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Nutrient Limitation of Primary Production in the York River, Virginia

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NUTRIENT LIMITATION OF PRIMARY PRODUCTION IN THE YORK RIVER, VIRGINIA

by

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ABSTRACT

Fifteen experiments, involving nutrient enrichment of water samples from the York River, Virginia as a means of studying limitation of phytoplankton production, were conducted between June 1962 and May 1963. Statistical analysis was employed to determine significant responses. The results were interpreted in the context of the annual phytoplankton cycle, described in terms of physical, morphological, and dynamic characteristics.

Two basic types of communities were recognized: blooms and interblooms. The former, characterized by high cell numbers, species diversity, and metabolic activity, responded significantly to enrichment responses in 24 hours. The interblooms, marked by lower cell numbers, diversity and activity gave little or no 24 hour responses. Consequently, the dynamic state of the samples must be included in interpretations bearing on the limiting status of particular nutrients.

Nitrate (sodium salt) and trace metals were accordingly interpreted to be limiting by deficiency throughout the year. Phosphate appeared to limit production only from mid-March through mid-September. Silicate and a vitamin mixture were observed to be generally non-limiting, while nitrate (ammonium salt) and a combination treatment were inherently inhibitory in the concentrations employed.

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INTRODUCTION

A discussion of water-mass metabolism can be facilitated by considering the interrelated biota as a system. Implicit in this distinction is the characteristic of homeostatic control resulting from an interaction of positive and negative forces (Beer 1959).

Included among these forces are the nutrients, considered to be a major factor limiting to production (Rabinowitch 1951). It is understood that certain chemical elements may control or limit production in two ways while other influences remain constant: first, a deficiency in an element implies a need; second, an excess results in inhibition.

Previous nutritional studies have undeniably established the dependence of phytoplankton dynamics on nitrogen, phosphorus, and silicon (e.g., Ketchum 1939; Edmondson and Edmondson 1947; Hutchinson 1957), as well as both iron and trace quantities of some of the heavier elements (Conover 1954; Ryther and Guillard 1959). Provasoli and Pintner (1953), working with unialgal cultures, showed the importance of cobalamin and thiamin in flagellate nutrition while Riley (1943) observed what appeared to be a direct relationship between production level and the number of different nutrients present.

The study presented here, partially based on similar experiments by Smayda (1963), is an attempt to determine through nutrient fertilization which elements exert a controlling influence on plankton production throughout one annual cycle in the York River, Virginia.

MATERIALS AND METHODS

Field

Water samples were obtained from a permanent station located 300 yards off the Virginia Institute of Marine Science pier. Prior work on this station is reported in Patten, Warinner and Eayrs (1961).

Sampling intervals were generally three weeks, with one exception of six weeks, and extended from June 1962 to May 1963 (Table 1). Although mean low water was 26 feet, sampling was carried out only at two and ten feet to observe effects on populations both above and below the mean annual five foot compensation depth (Patten, Young and Rutherford 1963).

From each depth a five-gallon water sample was collected in a chemically inert Van Dorn bottle and stored in polyethylene carboys. Aliquots were later removed from each carboy and analysed for chlorinity, extinction coefficient, dissolved oxygen and plankton density. At the time of sampling, temperatures at both depths were determined with a thermistor (Schiemer 1962).

Procedures and Experimental Design

A thorough homogenization of each carboy preceded the transfer of aliquots into 300 ml BOD bottles. Initial dissolved oxygen samples were taken first, then at intervals of every nine treatment bottles. Nutrients were added and the bottles sealed and shaken.

Concentrations for the enrichment media (Table 2) were partially based on Guillard's medium "F" (1959). Chloromycetin and penicillin were originally included as bacteriostats but inhibition of the Winkler reagents (Marshall and Orr 1958) led to their replacement by sodium nitrate (a

nitrogen source other than the inhibitory NH_4NO_3 appeared desirable).

The bottles were secured horizontally (Ohle 1957) to a wooden tray, covered with "hardware cloth", located in an outdoor concrete tank twice its area (Fig. 1). An inflow pipe at one end maintained a flow of ambient river water at approximately 150 gallons/minute. The water level above the tray was maintained at two feet by a standpipe 14 feet from the inflow.

Each sampling depth was represented by nine treatments (Table 2), each replicated four times. The overall design was a randomized block with treatments randomized in each of four blocks for the two levels of collection.

The first four experiments followed an original design of six hour exposures. This was modified in the fifth experiment to 24 hours, which then became standard procedure. An "evaluation" experiment was also performed to determine the effects of different exposure periods. This was indentical to the others in all respects but two: first, the water used came from one depth; second, one half of the experiment was terminated after 24 hours and the other half after 72 hours.

Extinction coefficients were determined from ambient water in the tank at the beginning and end of each experiment. Incident solar radiation (I_0) was recorded by an Eppley 10-junction pyrheliometer located about 80 feet from the tank and, with the aid of the mean extinction coefficent, the light incident on the tray (I_2) was computed. Maxium and minimum water temperatures were obtained from a thermometer placed directly on the tray.

Each experiment was terminated with the removal and subsequent fixation of the bottles with Winkler reagents.

Laboratory

All bottles used in these experiments were originally leached in sea water for 60 hours. Prior to each use they were thoroughly washed several times with tap water, followed by a rinse with 10 percent hydrochloric acid and then several rinses with distilled water. It was assumed that remaining impurities were equally represented in each treatment bottle.

Enrichment media were prepared with reagent grade chemicals and glass-distilled water which had been passed through an ion exchange resin. All solutions were stored in one-liter Pyrex bottles which were opened only at the time of each experiment. The vitamin mix was the only exception in that after mixing, it was autoclaved and frozen to minimize bacterial action. In additon to these precautions this mix was prepared twice during the twelve month period.

Chlorinity was determined by the Mohr method. Extinction coefficients were computed from optical densities, read on a Klett-Summerson colorimeter with neutral filter. Oxygen was determined by the Pomeroy-Kirschman-Alsterberg modification of the Winkler method.

Plankton density was estimated from counts in a Sedgwick-Rafter cell. Although counts were made within several hours of collection, refrigeration was employed to maintain samples.

Statistical Analysis

Oxygen values from each depth layout were analysed by an analysis of variance one-tail test (Snedecor 1956). When "F" values for treatments were significant, a multiple range test (Duncan 1955) was employed

to determine where significant mean differences existed.

RESULTS

1. COMMUNITY CHARACTERIZATION

In order to establish a context for assessing the effects of nutrient enrichment on the plankton system, it is necessary to provide a community identity for the period of investigation. Following are described some of the physical, morphological, and dynamic characteristics of the York River community, as well as the environment in which the enrichment experiments were conducted.

