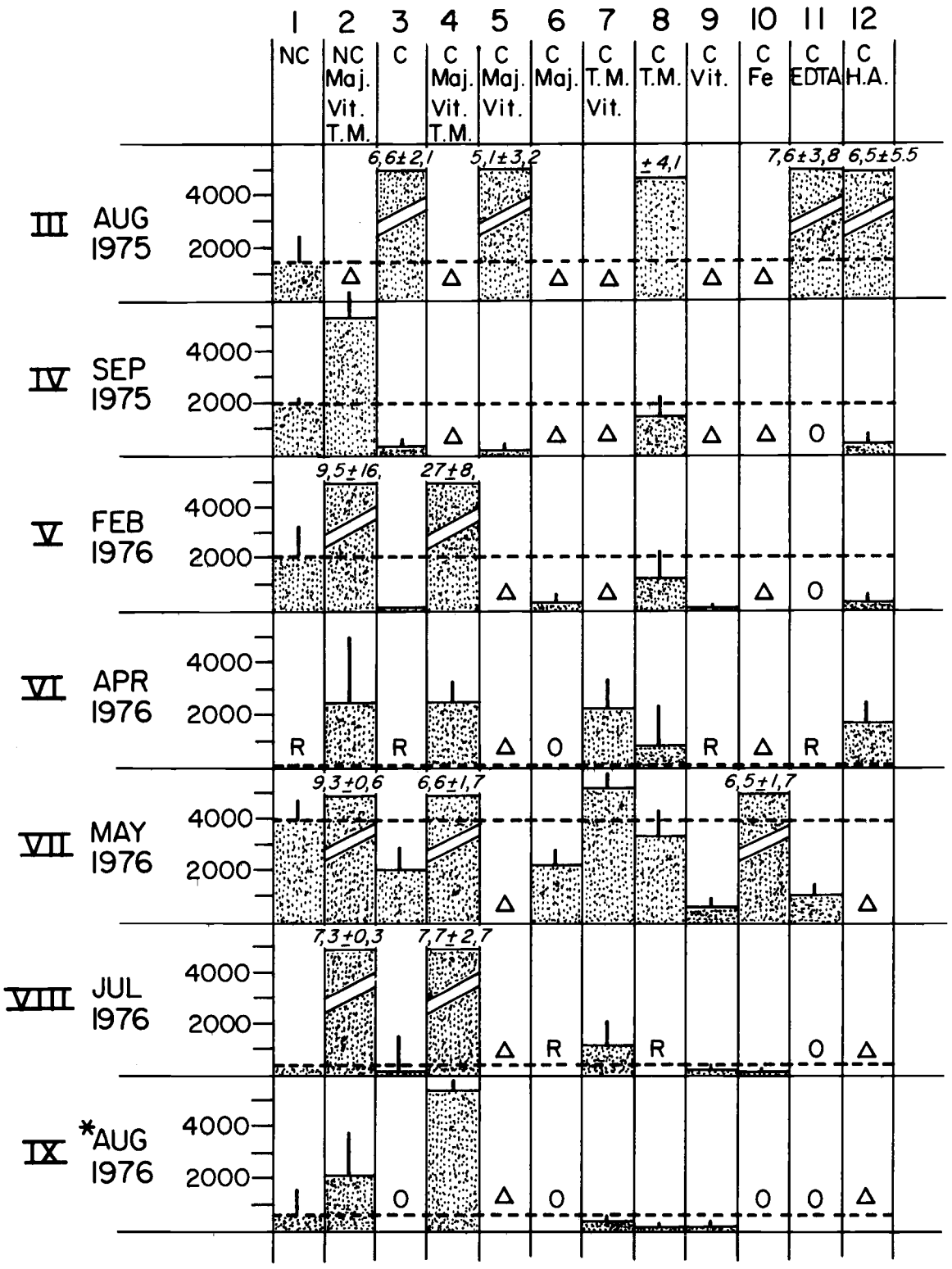
1 NC
2 NC Maj. Vit. T.M.
3 C
4 C Maj. Vit. T.M.
5 C Maj. Vit.
6 C Maj.
7 C T.M. Vit.
8 C T.M.
9 C Vit.
10 C Fe
11 C EDTA
12 C H.A.
III AUG 1975
IV SEP 1975
V FEB 1976
VI APR 1976
VII MAY 1976
VIII JUL 1976
IX *AUG 1976
4000
2000
6,6±2,1
5,1±3,2
±4,1
7,6±3,8
6,5±5.5
9,5±16,
27±8,
9,3±0,6
6,6±1,7
6,5±1,7
7,3±0,3
7,7±2,7
R
O
Δ

Figure 8.

with other phytoplankton which were themselves enhanced by the vitamin addition, rather than any direct inhibitory effects of vitamins.

The addition of trace metals usually caused a marked increase in cell numbers relative to the carbon-stripped control (C Control), and usually restored or nearly restored the cell numbers to those of the NC control. Addition of trace metals plus vitamins had an even more stimulatory effect than trace metals alone. In May 1976 the addition of iron was highly stimulatory to Thalassiosira production; in fact, iron alone induced a greater response than combined trace metals (which included iron in the same concentration). This suggests that competing species required other trace metals besides iron, thus giving Thalassiosira little competition in the absence of other trace metals; or alternatively, that some of the other trace metals were inhibitory to Thalassiosira. Unfortunately, the iron addition was only done once in the spring. The summer results indicate no effect of iron.

The chelator EDTA either inhibited the growth of Thalassiosira or showed no effect. Inhibition likely indicates that the EDTA competed for trace metals and made them less available to Thalassiosira. The moderate stimulation of Thalassiosira by humic acid addition is similar to trace metal stimulation, and is probably indicative of a trace metal content of the humic acid extract.

