THE EFFECTS OF ENERGY RELATED ACTIVITIES
ON THE PLANKTON OF THE CHESAPEAKE BAY

PROGRESS REPORT

Section 1

WORK IN PROGRESS

MASTER

Jay L. Taft

Chesapeake Bay Institute
The Johns Hopkins University
Baltimore, Maryland 21218

1 August 1975 - 31 July 1976

Prepared for the U.S. Energy Research and
Development Administration under Contract
No. E(11-1)-3279 on 1 April 1976.

NOTICE
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

TABLE OF CONTENTS

Section 1

Work in Progress

- A. General Introduction
- B. Release of Dissolved Organic Carbon by Phytoplankton
- C. Respiration and Nutrient Regeneration
- D. Bacterial Utilization of Labeled Compounds
- E. Effects of Heat and Chlorine on Natural Assemblages of Chesapeake Bay Phytoplankton
- F. Nutrient Flux between Sediment and Water

Section 2

Work Completed

- A. Taft, J. L. and W. R. Taylor. 1976. Phosphorus distribution in the Chesapeake Bay. Ches. Sci. 17: 67-73.
- B. Taft, J. L., W. R. Taylor and J. J. McCarthy. 1975. Uptake and release of phosphorus by phytoplankton in the Chesapeake Bay estuary, USA. Mar. Biol. 33: 21-32.
- C. Taft, J. L., M. E. Loftus and W. R. Taylor. 1976. Phosphate uptake from phosphomonoesters by phytoplankton in the Chesapeake Bay. Submitted to Limnology and Oceanography.
- D. Taft, J. L. 1976. Recovery of $^{14}\text{C}-\text{CO}_2$ at high flow rates from a CHN analyzer. Limnol. Oceanog. 21: 161-164.
- E. McCarthy, J. J., W. R. Taylor and J. L. Taft. 1975. The dynamics of nitrogen and phosphorus cycling in the open waters of the Chesapeake Bay. ACS Symposium, Philadelphia, Pa. pp. 664-681.
- F. Taft, J. L. and W. R. Taylor. 1976. Phosphorus dynamics in some coastal plain estuaries. Third Int. Est. Res. Conf. Galveston, Texas. In press.
- G. Hartwig, E. O. 1976. Nutrient cycling between the water column and a marine sediment. I. Organic carbon. Mar. Biol. In press.

*Reprints &
Preprints
Removed*

Table of Contents (continued)

- H. Hartwig, E. O. 1976. The impact of nitrogen and phosphorus release from a siliceous sediment on the overlying water. Third Int. Est. Res. Conf., Galveston, Texas. In press.
- I. McCarthy, J. J., W. R. Taylor and J. L. Taft. 1976. The nitrogenous nutrition of the plankton in the Chesapeake Bay. I. Nutrient availability and phytoplankton preferences. Submitted to Limnology and Oceanography.
- J. Grant, V. E., J. L. Taft, J. J. McCarthy and W. R. Taylor. 1976. Dissolved organic nitrogen measured in sea water by high temperature combustion and ultra violet photo-oxidation. Manuscript in preparation.
- K. Elliott, A. J. 1976. Methods for determining the concentrations and sources of pollutants in estuaries. CBI Special Report 50, Ref. 76-3.
- L. Taylor, W. R. Data report for the "AESOP" cruises will be forwarded when it returns from the printer.

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Energy Research and Development Administration (ERDA), nor any person acting on behalf of the Administration;

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness or usefulness of the information contained in this report, or that the use of any information, apparatus, method or process disclosed in this report may not infringe privately owned rights; or

B. Assumes that any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method or process disclosed in this report.

As used in the above, "person acting on behalf of the Administration" includes any employee or contractor of the Administration, or employee of such contractor, to the extent that such employee or contractor of the Administration, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Administration, or his employment with such contractor.

A. General Introduction

This continuing project is concerned with a comprehensive study of the Chesapeake Bay estuarine system. It encompasses the entire estuary over all seasons and combines field work with laboratory studies. It focuses on initial energy fixation by the planktonic primary producers and subsequent energy transfer to herbivorous and bacterial heterotrophs with attention to the impact of nuclear and fossil fuel energy technology on these processes.

The objectives stated for the contract period 1 August 1975 to 31 July 1976 were:

1. Identify productivity, light and nutrient relationships in previously collected data from the "AESOP" and "PROCON" cruises.
2. Evaluate organic carbon release by natural phytoplankton assemblages.
3. Enumerate, key and make biomass estimates of zooplankton in preserved quantitative samples.
4. Initiate studies of bacterial role in nutrient recycling in the bay.
5. Initiate studies concerned with the effects of several by-products of energy production by man on the plankton in Chesapeake Bay.

This document reports the status of our program with respect to these objectives, though not necessarily in the above order. It also reports on work completed in the form of reprints and manuscripts in preparation for publication.

Analysis of the productivity and nutrient data collected on "AESOP" and "PROCON" cruises is continuing through the use of a mixed dimension kinematic model, details of which are described in Document No. C00-3279-23. The purpose of this approach is to identify sources of nutrients in the estuary and determine the rates of nutrient flux into and out of the

euphotic zone. This is accomplished by dividing the estuary into two-layered segments and computing the fluxes between segments from measured nutrient concentrations and salt flux. The calculated nutrient flux rates will then be compared to biomass specific production rates to ascertain if nutrient flux is the major factor regulating productivity.

The analysis of preserved quantitative zooplankton samples by a consultant is underway and will be continued into the next contract period.

Other aspects of the work in progress are discussed in the following sections.

B. Release of Dissolved Organic Carbon by Phytoplankton.

Introduction

Release of newly fixed carbon by phytoplankton growing under less than optimal conditions may result in underestimation of primary production by the ^{14}C technique. The loss may be as much as 40% of carbon fixed in fresh water and oligotrophic ocean water (Fogg et al., 1969; Anderson and Zeutschel, 1970; Thomas, 1971). In nearshore marine waters about 10% or less of carbon fixed may be released (Hellebust, 1965; Anderson and Zeutschel, 1970; Thomas, 1971; Berman and Holm-Hansen, 1974) which does not seriously underestimate primary production. Although a smaller fraction of fixed carbon is released in coastal waters, the quantity released may be significant if total primary production is large.

The biological significance of phytoplankton released organic matter relates to its effect on the growth of other organisms and, therefore, on the succession of species in a natural plankton assemblage (Whittaker and Feeny, 1971). Carbohydrates, organic acids and amino acids, which exist in natural waters (Degens et al., 1964; Walsh, 1965a, b; Fogg et al., 1969; Jeffries, 1969; Riley and Segar, 1970; Schell, 1974; Shah and Wright, 1974; Wheeler et al., 1974; Al-Hasan et al., 1975; North, 1975) may directly support heterotrophic growth of phytoplankton and bacteria. In addition to energy sources, growth factors (e.g., vitamins), growth inhibitors and antibiotics may be released (Pratt et al., 1944; Steeman-Nielsen, 1955; Sieburth, 1968; Carlucci and Bowes, 1970a, b).

Release of organic carbon by phytoplankton frequently occurs under unfavorable or less than optimal growth conditions. For example, release may be stimulated by high light intensity which increases photorespiration (Watt, 1966; Berman and Holm-Hansen, 1974). Cells in the dark (Eppley and Sloan, 1965) or from deep low-light waters also released large fractions

of organic ^{14}C in some experiments (Fogg, 1958; Berman and Holm-Hansen, 1974), possibly due to senescence. A natural phytoplankton assemblage contains cells in various stages of growth and senescence. In estuarine waters tidal motion and wind mixing keep non-motile cells in suspension. Therefore, although it may be correlated with light intensity, release of recently fixed organic carbon by a natural assemblage cannot be related to cell nutrition or to change in the species composition of the assemblage.

In order to study dissolved organic carbon flux in complex natural systems, investigators have used several approaches. One approach attempts to measure in natural water or culture media the concentrations of compounds known to be synthesized by phytoplankton, such as glycolic acid (Fogg and Watt, 1965; Al-Hasan et al., 1975; Hellebust, 1965), glucose (Guillard and Wangersky, 1958), amino acids (Hellebust, 1965) and vitamins (Carlucci and Bowes, 1970a, b). The interpretation of this kind of data from natural waters is difficult for several reasons. If the organic compound is chemically detectable or perhaps abundant it may be accumulating because it is temporarily unimportant to the nutrition of the heterotrophic population. If it is not detectable, either it may not be entering the water, or it could be utilized rapidly at low concentration and, therefore, be very important to heterotrophic nutrition. From the standpoint of the phytoplankton, abundant release products could indicate decline and cell lysis of a past dominant species or rapid cell division resulting in leakage of intracellular materials.

Also, if the investigator is analyzing for a specific compound, glycolic acid for example, and another compound dominates in organic carbon flux from phytoplankton to bacteria, then little meaningful information is gained.

As a compromise, and for simplicity, the release of organic carbon from natural phytoplankton assemblages has been investigated through removal of inorganic ^{14}C from the filtrate of primary productivity experiments by acidification and aeration, followed by analyses of the residual radioactivity which is attributed to organic release products. This approach is subject to several failings. Inorganic ^{14}C may not be completely removed by acidification and aeration or it may contain nonvolatile radioactive contaminants (Thomas, 1971; Sharp and Renger, 1973; Berman and Holm-Hansen, 1974). The residual radioactivity blank increases in significance as the amount of fixed ^{14}C decreases which may account for relatively high organic carbon release rates in oligotrophic waters (Sharp and Renger, 1973). The low levels of radioactivity in phytoplankton release products may be close to background radiation, so investigators have increased detection sensitivity by adding relatively large amounts of ^{14}C (5 to 20 μCi per 100 ml) to natural water (Anderson and Zeutschel, 1970; Berman and Holm-Hansen, 1974) and by concentrating released organic ^{14}C from a large volume of sea water (Thomas, 1971; Berman and Holm-Hansen, 1974). Oxidizing organic ^{14}C contaminants in the inorganic ^{14}C stock and blank determinations improve the accuracy of these techniques (Berman and Holm-Hansen, 1974). Steeman-Nielsen (1975) suggests that poisons such as copper added as contaminants with inorganic ^{14}C to experimental incubations may artificially induce organic matter release. Contamination can be minimized by distilling commercially available inorganic ^{14}C into high quality distilled water.