Hydrography

Mean chlorinities (Fig. 2) at the two and ten foot sampling depths, respectively, were 11.37 and 11.67 o/oo with ranges of 9.71-12.80 and 10.14-12.88 o/oo. Temperature averaged 12.68 and 12.20 °C with ranges of 2.42-26.70 and 1.74-27.00 °C. Mean extinction coefficients were 0.72 and 0.85 m^{-1} with ranges of 0.29-1.38 and 0.34-1.64 m^{-1}. Dissolved oxygen averaged 8.18 and 7.67 mg O_2 l^{-1} with ranges of 5.30-11.39 and 4.52-10.91 mg O_2 l^{-1}. Annual mean differences of 0.30 o/oo, 0.48°C, 0.13 m^{-1}, 0.51 mg O_2 l^{-1} indicate a generally similar ambient medium at both depths throughout the year.

Tank Characteristics

Temperature observations denoted a mean daily range of 3.88 °C. This value was exceeded in only two experiments (6, 11), in each case due to a water supply failure of several hours. The mean annual extinction coefficient of 0.98 m^{-1} was 0.26 and 0.13 m^{-1} higher than those recorded at two and ten feet, respectively. Apparently, this was the result of

accumulated silt within the tank. Incident light (I_o) ranged from 95.6-733.2 with a mean of 332.2 langleys day^{-1}. The annual mean and range of light incident on the tray (I_2) were 186.6 and 50.9-394.3 langleys day^{-1}, indicating an average transmittance of 58 percent through the two feet of water above the tray (Fig. 2).

Plankton

Cell counts indicate the presence of two maxima within the experimental period. The first occurred early in August and corresponded to a red water condition which prevailed in the river at that time, while the second was observed late in March during a period of rising water temperatures, high winds and strong currents.

From the standpoint of seasonal dominance the μ-flagellates and dinoflagellates (collectively referred to as flagellates) were in greatest abundance from June through December and again in mid-April and May (Fig. 3). Throughout these periods the most significant forms, in descending order, were Chilomonas (?) sp., Gymnodinium sp., Cochlodinium sp., Massartia rotundata, Prorocentrum micans and Peridinium triquetrum. The major diatoms were Coscinodiscus asteromphalus (?) early in the summer, followed by Skeletonema costatum, Chaetoceros (affinis and/or compressus) and Asterionella japonica later in the fall. The order among the flagellates remained steady throughout this period with the exception of several weeks in August when the "red tide" outburst, due to Gymnodinium sp. and Cochlodinium sp., totally obscured the remaining flora.

Gradually declining water temperatures late in December (Fig. 2) preceded the change in dominance from flagellates to diatoms. Rhizosolenia fragilissima, S. costatum, A. japonica, C. (affinis and/or compressus) and

Nitzschia pungens var atlantica, in descending order, increased slowly and culminated in the late March maximum (Fig. 3). This peak was followed by return of the flagellate-dominated community. Throughout the winter the only flagellate observed with any regularity was Chilomonas (?) sp.

Morphologically, the quantitative aspects of phytoplankton distribution at the two depths were remarkably similar throughout the entire program, except that the two-foot depth occasionally had greater numbers. This is somewhat contray to the expected results (e. g., Patten 1963) which prompted use of a second depth.

Production Parameters

In addition to a morphological characterization of the community, several parameters were computed to aid in the establishment of a physiological or dynamic identity.

The first to be considered is respiration (ρ), which provides a measure of intrinsic metabolic activity under dark conditions. During the experimental period mean values for two and ten feet, respectively, were 0.27 and 0.26 mg O_2 l^{-1} day^{-1}. Three maxima were recorded (Fig. 3), two corresponding to the previously described peaks in cell numbers in August and March and the third occurring in October.

Gross production (π) provides an absolute measure of a community's photosynthetic activity. The means and ranges for two and ten feet were respectively 1.33 and 0.43-3.12, 1.26 and 0.40-3.23 mg O_2 l^{-1} day^{-1}. Seasonally, samples from both levels followed essentially similar patterns (Fig. 3). Comparison of the seasonal trends of π and ρ in Fig. 3 indicates that community metabolism and production followed closely the lead of the plankton constituents. That is, an increase in cell numbers promotes, in general, a higher intrinsic community metabolism which in turn contributes

to a positive modification of the production level. An exception is noted in that the occurrence of the production and respiration high in October coincided with a population level which was at a seasonal low. This may be due in part to the high insolation which was recorded during this experiment (Fig. 2).

In order to relate the observed production levels to available radiation at the tray surface, (π/I_2) was computed (Fig. 3). The means and ranges for two and ten feet respectively, were 0.92 and 0.12-3.39, 0.84 and 0.13-2.77 mg O_2 langley^{-1} x 100. Three periods of relatively high efficiency were observed during the fall and early spring. Each of these preceded the metabolic and photosynthetic maxima (Exps. 4, 7 and 13) by one experiment (3, 6 and 12).

2. STATISTICAL RESULTS

Analysis of variance indicated highly significant differences ($P<.01$) between treatment means in every experiment. Significant block differences ($P<.05$) occurred on only three occasions (Exps. 1, 2 and 14). That block differences were seldom noted is indicative of uniform conditions in the tank. The efficacy of the randomized block design is attested to by the fact that significant treatment differences were denoted with only four replications.

The multiple range test occasionally proved inadequate in that its ability to detect small mean differences was impaired whenever a large deviation from the control was assessed along with a small one in the opposite direction (e.g., attempting to detect significance in a minor stimulation of production when a large inhibition by another treatment was also present). This is because inherent in the multiple range test is the standard error of the mean, a statistic which varies with the range of

dissolved oxygen values represented.

The evaluation experiment was enlightening on this point in that it conclusively showed (Table 4) that two treatments, which in 24 hours produced a non-significant quantity of oxygen above a control, could become significant when the exposure was lengthened to 72 hours. It appears from this experiment that while most of the inhibition which will occur is accomplished within 24 hours, those treatments which stimulate production continue to do so over the extended period (Edmondson, 1955, recorded maximum at five days).

Following are the increments of change (i.e., mg O_2 l^{-1}) between the mean 72 hour and 24 hour dissolved oxygen concentration for each treatment (notations as in Table 4).

D	NH_4	SiO_3	Co	PO_4	L	Vit	TM	NO_3
-0.01	-0.03	0.24	1.46	2.04	1.84	1.84	1.96	2.19

It is noteworthy that two of the originally inhibitory treatments (NH_4 and SiO_3) remained relatively stable (Co and PO_4 appear to have experienced a lag which they may, at 72 hours, have been in the process of overcoming; Spencer 1954). On the other hand, the ultimately stimulatory treatments (TM and NO_3) continued to produce oxygen at a faster rate than the control.

Based on these considerations, results will be interpreted as significantly different from the control whenever reasonable proximity to significance is obtained in 24 hours.

3. NUTRIENTS

Graphic presentation of the nutrient data was achieved by plotting the difference between each treatment mean and that of the corresponding

light bottle control and representing the controls by horizontal lines (Fig. 4). The actual means are provided in Table 4. Although the enrichments were carried out on samples from two depths, the results do not generally indicate differences important enough to merit separate consideration by depth.