Chaetoceros

The genus Chaetoceros was present in all experiments, although it was quite rare in April 1976 (Figure 9). The important species

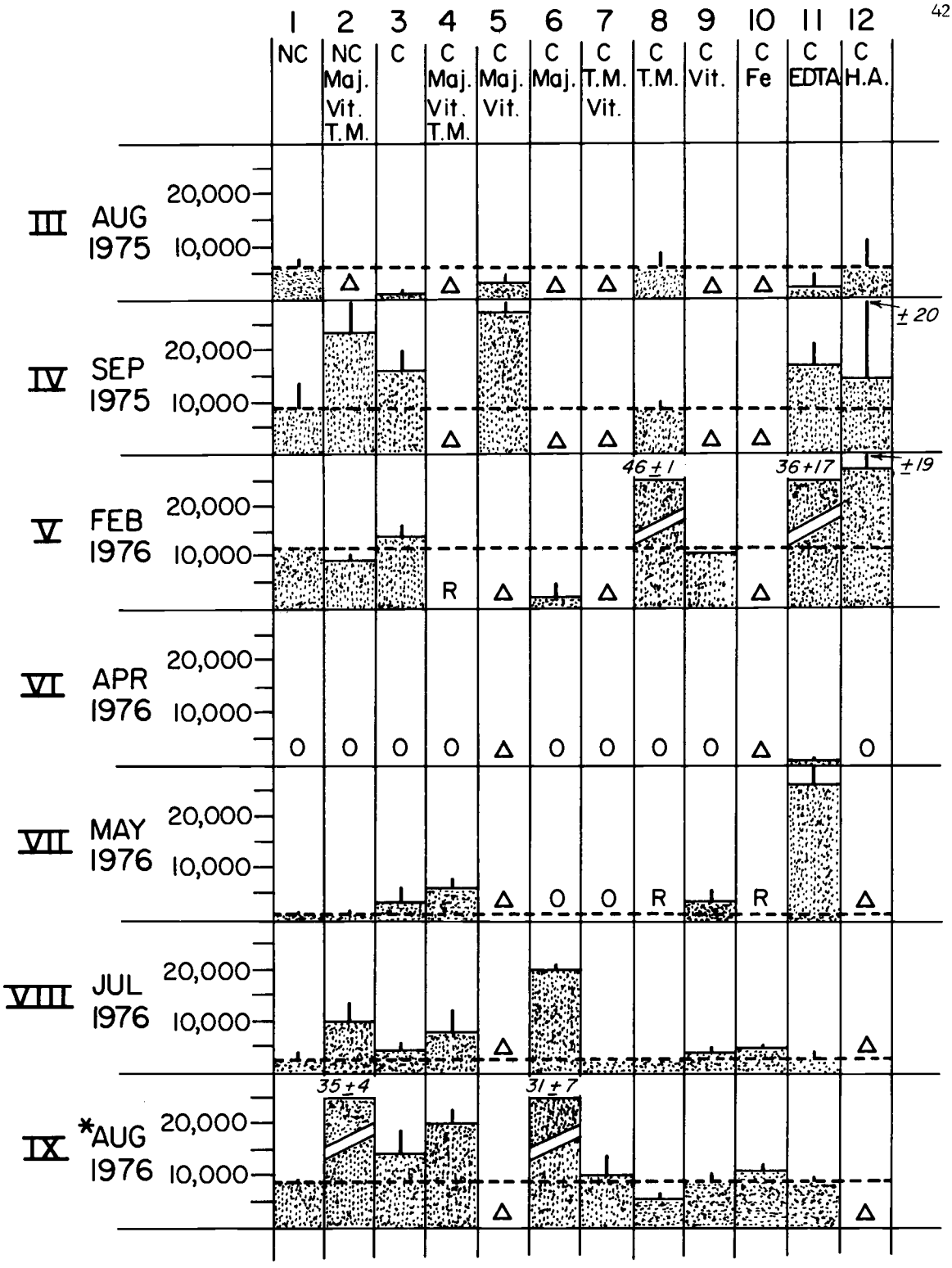

Figure 9. Chaetoceros yield. Cells/ml at fluorescence asymptote.

of this genus included C. socialis, debilis, constrictus, didymus, and compressus.

The Chaetoceros yield seems to be governed more by competition with other species for existing nutrient stocks, than direcly by nutrient treatments. During February, for example, the two treatments with additions of major nutrients plus micro-nutrients showed a reduced yield of Chaetoceros relative to the controls, while Thalassiosira and Asterionella yields were substantially enhanced. It appears most likely that the reduced yield of Chaetoceros was due to the depletion of limiting nutrients by these competing species, rather than the alternative, that Chaetoceros was directly inhibited by the nutrients added. Carbon treatment generally decreased the yield of all genera, except for Chaetoceros, which was generally slightly enhanced. EDTA had a generally inhibitory effect on all genera, except Chaetoceros, which was either enhanced or unaffected by EDTA. In April, Chaetoceros appeared only in the EDTA treatment, while the dominant genus, Asterionella, was reduced in numbers in this treatment and Thalassiosira did poorly. Chaetoceros seems adaptable to a range of nutrient conditions, and able to take advantage of conditions which other species find less than optimal. It is possible that some of this "adaptability" is simply a function of the greater number of species from this genus appearing in the experiments.

In the experiments of July and August 1976, Chaetoceros was enhanced by the addition of major nutrients alone, while the other genera present seemed to require the addition of micro-nutrients plus major nutrients for substantial enhancement of growth.

Chaetoceros appeared not to be as constrained by micro-nutrient requirements as other genera. Either it had no need for them (probably the case with vitamins), required very low concentrations, or could utilize forms apparently not available to the other species present in the experiments. The generally increased yield of Chaetoceros and reduced yield of Thalassiosira in the EDTA treatments suggests that chelated forms of trace metals may be less available to Thalassiosira while possibly still available to at least some species of Chaetoceros.

Asterionella

Asterionella (A. kariana) was present in significant numbers in February, April, May, July, and August of 1976 (Figure 10). In April it was clearly the dominant genus. In February Asterionella was numerically dominant, but less so than in April. It was not dominant in the other months.

