1980

Dissolved Carbohydrate Dynamics in the Sea

Curtis Michael Burney
University of Rhode Island

DISSOLVED CARBOHYDRATE DYNAMICS IN THE SEA

BY

CURTIS MICHAEL BURNEY

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN OCEANOGRAPHY

UNIVERSITY OF RHODE ISLAND

1980

ABSTRACT

Dissolved polysaccharide (PCHO), monosaccharide (MCHO) and total carbohydrate (TCHO) have been determined at two to four hour intervals in three marine environments, and related to several other chemical and biological parameters, to study the dynamics and biological interactions of dissolved carbohydrate (CHO).

Six vertical CHO and dissolved organic carbon (DOC) profiles from a preliminary study across the North Atlantic were compaired with those of particulate ATP (<3 um and >3 um size fractions), chlorophyll a, phaeopigments and dissolved oxygen. Carbohydrate peaks were associated with accumulations of >3 um organisms which were low in chlorophyll a, possibly indicative of collections of protozooplankton.

A total of ten follow up diel studies in an enclosed salt marsh and a 13 m^3 simulated estuarine ecosystem (MERL tank) show evidence of biologically mediated CHO fluctuations. During five of the six marsh studies, PCHO underwent periods of sustained accumulation commencing in the late morning or early afternoon and continuing through at least two sampling periods into the early evening. Accumulations during this time period seem to be related to the rate of primary production. They may result from the release of recently synthesized PCHO from phototrophs since their magnitude was a significant multiple linear function of daily solar radiation and mean daily temperature for the six studies spanning all seasons. Similiar periods of sustained release were not found in the MERL tank but a significant direct correlation between the rates of change of PCHO and phaeopigments in the combined data from all four tank studies suggests that zooplankton excretion was an important source of PCHO.

Bacterioplankton counts and PCHO levels, in one study, varied directly in the late morning and inversely through the afternoon and evening, indicating that the bacteria were active and able to respond rapidly and control the natural PCHO concentrations on a time scale of a few hours.

Data from two stations in the western Sargasso Sea, sampled at three or four hour intervals while following a drogued buoy, indicate that in-situ CHO concentrations were closely related to the phototrophic nanoplankton (PNAN), heterotrophic nanoplankton (HNAN) and heterotrophic bacterioplankton (HBAC) populations determined by epifluorescence microscopy. Significant inverse correlations of PCHO and of TCHO with PNAN numbers were found in two diel data sets from off the Carolina coast. Off central Florida, TCHO and/or PCHO were significant multiple linear functions of PNAN, HBAC and sometimes HNAN counts. Partial regression coefficients for PNAN and HNAN were always negative and positive, respectively. HBAC was not consistent in this respect but inverse relations dominated.

Very similiar relationships were found during a similiar drogue study in the Caribbean Sea. Significant inverse relations between PCHO and PNAN levels and between their apparent rates of change were found in the combined data from five diel studies. These correlations may reflect an inverse relation between the rates of cell division and CHO excretion by PNAN. A similiar inverse diel relation between the accumulation rates of TCHO and cell numbers was found in a nutrient replete Isochrysis galbana culture grown under natural sunlight. Fluctuations in HBAC populations were inversely and directly related to the apparent rates of change of PCHO and MCHO, respectively. This

appears to be partially due to bacterial extracellular enzymatic hydrolysis of PCHO to MCHO during periods of rapid bacterial reproduction, however actual net heterotrophic PCHO uptake by HBAC also occurred. The combined PNAN and HNAN or HBAC fluctuations accounted for a more significant proportion of the variation in the apparent rates of change of TCHO and PCHO than did any single population parameter, indicating that intimate interactions between these groups of planktonic microorganisms are important in the regulation of in-situ CHO in the Caribbean as well as in the Sargasso Sea.

Net hourly rates of in-situ TCHO release and uptake in the salt marsh frequently exceeded 40 ug C $L^{-1}h^{-1}$ (maximum of 70 and 98 ug C $L^{-1}h^{-1}$ for release and uptake respectively) in August, with rates up to 17 ug C $L^{-1}h^{-1}$ in February. Lower rates were found in the MERL tank, with maximum TCHO release and uptake of 35 ug C $L^{-1}h^{-1}$ in June and 5 ug C $L^{-1}h^{-1}$ in February.

Rate estimation from the open water measurements in the Sargasso and Caribbean Seas is tenuous because of the possibility of spatial patchiness. It is possible, however, that the observed CHO fluctuations in the Caribbean, primarily represent true temporal dynamics occurring in homogeneous water masses because sample sigma-t variations in each study were small and apparently unrelated to fluctuations in CHO or the populations of microbial plankton. There was no evidence of significant horizontal plankton patchiness. Assuming temporal dynamics, TCHO release and uptake rates averaged 7.4 and 5.4 ug C $L^{-1}h^{-1}$ with maximal values of 29 and 15 ug C $L^{-1}h^{-1}$, respectively. Away from the influence of shallow

waters, the total net apparent TCHO flux over 3 days in the Caribbean accounted for 33 and 48 % of the total net apparent DOC release and uptake, respectively. At one 48 h station with complete data, DOC release was 50% of net apparent primary production.

ACKNOWLEDGEMENTS

I am indebted to Dr. John McN. Sieburth for his unfailing support of both my family and my work and for organizing and including me in the three major research cruises which generated most of the data in this thesis. This work lacked for nothing by way of equipment, financial or moral support, thanks to Dr. Sieburth. I also thank Drs. Scott Nixon, Michael Pilson, Marilyn Harlin and Norris Wood for consultation and criticism of the manuscript. I would like to especially thank Ken Johnson for his considerable help and cooperation in many of the diel studies and for analyzing the DOC, total CO_2 and some of the MCHO samples.

A large number of other people have contributed data or assisted in the numerous separate studies which comprise this work. Melissa Hutchins and Gabriel Vargo provided inorganic nutrient data. Scott Nixon supplied the culvert plug used in the marsh studies, oxygen data from the first MERL experiment and the oxygen reaeration coefficients. Paul Davis, David Caron, James Fontaine, Michael Sieracki, Lorraine Coccozza and Germaine Reusch contributed nanoplankton and bacterioplankton counts. Richard Heffernan, Dennis Lavoie and Paula Willis Therrien provided ATP data. James Fontaine, Fredrick French III and Gabriel Vargo contributed chlorophyll and phaeopigment data. James Hannon and Roger Roussell served as marine technicians at sea and provided oxygen and salinity data and technical services. Paul Hargraves and Anthony Soldo supplied the Isochrysis and Uronema cultures, respectively. In addition to the aforementioned people, I am grateful to Roger Babbitt, Molly Baxter, Duncan Blanchard, Gary Hammond, Kenneth Hinga, Paul Johnson Jean Knapp, Peter LeMay, Evelyn

Lessard, Blaine McBurney, Janice Miller, Brian O'Halloran, Scott Sieburth, Steve Zeeman and the officers and crew of R/V Trident and R/V Endeavor for assistance with parts of this work. I thank Marge McCray for providing a steady stream of clean glassware and lively conversation in the lab.

Very special thanks go to Joyce Burney who willingly provided able technical assistance during several all night projects, both ashore and at sea, and encouraged me to bring my graduate student career to fruition.

This work has been supported by the Biological Oceanography Program of the National Science Foundation through grants GA41501X, OCE-7401537, OCE-7681779, and OCE-7826388 to John McN. Sieburth.

PREFACE

This dissertation is written in manuscript form in accordance with the requirements of the Graduate School of the University of Rhode Island and consists of three manuscripts and three appendices. The manuscripts containing unpublished data are intended for submission to Marine Biology.

The overall intent of this work was to investigate the role of dissolved polysaccharide, monosaccharide and total carbohydrate in a marsh, a simulated estuarine ecosystem (MERL) and the open sea, by following their diel cycles with short interval time series sampling and comparing these patterns with several other chemical and biological parameters measured simultaneously. It was hoped that this effort would yield useful information on the trophic interrelationships affecting the biological release and uptake of dissolved carbohydrate as well as the net rates of these processes.

Appendix I consists of two manuscripts published in Marine Chemistry by Johnson and Sieburth (1977) and Burney and Sieburth (1977) which give details of the carbohydrate analysis used in this work. A preliminary study in the open North Atlantic (published in Deep-Sea Research with K. M. Johnson, D. M. Lavoie and J. McN. Sieburth, 1979) which reports carbohydrate and ATP levels in vertical profiles and proposes biological explanations for the carbohydrate distributions, is included in Appendix II.

The first manuscript (to be published with K. Johnson and J. McN Sieburth), combines results from a total of ten diel studies in a Rhode Island salt marsh and a 13 m^3 seawater tank simulating Narragansett Bay (control tank No. 5 of the Marine Ecosystem Research

Laboratory, MERL). Carbohydrate levels and rates of change are compared seasonally and between areas, and hypotheses are proposed on the factors controlling their fluctuations.

The second manuscript (to be published with P. Davis, K. M. Johnson and J. McN. Sieburth) describes a study in the Sargasso Sea in which diel carbohydrate patterns are related to counts of phototrophic nanoplankton, heterotrophic nanoplankton and heterotrophic bacterioplankton by simple and multiple linear regression. The plankton data accounted for significant fractions of the carbohydrate variance in four of the five data sets.

Manuscript number 3 (to be published with P. Davis, K. M. Johnson and J. McN. Sieburth) reports a similiar study in the northwestern Caribbean Sea. The relationships between in-situ dissolved carbohydrate and the plankton groups were very similar to those found in the Sargasso Sea. The Caribbean study incorporated refinements not present in the Sargasso Sea effort which allowed the estimation of possible open ocean release and uptake rates. The relationship of the microbiota to dissolved carbohydrate levels and fluctuations appears to be both intimate and very dynamic in both nearshore and open ocean waters. The apparent rates of release and uptake of dissolved carbohydrate as determined in these studies far exceed the rates of microbial activity recorded in the literature for ^{14}C bottle assays.

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THE DIEL FLUX OF DISSOLVED CARBOHYDRATE IN A SALT MARSH AND A SUMULATED ESTUARINE ECOSYSTEM

ABSTRACT

The concentrations of total dissoved carbohydrate (TCHO), monosaccharide (MCHO) and polysaccharide (PCHO) were followed over a total of ten diel cycles in a salt marsh and a 13 m^3 seawater tank simulating an estuarine ecosystem. Their patterns are compared to those of total dissolved organic carbon (DOC), total CO_2, pH, O_2, chlorophyll *a*, phaeopigments, solar radiation and bacterioplankton. During five of the six marsh studies, PCHO underwent periods of sustained accumulation which started in the late morning or early afternoon and continued through at least two sampling periods into the early evening. This accumulation, which may represent release of recently synthesized PCHO from phototrophs, was much reduced on a cloudy day in August and was a significant linear function of daily solar radiation and mean daily temperature. Similar periods of sustained net PCHO release were not found in the tank although more transitory PCHO pulses occurred during the night and may have resulted from zooplankton excretion because they often occurred with phaeopigment peaks. The combined data from the four tank studies showed a significant direct correlation between the rates of change of PCHO and phaeopigments. Bacterioplankton counts and PCHO levels varied directly in late morning and inversely throughout the afternoon and evening, indicating that the bacteria were active and able to respond rapidly to, and control the natural PCHO concentrations on a time scale of a few hours. MCHO fluctuated to a much lesser extent than PCHO at both locations, with levels apparently maintained near the uptake threshold of the bacterioplankton. The overall mean CHO and DOC levels were two to three times higher in the salt marsh than

in the tank with concentrations in both systems generally highest in summer, lowest in winter, and intermediate in the fall samples. The mean contribution of TCHO to the total DOC varied from 11.7% in the marsh (winter) to 26.4% in the tank (summer). Mean values above 20% in the tank occurred on days with elevated chlorophyll a levels. Mean PCHO/TCHO ratios varied from 0.528 in the tank to 0.827 in the marsh. A significant positive correlation between PCHO and DOC was achieved in all but one of the marsh studies, but in none of the tank investigations.

Rates of net TCHO release ranged from 85 to 520 ug C $L^{-1}d^{-1}$ in the marsh and 16 to 109 ug C $L^{-1}d^{-1}$ in the tank. Net TCHO uptake varied from 45 to 419 ug C $L^{-1}d^{-1}$ and 31 to 131 ug C $L^{-1}d^{-1}$ in marsh and tank, respectively. For the salt marsh, TCHO accounted for from 9 to 33% of the net DOC release, 10 to 63% of the DOC uptake, 2 to 11% of net system production (based on CO_2) and 2 to 12% of net system respiration. In the tank, the TCHO flux accounted for 4 to 101% of the daily DOC release, 7 to 92% of net DOC uptake, 2 to 17% of net production and 6 to 29% of net respiration.

INTRODUCTION

There is a growing realization of the importance of free-living bacteria in marine ecology (Sorokin, 1971a,b; Hoppe, 1976; Sieburth, 1976, 1979; Sieburth et al., 1977; Larsson and Hagstrom, 1979). The vast majority of these bacterioplankters are not associated with particles but are free cells that utilize dissolved organic substrates (Williams, 1970; Azam and Hodson, 1977). Almost everything known about the rates of supply and uptake of these materials comes from studies on the release of ^{14}C labeled organic substances by phytoplankton and on the uptake of ^{14}C and ^{3}H labeled substrates by bacteria. Both of these types of studies employ bottle incubations and other sample handling which may introduce systematic errors or artifacts (Sieburth, 1979).