Carbon-14 incubations with natural populations are usually less than 24 hours long and only those compounds labeled during incubation are detected. Therefore, release of an unknown quantity of older organic materials may be detected.

In previous field studies we detected release of less than 10% of ^{14}C assimilated by natural phytoplankton assemblages back to the water as organic material during 2 to 53 hr incubations (Taylor, unpublished data; Taft and Taylor, unpublished data). This agrees generally with the findings of others for coastal waters (Hellebust, 1965; Anderson and Zeutschel, 1970; Thomas, 1971). The amount of ^{14}C labeled organic material detected is the net result of phytoplankton release and bacterial uptake and release. However, neither the total organic carbon released nor accurate flux rates of organic carbon from phytoplankton to bacteria can be determined. These problems have been cited by others attempting to quantitate organic carbon release by natural phytoplankton populations in fresh water (Fogg, 1958;) and sea water (Anderson and Zeutschel, 1970; Thomas, 1971). Bacterial uptake was thought to be negligible in one study because addition of antibiotics to the water did not alter organic carbon release (Anderson and Zeutschel, 1970). However, the effectiveness of the 6-8 hr antibiotic treatment was not independently evaluated.

The nature of organic carbon release is complicated by the physical stress a phytoplankter may encounter during filtration or water screening to exclude large zooplankters from the incubation chamber. Such stress may stimulate organic carbon release (McCarthy et al., 1974).

Another potential problem, which has not been studied successfully in natural plankton assemblages, is bacterial uptake, modification and release of algal released organic carbon. It has no doubt been disregarded both because of the complexity of meaningful study and because of the apparent insignificance of bacterial biomass and metabolic activity in the euphotic zone as compared to phytoplankton biomass and activity.

Status

We previously proposed to evaluate organic carbon release with high temperature oxidation of lyophilized sea water (Taft, 1976). High temperature oxidation is still the method of choice because it converts carbon bound in refractory compounds to CO_2 and because it separates ^{14}C from ^{32}P (and its radioactive decay products) used in the double-label experiments. However, due to a breakdown in the CHN analyzer, we have begun analyzing ^{14}C labeled organic materials from natural phytoplankton assemblages by an alternate method. This method will serve as a screening procedure only and final analyses will be accomplished by high temperature combustion. The procedure is to aerate 5 ml of glass fiber filtered sea water from the experimental vessel with 5% CO_2 in air for 30 minutes in a scintillation vial. This reduces the pH to about 5.8 and removes $^{14}\text{CO}_2$ from solution, as shown in Figure 1. Bubbling for a few minutes with air then removes excess CO_2 and restores alkaline pH. Then 10 ml of a 2:1 (U/V) solution of scintillation solution : Triton X-100 is added and the vial counted in a Tricarb 3320 scintillation spectrometer. In all cases the ^{32}P had decayed sufficiently to cause minimum interference with this screening procedure.

Data for recovery of ^{14}C labeled dissolved organic carbon (DOC) is shown in Figures 2 through 7. A detailed analysis of these data will not be made at this time, but a few general observations will be made. At stations 904N and 834G, release of ^{14}C -DOC was observed in each experiment. Results from the lower bay station (7070) and the continental shelf station contained poor replication and very low counts indicating that the screening procedure was not sufficiently sensitive for these regions of lower phytoplankton biomass. However, the sensitivity of the high temperature oxidation technique should permit useful results to be obtained.

Figure 1. Removal of $^{14}\text{CO}_2$ from sea water by purging with 5% CO_2 in air.

Figure 2-7. Dissolved organic carbon ^{14}C released by natural phytoplankton assemblages. Open circles represent data from water passed through 35 μm mesh; closed circles from water passed through 35 μm mesh to which 10 zooplankters per liter were added.

Figure 2

Figure 4

Figure 5

Figure 6

Figure 7

The particulate carbon, nitrogen and chlorophyll a data and phosphorus uptake data collected simultaneously with DOC release information is still being compiled and will not be discussed herein.

The DOC release data show that the addition of 10 zooplankters (between 253 and 405 μm in size) per liter did not influence DOC release except at station 834G in February 1975 (Figure 5). At some stations most DOC release occurred during daylight (Figures 2, 3 and 4) but at one station (Figure 6) the most dramatic release occurred during darkness. In one experiment (Figure 7) release seemed nearly continuous regardless of light level.

In an effort to characterize the released DOC, filtrates from several of the field studies were concentrated by hollow fiber dialysis and chromatographed with Sephadex G-10. In each case radioactivity was recovered in two peaks, one corresponding to molecular weight 600-700 and one corresponding to molecular weight 200-300. These initial experiments demonstrate that our method is sufficiently sensitive to detect several components but some refinements will be required to improve peak resolution.

C. Respiration and Nutrient Regeneration

1. Plankton Respiration

Introduction

Using the rate of oxygen consumption to determine the respiration rate of a water or sediment sample incubated in a darkened container is a standard technique. In most waters the respiration rate is too low and the standard Winkler or electrode measurement too imprecise to permit oxygen uptake to be determined except through long term incubations or concentrating the plankton (Strickland, 1960; Pomeroy, 1968), while in sediments, even those of low organic carbon content, this is not a problem (Pamatmat, 1968; Hartwig, 1974).

If one could measure the plankton respiration rate accurately and precisely it would be possible to estimate inorganic carbon (C), nitrogen (N) and phosphorus (P) regeneration in the water column by the use of a suitable respiratory quotient and C:N:P ratio. Using the basic idea of Carpenter (1965a) and Williams (P. J. LeB. Williams, personal communication) we have devised a photoelectric end-point detection system for use with the Winkler titration suitable for field use. According to Carpenter this end-point detection scheme is approximately 2.5 orders of magnitude more sensitive than visual methods. Carritt and Carpenter (1966) discuss more completely the chemistry and problems of the Winkler technique.

Status

In laboratory studies, using reagents as suggested by Carpenter (1965b), we have determined the precision of our method to be $\pm 0.4\%$ (95% confidence limits) extending over a wide range of standard concentrations (Fig. 1). In field measurements the precision decreased to $\pm 3.2\%$ on the first cruise

but on a subsequent cruise almost equalled the laboratory value of $\pm 0.4\%$. These values were obtained by analyzing the variability of all sea water sample oxygen concentrations measured on the two cruises, one in August, 1975 and the other in February, 1976, throughout the entire length of Chesapeake Bay. The oxygen concentrations ranged from 0.163 to 0.7179 mg-at $O_2 \ell^{-1}$ with the 95% confidence limits of individual means varying from 0.14% to 10%. The improved precision on the second cruise was due to three factors: 1) structural modifications made to the original apparatus; 2) slight alterations of the electronics and 3) the effect of oxygen concentration on precision. As seen from figure 2 precision varied inversely with oxygen concentration on the August cruise which also had the lowest oxygen concentrations measured of the two cruises. Precision was independent of oxygen concentration on the February cruise and this was due to the higher concentrations found in February, the refinements made to the instrument and improved experimental technique.

Using this analytical technique we have determined the respiration rate of natural water samples from Chesapeake Bay. The method used consisted of time series oxygen analysis of replicate water samples contained in glass-stoppered bottles of approximately 130 ml volume. The rate of oxygen uptake was calculated as b in the regression expression $Y = A + bX$ where X = time in hours; b = slope of regression line in units of mg-at $O_2 \ell^{-1} \text{ hr}^{-1}$; and A = Y intercept in units of mg-at $O_2 \ell^{-1}$. Tables 1 and 2 list the respiration rates measured on both cruises and the 95% confidence limits on the individual rates. All regressions were significant on the second cruise but not on the first, this was due both to lack of field experience with this method on the first cruise and to experimental procedure.

Examination of several of the regression plots from the August cruise, such as that represented in figure 3A, revealed that incubations may have been too long resulting in non-linearity, decreased rates and greater variability. During the February cruise the incubation times were shortened and regression plots appeared more linear (Fig. 3B). The mean variability (as 95% confidence limits) of regression rate plots were $\pm 57\%$ and $\pm 49\%$ for the August and February cruises, respectively, with a mean variability of all regression plots of $\pm 53\%$. This is in contrast to the precision of the replicate field determinations given above to be $\pm 3.2\%$ on the first cruise and $\pm 0.5\%$ on the second cruise, therefore, the variability seen in the respiration rate experiments was not due to analytical technique but most probably due to sampling technique, natural population inhomogeneities and experimental handling of samples. Improved sampling and experimental techniques coupled with further refinements to the titration apparatus should improve not only the reliability of individual titrations but also decrease the variability of the measured respiration rates.

In a series of other experiments water samples were run through a 35 μm mesh netting and in some cases further filtered through a 3 μm membrane filter before being put into experimental bottles. The results of these experiments are given in Table 1. Using 35 μm mesh one would remove all the larger zooplankton but retain most of the photosynthetic activity and biomass of the phytoplankton population. McCarthy et al. (1974) found during a one week study in the Chesapeake Bay that 93.4% of the chlorophyll a and 100% of the photosynthetic activity was in the less than 35 μm size fraction and over a two year period 89.6% of photosynthetic activity was in this fraction.

The less than 3 μm size fraction was examined to determine the participation of bacteria in the total respiration assuming that mainly bacteria would pass through a 3 μm membrane filter. This fraction contained approximately 9% as much chlorophyll a as the total plankton and 15% as much chlorophyll a as was in the less than 35 μm size fraction. The respiration rates in these experiments showed a substantial percentage (59%) of the total rate to be associated with the less than 3 μm size fraction. Bacterial attachment to particles is thought to be an active process and an important mechanism for bacterial scavenging of organic matter in dilute organic environments (Disalvo, 1973; Riley, 1963; Baylor and Sutcliffe, 1963; Oppenheimer and Vance, 1960; Paerl, 1974, 1975). These results indicate, however, that a substantial portion of bacterial activity is not associated with particles larger than 3 μm . The bacteria in the 3 μm filtrate may have been attached to small particles or on easily disrupted larger particles. Future experiments will examine bacterial density and total biomass differences in the various size fractions using bacterial counts and ATP analysis.