Asterionella seemed to respond positively to both major nutrient and micro-nutrient additions, but the actual response was complicated by interactions with competing species. During April, when Asterionella was the dominant genus, the cell numbers were greatly enhanced in both carbon-stripped and non-stripped water by the addition of a full range of nutrients. Cell numbers could be restored to NC Control values in April by trace metal or humic acid additions. In February, when there was more competition from Thalassiosira and Chaetoceros, the high numbers of Thalassiosira in the carbon-stripped water with complete nutrient addition seems to have curtailed the Asterionella

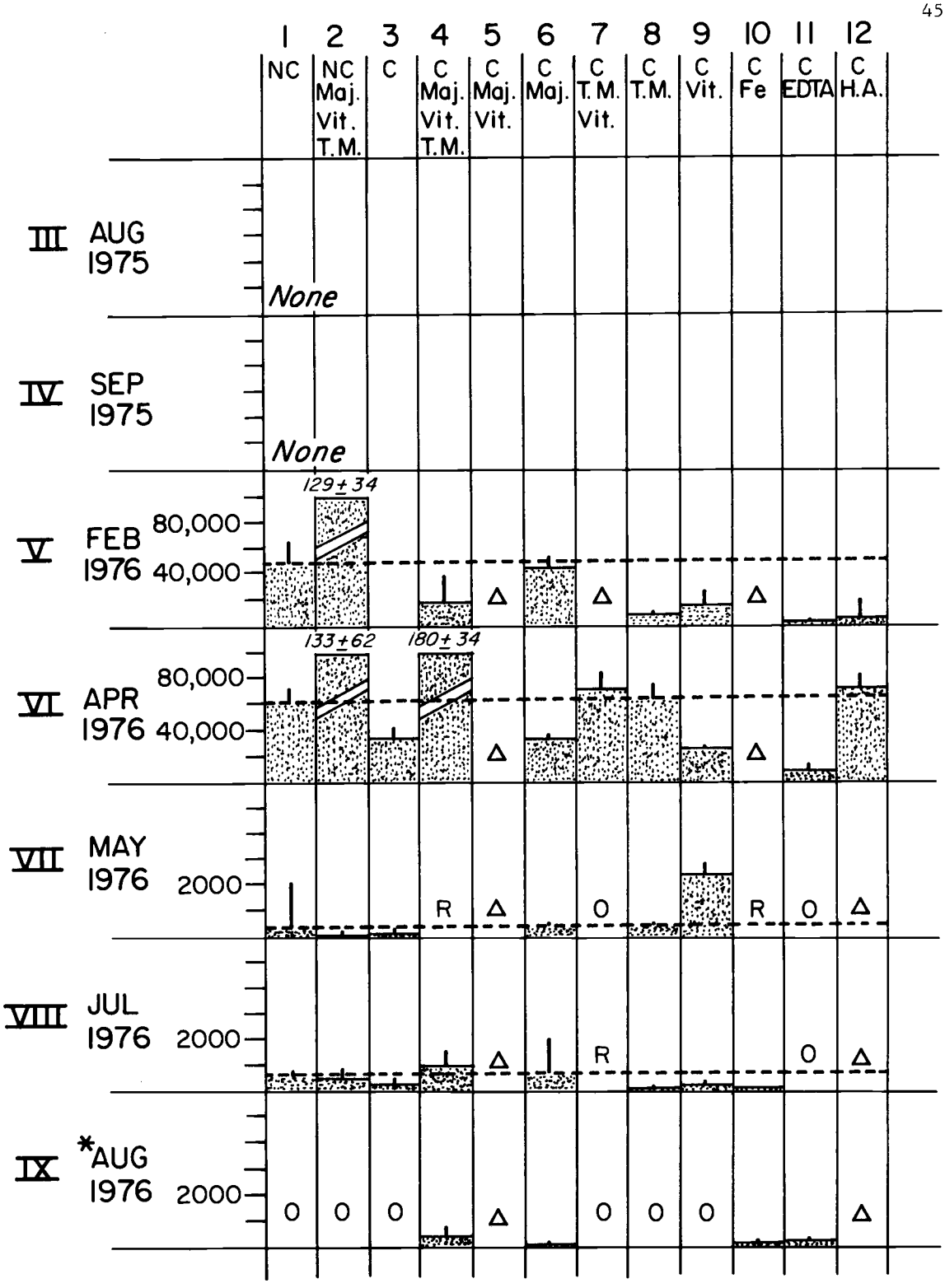

Figure 10. Asterionella yield. Cells/ml at fluorescence asymptote.

cell numbers which might have been expected. Vitamin addition did not increase the cell numbers relative to the C Control in April, as well as in July and August 1976. However, vitamin addition seemed to enhance cell numbers in February over the C Control, and it caused a marked increase in Asterionella production in May.

Rhizosolenia

Rhizosolenia (R. alata) appeared in only the May and July experiments (Figure 11). It clearly required vitamins, and did not show a need for any addition of trace metals. In May 1976, Rhizosolenia appeared in all three replicates of the vitamin treatment, and in no others. It was probably able to compete well in that treatment because Thalassiosira, the dominant genus in the May experiment, required the addition of trace metals for full growth. When trace metals were added along with vitamins, Thalassiosira dominated and apparently kept out Rhizosolenia. In July 1976, Rhizosolenia appeared (in relatively low numbers) in all the treatments which received a vitamin addition, and in the NC Control. This is reasonable evidence that there were vitamins in the natural sea water in July, and that these were at least partially removed by carbon stripping.

Skeletonema

Skeletonema (S. costatum) appeared in the May, July, and August 1976 experiments (Figure 12). This diatom seemed to require abundant vitamins and trace metals. It grew best in July and