Nitrate. As explained earlier, nitrate enrichment was not begun until Exp. 5. Greatest augmentation of production by this nutrient was recorded in Exps. 7 and 13-15, coincident with highs in production and respiration (Fig. 3). Exp. 13 alone was marked by a peak in cell numbers (Fig. 3). Exps. 5 and 6 exhibited no significant effect at the two-foot depth, but at ten feet production was increased slightly in 5 and inhibited in 6. The late fall and winter months (Exps. 8-12) were marked by a general immunity to nitrate enrichment. In general, nitrate did not appear to be limiting except possibly during October (Exp. 7) and late March-May (Exps. 13-15). That it may also have had limiting effects during the flagellate maximum in August (Exp. 4) is suggested by the response to ammonia during this period, which was comparable to that during Exps. 7 and 13 (see below).

Ammonium Nitrate. Contrary to expectation (Smayda 1963), the ammonium ion added as nitrate inhibited oxygen production throughout the program. Relatively speaking, least inhibition occurred in the summer (Exps. 5 and 6) and during the period from late fall to early spring (Exps. 8-12). Paradoxically, the greatest inhibitions coincided with the maximum stimulation from $NaNO_3$ (Exps. 7 and 13), as well as with the August flagellate peak (Exp. 4). Generally, a direct relationship appears to exist

between π, ρ, and high cell numbers, and the ability of this nutrient to reduce production.

Phosphate. Orthophosphate stimulated production only in August (Exps. 4 and 5) and April-May (Exp. 14 and at two feet only in Exp. 15), both periods characterized by flagellate dominance and high community metabolism. Oscillation from neutrality (Exps. 1-3, 6-8, 10, 11 and 15) to inhibition (Exps. 1, 9, 12 and 13) marked the remainder of the year. Although community metabolism was in its second fall peak during Exp. 7 (Fig. 3), phosphate inhibition is strongly suggested at this time. It appears that this nutrient limited production only during periods of flagellate abundance, but that inhibition occurred under variable floristic conditions.

Silicate. Another paradox presents itself in the form of an inverse relationship between the presence of diatoms and production enhancement from silicate enrichment. Throughout this period extending from mid-April (Exps. 6-14) inhibition was recorded in varying degrees in every instance. Relatively speaking, three periods of less pronounced inhibition occurred: Exps. 2-5, 8-11 and 14-15. These correspond, respectively, to periods of maximum flagellate numbers, minimum diatoms, and the decline of the diatoms in the spring (Fig. 3). The two periods of maximum production inhibition (Exps. 7-13) coincided with the fall and spring diatom maxima (Fig. 3).

Trace metals. Continuous oscillation from production levels indistinguishable from the control to levels distinctly inhibitory was a characteristic of this treatment in all but three experiments (7, 13 and 14). On these occasions, as with the nitrate treatment, production was increased coincident with highs in π, ρ and cell numbers (Fig. 3). Among

those experiments registering inhibition (1, 6, 9 and 12), there does not appear to be any correlation with other phenomena except that of preceding two of the production highs by one experiment (6 and 12).

Vitamins. With few exceptions (Exp. 14 and posibly also 7 and 15) this enrichment either had no effect or inhibited production. Exps. 1-3 suggest negative responses, although not statistically demonstrable; in Exps. 4-7 maximum inhibition occurred, coincident in part with the red w condition (Exp. 4). Disregarding Exp. 9 (at two feet) the remaining pr (Exps. 8-13) was statistically inseparable from the control.

Combined Media. Similar to ammonium nitrate in that in virtually every experiment production was inhibited, the most striking feature of combined treatment was a more or less moderate level of inhibition twice punctuated (Exps. 4-5 and 13) by negative departures.

DISCUSSION

In a certain sense, all natural systems possess a capacity for self-regulation (Beer 1959). Within the plankton system, the steady state condition can be conceived as an instantaneous balance between deviation-amplifying and deviation-counteracting (Maruyama 1963) tendencies. Biotic potential and carrying capacity perhaps represent the former, and environmental resistance the latter. Implicit in this distinction of homeostatic control at the system level is a generally narrow range of oscillation displayed by those factors exerting a constraining influence (e. g., predation, insolation, temperature, nutrients) since an extreme deviation would modify the system to the point of disruption. The occurrence, however, of synchronized factor deviation, possibly in a significant sequence, could produce a net reduction in the negative pressure normally exerted. This relaxation would then allow expression of the deviation-amplifying tendencies and result in initiation of the first stages of a bloom.

Previous observations on diatom flowering (Riley 1943) indicate that synchronization is responsible for the first stage of development: the lag phase. Riley considered this refractory period, of indefinite length and characterized by minimum net production and erratic oxygen evolution, to be represented by large numbers (as much as 20 percent) of senescent cells. He indicated that the retarded responses observed in the lag phase may result from a balance between the death rate of the irreversibly starved cells and the growth rate of the newly stimulated cells. Spencer (1954) suggested that a physiological adaptation may be involved. With passage of the second stage of development, that of logarithmic growth, the system must again come under the influence of the retarding forces since another temporary balance is reached,

this time in the form of a bloom.

The York River phytoplankton system apparently underwent similar flowering on three occasions: red water in August, fall bloom in October and spring bloom in March. On the basis of cell numbers, species diversity, production parameters, and enrichment responses, two broad classifications are applicable to this sytem: interbloom and bloom. The interblooms, beginning with the end of one bloom and extending to the initiation of another, possessed low cell numbers, low production, reduced diversity and indifferent enrichment responses. The fall bloom, coinciding with Morse's (1947) earlier description, exhibited (with the exception of high cell numbers) each definitive characteristic found in the more typical spring bloom, i. e., increases in efficiency, respiration, and gross production, as well as rapid and decisive enrichment responses. The red water differed primarily in its lack of species diversification, but it was similar enough in other respects to be considered a bloom.

Although present system theory (Bertalanffy 1950) relates stability directly to complexity, it appears in this study (Fig. 4: Exps. 4, 7 and 13) that the greatest short-term perturbations occurred during each bloom. Instead of stable self-regulation, artificially imposed changes in the bloom communities resulted in relatively violent deviations. The interbloom communities, on the other hand, responded midly or not at all.

According to Fager (1963), each distinctive period within the annual phytoplankton cycle represents a different temporal stage of development of the same community. Enrichment of any stage, then, is enrichment of one community, with the blooms representing an indefinite advance in metabolic and morphologic development beyond the base-level of the interblooms.

Essentially, then, any comparison of enrichment responses between periods can only be accomplished by extending the length of the interbloom observation, i.e., substituting time for activity, thereby simulating conditions during a bloom. Observations made during the bloom, on the other hand, substitute activity for time in that significant responses are obtained within 24 hours. Responses during these more active periods should give some indication of overall systemic limitations if no contradictions, in the form of significantly different results, are observed during the interblooms.