2. Planktonic Nutrient Regeneration

Introduction

As stated in section C of this report if one desires to calculate the amount of organic carbon degraded using a measured respiration rate, a suitable respiratory quotient (RQ) must be used. Commonly determined RQ values range from 0.27 in an intertidal sand flat to 1.6 for Chlorella using nitrate as a nitrogen source (Strickland, 1960; Teal and Kanwisher, 1961; Pamatmat, 1968). For the purposes of this report an RQ of 0.85 will be assumed with the realization that an RQ of ± 0.20 , which encompasses

most RQ measurements found in the literature, yields only a $\pm 25\%$ variability, which is much less than the $\pm 53\%$ mean variability of the respiration rates.

The incorporation of C:N:P atomic ratios into the calculation will yield inorganic nitrogen and phosphorus regeneration rates. The atomic ratios of Redfield et al. (1963) (106 C to 16 N to 1 P) were tested on recent Atlantic and Pacific Ocean field data and found satisfactory by Alvarez-Borrego et al. (1975) and will be used in this report.

Status

Respiration and nutrient regeneration rates for the various stations occupied on the two cruises are given in Tables 1 and 2. Rates calculated for surface waters from the stations on the first cruise (August) were not significantly different at the 5% level from each other. The mean respiration rate was 4.0×10^{-3} mg-at O_2 $\ell^{-1} \text{hr}^{-1}$, the mean inorganic N regeneration rate was 5.1×10^{-1} $\mu\text{g-at N } \ell^{-1} \text{hr}^{-1}$ and the mean inorganic phosphorus regeneration rate was 3.2×10^{-2} $\mu\text{g-at P } \ell^{-1} \text{hr}^{-1}$. Deeper water samples were collected and analyzed for respiration rates at only 3 of the 8 stations. The respiration rates were not significantly different at the 5% level than the surface water rates and have been included in the mean rates given above.

Of the 7 stations occupied during the February cruise the bottom waters were analyzed at 6. Only a mid-depth water sample was taken at the coastal station as the water was completely mixed. On the second cruise the experimental procedure and analytical technique had been improved upon, as discussed previously, and several significant differences were noted between bay stations and between the top and bottom water at one station.

However, for the purposes of this report all rates will be combined to give mean rates of respiration, inorganic N regeneration and inorganic P regeneration. This was done because the rates were highly variable and until further measurements are made we will average the rates. In February the mean respiration rate was 2.5×10^{-3} mg-at O_2 $\ell^{-1}hr^{-1}$, the mean inorganic N regeneration rate was 3.2×10^{-1} $\mu\text{g-at N } \ell^{-1}hr^{-1}$ and the mean inorganic P regeneration rate was 2.0×10^{-2} $\mu\text{g-at P } \ell^{-1}hr^{-1}$.

Examining the rates given in Tables 1 and 2 one sees that the winter rates (February) were 63% as great as the summer rates (August). The water temperature difference between February and August was approximately 25°C . If the Q_{10} of the organisms present was around 2 one would expect the February rates to be only 20% as high as the August rates, other factors being equal. This may imply that the thermal regime of Chesapeake Bay exerts a selection pressure on microbial communities. Thermal changes of the magnitude found in Chesapeake Bay were found by Sieburth (1967) to cause shifts in the thermal types of microbes present in Narragansett Bay, Rhode Island. Thermal selection may be a factor permitting maximum utilization of organic substrates throughout the year in water masses which undergo large temperature fluctuations.

An important aspect of the water column nutrient regeneration rate concerns its coupling with nutrient supply required for primary productivity. Using values for productivity of the Chesapeake Bay from Taylor and McCarthy (1973, unpublished) in August to be $51.8 \text{ gC m}^{-3}hr^{-1}$ and February to be $6.63 \text{ gC m}^{-3}hr^{-1}$ and using the assimilation ratio of C:N:P to be 106:16:1 yields a primary production requirement of $652 \mu\text{g-at N } \ell^{-1}hr^{-1}$ and $40.7 \mu\text{g-at P } \ell^{-1}hr^{-1}$ in August and a requirement for $83.4 \mu\text{g-at N}$

$\ell^{-1}\text{hr}^{-1}$ and $5.21\text{ }\mu\text{g-at P }\ell^{-1}\text{hr}^{-1}$ in February. Comparing the required nutrient inputs for primary productivity at the above rates to the regeneration data (Tables 1 and 2) shows that overall the coupling of primary productivity with water column nutrient regeneration is insignificant (0.078% of N and P requirement in August and 0.38% of N and P requirement in February). Therefore, even if the RQ value and atomic regeneration and assimilation ratios used were an order of magnitude off the overall insignificance would be the same.

The increased percentage in February is because February productivity was only 13% of the August rates while February respiration rates were 63% of the August values. All stations, except 0707V, 7070 and 904N (coastal water station, first bay station and furthestmost toward the head bay station) showed only twice the supply of required N and P in February as in August. In contrast to this, station 0707V, the coastal station, had an order of magnitude more supply of required N and P in August as in February. This is the only station where productivity was greater in February than in August while respiration in February was only 14% of the August value. Stations 904N and 7070, the two extreme bay stations, one near the head and the other near the mouth of the bay, had six to seven times greater supply of required N and P in February as in August. Therefore, it appears that for the major reach of the Chesapeake Bay that supply of required N and P via respiratory processes is relatively constant (varied by a factor of two) but at the extremes of the bay the supply via water column nutrient regeneration undergoes major fluctuations. Further measurements to be made in the coming year will look at these fluctuations and their causes.

Table 1. Respiration and Regeneration Rates - August Cruise.

Station	Sample		Respiration Rate	N Regeneration Rate	P Regeneration Rate
			$\pm 95\% \text{ }^1\text{C.L.}$ $\text{mg-at O}_2 \text{ } \ell^{-1} \text{ hr}^{-1} \times 10^{-3}$	$\pm 95\% \text{ C.L.}$ $\mu\text{g-at N } \ell^{-1} \text{ hr}^{-1} \times 10^{-1}$	$\pm 95\% \text{ C.L.}$ $\mu\text{g-at P } \ell^{-1} \text{ hr}^{-1} \times 10^{-2}$
Ø707V	Surface	² TP	3.4 ± 3.45	4.4 ± 4.4	2.7 ± 2.8
		³ <35 μm	1.2 ± 0.48	1.5 ± 0.62	0.96 ± 0.39
707Ø	Surface	TP	2.4 ± 1.3	3.1 ± 0.17	1.9 ± 1.0
	10 m	TP	2.1 ± 1.4	2.7 ± 0.18	1.7 ± 1.1
744	Surface	TP	4.6 ± 0.78	5.9 ± 1.0	3.7 ± 0.63
		<35 μm	4.1 ± 1.0	5.3 ± 1.3	3.3 ± 0.80
		⁴ <3 μm	2.3 ± 0.85	3.0 ± 1.1	1.8 ± 0.68
P-12	Surface	TP	2.5 ± 1.9	3.2 ± 2.4	2.0 ± 1.5
		<3 μm	1.2 ± 1.3	1.5 ± 1.7	0.96 ± 1.0
818P	Surface	TP	3.6 ± 1.1	4.6 ± 1.4	2.9 ± 0.88
834G	Surface	TP	4.6 ± 0.43	5.9 ± 0.55	3.7 ± 0.35
853F	Surface	TP	3.4 ± 1.8	4.4 ± 2.3	2.7 ± 1.4
		<3 μm	2.7 ± 0.92	3.5 ± 1.2	2.2 ± 0.74
	6 m	TP	7.1 ± 3.6	9.1 ± 4.6	5.7 ± 2.9
904N	Surface	TP	4.9 ± 0.92	6.3 ± 1.2	3.9 ± 0.74
	7 m	TP	5.2 ± 4.0	6.7 ± 5.1	4.2 ± 3.2

¹C.L. = confidence limits

²TP = total plankton

³<35 μm = plankton passing 35 μm mesh

⁴<3 μm = plankton passing 3 μm membrane filter.

Table 2. Respiration and Regeneration Rates - February Cruise.

Station	Sample		Respiration Rate	N Regeneration Rate	P Regeneration Rate
			$\pm 95\% \text{ }^1\text{C.L.}$ $\text{mg-at O}_2 \ell^{-1} \text{ hr}^{-1} \times 10^{-3}$	$\pm 95\% \text{ C.L.}$ $\mu\text{g-at N } \ell^{-1} \text{ hr}^{-1} \times 10^{-1}$	$\pm 95\% \text{ C.L.}$ $\mu\text{g-at P } \ell^{-1} \text{ hr}^{-1} \times 10^{-2}$
Ø707V	8m	² TP	0.60 ± 0.35	0.77 ± 0.45	0.48 ± 0.28
707Ø	1m	TP	3.5 ± 0.49	4.5 ± 0.63	2.8 ± 0.39
	10m	TP	2.8 ± 1.5	3.6 ± 1.9	2.3 ± 1.2
744	2m	TP	1.7 ± 0.88	2.2 ± 1.1	1.4 ± 0.71
	10m	TP	1.3 ± 0.84	1.7 ± 1.1	1.0 ± 0.67
804C	4m	TP	2.3 ± 1.4	3.0 ± 1.8	1.9 ± 1.1
	20m	TP	1.4 ± 0.77	1.8 ± 0.99	1.1 ± 0.62
Calvert Cliffs	Intake	TP	1.3 ± 0.56	1.7 ± 0.72	1.0 ± 0.45
	Discharge	TP	2.2 ± 1.0	2.8 ± 1.3	1.8 ± 0.80
834G	2m	TP	3.3 ± 1.7	4.2 ± 2.2	2.7 ± 1.4
	9m	TP	1.3 ± 0.67	1.7 ± 0.86	1.0 ± 0.54
904N	2m	TP	2.1 ± 0.79	2.7 ± 1.0	1.7 ± 0.63
	2m	TP	2.5 ± 0.73	3.2 ± 0.94	2.0 ± 0.59
	11m	TP	0.85 ± 0.024	1.1 ± 0.031	0.68 ± 0.019