Rates of supply and uptake of biologically important substances dissolved in seawater can be determined through direct analysis over diel cycles. Such studies can also provide information on the functional relationships of the different trophic components of the ecosystem when microbiological and chemical parameters are followed simultaneously. Dissolved carbohydrates (CHO) are important bacterial substrates which are released by phytoplankters and macrophytes (Hellebust,1974) and account for about 10 to 20% of the total dissolved organic carbon (DOC) in Narragansett Bay, RI (Burney and Sieburth, 1977). Previous studies (Collier et al., 1953; Walsh, 1965) have demonstrated large diel CHO fluctuations in inshore waters. The purpose of this study was to estimate net CHO release and uptake rates and to look for evidence of biological regulation of the CHO dynamics using measurements made at 2 or 3 h intervals over diel

cycles from a New England salt marsh and a 5-m deep seawater tank designed to simulate the Narragansett Bay estuary.

MATERIALS AND METHODS

The salt marsh sampling was conducted at a 6000 m^2 marsh embayment north of Bissel Cove on the West Passage of Narragansett Bay, Rhode Island. The area has been extensively described (Nixon and Oviatt, 1973; Nixon et al., 1976). The principle advantage of this location for diel studies is that all tidal exchange with Bissel Cove can be blocked by plugging a culvert, thus trapping a water mass in which to conduct time series measurements. A total of six 24-h samplings were conducted from shore near the culvert in the deepest part of the embayment (maximum of 1 m) on 18-19 August 1976 and 27-28 February, 12-14 August, 30-31 August and 11-12 October 1978.

The simulated estuarine ecosystem was control tank No. 5 of the Marine Ecosystems Research Laboratory (MERL) at the Narragansett Bay campus of the University of Rhode Island (Pilson et al., 1977). The tank holds a 5-m column of seawater above a 30-cm layer of Narragansett Bay sediment. Seawater is pumped from the bay at a rate of 0.48 $m^3 \cdot d^{-1}$ to replace the tank volume every 27 days and tidal resuspension of particulate matter is simulated by intermittent mechanical mixing. Four diel studies were conducted on 3-4 March and 19-20 September 1977, 3-4 February 1978 and 6-7 June 1979.

Samples were taken every 2 or 3 h from a depth of 1 m in the tank and just below the surface in the marsh. For oxygen, total carbon dioxide (TCO_2) and pH analyses, BOD bottles were filled by bubble-free siphon from the tank and with a 50-ml syringe from the marsh. The bottles were overflowed by several volumes and stoppered without bubbles. Other samples were collected in clean glass bottles mounted on a Wheaton (Millville, NJ) extension sampler which allowed

them to be opened and closed below the surface to avoid contamination from the surface film (DiSalvo, 1973). The samples were shielded from direct sunlight and returned to the laboratory where processing commenced within 15 min.

CHO and DOC samples were filtered by gravity through Gelman A/E glass fiber filters into glass bottles and frozen at -30°C. The filter holders were acid cleaned and pre-rinsed with sample. About 150 ml of sample was filtered and discarded before the aliquot for analysis was collected. All filters and sample bottles were precombusted at 450°C overnight. Monosaccharide (MCHO) was analyzed by the method of Johnson and Sieburth (1977). Total dissolved carbohyhdrate (TCHO) was determined, and polysaccharide (PCHO) estimated, according to Burney and Sieburth (1977). Four to six replicates were run for each analysis. Three to five DOC replicates per sample were analyzed by the wet persulphate method of Menzel and Vaccaro (1964) as modified by Kerr and Quinn (1975) using an Oceanography International ampule analyzer panel and a Beckman (Fullerton, CA) 315 infrared gas analyzer. After October, 1978, an Oceanography International (College Station, TX) total carbon system, model 254B, was used. The output of both analyzers was integrated with an Infotronics model CRS-208 automatic digital integrator (Columbia Scientific, Houston, TX).

In the first two salt marsh studies, CO_2 flux was estimated from pH measurements (Corning model 101 digital electrometer, Corning, NY) by the Beyers (1963) method using empirical pH-CO_2 curves obtained by titrating natural water samples with saturated CO_2 solutions. In later work, TCO_2 was determined directly by purging

acidified samples with nitrogen and determining CO_2 by infrared analysis using the same equipment as for DOC (Johnson et al., in prep.). Dissolved oxygen was determined by the Winkler method except in the first marsh study when a Yellow Springs (Yellow Springs, OH) model 51A oxygen meter was used. The manganous chloride and alkaline iodide solutions were added immediately to the Winkler samples in the field.

In the MERL work, particulate chlorophyll a and phaeopigments were determined fluorometrically on 90% acetone extracts (Strickland and Parsons, 1968) using an Aminco (Silver Spring, MD) fluorocolorimeter. They were not determined in the marsh since plankton accounts for a small fraction of total system metabolism (Nixon and Oviatt, 1973).

Salinity (American Optical, Keene, NH, hand refractometer) and temperature were recorded and integrated hourly solar radiation data from a roof-top pyrheliometer were provided by Eppley Laboratory in Newport, RI. For the last MERL study, suspended bacteria were counted using acridine orange staining and epifluorescence microscopy (Hobbie, Daley and Jasper, 1977).

Net apparent daily rates of release and uptake of CHO, DOC, TCO_2 and O_2 were calculated from the time series data by the single diurnal curve method of Odum and Hoskin (1958). The calculation involves integration of the area under hourly rate of change plots. The positive area represents net input and the negative portion is proportional to net uptake. Hourly oxygen rates were corrected for atmospheric exchange using empirical reaeration constants of 0.28 and 0.012 ml O_2 L^{-1} h^{-1} at 100%

saturation deficit, for the marsh and tank data, respectively (S. Nixon, personal commnication). Rates for TCO_2 were not corrected for atmospheric diffusion since this is insignificant relative to biological flux in areas of high productivity (Smith, 1973; Johnson et al., 1979). Oxygen flux was expressed in terms of carbon by assuming that 1 ml of O_2 represents 536 ug C (PQ=RQ=1).

RESULTS

Figure 1 presents the results of four multi-parameter diel studies on the Bissel Cove salt marsh. Data from two less extensive observations in the marsh, made on consecutive days, show the effects of a cloudy and sunny day (Fig. 2). Figure 3 gives the results of four multi-parameter diel studies in the MERL tanks. To facilitate comparisons, a standardized time scale (abscissa) was used in these figures, with 12:00 noon centrally located. This causes a break in the curves with the right and left segments representing the first and second day of each study, respectively. Error bars representing plus and minus one standard error of the mean were plotted on the TCHO, MCHO, DOC, CO_2, O_2, chlorophyll *a* and phaeopigment curves. When no bar appears, the standard error was smaller than the plot symbol. Figure 4 illustrates the multiple linear relationships of the total TCHO and PCHO accumulations occurring between 1040 and 2000 hours, with daily solar radiation and mean daily temperature, for the six marsh studies. Figure 5 shows the direct relationships of the rates of change of phaeopigment to TCHO and PCHO dynamics for the combined MERL data. Figure 6 compares bacterioplankton populations and PCHO concentrations in the June MERL study. The hourly rates of change of TCHO, PCHO and MCHO from six marsh diel cycles and four MERL ecosystem diel cycles are given in Figures 7 and 8, respectively.

Table 1 lists the mean TCHO, PCHO, MCHO and DOC values ($\pm$one standard deviation) for each diel cycle and the overall values for both locations. The mean contributions of TCHO to DOC and of PCHO to TCHO are presented for each study in Table 2. Correlation coefficients and significance levels derived from the MCHO, PCHO and

DOC data are given in Table 3. PCHO and TCHO were always positively correlated ($P<.005$) except for the first MERL study where $P=.052$. The daily net apparent flux of CHO and DOC with total system net production and respiration estimates based on the O_2 and CO_2 data for both the salt marsh and MERL tank are summarized in Table 4. The daily net rates of TCHO release and uptake compared to those for DOC and CO_2 based production and respiration for both the marsh and MERL tank are given in Table 5.

Figure 1: Comparison of four multi-parameter diel studies on the Bissel Cove salt marsh. Continued on next page.

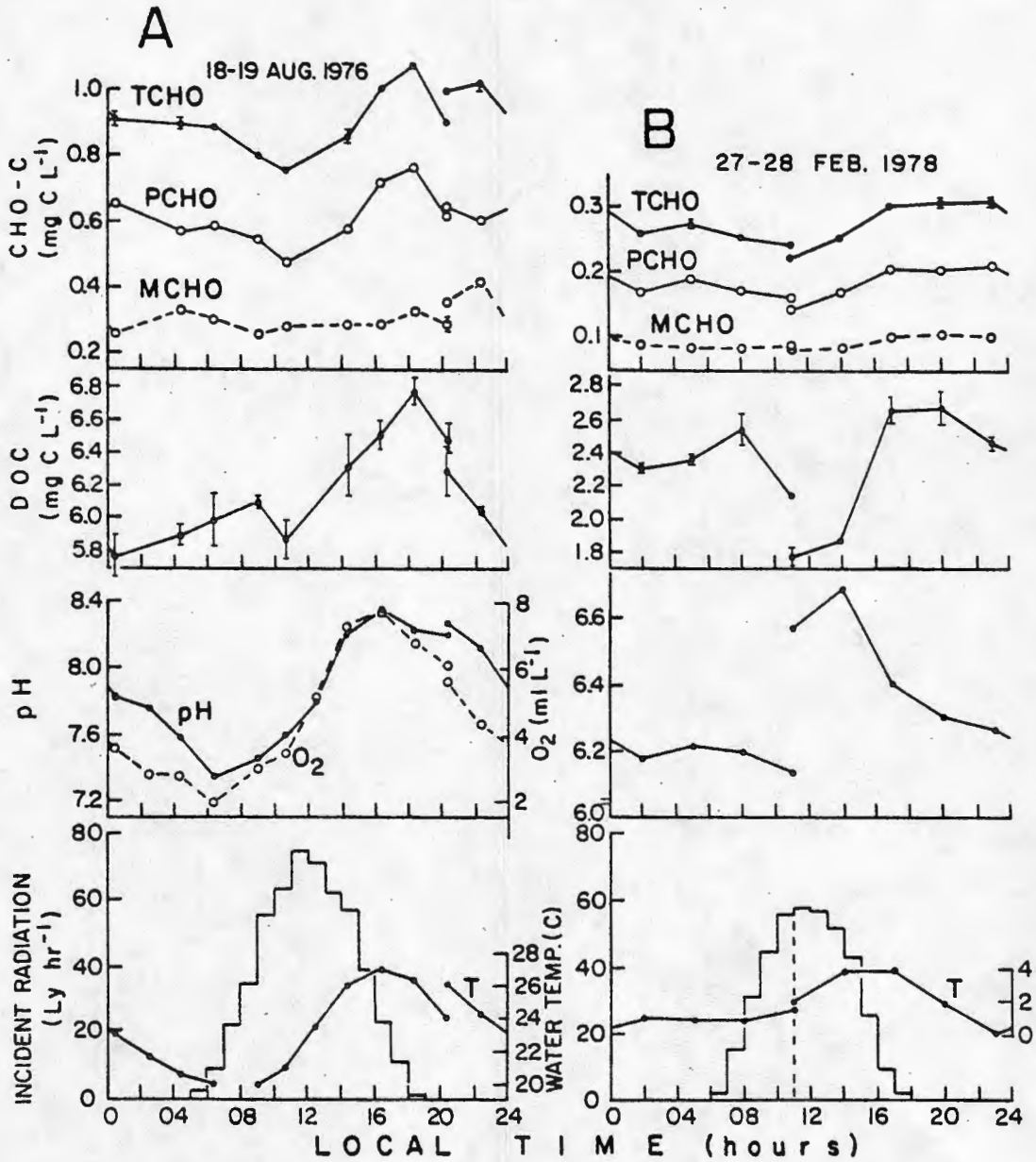
A
18-19 AUG. 1976
TCHO
PCHO
MCHO
CHO-C (mg C L⁻¹)
DOC (mg C L⁻¹)
pH
O2 (ml L⁻¹)
INCIDENT RADIATION (Ly hr⁻¹)
WATER TEMP.(C)
T
B
27-28 FEB. 1978
LOCAL TIME (hours)

Figure 1

Figure 1: Continued.