It is interesting to note the comparison between observations made by Spencer (1954), working with unialgal cultures of Nitzschia closterium forma minutissima, and those presented here. He found that

> . . . cells subcultured from the exponential phase [of growth] into fresh medium of identical composition immediately continue growth at the same rate. In contrast, cells which are subcultured from the exhaustion phase [following exponential phase] do not grow immediately but exhibit an initial stationary phase [lag]. Growth curves show an increasing lag time . . . related to the state of exhaustion of the parent culture. In addition, cells which have been stored in the exhaustion phase for several days show an initial phase of slow growth between the initial stationary phase and the onset of growth at the optimum exponential rate.

Special reference to Spencer's Fig.14, showing "variation of lag time with the conditions of the cells of the innoculum", is suggested.

Significant interbloom responses, strongly suggestive of inhibition, (e.g., ammonium nitrate, silicate, combined media) appear as exceptions to the general rule of indifference exhibited during the inactive period. Although the emphasis of the evaluation experiment may be somewhat reduced by its proximity to the spring bloom, it nevertheless indicates that 24 hour negative responses remained negative for at least 72 hours. This extended inhibition suggests that the interbloom may also exhibit irreversible

responses in 24 hours. It is likely that inhibition acts in a passive manner, halting metabolic activity, and as the control continues its gross production the treatment, therefore, becomes increasingly inhibitory. Stimulation, however, would be an active process, involving both an uptake and utilization of nutrients resulting in production above the control. Based on these considerations, an interbloom response may lend substance to conclusions drawn from bloom enrichments, while alone it is unable to conclusively demonstrate factor limitation.

Regardless of the stage of development, enrichment may have resulted in one of three responses: stimulation, inhibition, or indifference. The first two apply principally to the blooms, since indifference was found on but one occasion during a bloom (Fig. 4: Vitamins, Exp. 13). During the interbloom, inhibition and indifference were the most commonly observed responses, in that order.

Stimulation: significant increase in gross production above control. Only one conclusion may be drawn from an enrichment yielding this response: the provided nutrient was limiting to systemic production. Following enrichment, the nutrient may be utilized either directly by all or part of the community or indirectly as an aid in utilizing another, up to that time, unavailable nutrient.

Inhibition: significant decrease in gross production below a control.

1. Organisms have a need for the provided nutrient but prior to utilization, work involving oxygen uptake must be done to absorb and incorporate it. If in 24 hours this oxygen deficit could not be cancelled then this would appear as an inhibition.

2. The presence of high environmental nutrient concentrations

combined with an enrichment, may narcotize or poison all or part of the community.

3. The enrichment may itself be inherently toxic (see discussion of ammonium nitrate below), or the interaction of many nutrients in one mix may so drastically alter the environment as to overwhelm the community (see discussion of combined media below).

4. Organisms may require a nutrient but due to either a physiological lag (Hutchinson 1944), or a period of adaptation (Spencer 1954) no increase in production occurs.

Indifference: no significant change in gross production from that of the control.

1. Nutrient may be superfluous due to absence of organisms capable of utilizing it.

2. Organisms may have a need but are incapable of utilization in the absence of another factor unavailable at that time, e. g., nutrient, exocrine, or environmental stimuli.

3. Environmental effects, contributing to community structure and metabolism, may exert such a profound negative influence as to turn away all but the strongest stimuli.

Although all of the above are not specifically pertinent, each nutrient enrichment will be discussed in the light of its success or failure in evoking the above responses from the York River plankton community.

Nitrate. Significant stimulation during each bloom, as well as a suggestion of stimulation during red water, indicates this nutrient to be deficient and thereby generally limiting to the community production level. The inhibitory response noted in Exp. 6 may be the first sign of a lag

development prior to the fall bloom. Although the winter interbloom production level was never stimulated, it was also never inhibited. Based on the earlier discussion, this would indicate that the nutrient was not inhibitory during the interbloom, as well as suggesting that this nutrient may be needed throughout the year. The indifferent responses may be due to a period of adaptation by the community prior to utilizing the nutrient.

Ammonium Nitrate. Although this enrichment produced inhibition at all times, it is not likely that any inferences may be made regarding environmental limitation, since the fault apparently lies in the enrichment. The observed negative responses are probably due to either an excess of nitrogen (which was six times more abundant than in the previous treatment) or to toxicity resulting from the ammonium ion. Although Smayda (1963) obtained stimulatory results using ammonium chloride, Provasoli (1958) maintains that the ammonium ion is toxic when employed in an alkaline medium. Regardless of the fact that no record could be found of inhibitory effects resulting from excess nitrate, both the toxic ion and the excess nitrate may have combined to form an inhibitory agent.

Phosphate. Spencer (1954) observed that addition of "great concentrations" of phosphate to non-deficient diatoms resulted in its absorption and organic combination in the absence of light. Illumination then resulted in a production lag as the previously absorbed phosphate was returned to the medium. Active transport, involving oxygen utilization, may be the mechanism employed in the transfer of this nutrient to and from the medium. If so, a 24 hour observation, in a period of relative phosphate

abundance, would see an evening oxygen deficit which could not be filled during the lag of the following day. Oxygen utilization would undoubtedly be higher during a bloom when both cell numbers and phosphate concentrations would be higher. The stimulations observed during red water and again following the spring bloom are similar in that each occurred in relatively warm water dominated by flagellates. It may be that the flagellates, unlike the diatoms, thrive on high phosphate concentrations without suffering a lag. It may also be due to greater nutrient deficiencies brought about through higher temperatures (Provasoli 1958). In any case, the work cited by both Provasoli (1958) and Tranter and Newell (1963) indicates the fallacy of placing too much emphasis on absolute environmental concentrations as a guide toward explaining community dynamics. It appears, therefore, that phosphate may have been deficient from the spring bloom to red water, abundant during each bloom and possibly abundant during the winter interbloom.

Silicate. Contrary to prior evidence (Lund 1950; Hutchinson 1957; Ryther and Guillard 1959), enrichment with this nutrient contributed to negative production throughout the diatom-abundant winter months. Contrasting the inhibited fall and spring blooms with the indifferent flagellate-dominated red water, it becomes apparent that the effect is on diatoms. Harvey (1955) observed diatom inhibition at concentrations of 25 mg SiO_3-Si l^{-1} but did not provide a tolerance threshold. Although present enrichment concentrations were only 2.5 mg l^{-1}, inhibition may have been induced when environmental concentrations were also high. Sverdrup et al. (1942) indicates a seasonal silicate cycle which roughly

paralleled that of diatom abundance in the York River. If this can be extrapolated, it appears that silicate may have been most abundant throughout the winter months. In general, it does not appear that production suffers from silicate deficiency at any time in the year.