¹C.L. = confidence limits²TP = total plankton

Figure 1

REGRESSION EQUATION

$$y = 0.000 + 8.65 \times 10^{-2}$$

$$r^2 = 0.99997$$

95% Confidence Limits

Intercept = ± 0.002 ml ThiosulfateSlope = $\pm 0.034 \times 10^{-2}$ ($\pm 0.39\%$)

Figure 2

Figure 3

Figure 4

D. Bacterial Utilization of Labeled Compounds

Introduction

Waksman et al. (1937) reported that organic material released by phytoplankton in light was rapidly oxidized by bacteria in the dark. Jannasch (1967) states that "sea water must be considered a suboptimal medium" for the growth of bacteria and bacterial activity in the natural environment might have to be closely allied with the activity of phytoplankton (Zobell, 1946; Sieburth, 1968). However, bacteria have been isolated which can grow in the natural concentrations of organic matter found in sea water (Carlucci and Shimp, 1974), moreover, these bacteria not only survive, as is the case for some bacteria, but can grow in these low nutrient waters (Jannasch, 1967, 1969). Numerous additional reports of bacterial stimulation and inhibition by algal release products can be cited (Steeman-Nielsen, 1955; Allen and Dawson, 1960; Vaccaro et al., 1968; Fitzgerald, 1969; Berland et al., 1970; Allen, 1971; Bell and Mitchell, 1972; Belly et al., 1973; Bauld and Brock, 1974; Bell et al., 1974).

Release products of an alga may be inhibitory to one bacterium and stimulatory to another (Bell et al., 1974), this effect in nature could lead to the development of physiologically based phytoplankton-bacterial communities. To understand these communities which exist in culture and which may exist in nature, the response of bacterial isolates to specific algal extracellular products obtained from numerous algal species must be quantified. This response includes not only assimilation and mineralization of the algal extracellular product but also its conversion into metabolic by-products released by bacteria and the ability of the algal product to maintain bacterial viability.

The utilization of a wide spectrum of organic compounds by natural communities of bacteria is possible by virtue of the large array of enzymatic abilities they possess. Measurements of heterotrophic activity in aquatic environments is in common practice today using a variety of methods. The oxygen uptake or respirometric technique is exemplified by biological oxygen demand (BOD) measurements and has, to date, only been useful in environments exhibiting a high metabolic rate. Recently respirometric techniques have been developed using accurate, high precision burettes and photometric end-point detection permitting mesotrophic and oligotrophic waters to be examined and results using this technique are discussed in section C.

Additional techniques for studying heterotrophic activity are the uptake of inorganic carbon-14 ($^{14}\text{CO}_2$) and sulfur-32 sulfate ($^{32}\text{SO}_4^{=}$) (Steeman-Nielsen, 1960; Romanenko, 1964, 1965; Sorokin, 1966; Monheimer, 1974, 1975). The $^{32}\text{SO}_4^{=}$ and $^{14}\text{CO}_2$ uptake techniques are difficult to interpret because of the nonequivalence between their uptake and total heterotrophic activity. Another and more widely used method in unpolluted waters is the heterotrophic uptake of labeled organic carbon compounds as described by Parsons and Strickland (1962). They reasoned that one should measure the uptake of a required substrate, as in the $^{14}\text{CO}_2$ uptake method for photosynthesis, but for bacterial heterotrophs. $^{14}\text{CO}_2$ was replaced with a labeled organic substrate. Furthermore, they deduced that by using radioisotopic dilution, it would be possible to measure in situ substrate concentrations or obtain information on bacterial enzyme kinetics. Parsons and Strickland's (1962) kinetic approach to heterotrophic uptake was modified notably by Wright and Hobbie (1965) and Vaccaro and Jannasch (1967), and finally expanded to include not only uptake but the mineralization or

respiration of the labeled substrate (Hamilton and Austin, 1967; Hobbie and Crawford, 1969). All these methods retain the basic kinetic approach to the problem in contrast to the techniques of Kadota et al. (1966) and Williams and Askew (1968). Williams and Askew (1968) developed the technique for studying mineralization rates and turnover times for naturally occurring substrates independently and more thoroughly than Kadota et al. (1966). The interrelationship between the kinetic approach and the Williams and Askew technique, and the assumptions involved in both, are discussed by Wright (1973, 1974) and Thompson and Hamilton (1974).

The kinetic approach of Parsons and Strickland (1962) as modified by Hobbie and Crawford (1969) uses radioisotopic dilution in sealed vessels to measure both mineralization and uptake. The major assumptions of the kinetic approach are that: 1) uptake follows Michaelis-Menten kinetics; 2) added substrate tracer does not significantly increase the natural substrate concentration; 3) all the organisms in the sample taking up and respiring the tracer respond in a similar manner to the substrate; 4) substrate concentration should not decrease substantially in the course of an experiment; 5) no growth of organisms; 6) no induction of new transport systems; 7) no isotope effect; 8) no interference of uptake by other organics present; 9) no transport of the labeled substrate out of the cell; and 10) the assumption is made that there is no appreciable release of a modified dissolved organic label (as opposed to transport of the labeled substrate) back into the surrounding environment.

Although investigators concerned with heterotrophic activity in situ are aware of these assumptions there has been little experimental work performed to show adherence to these assumptions. Many natural communities appear to follow Michaelis-Menten kinetics but some do not (Vaccaro and

Jannasch, 1967; Hamilton and Preslan, 1970). By a simple mathematical model Williams (1973) showed that bacterial communities do not adhere to Michaelis-Menten kinetics at low substrate concentrations and observed values can be 25% higher than predicted and that increased diversity resulted in decreased applicability of the kinetic approach.

The main assumption of the Williams and Askew technique is that the tracer adds a negligible concentration of substrate to the sample compared to the natural substrate concentration, or, if it is not a negligible addition, then there is a proportionate increase in the rate of respiration. In order to know which case applies and to be able to measure the rate of respiration, the natural substrate concentration must be determined. As pointed out by Wright (1974), the amount of labeled substrate added is often of the same order of magnitude as the concentration of naturally occurring substrate. Therefore, the usual case for the assumption is the latter and the relationship between total substrate concentration and respiration is important.

The uptake velocity of substrate by the responsible bacterial species in the community follows Michaelis-Menten kinetics and is, therefore, not linear, although within narrow substrate limits it can be linearly approximated. The statistical non-significance of a percentage of the mineralization kinetic plots experimentally determined by numerous investigators (cited in Wright, 1974) is, in part, due to the ill defined relationship between substrate concentration and respiration. The chemical nature of the substrate, environmental conditions, "health" of bacteria and species of bacteria will affect this relationship. If respiration is related to transport, then respiration will be related, in a non-linear fashion, to substrate concentration as described with Michaelis-Menten kinetic equations.

A major omission in both the Williams and Askew approach and the kinetic approach is the lack of measurement of labeled dissolved organic carbon which has been taken up, metabolized, and then released back into the environment, i.e. metabolic by-products. Bacterial release of products such as vitamins, exoenzymes, organic acids, etc. is a well known occurrence described even in general microbiology textbooks. By-product release and non-detection will lead to an overestimate of turn-over time and a decrease in the estimate of the maximum velocity of uptake (V_m), in the half-saturation constant (K_s) and in the actual velocity of uptake (V) by not accounting for a portion of the label taken up and metabolized.

The list of labeled substrates utilized in heterotrophic studies is large, covering a wide spectrum of carbohydrates, organic acids and amino acids (Williams and Gray, 1970; Robinson et al. 1973; Thompson and Hamilton, 1973; Crawford et al., 1974; Wirsén and Jannasch, 1974; Wright, 1975). The measurement of respired $^{14}\text{CO}_2$ has greatly increased the accuracy of the method as the respired $^{14}\text{CO}_2$ can be a significant fraction of the total uptake depending upon the substrate used, the environmental conditions and the inferred bacterial population (Williams, 1970; Banoub and Williams, 1972; Robinson et al., 1973; Wright, 1973; Crawford et al., 1974; Wright, 1975). The basic technique, however, lacks background data relating various environmental conditions and different substrates to their effect on the heterotrophic process. This process includes not only the assimilation and respiration of the labeled carbon but also the release of labeled metabolic by-products.

Status

In these preliminary experiments the uptake of inorganic- $^{32}\text{PO}_4^{-3}$, labeled algal lysate and labeled algal extracellular product by natural populations were followed. In addition the uptake and respiration of uniformly labeled ^{14}C -glycine by a laboratory culture of bacteria was examined.

$^{32}\text{PO}_4^{-3}$ uptake was examined using a natural water sample taken during the August cruise. Uptake by total plankton in the light was compared with uptake by total plankton in the dark and uptake by the less than 3 μm size fraction in the dark. The results of this experiment are shown in figure 4. Total plankton uptake occurs at a decaying rate in both light and dark with uptake terminating after approximately 60 to 100 minutes as cell saturation was reached or all available $^{32}\text{PO}_4^{-3}$ was removed. The initial rapid uptake of $^{32}\text{PO}_4^{-3}$ exhibited under all three conditions was probably due to exchange across the cell membrane and not to actual net uptake (Taft et al., 1975). The less than 3 μm darkened fraction exhibited a different uptake pattern after the initial rapid uptake of $^{32}\text{PO}_4^{-3}$. Following this initial period there was a slow but exponential increase in the rate of $^{32}\text{PO}_4^{-3}$ uptake. The initial (8 minutes) and most rapid uptake by the less than 3 μm fraction was only 25% of the rate exhibited by either the light or dark total plankton sample, which were not significantly different from one another. The exponential increase in the rate of $^{32}\text{PO}_4^{-3}$ uptake resembles typical growth curves for bacteria in nutrient media. This uptake rate may indicate an exponentially growing population, an increasing rate of transport or an increasing formation rate of $^{32}\text{PO}_4^{-3}$ containing particles.