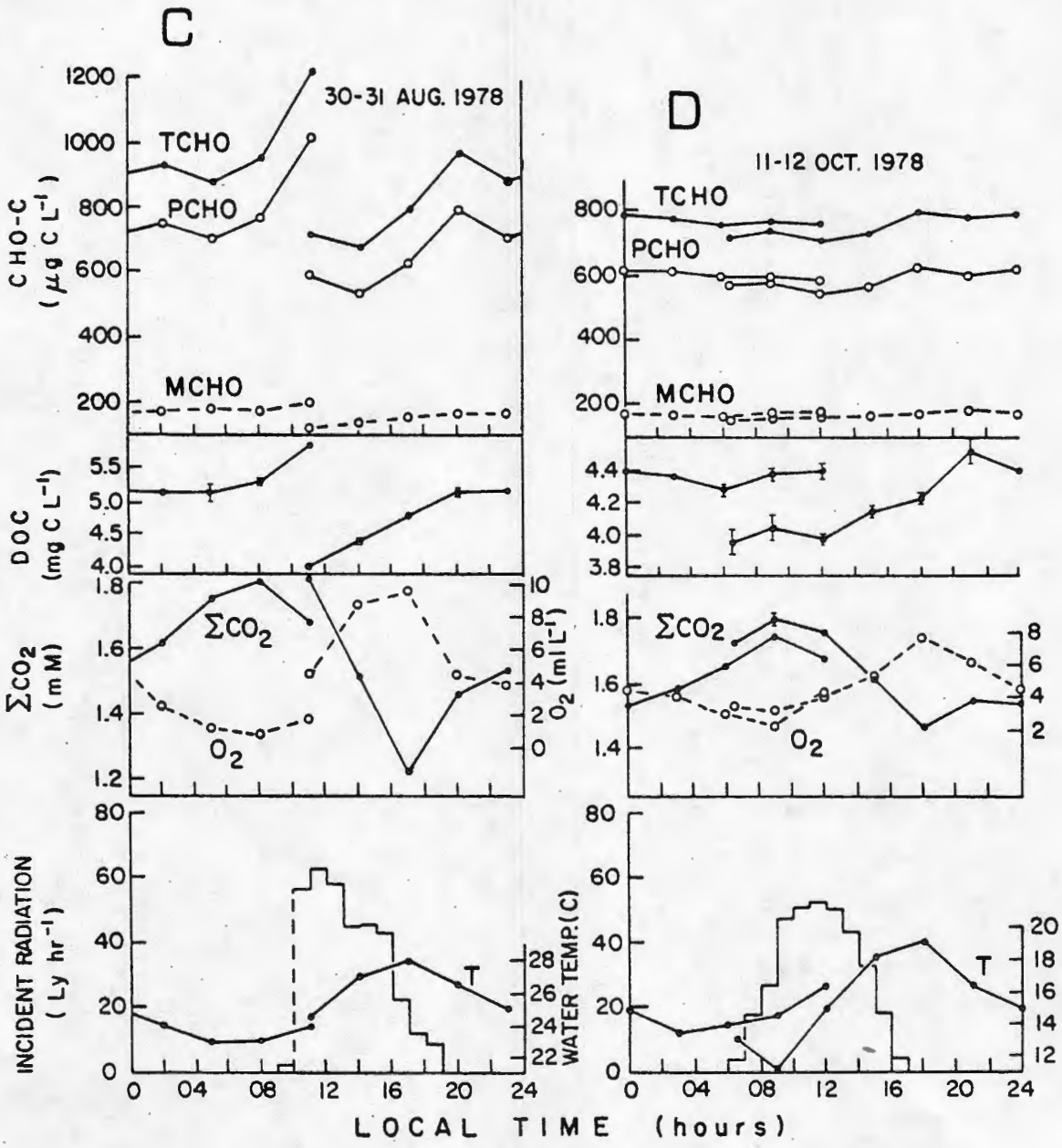

Figure 1

Continued.

Figure 2: Comparison of diel cycles of dissolved carbohydrates and DOC observed on consecutive cloudy and sunny days in the Bissel Cove salt marsh.

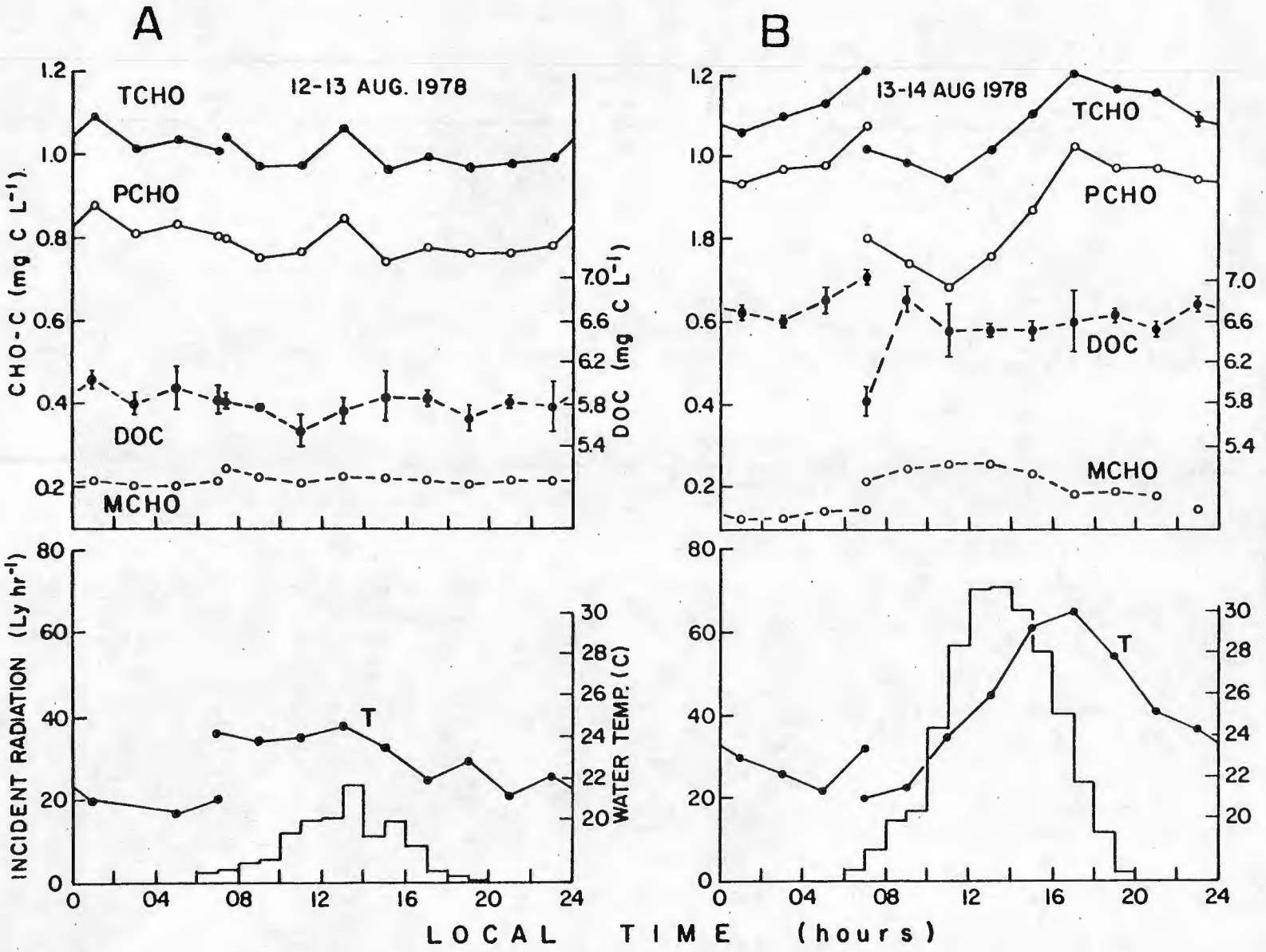
A
B
12-13 AUG. 1978
13-14 AUG 1978
TCHO
PCHO
DOC
MCHO
T
CHO-C (mg C L⁻¹)
DOC (mg C L⁻¹)
INCIDENT RADIATION (Ly hr⁻¹)
WATER TEMP. (C)
LOCAL TIME (hours)

Figure 2

Figure 3: Comparison of four multi-parameter diel studies on the MERL simulated estuarine ecosystem (control tank No. 5). Continued on next page.

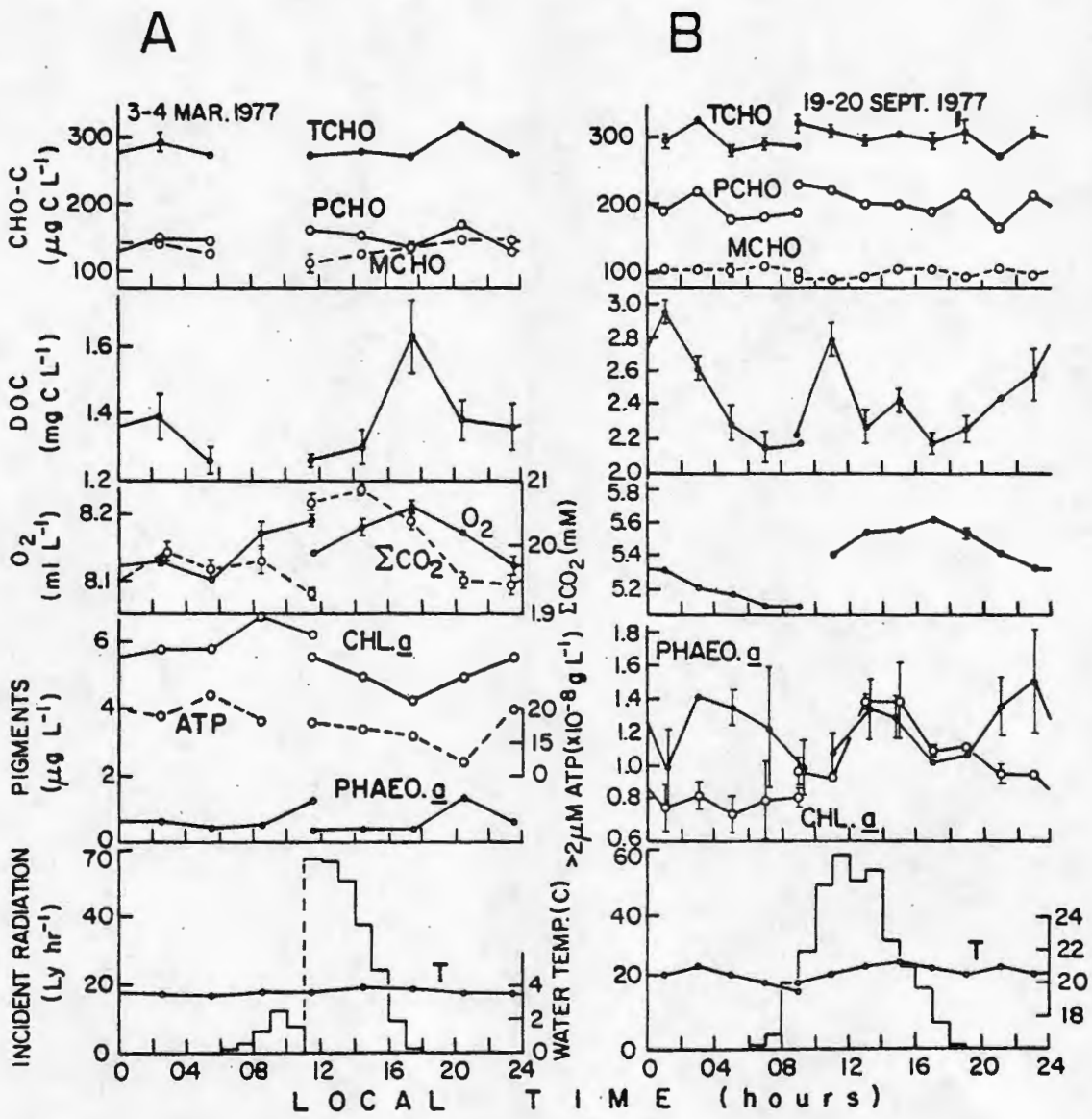
A
B
3-4 MAR. 1977
19-20 SEPT. 1977
TCHO
PCHO
MCHO
CHO-C (μg C L⁻¹)
DOC (mg C L⁻¹)
O2 (ml L⁻¹)
ΣCO2
ΣCO2 (mM)
CHL. a
ATP
PHAEO. a
PIGMENTS (μg L⁻¹)
>2μM ATP (x10⁻⁸ g L⁻¹)
INCIDENT RADIATION (Ly hr⁻¹)
T
WATER TEMP.(C)
LOCAL TIME (hours)

Figure 3

Figure 3: Continued

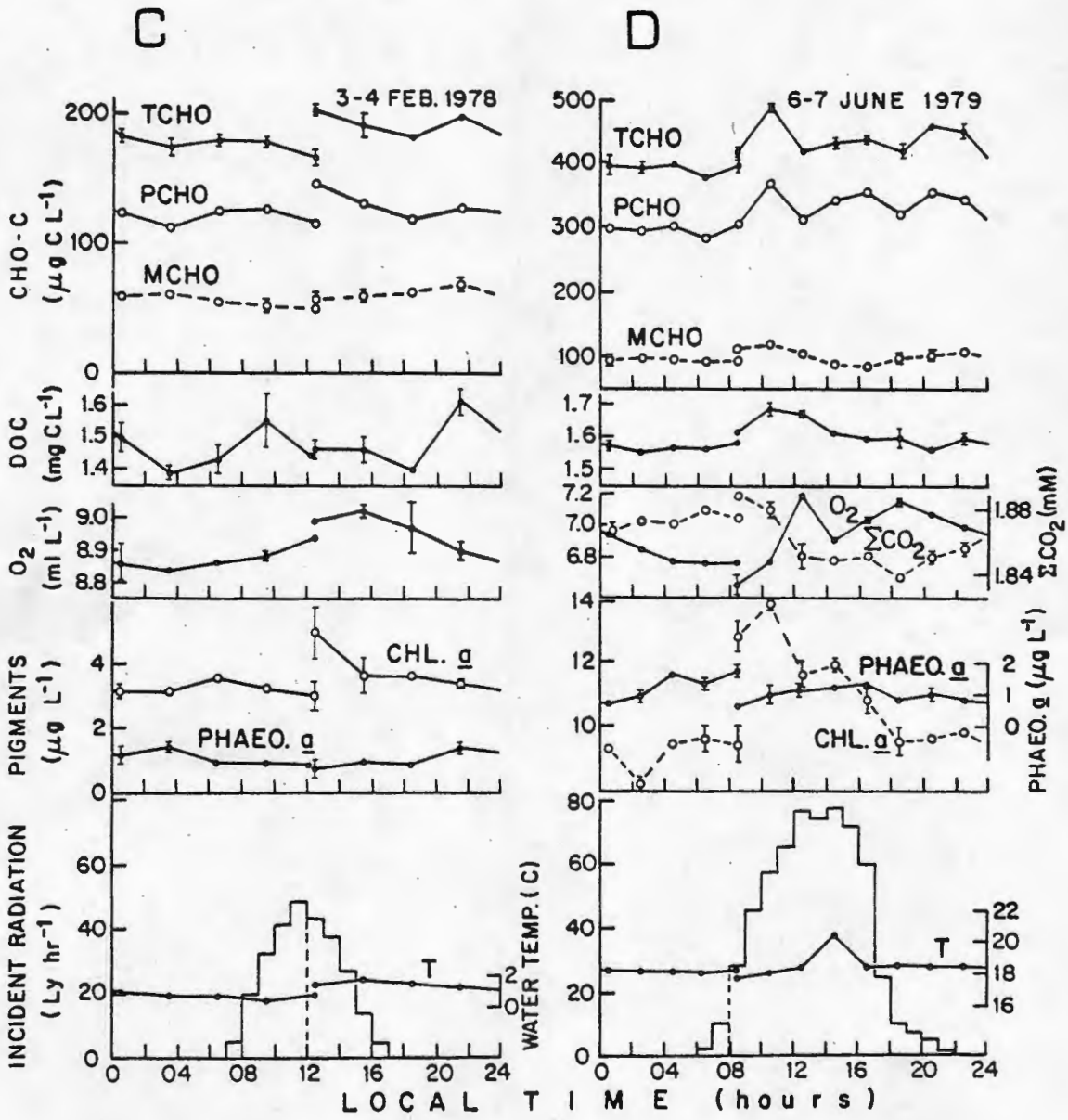

Figure 3

Continued.