Vitamins. On one occasion following the spring bloom, this nutrient mixture was observed to increase production; this may be due to either biotin or thiamin since Provasoli (1963) feels that vitamin B_{12} is never limiting in inshore waters. Evidently, enrichment caused inhibition during the period between red water and the fall bloom. This corresponds to the period of peak offshore B_{12} production (Menzel and Spaeth 1962) and a possible radical increase in B_{12}-producing bacteria following the decay of red water (Pintner and Provasoli 1958). The increased surface area of the bottles could also have increased bacterial growth (Zobell and Anderson 1936) so that during this period three sources of B_{12} may have been available, possibly contributing to an excess which reduced production. This excess would not be so apt to occur following the two other blooms since each occurred at a time when the offshore B_{12} peak had passed (Menzel and Spaeth 1962) as well as in considerably cooler water, thereby reducing the possibility of a bacterial outburst. Another possible explanation is that the excessive bacterial growth may have reduced the dissolved oxygen while the bacteria were utilizing the available B_{12} (Burkholder 1963). The beginning of the fall bloom may have reduced the quantity of available vitamins in the water, allowing a large enough supply to remain until it was drawn upon in the spring, thereby promoting the only deficit of the year. Based on these considerations, it does not appear as if these three vitamins

are limiting to production in the York River community, with the possible exception of a short period following the spring bloom.

Trace Metals. Enrichment with this nutrient mixture elicited production stimulations during each bloom, thereby indicating a possible overall community need. Support for such a conclusion may be found, as in the nitrate treatment, in the generally indifferent responses observed during the interblooms. Thomas (1959), Ryther and Guillard (1959) and Johnston (cited by Provasoli 1963) have all determined trace metals to be essential for attainment of higher production levels, whereas Smayda (1963) was unable to achieve a positive response at any time. Although the components of this mix have not been studied individually, Menzel and Ryther (1961) were able to isolate iron as the limiting element in the Sargasso Sea. It is possible that the indifferent response observed during red water may have been due to excessive chelation by organic nutrients, e.g., B_{12} (Shapiro 1957), present in the water at that time. The negative response prior to each bloom could well indicate community work involving the removal and storage of needed nutrients during the lag period.

Combined Media. Contrary to the additive effect witnessed by Riley (1943) and the beneficial responses observed by Thomas (1959) and Ryther and Guillard (1959), this treatment completely inhibited production. It was not possible to select the nutrient(s) responsible for these results. It may well be the result of an altogether too radical environmental change stemming from an excessive quantity of nutrients in such a small volume. The evaluation experiment indicated this enrichment to be less inhibitory than either ammonium nitrate or silicate, but with extended ob-

servation it appeared to reduce the production at an increasing rate.

On the basis of these observations, silicate was never deficient, nor were biotin, thiamin, or vitamin B_{12}, except for a short period following the spring bloom. Each of these may have inhibited production by excess in the experiments but this could not be positively demonstrated. Apparently, ammonium nitrate and the combined mix inhibited through some fault of the media. Both sodium nitrate and the trace metals are considered to be limiting to the system. With phosphate it appears that environmental concentrations may be abundant from mid-September to mid-March, while a deficiency may be present the remainder of the year.

Several possible refinements in technique became increasingly obvious as the study progressed. (i) Simultaneous fertilization of dark, as well as light bottles would contribute some understanding of the effects of enrichment on metabolism, as distinguished from production. (ii) The use of a compatible antibiotic would be invaluable in separating the bacterial and algal influences. (iii) Several more properly placed evaluation experiments could prove helpful in investigating the relationships between initial and long term enrichment responses. (iv) A better understanding of the absolute environmental nutrient concentrations would permit more reliable inferences to be made from the experimental responses. (v) Observation of the effects of different concentrations of a nutrient might give some insight into the role of excess in inhibition.

In conclusion, it will once again be stressed that enrichment of a plankton community as a method of studying nutrient limitation should not be done in ignorance of the dynamic state of that community. Since the

eventual response from an identical enrichment on two different developmental stages of the system may prove to be similar, the results obtained from short-term observations cannot be relied upon and may very well prove misleading.

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APPENDIX

Figure 1. Randomized block design; each horizontal row represents one replication for either two or ten feet.

Figure 2. Annual cycles for chlorinity(o/oo), temperature(^{o}C), extinction coefficients(m^{-1}), dissolved oxygen(mg l^{-1}), and solar radiation(langleys day^{-1}). Solid and dotted lines represent two and ten feet for all but insolation, where they indicate I_1 and I_2, respectively. Numbers at the top indicate individual experiments.

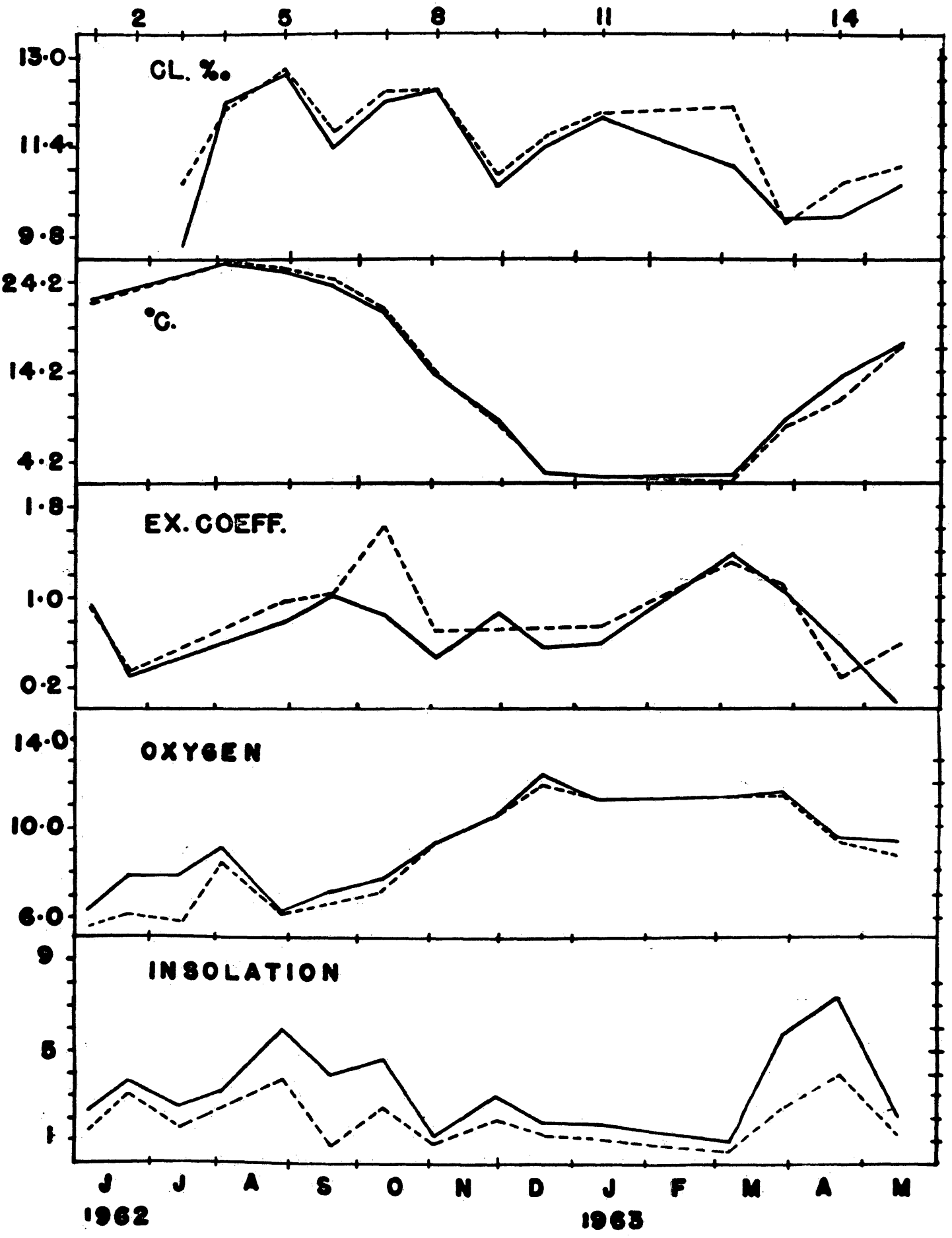
2
5
8
11
14
13·0
11·4
9·8
CL. ‰
24·2
14·2
4·2
°C.
1·8
1·0
0·2
EX. COEFF.
14·0
10·0
6·0
OXYGEN
9
5
1
INSOLATION
J
J
A
S
O
N
D
J
F
M
A
M
1962
1963