Aquatic bacteria are known to utilize PO_4^{-3} and when PO_4^{-3} concentrations are relatively high (0.1 to 0.5 $\mu\text{g-at P l}^{-1}$) may out-compete phytoplankton for available PO_4^{-3} (Watt and Hayes, 1963; Fuhs et al., 1972; Rhee, 1972). Comparing Ks values for bacterial versus phytoplankton uptake of PO_4^{-3} (6.7 to 12.0 $\mu\text{g-at P l}^{-1}$ vs 0.01 to 0.45 $\mu\text{g-at P l}^{-1}$) (Fuhs et al., 1972; Stross and Pemrick, 1974) reveals that at the PO_4^{-3} concentrations in Chesapeake Bay (0.00 to 0.45 $\mu\text{g-at l}^{-1}$) (Taft et al., 1975) phytoplankton uptake will dominate. Taft et al. (1975), using water samples collected from the Chesapeake Bay found Ks values for the contained natural populations of from 0.09 to 1.72 $\mu\text{g-at P l}^{-1}$ again indicating that PO_4^{-3} uptake is dominated by the phytoplankton population.

To examine the uptake of labeled algal lysate and labeled algal extracellular product a screened (50% light transmission), glass-stoppered bottle was filled one-third its total volume with a water sample which had been filtered through a 35 μm phytoplankton mesh to remove the larger zooplankton. To this natural assemblage labeled bicarbonate (H^{14}CO_3) and $^{32}\text{PO}_4^{-3}$ (2 μc and 0.5 μc respectively) were added as well as sterile sea water of the same salinity (15‰) to fill the bottle.

The bottle was incubated for 30 hours during which the phytoplankton regained their original total concentration (chlorophyll a concentration was 9% higher at end of incubation). The culture was gently filtered through a GF/C filter which was subsequently ground with a tissue homogenizer using 15‰ sterile sea water and 15 drops 90% acetone as an extractant. The extract was filtered through a GF/C to remove particulate debris, bubbled with air to remove acetone and frozen until used. The natural population filtrate, obtained from the first filtration above was

used as the labeled algal extracellular product and stored frozen. Initial uncorrected counts for the lysate and extracellular products were approximately 1.3×10^5 cpm ml⁻¹ and 1.5×10^4 cpm ml⁻¹, respectively.

The uptake of labeled material was done with both total plankton and the less than 3 μ m fraction of plankton. Five 100-ml samples of each fraction were incubated with 5 ml of lysate or 5 ml of extracellular product in milk dilution bottles. At selected time intervals 10 ml was removed from each bottle, filtered, placed into scintillation cocktail and counted. The results for lysate uptake are shown in figures 5 and 6. Figure 5A represents counts of the less than 3 μ m fraction taken on board ship at the time of the experiment, while figure 5B are the same vials counted 5-1/2 months later to allow time for disintegration of ³²P (half-life of 14.5 days). There is no a priori reason to believe that the total plankton counts would not show the same trend for counts taken on board ship (fig. 6 represents counts taken at 5-1/2 months for TP).

The differences in the two uptake curves for the less than 3 μ m (fig. 5) fraction are very significant. The counts are three orders of magnitude greater for the on board counts revealing that most uptake was of ³²P (organic and/or inorganic) and not ¹⁴C (organic and/or inorganic). Also note from figures 5A and B that the initial rate of label uptake was positive for ³²P but negative for ¹⁴C while figure 6 shows initial rate of ¹⁴C uptake for total plankton to be positive. At present no reason can be offered for this difference due to the preliminary nature of these experiments.

Figure 7 represents the results for the uptake of extracellular products taken on board ship. The 5-1/2 month counts revealed that there was no uptake of ¹⁴C-extracellular product, therefore, what is seen in figure 7

Figure 5

Figure 6

Figure 7

Extracellular Product Uptake 834 G

is the uptake of ^{32}P by the five replicates. comparing Figure 4 (inorganic $^{32}\text{PO}_4^{-3}$ uptake) with figures 5B, 7A and 7B one can infer that the ^{32}P uptake observed in the latter three figures probably does not represent uptake of inorganic ^{32}P as the shape of both the TP and the less than 3 μm uptake curves does not closely resemble the uptake curves for inorganic $^{32}\text{PO}_4^{-3}$ (fig. 4).

That there was no uptake of extracellular- ^{14}C could be due to: 1) no release of extracellular material; 2) release products unassimilable; or 3) release products produced in incubation bottle were utilized rapidly by the bacterial population in the water sample yielding no organic label in the filtrate. We believe that the latter explains why we saw no ^{14}C -uptake. Algal release and the biodegradability of some release products is a well documented occurrence (see section B and E this report). This coupled with the fact that we observed release of algal product on this cruise (section B) leads us to conclude that although there was extracellular production of organic matter by the phytoplankton in the incubation bottle, after 30 hours incubation the bacterial population in the bottle had utilized all but the more refractory compounds.

In the final labeling experiment the respiration as well as uptake of uniformly labeled glycine- ^{14}C by a laboratory culture of bacteria were examined using a modified form of the gas sparging technique of Taft (1975). We desire, in the end, to examine not only respiration and uptake but also excretion of organics by bacteria, therefore, minimal alteration of the media is desired. The results shown in figure 8 reveal that the technique which involved displacing the $^{14}\text{CO}_2$ in solution with a 5% CO_2 mixture in air, is potentially suitable for such studies especially when the investigator does not want to alter the pH to such extremes as in the methods of

Figure 8

Hobbie and Crawford (1969) and Williams and Askew (1968). The final pH of sparged media in this experiment was 6.0 to 6.1 which represents four orders of magnitude fewer hydrogen ions present than used in other techniques ($\text{pH} = 2.0$), thereby reducing the chance of hydrolysing peptides or polysaccharides in an acid environment as well as reducing the chance of lysing cells. This preliminary experiment reveals that the technique may be appropriate for these studies and will in the near future be used to look at bacterial excretion of labeled extracellular products.

E. Effects of Heat and Chlorine on Natural Assemblages of Chesapeake Bay Phytoplankton.

Introduction

Several studies have dealt with the effects of passage through an electric power generating plant on phytoplankton (Morgan and Stross, 1969; Hamilton et al., 1970; Carpenter et al., 1974a; Carpenter, 1973; Carpenter et al., 1972) and zooplankton (Heinle, 1969; Carpenter et al., 1974b). During passage through power plant cooling condensers the plankton are subjected to physical abrasion, rapidly increased temperature and, at certain times, are exposed to biocides such as chlorine which are added to cooling water to prevent attached organisms from growing within the plumbing.

Physical abrasion and temperature increase may (Morgan and Stross, 1969) or may not (Carpenter et al., 1972) reduce phytoplankton productivity and, in cold seasons, elevated temperature may stimulate productivity (Morgan and Stross, 1969; Gurtz and Weiss, 1974; Brooks, 1974). Raising temperature by an average of 5.5°C in pools of natural brackish water increased dinoflagellate numbers and the numbers of larger cells (Carpenter, 1973). The populations also became more diverse and matured earlier than control populations at ambient temperature.

An organism which survives the initial temperature perturbation during entrainment may suffer physiological alterations if it remains in a heated effluent. Naylor (1965) and Coutant (1969) discuss temperature effects on metabolism, reproduction and behavior of fishes and some invertebrates. It is unlikely that an entrained phytoplankter would remain in an effluent plume for periods as extensive as those experienced by benthic or attached organisms but short exposure to a 5°C increase could provoke alterations

in biological functions of such an organism. Drost-Hansen and Thorhaug (1967) and Dorst-Hansen (1969) even suggest that alterations in water structure at temperatures between 29 and 33°C cause profound changes in membrane systems and, possibly, macromolecules such as enzymes. If this is correct, temperature elevation of 5°C in power plant cooling water from 26 to 31°C might induce chronic physiological alterations in phytoplankton through changes in water structure.

Chlorination dramatically reduces phytoplankton biomass and productivity in power plant discharges (Hamilton et al., 1970; Carpenter et al., 1972; Fox and Moyer, 1975) even when the amount added is 0.1 mg liter⁻¹ and the residual chlorine in solution is less than 0.05 mg liter⁻¹.

Copper sulfate concentrations of 12.5 µM are toxic to phytoplankton in the absence of metal chelating organic compounds (Palmer and Maloney, 1955). This is about 200 times and ambient total copper concentration and is about 10³ times the dissolved copper concentration in upper Chesapeake Bay. Heavy metals, such as copper (Roosenburg, 1969), may be leached from condenser tubes and be carried out of the plant with adverse effects on organisms that have not passed through the power plant as well as on those which have.

Most studies of the effects of power plant entrainment on phytoplankton reported in the open literature have considered only the organisms which actually passed through condensers and have ignored the effects on organisms which never entered the power plant but are mixed into the discharge plume. This is probably due to the extremely large number of samples which must be collected to detect small changes in conveniently measured parameters such as chlorophyll a or number of organisms (Carpenter et al., 1974a). A 2 to 1 dilution of discharge water with new natural water results in the

organisms in a volume equal to the entrained volume being subjected to perturbations averaging one-half the magnitude endured by organisms actually entrained. However, since discharge dilution occurs at non-linear (logarithmic) rates, simply averaging perturbation magnitudes within a large water volume is not realistic on small scales. Also, the time spent in the discharge plume should influence the magnitude of a perturbation upon the planktonic organisms (Schubel and Auld, 1973; Schubel, 1973).

This year we initiated a study of the effects of experimentally induced environmental perturbations on those physiological parameters of natural phytoplankton assemblages from Chesapeake Bay for which we already have base line data. These parameters are carbon fixation rate, orthophosphate uptake, ammonium uptake, alkaline phosphatase activity, ATP, in vivo fluorescence and release of dissolved organic matter. The sensitivity of the measurements made within a captured sample should enable us to detect small changes in the biological functions of the exposed organisms. Thus, we hope to avoid the sampling problems noted by Carpenter et al. (1974a). In addition to the physiological measurements we also measured phytoplankton biomass by chlorophyll a and change in relative bacteria numbers.