Figure 4: The total observed TCHO (closed circles) and PCHO (open circles) accumulation occurring between 1040 and 2000 hrs in the six marsh studies as a function of the estimated rates (EST) derived from multiple linear relationships incorporating daily solar radiation (total rad,ly d^{-1}) and mean daily temperature (T ^{o}C). The line represents a perfect 1:1 relationship.

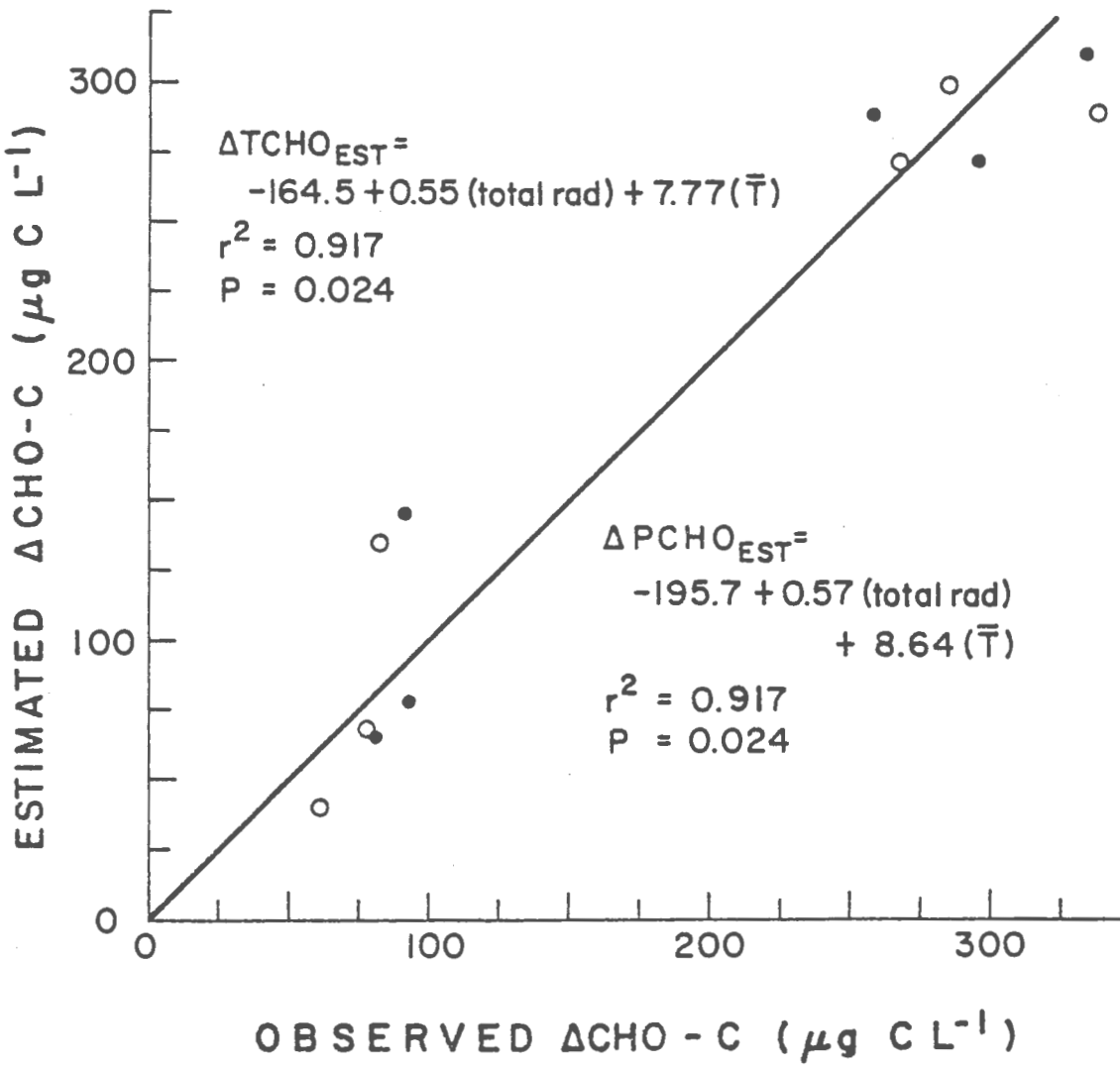
ESTIMATED ΔCHO-C (μg C L⁻¹)
OBSERVED ΔCHO - C (μg C L⁻¹)
0
100
200
300
ΔTCHO_EST =
-164.5 + 0.55 (total rad) + 7.77 (T̄)
r² = 0.917
P = 0.024
ΔPCHO_EST =
-195.7 + 0.57 (total rad)
+ 8.64 (T̄)
r² = 0.917
P = 0.024

Figure 4

Figure 5: Correlations of the rates of change of PCHO and of TCHO to phaeopigment fluctuations in the combined data from the four MERL tank studies. Continued on next page.

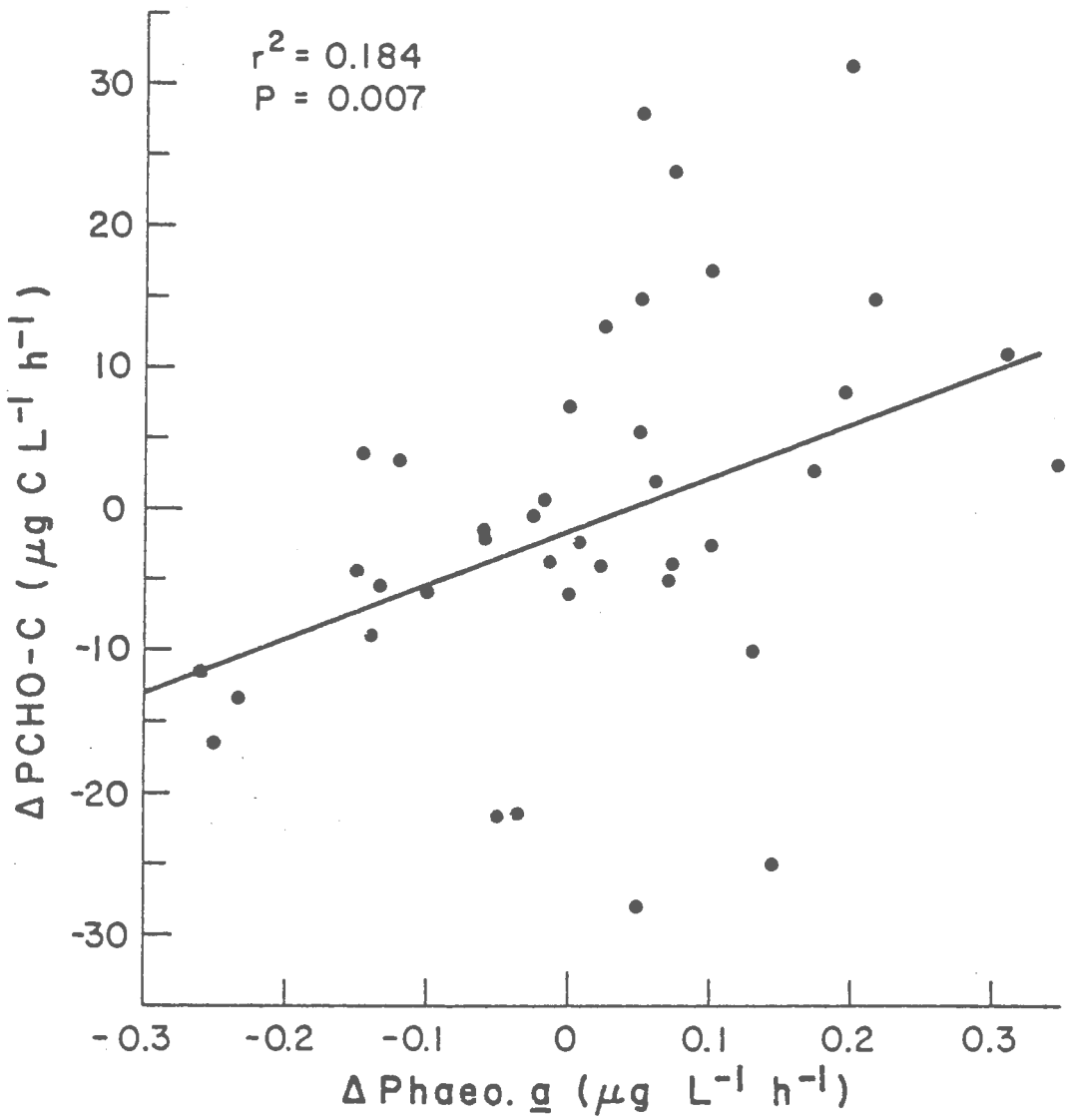
r² = 0.184
P = 0.007
30
20
10
0
-10
-20
-30
-0.3
-0.2
-0.1
0
0.1
0.2
0.3
ΔPCHO-C (μg C L⁻¹ h⁻¹)
Δ Phaeo. g (μg L⁻¹ h⁻¹)

Figure 5

Figure 5: Continued.

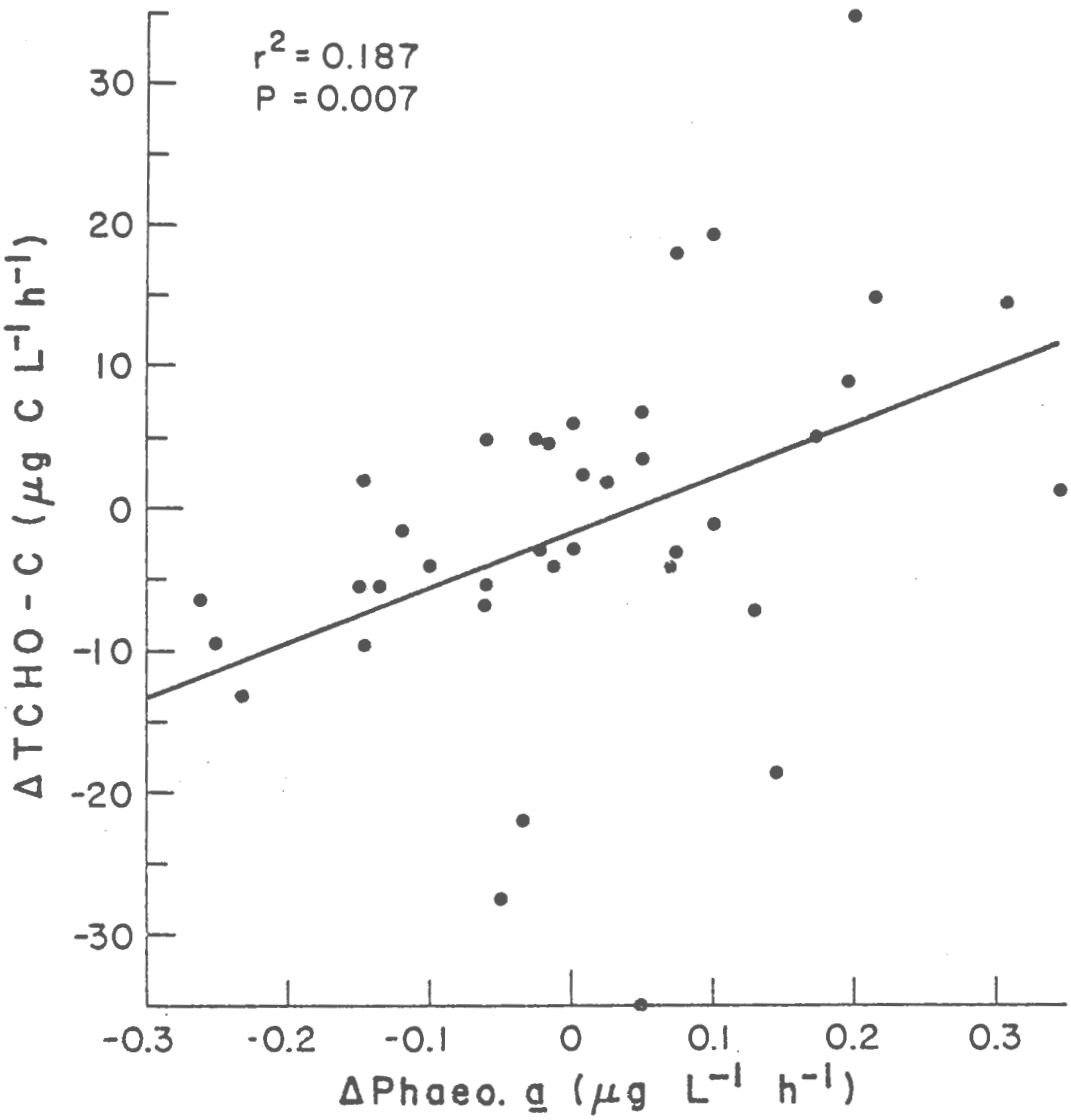

Figure 5

Continued.