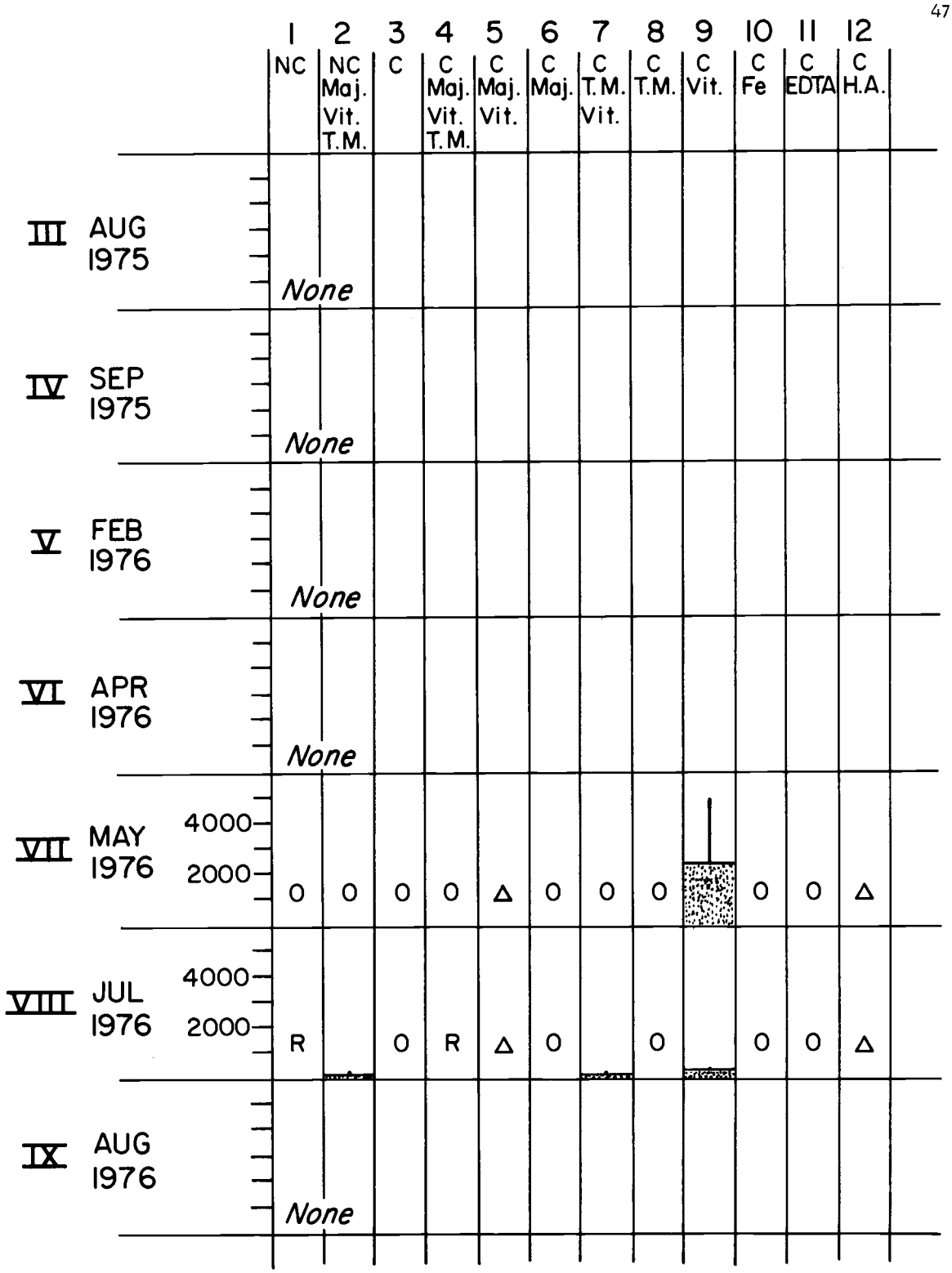

Figure 11. Rhizosolenia yield. Cells/ml at fluorescence asymptote.

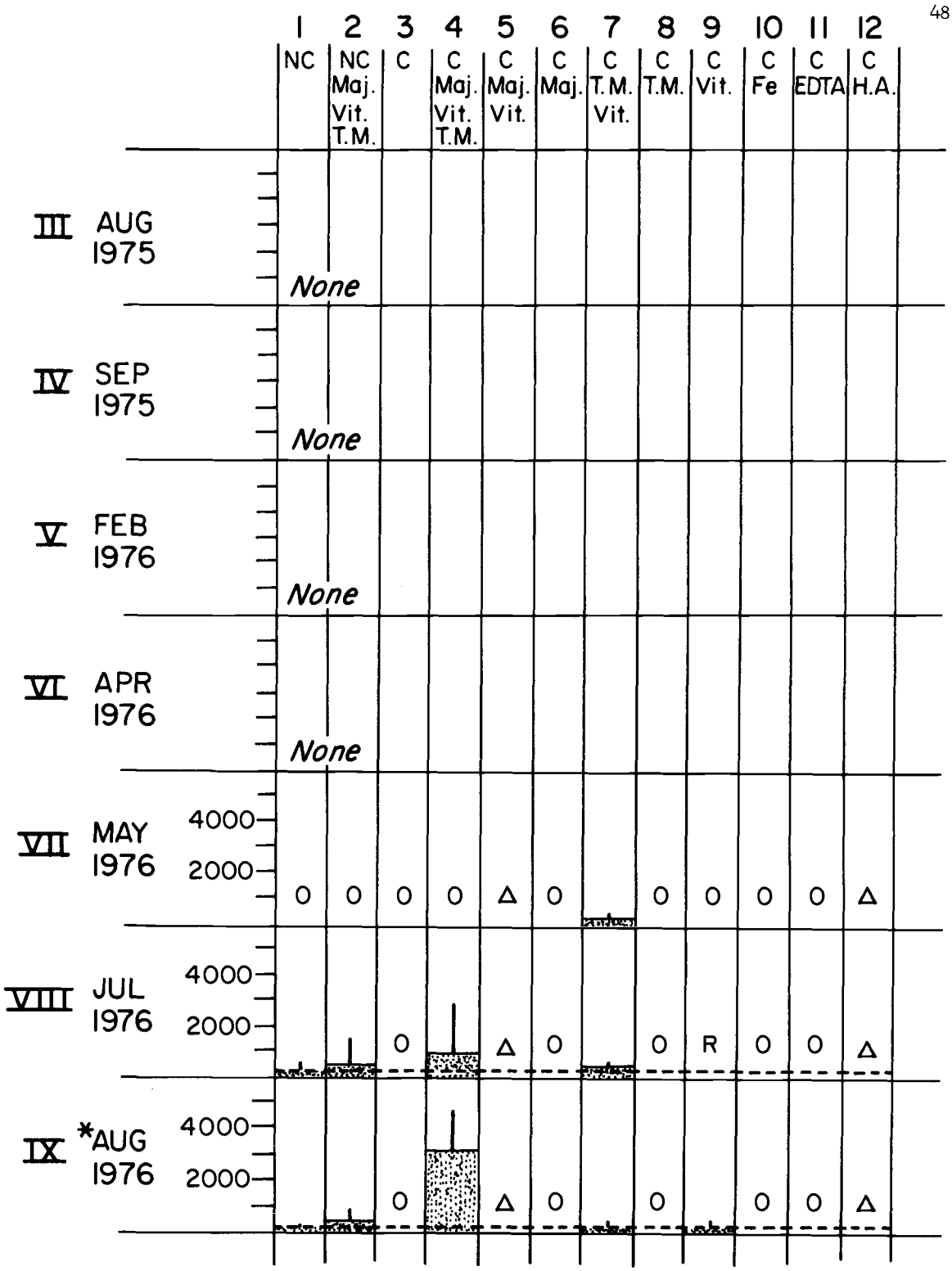

Figure 12. Skeletonema yield. Cells/ml at fluorescence asymptote.