Status

The perturbations evaluated to date are 5°C to 20°C temperature elevation and additions of chlorine up to 0.55 ppm. These experiments were conducted at three stations in February 1976. Figures 1 and 2 show the sample heating and cooling rates on logarithmic scales. At station 0707V on the continental shelf surface was heated over 5 hours to 12°C above ambient and cooled over 4 hours to near ambient. The temperature in the "control" carboy was slightly elevated from sitting on deck. At station

7070 in the lower bay a carboy of surface water was equilibrated with the air temperature on deck ($\sim 3^{\circ}\text{C}$ above water temperature). One subsample was then heated quickly to 23°C and maintained at 21°C for 60 minutes after which it was placed in an incubator cooled with near surface water. The control subsample was drawn from the carboy and placed directly in the incubator.

In each experiment primary productivity and chlorophyll a concentrations were measured before and after exposure to elevated temperature. The raw data in Table I show that ^{14}C uptake was nearly identical in heated water and in the controls, even up to 17 hours after exposure at station 0707V. Chlorophyll a values were slightly lower in the discharge plume.

Additional experiments of this nature will be conducted in spring and summer so detailed data analysis will be withheld until they are completed. However, these few data suggest that our temperature elevation experiments do not approximate conditions experienced by phytoplankton passing through a power plant.

Figure 3 shows the results of chlorine addition on ^{14}C uptake at station 904N. As noted by many other investigators, chlorine addition sharply reduces ^{14}C uptake. However, chlorine is not employed at Calvert Cliffs and thus cannot account for reduced ^{14}C uptake in discharge waters.

Figure 1. Heating and cooling rates for natural water collected from station Ø707V.

Figure 2. Heating and cooling rates for natural water from station 7070

Figure 3. Effect of chlorine on primary productivity (^{14}C -uptake) of natural phytoplankton from station 904N.

Table 1. ^{14}C uptake by and Chlorophyll a content of natural phytoplankton assemblages experimentally heated and passing through a nuclear power plant.

Station	Ø707V		707Ø		Calvert Cliffs	
	<u>Control</u>	<u>Heat</u>	<u>Control</u>	<u>Heat</u>	<u>Intake</u>	<u>Discharge</u>
Immediately After Heating and Cooling						
^{14}C Uptake DPM/liter	5340	7310	148300	145690	21490	11620
$\mu\text{g Chl}_a/\text{liter}$	1.6	1.5	3.2	3.1	4.7	4.0
	1.1	1.3				
17 Hours After Heating and Cooling						
^{14}C uptake DPM/liter	21650	22620				

F. Nutrient Flux Between Sediment and Water

Introduction

Marine benthic communities receive their organic matter from sinking detritus, sinking organisms and benthic autotrophic production. Metabolism, diffusion and resuspension (Teal and Kanwisher, 1961; Pamatmat, 1971, 1973; Smith, 1973; Hartwig, 1974, 1976) are the major routes of organic matter loss from the benthos, whereas burial is not considered a loss because the organic matter remains in the sediment.

Metabolism results in the assimilation of a portion of the organic matter, the release of another portion and the mineralization of the remainder. Natural bacterial assemblages metabolizing under aerobic conditions mineralize 8-57% of the total organic carbon taken up (Hobbie and Crawford, 1969; Williams, 1970), the remainder being incorporated into new cell material or partially mineralized and released. Many factors determine the rate at which these metabolic products participate in sediment-water nutrient exchange. Diffusion rate, bioturbation, resuspension, metabolic rate, photosynthetic rate, sorption reactions, redox and pH (Carritt and Goodgal, 1954; Hayes, 1964; Pomeroy et al., 1965; Lee, 1970; Mortimer, 1971; Hale, 1974; Hartwig, 1974, 1976; Nichols, 1974) are the more important of these factors.

In shallow coastal waters benthic nutrient exchange can influence the water nutrient chemistry and thereby the productivity of the overlying water (Pomeroy et al., 1965; Hale, 1974; Rowe et al., 1975). The impact of nutrient regeneration on phytoplankton productivity depends on several factors, including the rate of release of the nutrient from the sediment, the depth of the water, the degree of mixing of the overlying water, the in situ concentration of the nutrient, and the rate of uptake of the nutrient by the phytoplankton.

In deep waters, such as over the Santa Barbara Basin, California, the importance of benthic nitrogen release to the phytoplankton nitrogen requirement in the upper waters was shown to be negligible, providing only 0.4% of the nitrogen required (Rittenberg, et al., 1955). In Doboy Sound, Georgia, which has a mean depth of 2 m at mean high water and a mean tidal amplitude of 2 m, Pomeroy et al. (1965) found that the sediment acted to stabilize the phosphate concentration in the overlying water to $1 \mu \text{mol l}^{-1}$. Hale (1974) concludes from direct measurements of nitrogen and phosphorus released from Narragansett Bay sediments (a shallow bay, average depth 8.8 m) that in June, 80% of the nitrogen requirement and more than 200% of the phosphorus requirement of Skeletonema costatum (a major component of the phytoplankton community) is released from the bay sediments. In the period of August through November, 780% of the nitrogen requirement was released from bay sediments. Rowe et al. (1975) calculated that over the New York Bight benthic nutrient regeneration accounted for more than 200% of the nitrogen required by phytoplankton photosynthesis. The calculations of Rowe et al. (1975) were based on many assumptions but show the contribution sediment nutrient regeneration can make even in deeper (80 m) waters.

Status

Gravity cores were taken on the August and February cruises at stations 818P and 834G. In August the entire core, with overlying water, was capped on the top and bottom and incubated on deck at ambient air temperature. The bottom water was anaerobic at this time and smelled of hydrogen sulfide. the entire core at this time of year was black and gelatinous. At each sampling time three cores were sacrificed, the

overlying water siphoned off, and nutrient concentrations determined in this water. The values were corrected for the volume of water overlying the core mud, the surface area of sediments and the results expressed as $\mu\text{g-at of nutrient m}^{-2}\text{hr}^{-1}$. The flux rates for these experiments varied by as much as an order of magnitude for replicate cores. This variability we believe to be due to disturbance of the sediment during the coring process and while siphoning out sample water. Hartwig (1975) reported that disturbance of the surface layer of sediment causes changes in the nutrient levels in the water above the sediments.

As the only corer available, at present, is a gravity corer, on the February cruise it was decided to look at nutrient flux by a method not dependent upon undisturbed sediment samples. Two gravity cores were taken, their overlying water siphoned off and approximately the top 5 cm of each core mixed together in a beaker. At this time of year the entire water column is aerobic and the top 1 cm or so of sediment is brown as opposed to the black color of the deeper sediments. After mixing, six sediment portions were scooped into replicate bottles and to this was added 100 ml of bottom water, taken with a van Dorn bottle at the sampling site. The bottles were stoppered, shook for 30 seconds and then either sampled immediately or incubated horizontally at room temperature in the laboratory on a moving belt similar to a roll-tube culture.

Sampling consisted of allowing the sediment to settle for 10 minutes before removing a top water aliquot and filtering through a GF/C. The filtrate was then diluted 10 to 1 and nutrient concentrations determined. After sampling the bottles were frozen and brought to the shore-based laboratory where the dry weight of sediment was determined after vacuum

evaporation of its contained water. The nutrient concentrations were corrected for the bottom water nutrient concentrations, the 10 to 1 dilution and the dry weight of mud and expressed as $\mu\text{g-at of nutrient (gm dry wt of mud)}^{-1}$. The results are shown in figures 9 and 10.

Although the rates were significantly different at the two stations there appear to be similarities in nutrient flux processes. Initial disturbance of the sediment and its resuspension into overlying water causes a release of all nutrients. After its initial release the concentration of PO_4^{-3} decreases drastically probably due to reprecipitation as an iron salt in the aerobic environment. NH_4^+ , after its initial release, slowly disappears probably due to the multiple effects of chemical binding, biological uptake and nitrification. After 12 hours both NO_3^- and NO_2^- exhibit increases in concentration. Their increases are not stoichiometrically related to the decrease of NH_4^+ , although in both cases the percent of NH_4^+ decrease that is accounted for as $\text{NO}_3^- + \text{NO}_2^-$ is approximately 30% indicating that nitrification occurs in these sediments.

As was shown in section D of this report, nutrient regeneration in the water column is minimal, therefore, there must be another source of nutrient to sustain primary productivity when the level of nutrients in the water column is near zero. The above experiments indicate that, as in other marine and estuarine waters (Hale, 1974; Hartwig, 1975; Rowe et al., 1975) the sediments of Chesapeake Bay may well act as a nutrient source under certain conditions.

Station 818 P
February Cruise

Figure 1

Station 834 G
February Cruise

Figure 2

LITERATURE CITED

- Al-Hasan, R. H., S. J. Coughlan, A. Pant and G. E. Fogg, 1975. Seasonal variations in phytoplankton and glycollate concentrations in the Menai Straits, Anglesey. *J. Mar. Biol. Ass. U. K.* 55: 557-565.
- Allen, H. L., 1971. Primary productivity, chemo-organotrophy, and nutritional interactions of epiphytic algae and bacteria on macrophytes in the littoral of a lake. *Ecol. Monographs* 41: 97-127.
- Allen, M. B. and E. Y. Dawson, 1960. Production of antibacterial substances by benthic tropical marine algae. *J. Bacteriology* 79: 459-460.
- Alvarez-Borrego, S., D. Guthrie, C. H. Culberson and P. K. Park. 1975. Test of Redfield's model for oxygen-nutrient relationships using regression analysis. *Limnol. Oceanogr.* 20: 795-805.
- Anderson, G. C. and R. P. Zeutschel, 1970. Release of dissolved organic matter by marine phytoplankton in coastal and offshore areas of the northeast Pacific Ocean. *Limnol. Oceanogr.* 15: 402-407.
- Banoub, M. W. and P. J. LeB. Williams, 1972. Measurements of microbial activity and organic material in the western Mediterranean Sea. *Deep-Sea Res.* 19: 433-444.
- Bauld, J. and T. D. Brock, 1974. Algal excretion and bacterial assimilation in hot spring algal mats. *J. Phycol.* 10: 101-106.
- Baylor, E. R. and W. H. Sutcliffe. 1963. Dissolved organic matter in seawater as a source of particulate food. *Limnol. Oceanogr.* 8: 369-371.
- Bell, W. and R. Michell, 1972. Chemotactic and growth responses of marine bacteria to algal extracellular products. *Biol. Bull.* 143: 265-277.
- Bell, W. H., J. M. Lang and R. Mitchel, 1974. Selective stimulation of marine bacteria by algal extracellular products. *Limnol. Oceanogr.* 19: 833-839.