Figure 3. Annual phytoplankton cycle at two and ten feet; each unit represents 1 x 10^3 cells ml^{-1}, with diatoms and flagellates indicated by solid and dotted lines, respectively. Gross production(mg O_2 l^{-1} day^{-1}), respiration(mg O_2 l^{-1} day^{-1}) and production efficiency(mg O_2 l^{-1} langley day^{-1} x 100) at two and ten feet(solid and dotted lines respectively). Numbers at top indicate individual experiments.

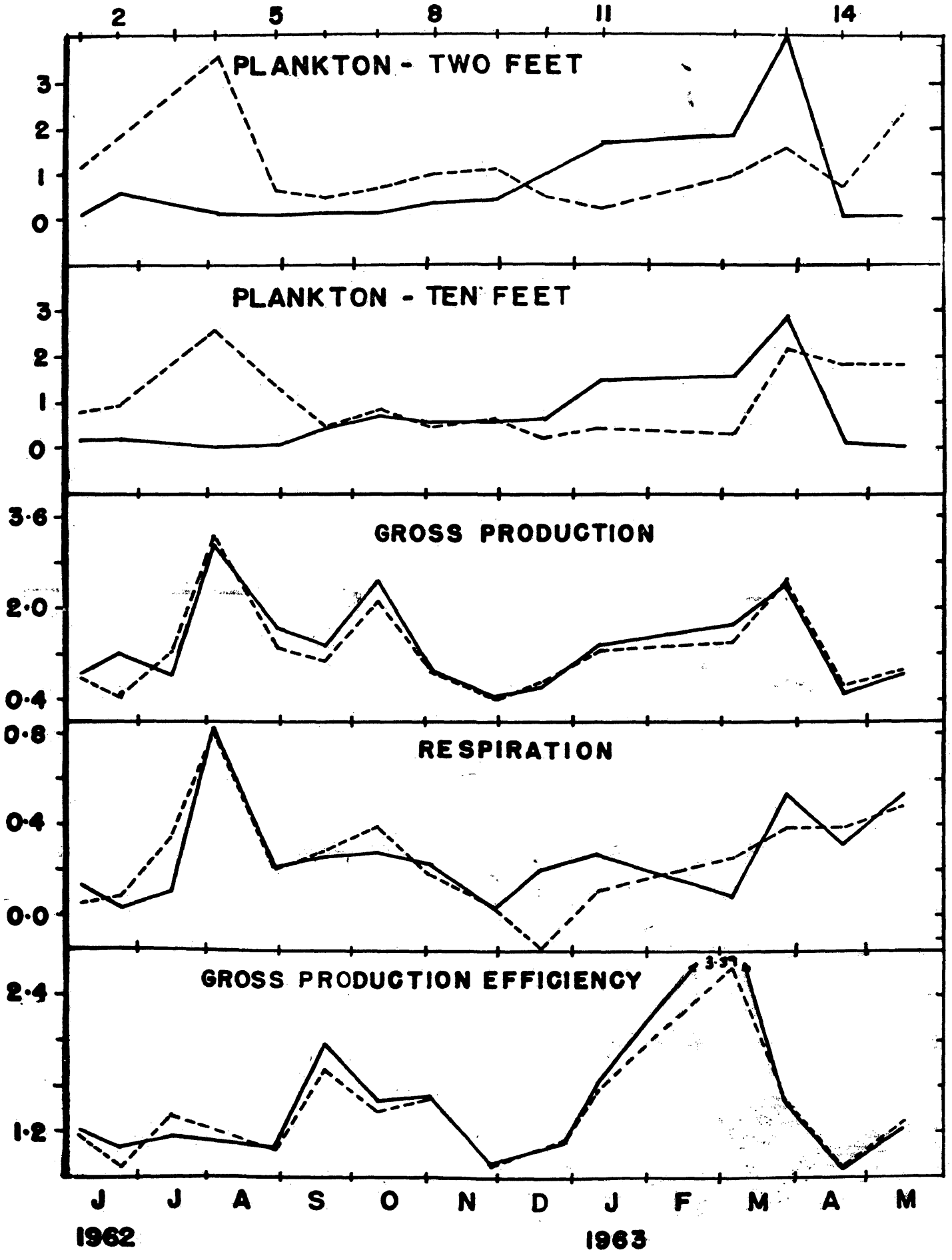
2
5
8
11
14
PLANKTON - TWO FEET
3
2
1
0
PLANKTON - TEN FEET
3
2
1
0
3·6
2·0
0·4
GROSS PRODUCTION
0·8
0·4
0·0
RESPIRATION
2·4
1·2
3·57
GROSS PRODUCTION EFFICIENCY
J
J
A
S
O
N
D
J
F
M
A
M
1962
1963

Figure 4. Enrichment results from two and ten feet(solid and dotted lines, respectively); marked by an open circle whenever response was significantly different($P < .05$) from light bottle control. Sodium nitrate($NaNO_3$), ammonium nitrate(NH_4NO_3), phosphate(PO_4), silicate(SiO_3). Numbers at the top indicate individual experiments.