August in carbon-stripped water which recieved the full addition of major plus micro-nutrients, while carbon-stripping with no additions eliminated Skeletonema from the final population. That the Skeletonema yield was somewhat higher in the carbon-stripped water with full nutrients added than in the non-stripped water with full nutrients added, is a hint of evidence of inhibitory trace substances in natural sea water.

In July and August (months which appear from data on final fluorescence yields to have trace metals available for growth), Skeletonema appeared only in the treatment with vitamins plus trace metals. These results support the conclusion that Skeletonema requires an abundance of vitamins and trace metals for growth.

DISCUSSION

The taxonomic composition of the developing phytoplankton populations was affected in a systematic and generally predictable fashion by the availability of micro-nutrients (trace metals and vitamins). Taxonomic composition was not much affected by the major nutrients (nitrate, phosphate, and silicate), although the overall yields of phytoplankton biomass were during some seasons. The micro-nutrients thus appear certain to be of considerable importance in determining phytoplankton species distribution and seasonal abundance ("successional") patterns. It appears that while the overall seasonal abundances of total phytoplankton biomass may be regulated to some extent by the major nutrients, the taxonomic composition of the phytoplankton population may be in large part under micro-nutrient control.

In my experiments, Thalassiosira was controlled to a considerable extent by the availability of trace metals. Rhizosolenia was controlled by vitamins, but not by trace metals, and Skeletonema was controlled by the availability of trace metals and vitamins together. Chaetoceros was least sensitive to the availability of micro-nutrients, and gained a competitive advantage when their availability was limited. While the micro-nutrient effects on these genera are fairly clear, the effects are often complicated by interactive effects of the various species present.

Ryther and Kramer (1961) reported that two Chaetoceros species required only low iron concentrations (6.5 to 26 μg/L) for full growth, while Skeletonema required 130 μg/L iron for full growth.

This is consistent with my experimental results, which showed Skeletonema, but not Chaetoceros, to be affected by removal of trace metals. That iron can cause species shifts in natural phytoplankton populations is also indicated by the work of Morton and Lee (1974). These authors showed that the addition of iron to fresh-water algal cultures could cause a shift in algal dominance (from greens to blue-greens) without causing a significant change in the total final biomass yield.

The micro-nutrients appeared to have a direct effect on growth rates and lag phases, being important for the achievement of maximal population growth rates and minimal lags, while the major nutrients had no such effect on growth rates or lags. Both the major nutrients and micro-nutrients, however, did affect the final biomass yields.

Two consistent overall community effects of stripping sea water with activated carbon were: 1) to increase the time required for the phytoplankton community to reach a given fluorescence (not significant in August 1975 and August 1976) (Figure 6), and 2) to reduce the exponential growth rate (with the exception of August 1975) (Figure 7). The August 1975 data are indeed exceptional, and will be discussed later. Final fluorescence-based yields were affected by caron stripping during the spring 1976 experiments, but not during the other experiments, suggesting some seasonal effects. All of the above effects could be overcome through the addition of various nutrient combinations. The fact that restoration was complete indicates that no other unknown or exotic trace substances in the sea water (which would likely have been removed to some extent by the

activated carbon) were required by the overall phytoplankton communities.

In the experiments where trace metals plus vitamins were added to the carbon-stripped water, the time required to reach a given fluorescence (a function of both lag and growth rate) (Figure 6) was in all cases restored to, or enhanced beyond, the values achieved in the non-carbon-stripped controls. The addition of the major nutrients by themselves in no case had any restorative effect on this parameter. The effects of trace metals and vitamins appeared to be additive, together giving greater enhancement than either did alone. The addition of the major nutrients by themselves, in no case restored the reduced growth rates in the carbon-stripped water to that of the non-stripped controls, while micro-nutrients sometimes did. The lack of growth rate data from the summer of 1976, and the lack of trace metal-plus-vitamin treatments in the first three experiments, makes it impossible to conclusively attribute all the restoration of growth rates to the micro-nutrients, however the data are consistent with this interpretation. The effects of trace metals and vitamins on the time to reach a given fluorescence, together with the growth rate data, make compelling the conclusion that micro-nutrients, and not major nutrients, can affect the lag and/or exponential growth phases in closed cultures.

From the above observations, and from the data on the population composition, the following conclusions can be drawn: 1) substances identical or similar in action to the trace metal and vitamin additions were removed to some extent from the sea water by

treatment with activated carbon; and 2) these substances are required for maximal growth rates and/or minimal lags.

The observed changes in community growth rates and/or lags induced by the removal and subsequent addition of micro-nutrients probably can be attributed to any or all of three main possibilities. First, the absence of a micro-nutrient might block a particular enzymatic pathway common to all species in the community (due to an inactive enzyme lacking its metal or vitamin co-enzyme). The metabolic end product then must be synthesized by a less direct and slower pathway. Secondly, the most rapidly growing species in the community might be inhibited by the lack of micro-nutrients, but slower growing species might still be able to grow. The community growth rate is thus controlled by the slower growing species. Finally, vitamins which are absent might have to be synthesized by bacteria or by a portion of the phytoplankton in the system. The growth rate would then be limited by the rate of vitamin production. Trace metals, which cannot be biologically produced, are sometimes made biologically available through the excretion of highly specific chelators by microorganisms (Corbin and Bulen, 1969; Ito and Neilands, 1958; Ratledge and Chaudhry, 1971), and this might have an effect similar to the example of vitamin production controlling community growth rates. The negligible or negative effects of EDTA on my experimental populations would tend to discount this latter possibility, although different chelators may be expected to have different biological effects.