- Belly, R. T., M. R. Tansey and T. D. Brock, 1973. Algal excretion of ^{14}C -labeled compounds and microbial interactions in Cyanidium caldarium mats. J. Phycol. 9: 123-127.
- Berland, B. R., D. J. Bonin and S. Y. Maestrini, 1970. Study of bacteria associated with marine algae in culture. 3. Organic substances supporting growth. Mar. Biol. 5: 68-76.
- Berman, T. and O. Holm-Hansen, 1974. Release of photoassimilated carbon as dissolved organic matter by marine phytoplankton. Mar. Biol. 28: 305-310.
- Brooks, A. S. 1974. Phytoplankton entrainment studies at the Indian River estuary, Delaware, p. 113-122. In: Proceedings of the Second Workshop on Entrainment and Intake Screening, L. D. Jenson (ed.). Johns Hopkins University, Baltimore, 347 pp.
- Carlucci, A. F. and P. M. Bowes, 1970a. Production of vitamin B₁₂, thiamine and biotin by phytoplankton. J. Phycol. 6: 351-357.
- Carlucci, A. F. and P. M. Bowes, 1970b. Vitamin production and utilization by phytoplankton in mixed culture. J. Phycol. 6: 393-400.
- Carlucci, A. F. and S. L. Shimp, 1974. Isolation and growth of a marine bacterium in low concentrations of substrate. In: Effect of the Ocean Environment on Microbial Activities, R. R. Colwell and R. Y. Morita (eds.), Univ. Park Press, Baltimore, Maryland, pp. 363-367.
- Carpenter, E. J. 1973. Brackish-water phytoplankton response to temperature elevation. Est. Coast. Mar. Sci. 1: 37-44.
- Carpenter, E. J., B. B. Peck and S. J. Anderson. 1972. Cooling water chlorination and productivity of entrained phytoplankton. Mar. Biol. 16: 37-40.

- Carpenter, E. J., S. J. Anderson and B. B. Peck. 1974a. Copepod and chlorophyll a concentrations in receiving waters of a nuclear power station and problems associated with their measurement. *Est. Coast. Mar. Sci.* 2: 83-88.
- Carpenter, E. J., B. B. Peck and S. J. Anderson. 1974b. Survival of copepods passing through a nuclear power station on Northeastern Long Island Sound, U.S.A. *Mar. Biol.* 24: 49-55.
- Carpenter, J. H. 1965a. The accuracy of the Winkler method for dissolved oxygen analysis. *Limnol. Oceanogr.* 10: 135-140.
- Carpenter, J. H. 1965b. The Chesapeake Bay Institute technique for the Winkler dissolved oxygen method. *Limnol. Oceanogr.* 10: 141-143.
- Carritt, D. E. and J. H. Carpenter. 1966. Comparison and evaluation of currently employed modifications of the Winkler method for determining dissolved oxygen in seawater; NASCO report. *Jour. Mar. Res.* 24: 286-318.
- Carritt, D. E. and S. Goodgal. 1954. Sorption reactions and some ecological implications. *Deep-Sea Res.* 1: 224-243.
- Coutant, C. C. 1969. Temperature, reproduction and behavior. *Ches. Sci.* 10: 261-274.
- Crawford, C. C., J. E. Hobbie and K. L. Webb, 1974. The utilization of dissolved free amino acids by estuarine microorganisms. *Ecology* 55: 551-563.
- Degens, E. T., J. H. Reuter and K. N. F. Shaw. 1964. Biochemical compounds in offshore California sediments and sea waters. *Geochim. Cosmochim. Acta* 28: 45-66.
- DiSalvo, L. H. 1973. Contamination of surfaces by bacterial neuston. *Limnol. Oceanogr.* 18: 165-168.

- Drost-Hansen, W. 1969. Allowable thermal pollution limits - a physico-chemical approach. *Ches. Sci.* 10: 281-288.
- Drost-Hansen, W. and A. Thorhaug. 1967. Temperature effects in membrane phenomena. *Nature* 215: 506-508.
- Eppley, R. W. and P. R. Sloan, 1965. Carbon balance experiments with marine phytoplankton. *J. Fish. Res. Bd. Can.* 22: 1083-1097.
- Fitzgerald, G. P. 1969. Some factors in the competition or antagonism among bacteria, algae and aquatic weeds. *J. Phycol.* 5: 351-359.
- Fogg, G. E., 1958. Extracellular products of phytoplankton and the estimation of primary production. *Rapp. P-V Reun. Cons., Perm. Int. Explor. Mer.* 144: 56-60.
- Fogg, F. E., D. J. Eagle and M. E. Kinson, 1969. The occurrence of glycollic acid in natural waters. *Vern. Internat. Verein. Limnol.* 17: 480-484.
- Fogg, G. E. and W. D. Watt, 1965. The kinetics of release of extracellular products of photosynthesis by phytoplankton. In: *Primary Productivity in Aquatic Environments*, C. R. Goldman (ed.). 1st Ital. Idrobiol., 18 Supple. University of California Press, Berkeley, pp. 167-174.
- Fox, J. L. and M. S. Moyer. 1975. Effect of power plant chlorination on estuarine productivity. *Ches. Sci.* 16: 66-68.
- Guillard, R. R. L. and P. J. Wangersky, 1958. The production of extracellular carbohydrates by some marine flagellates. *Limnol. Oceanogr.* 3: 449-454.
- Gurtz, M. E. and C. M. Weiss. 1974. Effect of thermal stress on phytoplankton productivity in condenser cooling water. In: *Thermal Ecology* (J. W. Gibbons and R. R. Sharite, ed). AEC Symposium Series. 686 pp.

- Hale, S. S. 1974. The role of benthic communities in the nutrient cycles of Narragansett Bay. M. S. Thesis, Univ. Rhode Island, Kingston, Rhode Island, 123 pp.
- Hamilton, R. D. and K. E. Austin, 1967. Assay of relative heterotrophic potential in the sea: the use of specifically labeled glucose. Can J. Microbiol. 13: 1165-1173.
- Hamilton, D. H., D. A. Flemer, C. W. Keefe and J. A. Mihursky. 1970. Power plants: effects of chlorination on estuarine primary production. Science 169: 197-198.
- Hamilton, R. D. and J. E. Preslan, 1970. Observations on heterotrophic activity in the eastern tropical Pacific. Limnol. Oceanogr. 15: 395-401.
- Hartwig, E. O. 1974. Physical, chemical and biological aspects of nutrient exchange between the marine benthos and the overlying water. Ph.D. Dissertation, Univ. Calif., San Diego, Scripps Inst. Oceanogr., La Jolla, Calif., 174 pp.
- Hartwig, E. O. 1975. The impact of nitrogen and phosphorus release from a siliceous sediment on the overlying water. In: M. L. Wiley (ed.), Third Internat'l. Estuarine Res. Fed. Conference (1975, in press).
- Hartwig, E. O. 1976. Nutrient cycling between the water column and a marine sediment. 1. Organic Carbon. Mar. Biol. 34: 285-295.
- Hayes, F. R. 1964. The mud-water interface. Oceanogr. Mar. Biol. Ann. Ref. 2: 121-145.
- Hellebust, J. A., 1965. Excretion of some organic compounds by marine phytoplankton. Limnol. Oceanogr. 10: 192-206.
- Hobbie, T. E. and C. C. Crawford, 1969. Respiration corrections for bacterial uptake of dissolved organic compounds in natural waters. Limnol. Oceanogr. 14: 528-532.

- Jannasch, H. W. 1967. Growth of marine bacteria of limiting concentrations of organic carbon in seawater. *Limnol. Oceanogr.* 12: 264-271.
- Jannasch, H. W., 1969. Estimations of bacterial growth rates in natural waters. *J. Bacteriology* 99: 156-160.
- Kadota, H., Y. Hata and H. Miyoshi, 1966. A new method for estimating the mineralization activity of lake water and sediment. *Mem. Res. Inst. Food Sci., Kyoto Univ.* 27: 28-30.
- Lee, G. F. 1970. Factors affecting the transfer of materials between water and sediments. Literature review no. 1. Univ. of Wisconsin Water Resources Center Eutrophication Program. Madison, Wisc. 50 pp.
- McCarthy, J. J., W. R. Taylor and M. E. Loftus, 1974. Significance of nanoplankton in the Chesapeake Bay estuary and problems associated with measurement of nanoplankton productivity. *Mar. Biol.* 24: 7-16.
- Morgan, R. P. and R. G. Stross. 1969. Destruction of phytoplankton in the cooling water supply of a steam electric station. *Ches. Sci.* 10: 165-171.
- Mortimer, C. H. 1971. Chemical exchange between sediments and water in the Great Lakes: speculations on probable regulatory mechanisms. *Limnol. Oceanogr.* 16: 387-404.
- Naylor, E. 1965. Effects of heated effluents upon marine and estuarine organisms. *In*: *Advances in Marine Biology*, H. Barnes, ed., p. 63-103.
- Nichols, F. H. 1974. Sediment turnover by a deposit-feeding polychaete. *Limnol. Oceanogr.* 19: 945-951.
- North, B. B. 1975. Primary amines in California coastal waters: Utilization by phytoplankton. *Limnol. Oceanogr.* 20: 20-27.