Figure 6: Comparison of the diel patterns of PCHO and BAC in MERL tank No. 5, 6-7 June 1979.

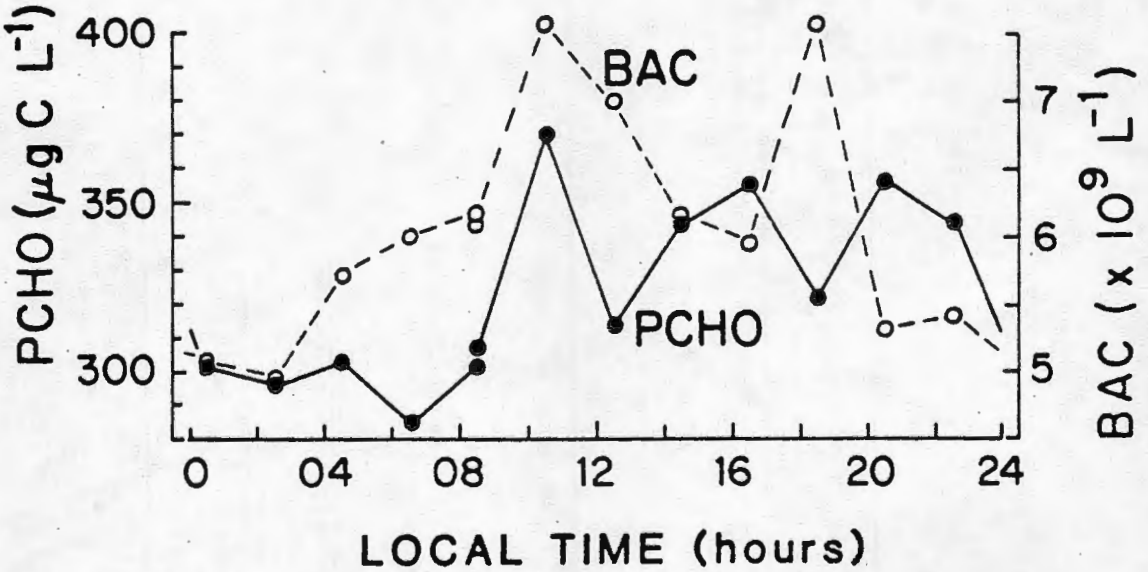
PCHO (μg C L⁻¹)
400
350
300
BAC
PCHO
7
6
5
BAC (x 10⁹ L⁻¹)
0
04
08
12
16
20
24
LOCAL TIME (hours)

Figure 6

Figure 7: Hourly rates of change of TCHO (filled circles), PCHO (open circles) and MCHO (triangles) for six diel cycles in the Bissel Cove salt marsh.

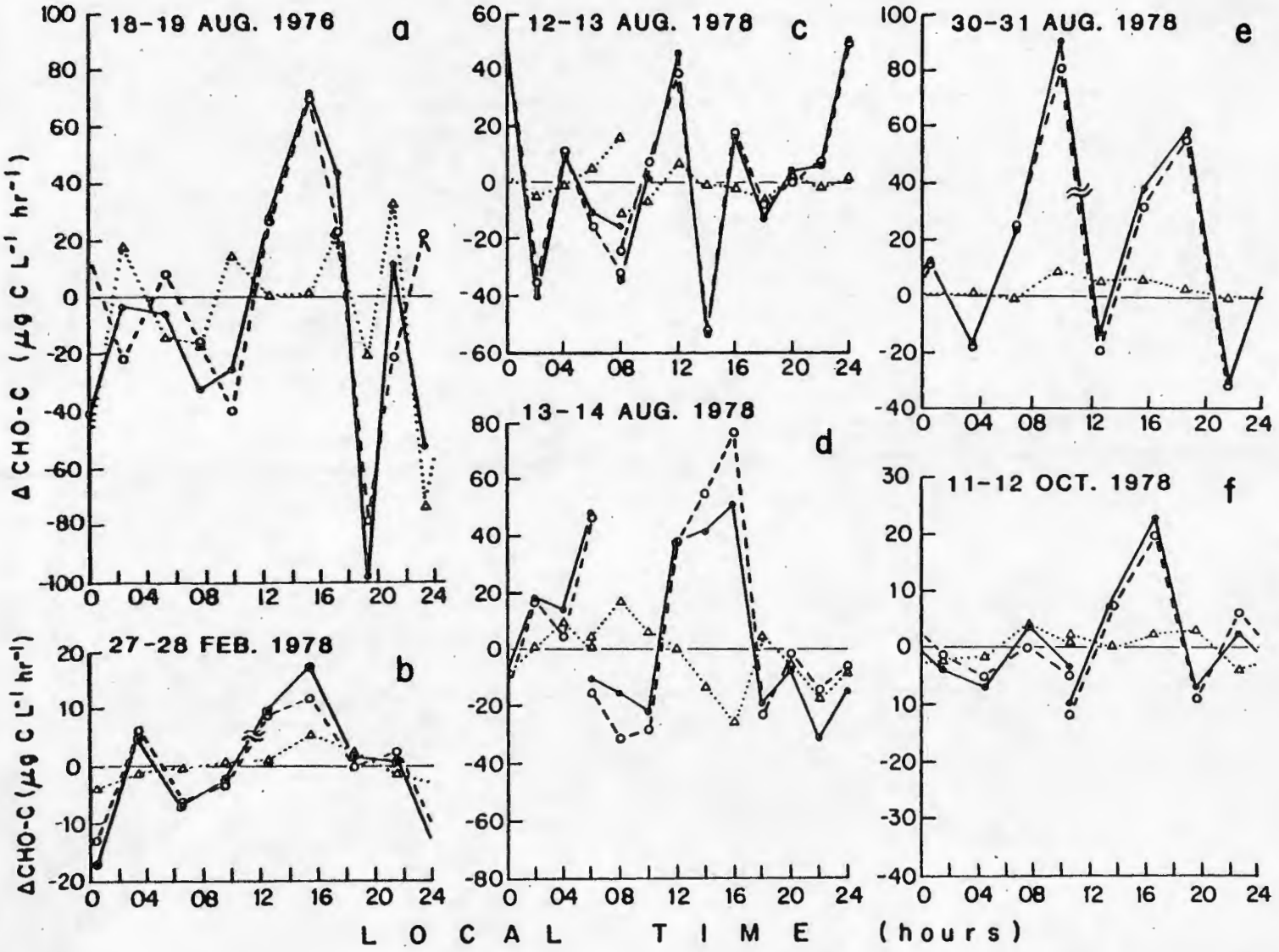
18-19 AUG. 1976
a
12-13 AUG. 1978
c
30-31 AUG. 1978
e
13-14 AUG. 1978
d
27-28 FEB. 1978
b
11-12 OCT. 1978
f
ΔCHO-C (μg C L⁻¹ hr⁻¹)
LOCAL TIME (hours)

Figure 7

Figure 8: Hourly rates of change of TCHO, PCHO and MCHO for four diel cycles in the MERL ecosystem. Symbols as in Fig. 7.

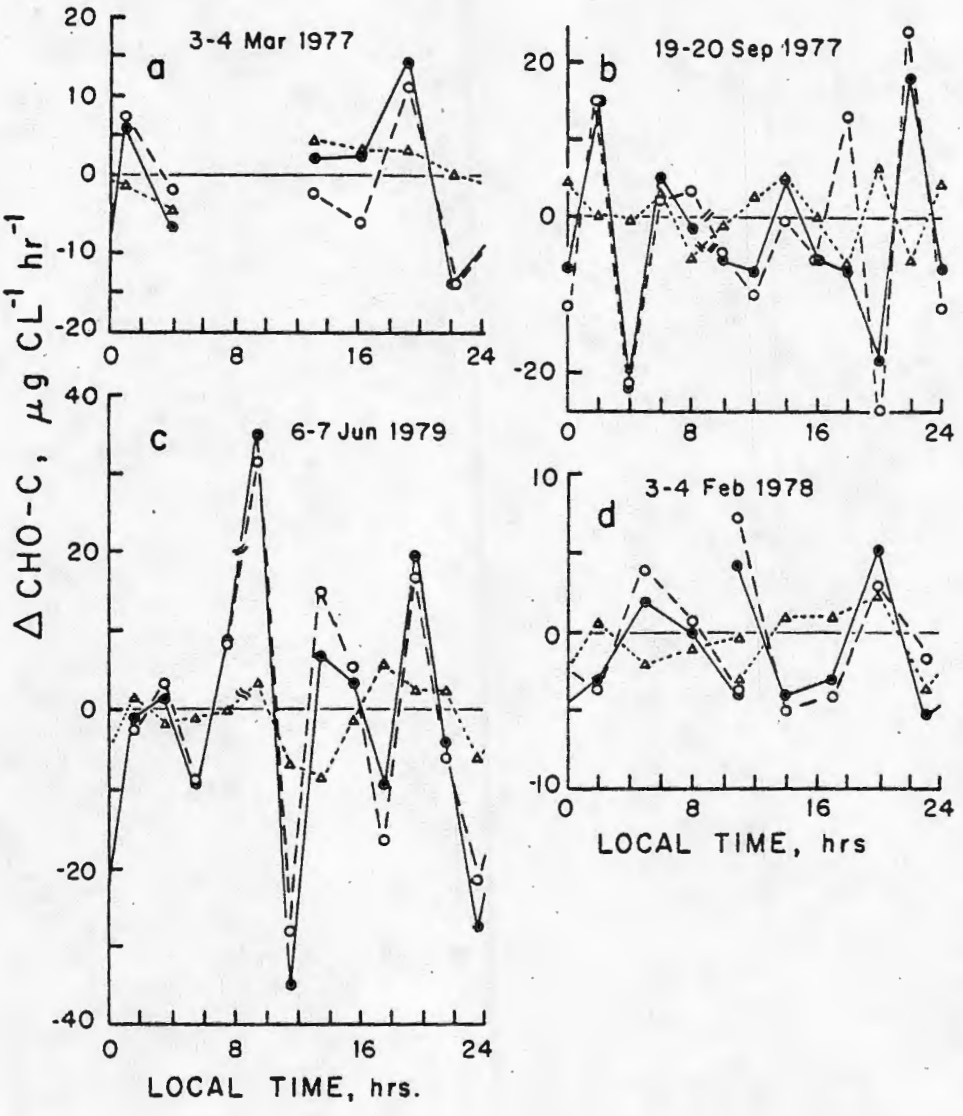
a
3-4 Mar 1977
b
19-20 Sep 1977
c
6-7 Jun 1979
d
3-4 Feb 1978
ΔCHO-C, μg CL⁻¹ hr⁻¹
LOCAL TIME, hrs.
LOCAL TIME, hrs

Table 1. Daily and overall means for CHO and DOC values (± one standard deviation) obtained in the Bissel Cove salt marsh and the MERL tank diel studies.

Survey Dates	TCHO±SD	PCHO±SD	MCHO±SD	DOC±SD
	ug C L^{-1}			
Salt Marsh				
18-19 Aug 1976	920±100	613± 80	307±47	6190±314
27-28 Feb 1978	268± 31	178± 23	90± 9	2310±326
12-13 Aug 1978	1009± 41	793± 40	216±10	5800±123
13-14 Aug 1978	1096± 84	910±121	186±50	6670±160
30-31 Aug 1978	898±161	729±140	170±23	5000±541
11-12 Oct 1978	755± 30	587± 25	168± 9	4240±189
MERL				
03-04 Mar 1977	284± 16	150± 14	134±12	1370±127
19-20 Sep 1977	301± 15	201± 19	100±17	2413±256
03-04 Feb 1978	185± 11	126± 9	59± 6	1468± 68
06-07 Jun 1979	422± 31	323± 27	99± 9	1597± 41
Overall				
Bissel Cove	849± 81	655± 80	194±30	5170±279
MERL	308± 20	212± 19	96± 8	1780±150

Table 2. Daily and overall mean contributions of TCHO to DOC and of PCHO to TCHO in Bissel Cove salt marsh and the MERL tank.

Survey dates	TCHO/DOC±SD	PCHO/TCHO±SD
Salt Marsh		
18-19 Aug 1976	.149±.013	.666±.038
27-28 Feb 1978	.117±.010	.664±.015
12-13 Aug 1978	.174±.006	.786±.012
13-14 Aug 1978	.164±.012	.827±.058
30-31 Aug 1978	.179±.015	.810±.015
11-12 Oct 1978	.178±.005	.778±.009
MERL		
03-04 Mar 1977	.208±.020	.528±.038
19-20 Sep 1977	.126±.013	.688±.035
03-04 Feb 1978	.126±.008	.679±.026
06-07 Jun 1979	.264±.017	.765±.019
Overall		
Salt Marsh	.161±.023	.758±.071
MERL	.181±.064	.677±.083

Table 3. Correlation coefficients and levels of significance (one tailed t test) between MCHO, PCHO and DOC.