The addition of a full suite of nutrients (macro plus micro)

always substantially increased the final yields (Figure 5), except in the anomalous August 1975 experiment. This indicates that (except in August 1975) cessation of growth in the controls was caused by the exhaustion of one or more nutrients. Unlike the growth rates, which were always controlled by the micro-nutrients and never by the major nutrients, the final yield apparently was sometimes controlled by major nutrients. During the summer of 1976, final yields were clearly limited by major nutrients alone. Nutrient analyses showed that nitrate plus nitrite was almost absent from the beginning of these experiments, and the addition of major nutrients alone produced yields as high as the complete addition of all nutrients.

The addition of trace metals plus vitamins increased the final yield above that of the NC Control during the spring (Figure 5), suggesting a micro-nutrient limitation in the unstripped sea water. In February, only the combined addition of major nutrients and micro-nutrients increased the final yield. Thus, during the April, May, and possibly February experiments, there was evidence of a limitation of biomass caused by micro-nutrients. Trace metals, perhaps mainly iron, are strongly implicated in this limitation.

Most models of marine ecosystems (Riley, 1946; Steele, 1974; Walsh and Dugdale, 1971; Wroblewski, 1976) assume a major nutrient (usually nitrogen) control of phytoplankton growth. My evidence that micro-nutrients may play a significant role in determining phytoplankton growth characteristics, especially growth rates, suggests that the assumption of nitrogen control of growth may be an inaccurate simplification. At the very least, it is apparent

that more work to determine the actual nutrient factors which control phytoplankton growth is essential, as this is a basic part of most marine ecosystem models.

Though our experimental evidence seems clear, caution must be used in extrapolating this trace metal limitation to the field. Lewin and Chen (1973) found that within 36 hours of collection, about half of the soluble iron (iron which passed through a filter of 0.45 μm nominal pore size) became particulate (would not pass through a 0,45 μm filter). This suggests that the filtration process in my experiments may have removed about half of the iron. Hem (1974) reports that chemical solubility models suggest that other metal ions tend to coprecipitate with ferric hydroxide. If this is so, other trace metal concentrations might also have been reduced by my storage and filtration procedures, and during the time course of my experiments. The absence of trace metal limitation of final biomass yields in the summer experiments is good evidence of the lack of such a limitation in the field, but a demonstrated trace metal limitation in the winter and spring experiments might or might not indicate an actual limitation in the field.

Menzel et al. (1963) performed nutrient addition experiments with natural phytoplankton populations from the Saragasso Sea, and found that although addition of nitrate plus phosphate produced as high a final yield as the addition of nitrate plus phosphate plus iron, the population with the iron grew faster to its peak yield. These data are consistent with my findings that although the major nutrients can control final yield, micro-nutrient limitation might

prevent an optimal growth rate from being realized.

Phytoplankton in pure culture have been shown to be both rate- and yield-limited by vitamins. Unfortunately, most workers have not differentiated between these two different effects. Daisley (1957) suggested that the major importance of vitamins in the marine ecosystem may be their influence on growth rate, rather than their effect on yield. The vitamin bioassay technique of Carlucci (1966; 1967) is based on the fact that pure cultures of vitamin-requiring phytoplankton grow at a rate proportional to the concentration of the required vitamin in the media. Carlucci and Silbernagel (1969) showed that cultures of Thalassiosira pseudonana (= Cyclotella nana), Amphidinium carteri, and Monochrysis lutheri were both rate- and yield-limited by vitamin B_{12}, biotin, and thiamin, respectively. Droop (1961), on the other hand, found a yield limitation of Monochrysis lutheri by vitamin B_{12}, but found no rate limitation. Ford (1958) showed that the rate of growth of Ochromonas malhamensis responded to the levels of vitamin B_{12} in the media. The work of both Carlucci and Ford tends to support my findings that vitamins can affect growth rates in closed systems, while the work of Droop appears to conflict with the others and does not agree with my findings.

The apparent paradox of a constant but reduced exponential growth rate in closed cultures controlled by presumably diminishing concentrations of vitamins, has not been explained, or, it would appear, even noticed until now. Carlucci and Silbernagel (1969) report that the first two or three days of growth of their vitamin-

limited cultures gave similar growth rates, and then changed to rates of growth proportional to the concentration of added vitamin. Perhaps this is a clue, suggesting that the response of the cellular mechanism to altered ambient vitamin concentrations is quite sluggish, possibly due to the need of the cells to employ different metabolic pathways depending on the availability of vitamins as co-enzymes.

My experiment conducted during August 1975 produced results which, except for some similarities to August 1976, were unlike the relatively consistent results from the other experiments. The August 1975 results are unique in the following respects: 1) the specific growth rate (Figure 7) was not reduced by carbon stripping as it was in the other experiments, but rather was enhanced relative to that of the NC Control; 2) the addition of trace metals to the carbon-treated water had no effect on the growth rate, while the addition of the major nutrients plus vitamins reduced the carbon-treated growth rate to a value slightly below that of the C Control; 3) the final yields (Figure 5) were not significantly affected by any of the treatments, although the full combination of all nutrients was not tested and scatter among replicates was surprisingly high; and 4) although the populations consisted mainly of Thalassiosira and Chaetoceros during August, the usually clear species abundance trends were absent (Figures 8 and 9). Both August 1975 and August 1976 showed only a small increase in "lag" from carbon-stripping.

These results are of considerable interest, because the high levels of major nutrients measured in the water suggest that the

water was recently upwelled from off the coast and transported into Yaquina Bay on the high tide. Barber et al., (1971) found that freshly upwelled water gave bioassay results which were quite different from the results obtained with non-upwelled surface water. Their results indicated that stripping of "normal" sea water with activated carbon reduced the rate of growth of the phytoplankton inoculum, but in freshly upwelled water carbon stripping only slightly reduced the growth rate. In my August 1975 samples, carbon stripping actually enhanced the growth rate. Growth in non-carbon-stripped upwelled water was considerably slower than in unstripped "normal" water, in the Barber et al. results. When they added trace metals and/or EDTA to non-carbon-stripped upwelled water, the growth rates were elevated to rates similar to those of the unstripped "normal" water. Their conclusion was that newly upwelled water must first be biologically "conditioned" by the phytoplankton (presumably by making chelating compounds) before the trace metals are in the proper physico-chemical form to support rapid phytoplankton growth. An alternative explanation, however, is that copper ions in the freshly upwelled water must be detoxified by biologically produced chelators before full growth is possible. Because growth was actually enhanced in the C Control in August 1975 (relative to NC Control), the detoxification alternative is appealing.