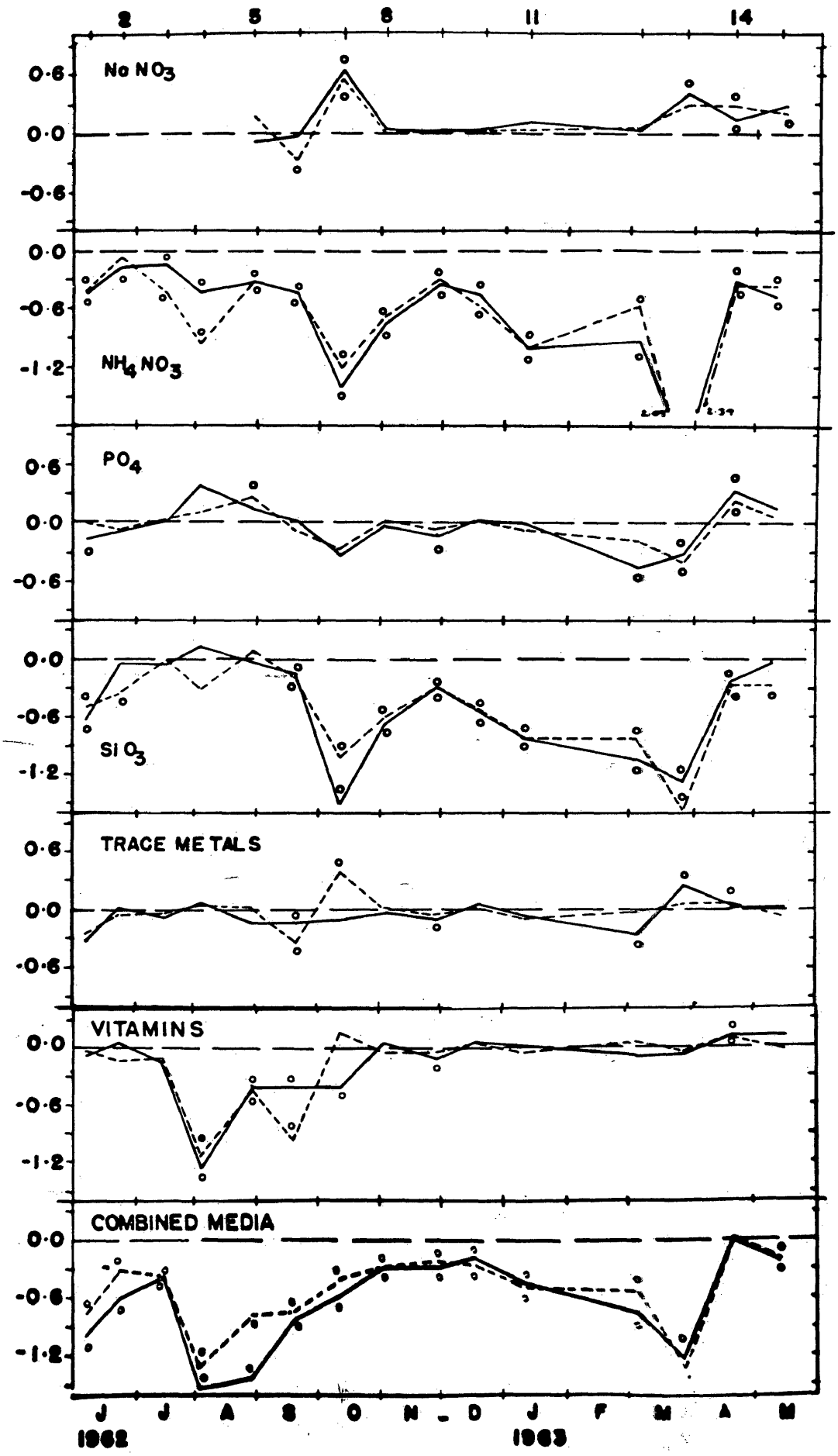
2
5
8
11
14
No NO3
0·6
0·0
-0·6
NH4 NO3
-1·2
PO4
SiO3
TRACE METALS
VITAMINS
COMBINED MEDIA
J
J
A
S
O
N
D
J
F
M
A
M
1962
1963

TABLE 1. Experimental dates between June 1962 and May 1963

*

EXPERIMENTS	DATES
1	Jun 6-7, 1962
2	Jun 23-24, "
3	Jul 14-15, "
4	Aug 3-4, "
5	Aug 29-30, "
6	Sep 19-20, "
7	Oct 11-12, "
8	Nov 2-3, "
9	Nov 29-30, "
10	Dec 18-19, "
11	Jan 11-12, 1963
12	Mar 5-6, "
Evaluation	Mar 20-23, "
13	Mar 27-28, "
14	Apr 20-21, "
15	May 15-16, "

TABLE 2. Nutrient media adapted from Guillards's (1959) medium "F". Seven treatments plus a dark and a light bottle control constitute a replication.

TREATMENTS	CONCENTRATIONS
1. $NaNO_3$	1.28 mg-at NO_3-N l^{-1}
2. NH_4NO_3	1.60 " NH_4-N " 5.60 " NO_3-N "
3. $NaH_2PO_4 \cdot H_2O$	0.16 " PO_4-P "
4. $Na_2SiO_3 \cdot 9H_2O$	2.25 " SiO_3-Si"
5. Trace Metals	
$CuSO_4 \cdot 5H_2O$	0.07 ug-at Cu l^{-1}
$ZnSO_4 \cdot 7H_2O$	0.15 " Zn "
$CoCl_2 \cdot 6H_2O$	0.08 " Co "
$MnCl_2 \cdot 4H_2O$	0.18 " Mn "
$FeCl_3 \cdot H_2O$	0.02 " Fe "
$NaMoO_4 \cdot 2H_2O$	0.05 " Mo "
H_3BO_3	97.00 " "
Di-Na-EDTA	8.70 " "
6. Vitamins	
Thiamin.HCl	0.20 mg l^{-1}
Biotin	1.00 ug "
B_{12}	0.15 " "
7. Complete Medium Contained all of the above in the same concentrations, except for NH_4NO_3, replaced by $NaNO_3$ in Exp. 5.	
8. Dark Bottle	
9. Light Bottle(control)	

TABLE 3. Production parameters computed for each experiment at two a ten feet. Gross production(π) in mg O_2 l^{-1} day^{-1}, respiration(ρ) in O_2 l^{-1} day^{-1}, and gross production efficiency(π/I_2) in mg O_2 l^{-1} lan x 100.