Survey Dates	MCHO vs PCHO		PCHO vs DOC		MCHO vs DOC	
Salt Marsh						
18-19 Aug 1976	.1845	n/s	.6881	.010	.0957	n/s
27-28 Feb 1978	.8590	.002	.8061	.004	.7345	.012
12-13 Aug 1978	-.0529	n/s	.5302	.031	.0843	n/s
13-14 Aug 1978	-.8246	<.001	.4200	n/s	-.4447	n/s
30-31 Aug 1978	.8712	.001	.8973	<.001	.9905	<.001
11-12 Oct 1978	.3981	n/s	.6857	.010	.7615	.003
MERL						
03-04 Mar 1977	-.2520	n/s	-.4014	n/s	.4516	n/s
19-20 Sep 1977	-.7312	.002	.1839	n/s	.0721	n/s
03-04 Feb 1978	-.1794	n/s	.3182	n/s	.1541	n/s
06-07 Jun 1979	.2510	n/s	.4574	n/s	.5641	.022

n/s listed when P>.05

Table 4. Daily net apparent flux of CHO, DOC, O_2 and TCO_2 expressed as ug C $L^{-1}d^{-1}$.

Dates	TCHO R/U	MCHO A/D	PCHO A/D	DOC R/U	O_2 P/R	CO_2 P/R
Salt Marsh						
18 Aug 1976	293/419	114/215	285/310	907/964	2743/2974	2685/3411
02 Feb 1978	88/65	25/20	68/50	1008/638	n	n
12 Aug 1978	189/214	22/51	186/182	620/610	n	n
13 Aug 1978	370/168	68/133	418/150	1559/359	n	n
30 Aug 1978	520/173	64/12	468/173	1559/275	2027/4977	7540/5956
11 Oct 1978	85/45	28/18	77/48	551/176	2102/2550	3604/2823
MERL						
03 Mar 1977[a]	39/31	27/11	31/40	291/245	87/42	1657/495
19 Sep 1977	54/87	32/24	63/105	1240/1190	107/267	n
03 Feb 1978	16/40	10/20	22/36	174/184	69/89	n
06 Jun 1979	109/131	25/43	112/117	108/143	348/273	624/456

R/U= release/uptake, A/D= accumulation/dissapearance
P/R= production/respiration, a= Integrated over 18 hrs only
n= no data

Table 5. Daily net TCHO rates as percentages of DOC flux and net aparent system production based on TCO_2 flux.

Date	TCHO release / DOC release percent	TCHO uptake / DOC uptake percent	TCHO release / Production percent	TCHO uptake / Respiration percent
Salt Marsh				
8/18/76	32	43	11	12
2/27/78	9	10	n	n
8/12/78	30	35	n	n
8/13/78	24	47	n	n
8/30/78	33	63	7	3
10/11/78	15	26	2	2
MERL				
3/03/77	13	13	2	6
9/19/77	4	7	n	n
2/03/78	9	22	n	n
6/06/79	101	92	17	29

n = no data.

DISCUSSION

Results from each of the salt marsh studies in Figure 1 show evidence of highly significant TCHO accumulation, primarily due to net PCHO release, commencing in the late morning or early afternoon and continuing through at least two sampling periods into the early evening. Except for the 30-31 August, 1978, study (Fig. 1-C), TCHO and PCHO usually reached their daily maxima at the end of this period of sustained release. The maximum rates of PCHO accumulation in this time period occurred during or just after the period of maximum net system production indicated by the O_2, pH and TCO_2 data (Fig. 1). Saunders (1969) found that the maximum rates of phytoplankton excretion lagged the maximum photosynthetic rate by two hours in Frans Lake. No period of PCHO accumulation sustained over more than one sampling interval occurred on a cloudy day in August (Fig. 2-A), however a very pronounced PCHO increase from 1100 to 1700 hours appeared on the following clear day (Fig. 2-B), suggesting an association between the photosynthetic rate and PCHO release. The period of net PCHO release from 1100 to 1300 hours on the cloudy day (Fig. 2-A) started at the same time as the period of sustained release on the following day but the former was probably curtailed due to light limitation of photosynthesis. Morris and Skea (1978) have shown that sub-optimal light intensities cause phytoplankton to incorporate a smaller fraction of their photoassimilated carbon into PCHO. For the six marsh studies, total TCHO and PCHO accumulations in the 1040 to 2000 time period were significant functions of daily solar radiation and mean daily temperature (Fig. 4). This association with solar radiation suggests that these accumulations resulted from net

release of recently fixed PCHO.

The recent photosynthetic origin of the PCHO which accumulates through the afternoon in the marsh is indicated, but the mechanism causing its extracellular release is unknown. Phytoplankton and macroalgae contain maximal cellular CHO levels in the afternoon (Gibson, 1978; Oohusa et al., 1978) when a large proportion of their photosynthate is incorporated into PCHO (Morris and Skea, 1978). Its release during this time may be due to leakage or release of excess PCHO storage product.

During two summer marsh studies, periods of sustained PCHO release occurred in the morning from 0500 to 1100 hours (Fig. 1-C) and from 0100 to 0700 hours (Fig. 2-B). These early morning accumulations did not occur consistently and their source is unknown but they are probably caused by different processes than those responsible for the afternoon-evening PCHO peaks.

The MCHO cycles in the marsh Figs. 1 and 2) show much smaller net fluctuations relative to PCHO. Simple sugars are usually not major extracellular products of algae (Hellebust, 1974) and are probably maintained at or near the threshold concentration for heterotrophic uptake by the bacterial populations (Jannasch, 1967). This threshold concentration seems to vary seasonally between 100 and 200 ug C L^{-1} in summer and about 50 ug C L^{-1} in winter. The latter figure is similar to the minimum MCHO concentrations measured in the open North Atlantic (Burney et al., 1979).

In the MERL tank (Fig. 3), PCHO and TCHO did not show large excursions as in the marsh. With the possible exception of the June study (Fig. 3-D) there was a complete lack of the sustained afternoon

PCHO increases which were characteristic of the marsh. This may be due to lower total system net production in the tank.

In all MERL studies, there was some evidence of a metazooplankton mediated release of PCHO with peaks occurring during the night. These transient pulses may have been caused by either excretion or the sloppy feeding of zooplankton (Conover, 1966; Lampert, 1978; Burney et al., 1979). Phaeopigment accumulations resulting from herbivorous metazooplankton grazing activity (Lorenzen, 1967; Shuman and Lorenzen, 1975) appeared with PCHO peaks at 2030 in the March study (Fig. 3-A) and at 2330 and 0300 in September (Fig. 3-B). Smaller PCHO, MCHO and phaeopigment peaks occurred at 2130 in the February survey (Fig. 3-C). The ATP data in Fig. 3-A indicates a drop in the biomass of the >2 um size fraction that could have been caused by the killing of prey by metazooplankton, which was coincident with the PCHO and phaeopigment peaks at 2030. The highly significant direct correlations of the rates of change of PCHO and TCHO with those of phaeopigments (Fig. 5) further indicates the importance of zooplankton feeding and/or excretion on CHO dynamics in the tank.

In the June MERL study the bacterioplankton population also appeared to influence the PCHO levels although the relation was variable and the early morning relationship was non-significant (Fig. 6). Between 0830 and 1030, bacteria apparently grew in response to a pulse of PCHO, MCHO and DOC, but from 1230 until 2230, bacterioplankton counts and PCHO concentration were inversely related ($r=-.856$, $P=.015$). This indicates that the bacteria were highly active through most of the day and were able to rapidly respond to, and possibly conrol, the concentration of natural PCHO substrates on a

time scale of a few hours.

As expected, the overall mean CHO and DOC concentrations (Table 1) were two to three times higher in the salt marsh than in the MERL tank. Both systems also followed a seasonal pattern with the highest concentrations in summer, lowest in winter and intermediate levels in the fall samples, although MCHO and DOC deviated slightly from this in the MERL tank. The overall contribution of TCHO to the total DOC was significantly larger ($P=.012$) in the tank than in the marsh even though the September and February MERL values were lower than most of those from the marsh (Table 2). TCHO carbon accounted for more than 20% of the DOC in the tank in the March and June surveys. The tank also contained significantly higher chlorophyll a concentrations on these dates indicating that the increased proportion of TCHO in the DOC may have been associated with the increased phytoplankton biomass. The TCHO was composed primarily of PCHO in all studies (Table 2) which accounted for slightly over half to more than 80% of the total. Overall a significantly larger ($P<.001$) fraction of the TCHO was polymeric in the salt marsh than in the MERL ecosystem which agrees with the findings of Wheeler (1976) and the higher proportion of PCHO found near land in the North Atlantic (Burney et al., 1979).

A significant ($P<.05$) positive correlation between PCHO and DOC was achieved in all but one of the marsh investigations (Table 3) but in none of the MERL studies. In the marsh, other organic comounds apparently follow patterns of release and uptake similar to that of PCHO , while in the MERL tank other types of organic matter seem to fluctuate independently. MCHO showed no consistent correlation with DOC in either location, however significant positive relationships

occurred three times in the marsh and once at MERL. The factors which cause the apparent coupling of MCHO and DOC fluctuations in some instances and not in others remain obscure. The relation of MCHO to PCHO was also variable. When significance was achieved, the confidence levels were very high and some of the correlations were inverse. Inverse variations of these constituents occurred to some extent in all ten studies, regardless of the overall correlation coefficients (Figs. 7-8). This may be due in part to interconversion of PCHO and MCHO. PCHO can be converted to MCHO through bacterial extracellular enzyme hydrolysis (Khailov, 1968). Bacteria can also assimilate MCHOs and rapidly excrete large molecules made from them (Nalewajko and Lean, 1972; Dunstall and Nalewajko, 1975). Of course, interconversion cannot account for all of the opposite variation in MCHO and PCHO, especially when one increases more than the other declines. The effect could be due simply to variations in the differential rates of biological release and uptake of the two components. However, the possibility of interconversion affects the interpretation of the PCHO and MCHO rates.

Table 4 and Figures 7-8 express net TCHO flux as rates of biological release and uptake assuming that non-biological processes are insignificant on the time scales involved. The more general terms accumulation and disappearance are used for the MCHO and PCHO rates to include the possibility that they are affected by the conversion of one form to the other, as well as by true biological release and uptake. Hourly net TCHO uptake rates based on direct time series measurements (Figs. 7-8) suggest far greater <u>in situ</u> bacterial activity than is indicated by the kinetic, heterotrophic potential

approach (methods of Parsons and Strickland, 1962; Wright and Hobbie, 1966). Maximum heterotrophic potential uptake velocities (Vm) rarely exceed 1 ug C L^{-1} h^{-1} (see Hoppe, 1978) and actual rates (V) can be only 1 to 10% of Vm (Crawford et al., 1974). We frequently found TCHO uptake rates exceeding 20 ug C L^{-1} h^{-1} in summer and in the 5-10 ug C L^{-1} h^{-1} range in winter (Figs. 7-8). Studies measuring direct uptake of ^{14}C or ^{3}H labeled glucose (Dietz et al., 1976; Meyer-Reil et al., 1978) indicate maximum rates of 5 to 8 ug C L^{-1} h^{-1} from nearshore marine waters which are similar to the majority of our MERL data (Fig. 8). Daily rates of TCHO release and uptake in the salt marsh (Table 4) ranged from 85 to 520 ug C L^{-1} d^{-1} and 45 to 419 ug C L^{-1} d^{-1}, respectively, with lower rates in the fall and winter than in summer. Walsh (1965) calculated TCHO release equivalent to 265 ug C L^{-1} d^{-1} in a 2 m basin of a productive coastal pond (August) which is in the range of our summer values. Rates of PCHO accumulation and disappearance were 2.5 to 8.5 and 1.1 to 14.4 times greater than the respective rates for MCHO. TCHO flux in the MERL tank was considerably lower than in the marsh, ranging from 16 to 109 ug C L^{-1} d^{-1} for net release and 31 to 131 ug C L^{-1} d^{-1} for net uptake. PCHO rates were again higher than those of MCHO but not by as much as in the marsh, with the former exceeding the latter by factors of 1.1 to 4.5 for accumulation and 1.8 to 4.4 for disappearance.

In all cases, the sum of the rates of accumulation and disappearance of MCHO and PCHO exceed the corresponding rates of TCHO release and uptake. Sometimes the PCHO rates alone are greater than

the associated TCHO flux. This happens because the hourly TCHO rates, from which the daily values are calculated, are the algebraic sums of the hourly MCHO and PCHO rates (Figs. 7-8). When the latter values are of opposite sign, one or both of their absolute values must exceed that of their sum.

The estimates of net daily production and respiration (Table 4) present a problem. Although several studies are in fair agreement, all of the values based on CO_2 flux exceed (sometimes drastically) those based on O_2 rates. This happened even in the last MERL survey and all of the marsh studies where TCO_2 (or pH) and O_2 showed the expected highly significant ($P<.01$) correlations. The discrepancy was greatest in the first MERL investigation (Fig. 4-A) where unknown processes caused O_2 and CO_2 to vary directly through much of the study. With the possible exception of this case, we believe the values based on TCO_2 measurements to be more accurate since TCO_2 is less affected by atmospheric exchange (Smith, 1973; Johnson et al., 1979) than is oxygen. TCO_2 rates do not require the assumption of photosynthetic and respiratory quotients for conversion to carbon units. This subject will be discussed in more detail elsewhere (Johnson et al., in prep.).