The absence of the usual trace metal-dependent trends in Thalassiosira growth in my August 1975 experiment suggests that trace metals were not limiting in any treatments. This would explain why the overall phytoplankton growth rate was not reduced by carbon

stripping, but does not explain why the growth rate was actually increased by carbon stripping, nor does it explain why the growth rate was reduced relative to the C Control by the addition of major nutrients plus vitamins. These observations suggest the existence in the August water of toxicants of some sort, and/or nutrients in inhibiting concentrations. The large amount of scatter among replicates which characterized the August data further suggests some sort of stress on the populations. The techniques used in this experiment were the same as those used in the other experiments. It is of some interest that sea water collected about this time of year along the Oregon coast has been known to often give anomalous experimental results and sometimes support only poor growth of unialgal laboratory cultures (H. C. Curl, Jr., personal communication).

To the best of my knowledge, mine is the first systematic study to show the distinction between the action of major nutrients and the action of micro-nutrients on the growth and species composition of phytoplankton communities. There are probably three major reasons why this distinction has not been shown in the past. First, some studies which might have detected this phenomenon did not clearly distinguish between growth rate and final yield effects (for example, Smayda, 1974). Secondly, most nutrient addition studies have been carried out with pure or unialgal assay cultures under small, short-term batch conditions, rather than with large-volume, natural populations for long time periods; hence these studies would miss the species competition phenomena in which the micro-nutrients have been shown to play an important role. Even experiments with natural

populations have almost always been of short duration, and species composition changes are rarely examined. Finally, the technique of stripping and adding micro-nutrients, along with the use of very low initial natural phytoplankton inocula, has added considerable power to our ability to assess the effects of naturally occurring trace metals and dissolved organic matter on the development of phytoplankton communities.

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APPENDIX

APPENDIX I. Final Fluorescence Yields. Median/ 1/2 range.

Exp. no.	Date Begun	Non-Carbon		Carbon Treated									
		None	Maj., Vit., T.M.	None	Maj., Vit., T.M.	Maj. Vit.	Maj.	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug.	37/8		43/12		45/3		47/14		42/5			42/5
IV	Sep.	16/2	29/3	22/1		29/2		17/4		17/2			18/3
V	Feb.	36/2	118/15	35/8	120/18		22/4	24/4	22/5	23/5			28/4
VI	Apr.	26/3.5	123/3	10/2.5	168/6		13/.3	11/1.2	17/1.8	27/0		34/4.3	33/.3
VII	May	17/2	50/1	6.3/.3	48/3		42/.7	19/2,5	13/2	17/3	21.5/3	23/3	
VIII	July	1.3/.3	39/1	1.5/.5	33/4		47/4	1.7/.2	1.5/.3	1.4/.2	1.7/.3	1.7/.5	
IX*	Aug.	3.1/.1	37/5.8	3.0/.3	32.5/.3		39/3.0	3.2/.3	3.5/.6	2.0/.0	2.9/.6	2.9/.4	

* Mean of 2 Rep.

APPENDIX II. Days to fluorescence threshold (0.5).

		Median/ 1/2 Range											
		Non-Carbon		Carbon Treated									
Exp. no.	Date begun	None	Maj., Vit., T.M.	None	Maj., Vit., T.M.	Maj. Vit.	Maj.	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug.	5.3/ .6		6.2/ .3		4.8/ .3		7.6/ .2	6.3/ .5	6.4/ .4			
IV	Sept.	3.7/ .1	3.6/ .2	4.8/ .3	3.7/ .1			4.8/ .2		5.6/ .4			3.8/ .3
V	Feb.	12.6/ .2	12.6/ .1	21.6/ .7	11.8/ .4		18.9/ 1.0	22.3/ .5	16.3/ 0	18.8/ .2			16.2/ .4
VI**	April	8.6/ .1	8.3/ .0	12.2/ .2	8.6/ .2		12.1/ .2	18.0/ .5	9.3/ .3	11.0/ .2		8.4/ .4	10.0/ .1
VII	May	5.0/ .2	4.4/ .2	8.4/ .6	3.8/ .3		6.8/ .2	10.8/ .4	7.8/ 1.1	5.3/ .3	5.2/ .1	3.7/ .1	
VIII	July	5.6/ 0	5.6/ .3	9.4/ .4	4.8/ .1		7.4/ .2	9.6/ .2	6.7/ .2	6.8/ .2	9.6/ .1	5.3/ .1	
IX*	Aug.	5.6/ 0	5.2/ .4	6.1/ .2	6.3/ .1		6.7/ .6	6.3/ .1	5.6/ .6	7.8/ .3	5.6/ .1	5.2/ .5	

** Fl = 5

* Mean of 2 replicates.

APPENDIX III. Specific growth rates (doublings/day).

Median/
1/2 Range

Exp. no.	Date begun	Non-Carbon		Carbon Treated									
		None	Maj., Vit., T.M.	None	Maj., Vit., T.M.	Maj. Vit.	Maj.	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug.	.88/ .17		1.31/ .17		.79/ .04		1.08/ .07		1.18/ .07			1.11/ .20
IV	Sept.	1.54/ .06	1.83/ .20	.98/ .07		1.57/ .15		.84/ .07		1.01/ .15			1.11/ .07
V	Feb.	.93/ .04	.81/ .02	.63/ .05	.92/ .02		.68/ .04	.84/ .14	.72/ .14	.74/ .04			.74/ .12
VI	April	1.00/ 1.6	1.08/ .04	.75/ .05	1.10/ .04		.70/ .03	.75/ .02	.68/ .05	1.00/ .03		.99/ .1	.91/ .02
VII	May	1.02/ .11	1.55/ .07	.51/ .02	1.37/ .02		.70/ .06	.31/	2.00/ .10	1.04/ .14	1.11/ .13	1.65/ .12	
VIII	July		1.30/ .36		1.34/ .12		.89/ .07						
IX*			1.54/ .16		1.41/ .01		1.28/ .29						

* Mean of 2 replicates.

APPENDIX IV. Thalassiosira. Thousands of cells/ml.