- Oppenheimer, C. H. and M. H. Vance. 1960. Attachment of bacteria to surfaces of living and dead micro-organisms in marine sediments. *Zeitschrift fur Allg. Microbiol.* 1: 47-52.
- Paerl, H. W. 1974. Bacterial uptake of dissolved organic matter in relation to detrital aggregation in marine and freshwater systems. *Limnol. Oceanogr.* 19: 966-972.
- Paerl, H. W. 1975. Microbial attachment to particles in marine and freshwater ecosystems. *Microbial. Ecol.* 2: 73-83.
- Palmer, C. M. and T. E. Maloney. 1955. Preliminary screening for potential algicides. *Ohio Jour. Sci.* 1: 1-8.
- Pamatmat, M. M. 1968. Ecology and metabolism of a benthic community on an intertidal sandflat. *Int. Revue ges. Hydrobiol.* 53: 211-298.
- Pamatmat, M. M. 1971. Oxygen consumption by the seabed. VI. Seasonal cycle of chemical oxidation and respiration in Puget Sound. *Int. Rev. Gesamten Hydrobiol.* 56: 769-793.
- Parsons, T. R. and J. D. H. Strickland, 1962. On the production of particulate organic carbon by heterotrophic processes in sea water. *Deep-Sea Res.* 8: 211-222.
- Pomeroy, L. R. and R. E. Johannes. 1968. Occurrence and respiration of ultraplankton in the upper 500 meters of the ocean. *Deep-Sea Res.* 15: 381-391.
- Pomeroy, L. R., E. E. Smith and C. M. Grant. 1965. The exchange of phosphate between estuarine water and sediments. *Limnol. Oceanogr.* 10: 167-172.
- Pratt, R. and others, 1944. Chlorellin, an antibacterial substance from Chlorella. *Science* 99: 351-352.

- Redfield, A. C., B. H. Ketchum and F. A. Richards. 1963. The influence of organisms on the composition of sea-water, 26-77. In: M. N. Hill (ed.), The Sea, Vol. 2, Interscience, New York.
- Riley, G. A. 1963. Organic aggregates in sea water and the dynamics of their formation and utilization. Limnol. Oceanogr. 8: 372-378.
- Riley, J. P. and D. A. Segar, 1970. The seasonal variation of the free and combined dissolved amino acids in the Irish Sea. J. Mar. Biol. Ass. U. K. 50: 713-720.
- Robinson, G. G. C., L. L. Hendzel and D. C. Gillespie, 1973. A relationship between heterotrophic utilization of organic acids and bacterial populations in West Blue Lake, Manitoba, Limnol. Oceanogr. 18: 264-269.
- Roosenburg, W. H. 1969. Greening and copper accumulation in the American oyster, Crassostrea virginica, in the vicinity of a steam electric generating station. Ches. Sci. 10: 241-252.
- Rowe, G. T., C. H. Clifford and K. L. Smith, Jr. 1975. Benthic nutrient regeneration and its coupling to primary productivity in coastal waters. Nature 255: 215-217.
- Schell, D. M., 1974. Uptake and regeneration of free amino acids in marine waters of southeast Alaska. Limnol. Oceanogr. 19: 620-670.
- Schubel, J. R. 1973. Effects of exposure to time-excess temperature histories typically experiences at power plants on the hatching success of fish eggs. CBI Spec. Rept. 32. Johns Hopkins University, Baltimore, 37 pp.
- Schubel, J. R. and A. Auld. 1973. Effects of exposure to time-excess temperature histories typically experienced at power plants on the hatching success of blueback herring (Alosa aestivalis) eggs. CBI Special Report 31, Ref. 73-4.

- Shah, N. M. and R. T. Wright, 1974. The occurrence of glycollic acid in coastal sea water. *Mar. Biol.* 24: 121-124.
- Sharp, J. H., 1974. Improved analysis for "particulate" organic carbon and nitrogen from seawater. *Limnol. Oceanogr.* 19: 984-989.
- Shapr, J.H. and E. H. Renger, 1973. Extracellular production of organic matter by marine phytoplankton. *Inst. Mar. Resour. Progr. Rep.* July 1972 - June 1973, pp. 69-72.
- Sieburth, J. McN., 1968. The influence of algal antibiosis on the ecology of marine micro-organisms. In: *Advances in Microbiology of the Sea*, Vol. 1, M. R. Droop and E. J. F. Wood (eds.), Academic Press, London, pp. 63-94.
- Smith, K. L., Jr. 1973. Respiration of a sublittoral community. *Ecology* 54: 1065-1075.
- Steeman-Nielsen, E., 1955. The production of antibiotics by plankton algae and its effect upon bacterial activities in the sea. *Deep-Sea Res.* 3: 281-289.
- Steeman-Nielsen, E., 1975. Marine photosynthesis with special emphasis on the ecological aspects. *Elsevier Oceanogr. Ser.*, Vol. 13, New York, 141 pp.
- Strickland, J. D. H. 1960. Measuring the production of marine phytoplankton. *Fish. Res. Bd. Can. Bulletin* 122.
- Taft, J. L. 1976. Recovery of $^{14}\text{C-CO}_2$ at high flow rates from a CHN analyzer. *Limnol. Oceanogr.* 21: 161-164.
- Teal, J. M. and J. Kanwisher. 1961. Gas exchange in a Georgia salt marsh. *Limnol. Oceanogr.* 6: 388-399.
- Thomas, J. P. 1971. Release of dissolved organic matter from natural populations of marine phytoplankton. *Mar. Biol.* 11: 311-323.

- Thompson, B. and R. D. Hamilton. 1974. Some problems with heterotrophic uptake methodology. In: Effect of the Ocean Environment on Microbial Activities, R. R. Colwell and R. V. Morita (eds.), Univ. Park Press, Baltimore, Maryland, pp. 566-575.
- Vaccaro, R. F. and H. W. Jannasch, 1967. Variations in uptake kinetics for glucose by natural populations in seawater. *Limnol. Oceanogr.* 12: 540-542.
- Vaccaro, R. F., S. E. Hicks, H. W. Jannasch and F. G. Carey, 1968. The occurrence and role of glucose in seawater. *Limnol. Oceanogr.* 13: 356-360.
- Waksman, S. A., J. L. Stokes and M. R. Butler, 1937. Relation of bacteria to diatoms in seawater. *J. Mar. Biol. Assoc. U. K.* 22: 359-373.
- Walsh, G. E., 1965a. Studies on dissolved carbohydrate in Cape Cod waters. K. General Survey. *Limnol. Oceanogr.* 10: 570-576.
- Walsh, G. E., 1965b. Studies on dissolved carbohydrate in Cape Cod waters. II. Diurnal fluctuations on Oyster Pond. *Limnol. Oceanogr.* 10: 577-582.
- Watt, W. D., 1966. Release of dissolved organic material from the cells of phytoplankton populations. *Proc. Roy. Soc. Ser. B* 164: 521-551.
- Whaley, R. C., J. H. Carpenter and R. L. Baker, 1966. Nutrient data summary 1964, 1965, 1966: Upper Chesapeake Bay (Smith Point to Turkey Point) Potomac, South, Severn, Magothy, Back, Chester and Miles Rivers; and Eastern Bay. CBI Spec. Rep. 12, Ref. 66-4.
- Wheeler, P. A., B. B. North and G. C. Stephens, 1974. Amino acid uptake by marine phytoplankters. *Limnol. Oceanogr.* 19: 249-259.
- Whittaker, R. W. and P. P. Feeny, 1971. Allelochemicals: Chemical interactions between species. *Science* 171: 757-770.

- Wiebe, W. and K. Bancroft, 1975. Use of the adenylate energy charge ratio to measure growth state of natural microbial communities. *Proc. Nat. Acad. Sci.* 72: 2112-2115.
- Williams, P. J. LeB. 1970. Heterotrophic utilization of dissolved organic compounds in the sea. I. Size distribution of population and relationship between respiration and incorporation of growth substrates. *J. Mar. Biol. Ass. U. K.* 50: 859-870.
- Williams, P. J. LeB. 1973. The validity of the application of simple kinetic analysis to heterogenous microbial populations. *Limnol. Oceanogr.* 18: 159-165.
- Williams, P. J. LeB. and C. Askew. 1968. A method of measuring the mineralization by microorganisms of organic compounds in sea water. *Deep-Sea Res.* 15: 365-375.
- Williams, P. J. LeB. and R. W. Gray, 1970. Heterotrophic utilization of dissolved organic compounds in the sea. II. Observations on the responses of heterotrophic marine populations to abrupt increases in amino acid concentration. *J. Mar. Biol. Ass. U. K.* 50: 871-881.
- Wirsén, C. O. and H. W. Jannasch, 1974. Microbial transformation of some ¹⁴C-labeled substrates in coastal water and sediment. *Microb. Ecol.* 1: 23-37.
- Wright, R. T. 1973. Some difficulties in using ¹⁴C organic solutes to measure heterotrophic bacterial activity. In: Belle W. Baruch Library in Marine Science, Vol. 1. *Estuarine Microbial Ecology*, H. L. Stevenson and R. R. Colwell (eds.), Univ. South Carolina Press, Columbia, South Carolina, pp. 199-217.

- Wright, R. T. 1974. Mineralization of organic solutes by heterotrophic bacteria. In: Effect of the ocean Environment on Microbial Activities R. R. Colwell and R. Y. Morita (eds.), Univ. Park Press, Baltimore, Maryland, pp. 546-565.
- Wright, R. T., 1975. Studies on glycollic acid metabolism by freshwater bacteria. *Limnol. Oceanogr.* 20: 626-633.
- Wright, R. T. and J. E. Hobbie. 1965. The uptake of organic solutes in lake water. *Limnol. Oceanogr.* 10: 22-28.
- Zobell, C. E. 1946. Marine Microbiology. *Chronica Botanica*.