*	π 2	π 10	ρ 2	ρ 10	π/I_2 2	π/I_2 10
1.	0.85	0.76	0.13	0.05	0.06	0.54
2.	1.20	0.42	0.03	0.08	0.39	0.13
3.	0.81	1.25	0.11	0.35	0.52	0.81
4.	3.12	3.28	0.83	0.81	-	-
5.	1.46	1.33	0.22	0.21	0.38	0.36
6.	1.34	1.08	0.26	0.29	1.77	1.42
7.	2.46	2.12	0.28	0.40	0.01	0.87
8.	0.91	0.89	0.23	0.17	1.08	1.06
9.	0.43	0.40	0.03	0.04	0.21	0.20
10.	0.63	0.71	0.20	-0.14	0.49	0.55
11.	1.34	1.23	0.27	0.12	1.28	1.18
12.	1.73	1.41	0.09	0.26	3.39	2.77
Evaluation 24 Hrs	1.67	-	0.22	-	0.89	-
72 Hrs.	3.52	-	0.46	-	0.50	-
13.	2.41	2.51	0.54	0.39	1.01	1.06
14.	0.50	0.63	0.31	0.39	0.12	0.15
15.	0.38	0.92	0.53	0.48	0.67	0.74

TABLE 4. Summary of multiple range tests. Any two means overscored by the same line are not significantly different. Any two means not overscored by the same line are significantly different. Sodium nitrate(NO_3), ammonium nitrate(NH_4), phosphate(PO_4), silicate(SiO_3), trace metals(TM), vitamins (Vit), combined media(Co), dark(D), light bottle control(L).

1. (2)	5.03	5.17	5.37	5.57	5.71	5.86	5.94	6.02	
	Co	D	SiO_3	NH_4	TM	PO_4	Vit	L	
(10)	4.47	4.47	4.73	4.82	4.98	5.19	5.23	5.23	
	D	Co	SiO_3	NH_4	TM	Vit	L	PO_4	
2. (2)	6.70	7.29	7.71	7.85	7.90	7.92	7.96	8.00	
	D	Co	NH_4	SiO_3	L	TM	Vit	PO_4	
(10)	5.08	5.14	5.19	5.36	5.43	5.43	5.44	5.50	
3. (2)	6.71	7.14	7.34	7.37	7.44	7.45	7.52	7.52	
	D	Co	Vit	NH_4	TM	SiO_3	PO_4	L	
(10)	4.48	5.29	5.33	5.62	5.68	5.71	5.73	5.76	
	D	NH_4	Co	Vit	TM	SiO_3	L	PO_4	
4. (2)	7.25	8.82	9.14	9.93	10.37	10.44	10.51	10.77	
	D	Co	Vit	NH_4	L	TM	SiO_3	PO_4	
(10)	6.64	8.60	8.79	8.95	9.61	9.92	9.96	10.03	
5. (2)	5.09	5.11	6.15	6.24	6.43	6.45	6.51	6.55	6.69
	D	Co	Vit	NH_4	TM	NO_3	SiO_3	L	PO_4
(10)	5.01	5.54	5.92	6.02	6.34	6.35	6.43	6.51	6.61
6. (2)	5.88	6.40	6.55	6.79	7.05	7.08	7.18	7.22	7.24
	D	Co	Vit	NH_4	SiO_3	TM	NO_3	L	PO_4
(10)	5.32	5.42	5.56	5.98	6.05	6.11	6.17	6.31	6.40
	D	Vit	Co	NH_4	TM	NO_3	SiO_3	PO_4	L
7. (2)	6.46	7.42	7.52	8.34	8.51	8.59	8.83	8.92	9.58
	D	SiO_3	NH_4	Co	Vit	PO_4	TM	L	NO_3

TABLE 4.(cont.)

(10)	5.74	6.66	6.84	7.46	7.59	7.86	8.03	8.26	8.41
	D	NH_4	SiO_3	Co	PO_4	L	Vit	TM	NO_3
8. (2)	8.14	8.30	8.38	8.75	9.01	9.02	9.05	9.09	9.11
	D	NH_4	SiO_3	Co	PO_4	TM	L	NO_3	Vit
(10)	8.11	8.30	8.40	8.71	8.95	9.00	9.00	9.01	9.03
	D	NH_4	SiO_3	Co	Vit	PO_4	L	NO_3	TM
9. (2)	9.57	9.66	9.71	9.72	9.88	9.90	9.91	10.01	10.03
	D	NH_4	SiO_3	Co	PO_4	Vit	TM	L	NO_3
(10)	9.57	9.66	9.67	9.73	9.91	9.92	9.97	9.97	9.97
	D	NH_4	SiO_3	Co	TM	Vit	PO_4	L	NO_3
10. (2)	11.19	11.37	11.38	11.63	11.82	11.87	11.90	11.90	11.91
	D	NH_4	SiO_3	Co	L	NO_3	Vit	PO_4	TM
(10)	10.03	10.37	10.55	11.48	11.76	11.76	11.77	11.79	11.81
	D	NH_4	SiO_3	Co	L	PO_4	TM	NO_3	Vit
11. (2)	10.03	10.37	10.55	10.92	11.31	11.36	11.37	11.39	11.49
	D	NH_4	SiO_3	Co	TM	PO_4	L	Vit	NO_3
(10)	10.15	10.37	10.56	10.88	11.31	11.32	11.33	11.38	11.44
	D	NH_4	SiO_3	Co	PO_4	TM	Vit	L	NO_3
12. (2)	10.29	10.98	11.09	11.26	11.57	11.77	11.92	12.02	12.05
	D	SiO_3	NH_4	Co	PO_4	TM	Vit	L	NO_3
(10)	10.19	10.78	11.03	11.08	11.41	11.59	11.60	11.65	11.66
	D	SiO_3	NH_4	Co	PO_4	TM	L	Vit	NO_3
13. (2)	10.08	10.40	11.22	11.28	12.18	12.42	12.49	12.63	12.91
	D	NH_4	SiO_3	Co	PO_4	Vit	L	TM	NO_3
(10)	10.10	10.27	11.04	11.27	12.20	12.56	12.61	12.68	12.90
	D	NH_4	SiO_3	Co	PO_4	Vit	L	TM	NO_3
14. (2)	8.26	8.44	8.55	8.76	8.76	8.77	8.89	8.90	9.09
	D	NH_4	SiO_3	Co	L	TM	Vit	NO_3	PO_4

TABLE 4.(cont.)

(10)	8.02	8.25	8.38	8.65	8.66	8.72	8.76	8.88	8.94
	D	NH_4	SiO_3	L	Co	TM	Vit	PO_4	NO_3
15. (2)	7.88	8.22	8.48	8.70	8.71	8.74	8.83	8.87	9.00
	D	NH_4	Co	SiO_3	L	TM	Vit	PO_4	NO_3
Evaluation:									
24 Hrs.	10.35	10.67	10.71	11.00	11.78	12.02	12.03	12.11	12.12
	D	NH_4	SiO_3	Co	PO_4	L	Vit	NO_3	TM
72 Hrs.	10.34	10.36	10.95	12.46	13.82	13.86	13.87	14.02	14.30
	D	NH_4	SiO_3	Co	PO_4	L	Vit	TM	NO_3