		Median/ 1/2 Range											
		Non-Carbon		Carbon Treated									
Exp. no.	Date begun	None	Maj., Vit., T.M.	None	Maj., Vit., T.M.	Maj. Vit.	Maj.	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug.	1.4/ 1.0		6.6/ 2.0		5.1/ 3.0		7.6/ 3.7		3.6/ 4.4			6.5/ 2.7
IV	Sept.	1.9/ .4	5.4/ 1	.4/ .1		.2/ .2		0.0/ 0		1.6/ .7			.5/ .5
V	Feb.	2.2/ 1.1	95/ 16	0.1/ .7	27/ 8		.3/ 0.4	0.0/ 0	0.1/ .1	1.3/ .9			.4/ .3
VI	April	R/ 1.3	2.5/ 5.7	R/ 0	2.5/ .9		0/ 0	R/ 0	R/ 0	.8/ 1.6		2.3/ 1.2	1.8/ .7
VII	May	3.9/ .8	9.3/ .6	2.1/ .8	6.5/ 1.7		2.3/ .6	1.1/ .3	.6/ .3	3.4/ .7	6.5/ 1.7	5.3/ .5	
VIII	July	0.5/ .05	7.3/ .3	0.1/ 1.5	7.7/ 2.7		R/ .16	0.0/ .6	0.3/ .3	R/ .3	0.1/ .2	1.2/ 1.0	
IX*	Aug.	0.7/ .08	2.2/ 1.5	0/ 0	5.5/ .4		0/ 0	0/ 0	.17/ .17	0.2/ .00		0.4/ .16	

* Mean of 2 replicates.

APPENDIX V. Chaetoceros. Thousands of cells/ml.

		Median/ 1/2 Range											
		Non-Carbon		Carbon Treated									
Exp. no.	Date begun	None	Maj., Vit., T.M.	None	Maj., Vit., T.M.	Maj. Vit.	Maj.	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug.	5.8/ 1.7		0.6/ 0.3		3.1/ 1.3		2.8/ 4.5		5.0/ 3.6			6.0/ 4.6
IV	Sept.	8.8/ 5	23.5/ 7	16.0/ 4		27.4/ 6		17.2/ 5		8.7/ 2			13.9/ 20
V	Feb.	12.3/ .3	9.5/ 1.0	13.8/ 2.2	R/ 1.3		2.2/ 2.2	36/ 17	11/ .5	47/ .6			27/ .9
VI	April	0.0	0.0	0.0	0.0		0.0	.2/ .2	0.0	0.0		0.0	0.0
VII	May	.9/ .5	.08/ .13	2.9/ 2.3	6.4/ 1.6		0/ .7	26/ 8	3.2/ 2.5	.24/ .12	.08/ .06	0/ 0	
VIII	July	2.5/ .9	9.6/ 4.6	4.2/ 1.0	7.5/ 8.2		20.5/ 1.0	2.2/ 2.0	3.5/ .9	2.9/ 1.0	4.7/ .7	1.7/ .7	
IX*	Aug.	8.5/ 1.2	34.7/ 3.6	14.0/ 3.5	20.1/ 4.9		31.4/ 6.8	9.7/ .3	9.2/ 1.5	5.4/ 1.0	10.8/ .8	10.0/ 4.1	

* Mean of 2 replicates.

APPENDIX VI. Asterionella. Thousands of cells/ml.

Exp. no.	Date begun	Median/ 1/2 Range											
		Non-Carbon		Carbon Treated									
		None	Maj., Vit., T.M.	None	Maj., Vit., T.M.	Maj. Vit.	Maj.	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug.	None											
IV	Sept.	None											
V	Feb.	50/ 15	129/ 34	.4/ 1.9	19/ 22		45/ 9.5	.6/ .8	15/ 11	7.8/ .5			5.0/ 16
VI	April	61/ 12	133/ 62	33/ 8	180/ 34		33/ 4	5.7/ 4.9	26/ 3.3	66/ 10		71/ 13	72/ 9
VII	May	.5/ 2.2	.1/ .1	.2/ .3	0/ .16		.4/ .2	0/ .7	2.4/ .5	.3/ .2	R/ .1	0/ 0	
VIII	July	0.7/ .1	.5/ .5	.3/ .2	1.0/ .6		.8/ 1.3	0/ 0	.2/ .2	.1/ .1	.1/ .2	0/ .2	
IX*	Aug.	0.0/ 0	0.0/ 0	0.0/ 0	.4/ .4		.1/ .1	.3/ .1	0.0/ 0	0.0/ 0	.1/ .1	0.0/ 0	

* Mean of 2 replicates.

APPENDIX VII. Rhizosolenia. Thousands of cells/ml.

		Median/ 1/2 Range											
		Non-Carbon		Carbon Treated									
Exp. no.	Date begun	None	Maj., Vit., T.M.	None	Maj., Vit., T.M.	Maj. Vit.	Maj.	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug.	None											
IV	Sept.	None											
V	Feb.	None											
VI	April	None											
VII	May	0.0	0.0	0.0	0.0		0.0	0.0	2.4/ 2.6	0.0	0.0	0.0	
VIII	July	R	0.1/ 0.2	0.0	R		0.0	0.0	0.3/ 0.1	0.0	0.0	0.1/ 0.1	
IX	Aug.	None											

APPENDIX VIII. Skeletonema. Thousands of cells/ml.

		Median/ 1/2 Range											
		Non-Carbon		Carbon-Treated									
Exp. no.	Date begun	None	Maj., Vit., T.M.	None	Maj., Vit., T.M.	Maj. Vit.	Maj.	EDTA	Vit.	T.M.	Fe	T.M. Vit.	Humic acid
III	Aug.	None											
IV	Sept.	None											
V	Feb.	None											
VI	April	None											
VII	May	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.2/ 0.1	
VIII	July	0.3/ 0.3	0.5/ 1.0	0.0	1.0/ 2.0		0.0	0.0	0.0/ 0.1	0.0	0.0	0.5/ 0.1	
IX*	Aug.	0.2/ 0.2	0.5/ 0.5	0.0	3.1/ 1.7		0.0	0.0	0.3/ 0.3	0.0	0.0	0.3/ 0.1	

* Mean of 2 replicates.