In the salt marsh, net daily TCHO release accounted for 9 to 33% of the net DOC release and 10 to 63% of the DOC uptake (Table 5), with the largest fractions found in summer, smallest in winter, and intermediate values in fall. Net TCHO release amounted to 2-11% of the net system production based on CO_2 flux. Walsh (1965) estimated that from 14 to 44.9% of assimilated carbon was released as TCHO in Oyster Pond (summer), however his O_2 based production

values were considerably lower, and his CHO method was less reliable than ours (Burney and Sieburth, 1977). We estimate that daily TCHO uptake accounted for 2 to 12% of the net system respiration in the marsh.

The contribution of TCHO to the daily DOC flux showed extreme seasonal variability in the MERL tank (Table 5). TCHO accounted for from 4 to 101% of the net DOC release and from 7 to 92% of its net uptake. Values exceeding 100% are possible and indicate that another important fraction of the DOC varied inversely with the TCHO over part of the study. This lack of a direct apparent coupling of the DOC and TCHO flux in the simulated estuary appears to indicate a functional dissimilarity between this system and the marsh. The June rates of net TCHO release and uptake in the tank (Table 5) constituted the largest fractions of the net production and respiration found in any of the studies at either location. The low molar ratios of nitrate plus ammonia to phosphate (0.5 to 2.6) in this study (Burney, unpublished) may indicate nitrogen limitation which is known to increase CHO excretion by phytoplankton (Myklestad and Haug, 1972).

The multi-parameter diel sampling of confined water masses at 2 to 3 h intervals can yield useful information on the interaction of ecosystem components as well as on their net rates of change. As discussed by Sournia (1974), the ecological significance of fluctuations over diel cycles may be at least as important as those over annual periods. This study sought to contribute to our knowledge of the role of dissolved organic matter in the sea by studying the in situ diel flux of one of its biologically labile constituents, dissolved carbohydrate. This approach has also been extended to the

open sea and reports are in preparation.

ACKNOWLEDGEMENTS

I thank K.Johnson for analysis of the DOC, CO_2 and some of the MCHO samples, J. Fontaine for the bacterioplankton counts, R. Heffernan for the ATP data and S. Nixon for the loan of the culvert plug used in the marsh work. Oxygen and pigment data from the first MERL study were provided by S. Nixon and G. Vargo, respectively. J. Burney, P. Davis, J. Fontaine, G. Hammond, R. Heffernan, K. Johnson and E. Lessard assisted with field sampling. The comments and discussion of J. Sieburth, S. Nixon and M. Pilson are gratefully acknowledged. This work was supported by the Biological Oceanography Program of the National Science Foundation through grants OCE-7681779 and OCE-7826388 to J.McN. Sieburth.

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DIEL DEPENDENCE OF DISSOLVED CARBOHYDRATE CONCENTRATIONS UPON THE POPULATIONS OF NANOPLANKTON AND BACTERIOPLANKTON IN THE WESTERN SARGASSO SEA

ABSTRACT

Significant correlations between dissolved polysaccharide (PCHO) and total carbohydrate (TCHO) concentrations and components of the less than 20 um microbial plankton have been found at two stations in the western Sargasso Sea. These were sampled at three or four hour intervals, over diel cycles while following a drogued buoy. An attempt was made to sample on two pre-selected isotherms (approximating isopycnals) at each station. Significant inverse simple correlations of TCHO and PCHO with phototrophic nanoplankton (PNAN) populations were found along both isotherms at a station off the Carolina coast. Off central Florida, TCHO and/or PCHO were significant multiple linear functions of PNAN, heterotrophic bacterioplankton (BAC) and sometimes heterotrophic nanoplankton (HNAN) counts. Partial regression coefficients for PNAN were always negative and those for HNAN were positive. BAC was not consistent in this respect but the dominant trend was toward inverse relationships. The combined data from these two stations (n=34) produced very similar multiple linear relations. PNAN and sometimes HNAN excretion appear to be important sources of dissolved carbohydrate. No correlation between dissolved carbohydrate and any of the less than 20 um plankton components was found in the continental slope water entrained in a recently formed Gulf Stream cold core ring but a direct correlation between DOC and phaeopigments was found which suggests a relationship between metazooplankton excretion and DOC release.

INTRODUCTION

The bulk of the dissolved organic matter (DOM) in the open sea results from in-situ phytoplankton production (Duursma, 1961; Degens, 1970). Although some controversy remains over the amounts, it appears that roughly 25% of phytoplankton production may enter the dissolved pool (Sieburth, 1976, 1979) through a combination of active excretion (Fogg, 1977), autolysis (Sharp, 1977) and the feeding and excretion of consumers (Lampert, 1978; Burney et al., 1979). Most of this material appears to be rapidly consumed by bacterioplankton (Larsson and Hagstrom, 1979). Because the producers, releasers and consumers are all members of the microbial plankton, it is reasonable to expect that a set of microbiological parameters could be identified which would account for a significant fraction of the DOM fluctuations observed in the field. Since a large part of the total DOM consists of poly-condensed humic materials (Degens, 1970) which are not direct products of the phytoplankton and appear to be relatively resistant to bacterial attack (Williams, 1971), it is important to study a class of primary metabolites such as carbohydrates rather than the total DOC.

The concentration of total dissolved carbohydrate (TCHO) in natural waters is definitely influenced by in-situ biological processes. Levels decline during phytoplankton blooms and increase afterwards (Wangersky, 1959; Walsh, 1965a). TCHO and chlorophyll a levels in Oyster Pond (Cape Cod, MA) were inversely correlated over an annual cycle when sampled in the morning at weekly intervals (Walsh, 1966). A significant direct correlation was found between bacterial numbers (direct counts) and glucose concentrations in a seasonal study of waters overlying sandy beaches (Meyer-Reil et al., 1978).

Bacterial number and biomass were inversely related to glucose and fructose turnover times in a diel study in the Baltic (Meyer-Reil et al., 1979) but not with measured glucose or fructose concentrations.

While these studies suggest the importance of particular groups within the microbial plankton, an integrated attempt to relate in-situ carbohydrate flux to plankton dynamics must consider the dominant phototrophic, phagotrophic and osmotrophic components of the microbial community, because of their interdependence (Sieburth et al., 1978; Sieburth, 1979). In a preliminary study in the North Atlantic (Burney et al., 1979) vertical profiles showed TCHO peaks associated with accumulations of >3 um sized microorganisms which were low in chlorophyll a, suggestive of protozooplankton, while profiles of bacterial sized ATP (<3 um) usually showed maxima at the depths of TCHO minima suggesting an inverse relationship. Biological processes affecting CHO flux in two coastal ecosystems have been suggested (No. 1, this series). This report presents results of an oceanic diel study in which carbohydrate concentrations are related by linear regression analysis to the dominant microbial populations of the three trophic groups, the heterotrophic nanoplankton (HNAN), phototrophic nanoplankton (PNAN), and heterotrophic bacterioplankton (BAC).

MATERIALS AND METHODS

Niskin bottle samples, from the depths of two isotherms, were taken every 3 or 4 h over 24 or 48 h periods at each of three diel stations in the western North Atlantic (5-21 June 1977) while following a parachute drogued buoy (Volkmann et al., 1956) during R/V Endeavor Cruise 009. Station 1 was located near the center of a Gulf Stream cold core ring. Station 2 was in the western Sargasso Sea off North Carolina and Station 3 was just east of the Gulf Stream off the coast of central Florida. These locations are shown in Fig. 1 with the diel stations 1, 2 and 3 corresponding to stations 4, 8 and 13, respectively, in the ship's log. Prior to the start of each diel study, multiple samples from a hydrocast through the euphotic zone were analyzed for dissolved oxygen, chlorophyll a, phaeopigments, particulate ATP (<1 um) and dissolved monosaccharide (MCHO) in order to locate biologically active layers on which to conduct the diel investigations. The temperatures of each selected layer were determined from a simultaneous Sippican (Marion, MA) expendable bathythermograph (XBT) profile. The depths of these isotherms were relocated by XBT prior to each sampling and Niskin bottles were vertically positioned by winch meter readings. Table 1 gives ship positions at the beginning and end of each station, isotherm temperatures and biological characteristics for each sampled layer. The shallower and deeper isotherms at each station are designated S and D, respectively. No data from station 1-D are presented due to accidental sample loss. By sampling on isotherms (approximating isopycnals) rather than isobaths, we attempted to monitor true biological dynamics rather than fluctuations caused by internal wave

passage (Denman, 1977). To clean the samplers and prevent neuston contamination, the Niskin bottles were scrubbed in N/10 HCl before each cast (Burney et al., 1979), rinsed rapidly by lowering to 200 m, and then raised to sampling depth and closed.

Samples for carbohydrate and DOC analyses (100 ml) were immediately filtered by gravity though precombusted (480°C, 12 h) Gelman glass fiber filters into precombusted glass bottles and stored at -20°C for analysis ashore. The glass filter holders were soaked in N/10 HCl between casts and thoroughly rinsed with distilled water and at least 100 ml of the next sample before reuse. Each sample was analyzed for MCHO in quintuplicate and TCHO in quadruplicate by the methods of Johnson and Sieburth (1977) and Burney and Sieburth (1977), respectively. Polysaccharide (PCHO) concentrations were determined by difference. DOC was analyzed in quintuplicate by the method of Menzel and Vaccaro (1964) as modified by Kerr and Quinn (1975) using an Oceanography International ampule panel and a Beckman 315 infrared CO_2 analyzer.

Samples for bacterioplankton (BAC) and nanoplankton (NAN) enumeration were passed through 20 um Nytex screen and stored in 2% formalin at 5°C for a maximum of two days. Aliquots were stained with a 0.1% (W:V) acridine orange solution (Hobbie, Daley and Jasper, 1977) for 3 min in a 15-ml capacity Millipore filter chimney and then drawn through 0.2 um or 1.0 um Nuclepore filters prestained with irgalan black (Watson et al., 1977) for BAC or NAN counting, respectively. Cells on the filters were counted using an Olympus (Tokyo) Vanox epifluorescence microscope at 1000x for BAC and 400x for NAN. Phototrophic nanoplankton (PNAN) were enumerated by counting the

autofluorescent cells in unstained preparations. At least 300 cells were counted for each determination. Numbers of heterotrophic nanoplankton (HNAN) were calculated from the difference between PNAN and the total acridine orange stained NAN counts.

Chlorophyll a and phaeopigments were determined fluorometrically on 90% acetone extracts (Strickland and Parsons, 1968) using an Aminco (Silver Spring, MD) fluoro-colorimeter. Particulate ATP which was passed and retained by 1 um and 0.2 um pore size Nuclepore filters, respectively, was analyzed at sea with a DuPont (Wilmington, DE) luminescent biometer and enzyme system (Burney et al., 1979). Salinity was determined by induction salinometer (Plessy model 6230N, San Diego, CA) and dissolved oxygen by the Winkler method (Carritt and Carpenter, 1966). Linear correlation and regression analysis was used to determine the relation of the biological data to each of the CHO and DOC variables.

Figure 1: The track of R/V Endeavor Cruise 009 showing all sampling locations. The diel stations 1, 2 and 3 in this report correspond to stations 4, 8 and 13 in the ships log.

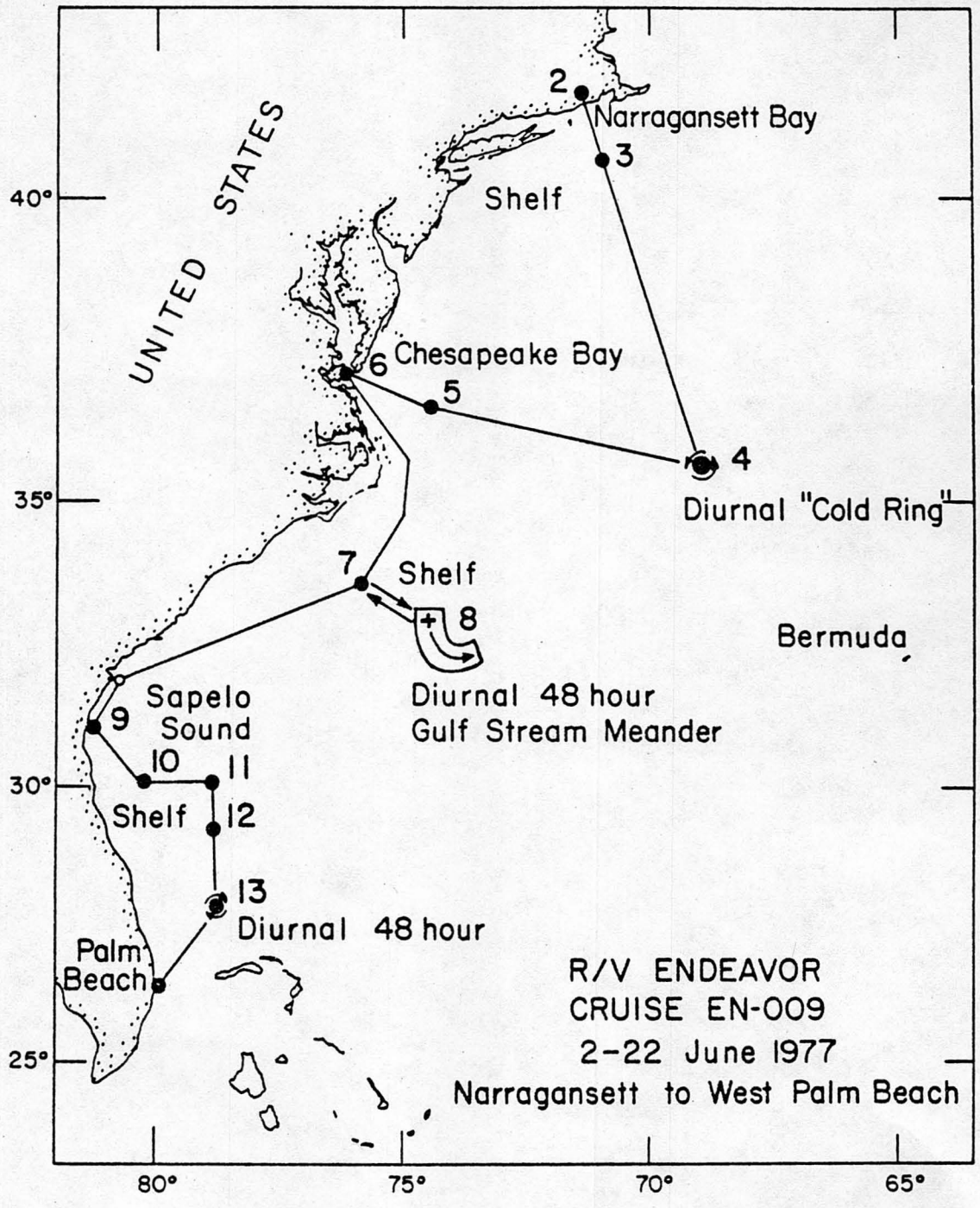
UNITED STATES
2
Narragansett Bay
3
Shelf
40°
6
Chesapeake Bay
5
4
Diurnal "Cold Ring"
35°
7
Shelf
8
Bermuda
Diurnal 48 hour
Gulf Stream Meander
Sapelo
Sound
9
10
11
30°
Shelf
12
13
Diurnal 48 hour
Palm
Beach
R/V ENDEAVOR
CRUISE EN-009
2-22 June 1977
Narragansett to West Palm Beach
25°
80°
75°
70°
65°

Figure 1

Table 1. Station data and layer characteristics.

Station*/ Area	Date (1977)	Time (local)	Position	Date (1977)	Time (local)	Position	Layer	Isotherm temp. (°C)	Biological Characteristics of layer
1 Gulf Stream cold core ring	6 June	1000	35°40.1'N 69°20.0'W	7 June	0000	35°31.6'N 68°47.7'W	S	21.7	ATP maximum
2 Western Sargasso Sea	12 June	0600	32°41.2'N 74°31.6'W	14 June	0600	32°07.0'N 73°52.0'W	S D	20.8 19.6	ATP maximum Chlorophyll *a* maximum
3 East of Gulf Stream off central Florida	19 June	1000	27°42.0'N 78°56.1'W	20 June	1000	27°44.7'N 78°59.3'W	S D	26.5 24.6	MCHO maximum ATP maximum

*Stations 1, 2 and 3 correspond to stations 4B, 8B and 13B, respectively, in the ship's log.

RESULTS

Figures 2-6 present results from stations 1-S, 2-S, 2-D, 3-S and 3-D. Figure 3 presents two consecutive 24 hr studies. Mean standard errors for the TCHO, MCHO and DOC analyses were 4.0 $\pm$2.1, 2.7 $\pm$1.5, and 56 $\pm$19 ug C L^{-1}, respectively. Standard errors for the chlorophyll *a* and phaeopigment determinations averaged .01$\pm$.01 ug L^{-1}. For the PNAN, HNAN and BAC, mean values $\pm$15% approximates 99% confidence intervals based on a total count of at least 300 cells per determination (Cassell, 1965). DOC and TCHO curves were similar in all cases except for station 2-D and the last 24 h of station 2-S (Figs. 3-B and 4). None of the CHO or DOC data were significantly ($P<.05$) correlated with salinity but the TCHO-salinity relation in Fig. 1 (station 1-S) was significant at the .94 level. Free bacterioplankton numbers were on the order of 10^8 L^{-1} while PNAN and HNAN counts were on the order of 10^6 L^{-1}. All three groups showed substantial fluctuations except for BAC at station 1-S (Fig. 2). Figure 7 shows the direct relation of DOC and phaeopigment concentrations found only at station 1. Figures 8-14 illustrate simple and multiple correlations of TCHO and PCHO concentrations with PNAN, BAC and HNAN populations from both layers at stations 2 and 3. No significant correlations between these parameters occurred at station 1. Figures 8 and 9 show the relationships of TCHO and PCHO to PNAN at station 2-S and 2-D, respectively. The slopes were all negative and significantly different from zero ($P<.05$). Figure 10 illustrates simple correlations of PCHO with PNAN and with BAC at station 3-S. Neither slope significantly differs from zero, however the multiple regression of PNAN and BAC on PCHO (Fig. 11) accounts for

a highly significant fraction of the observed PCHO variance. The partial regression coefficients for PNAN and BAC were negative and positive, respectively, and both were significantly different from zero ($P<.05$). Figure 12 illustrates the simple correlations of TCHO and PCHO with PNAN, BAC and HNAN at station 3-D. None of the individual relationships was significant, however multiple regressions including all three plankton groups (Fig. 13) accounted for over 90% of the observed PCHO and TCHO variance. Partial regression coefficients in both relationships were negative for PNAN and BAC and positive for HNAN. Similar relationships (excluding the absolute values of the regression coefficients) were found in the combined data from stations 2 and 3 (Fig. 14) except that the HNAN term was nonsignificant in the PCHO regression. Although these relationships were highly significant, they both tended to underestimate the observed TCHO and PCHO levels at high concentrations and overestimate them at low concentrations.

Figure 2: Comparison of the diel cycles of dissolved carbohydrates and organic carbon with those of the microbial plankton and pigments at station 1-S (21.7°C isotherm).

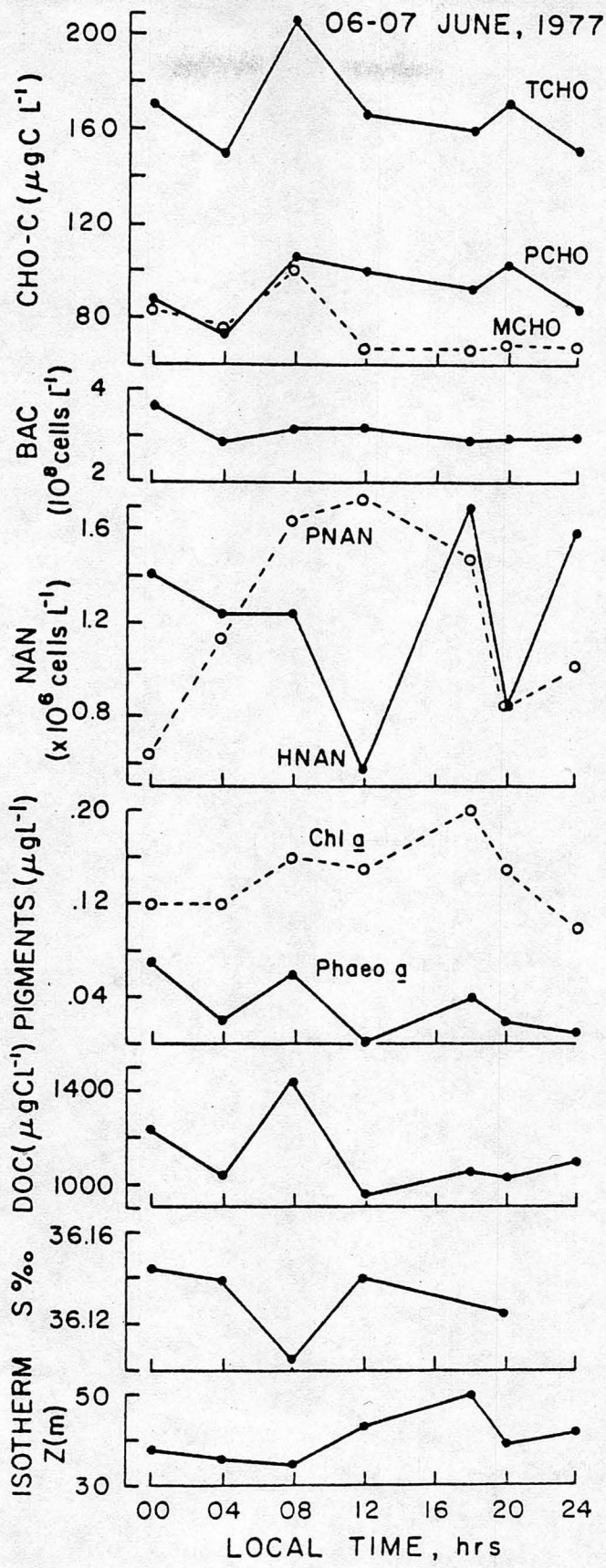
06-07 JUNE, 1977
CHO-C (μg C l⁻¹)
200
160
120
80
TCHO
PCHO
MCHO
BAC (10⁸ cells l⁻¹)
4
2
NAN (x 10⁶ cells l⁻¹)
1.6
1.2
0.8
PNAN
HNAN
PIGMENTS (μg l⁻¹)
.20
.12
.04
Chl a
Phaeo a
DOC (μg Cl⁻¹)
1400
1000
S ‰
36.16
36.12
ISOTHERM Z(m)
50
30
00
04
08
12
16
20
24
LOCAL TIME, hrs

Figure 2

Figure3: Comparison of two consecutive diel cycles of dissolved carbohydrates and organic carbon with those of the microbial plankton and pigments at station 2-S (20.8^{o}C isotherm).

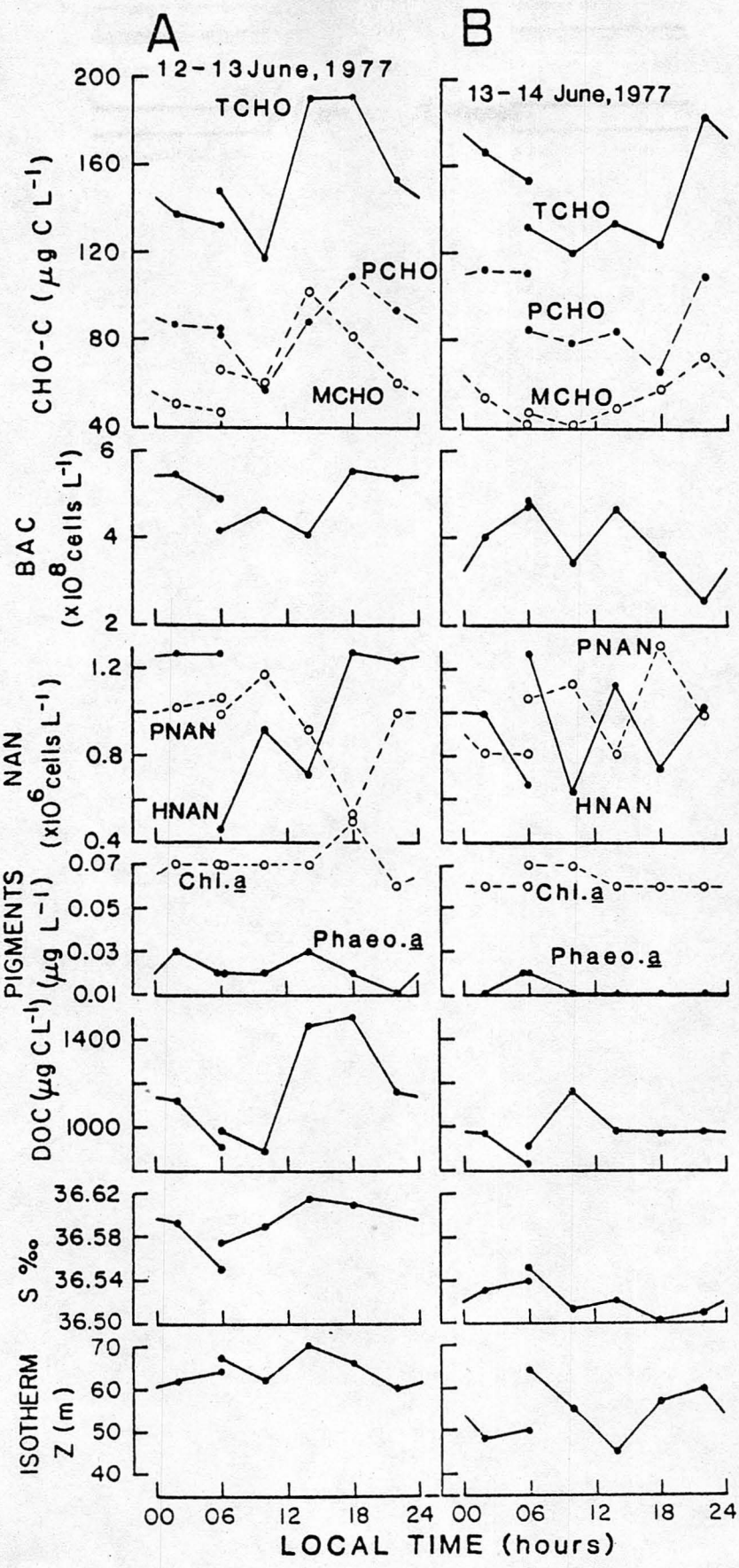
A
B
12-13 June, 1977
13-14 June, 1977
TCHO
PCHO
MCHO
CHO-C (μg C L⁻¹)
BAC (x10⁸ cells L⁻¹)
PNAN
HNAN
NAN (x10⁶ cells L⁻¹)
Chl.a
Phaeo.a
PIGMENTS (μg L⁻¹)
DOC (μg C L⁻¹)
S ‰
ISOTHERM Z (m)
LOCAL TIME (hours)

Figure 3

Figure 4: Comparison of the diel cycles of dissolved carbohydrates and organic carbon with those of the microbial plankton and pigments at station 2-D (19.6^{o}C isotherm).

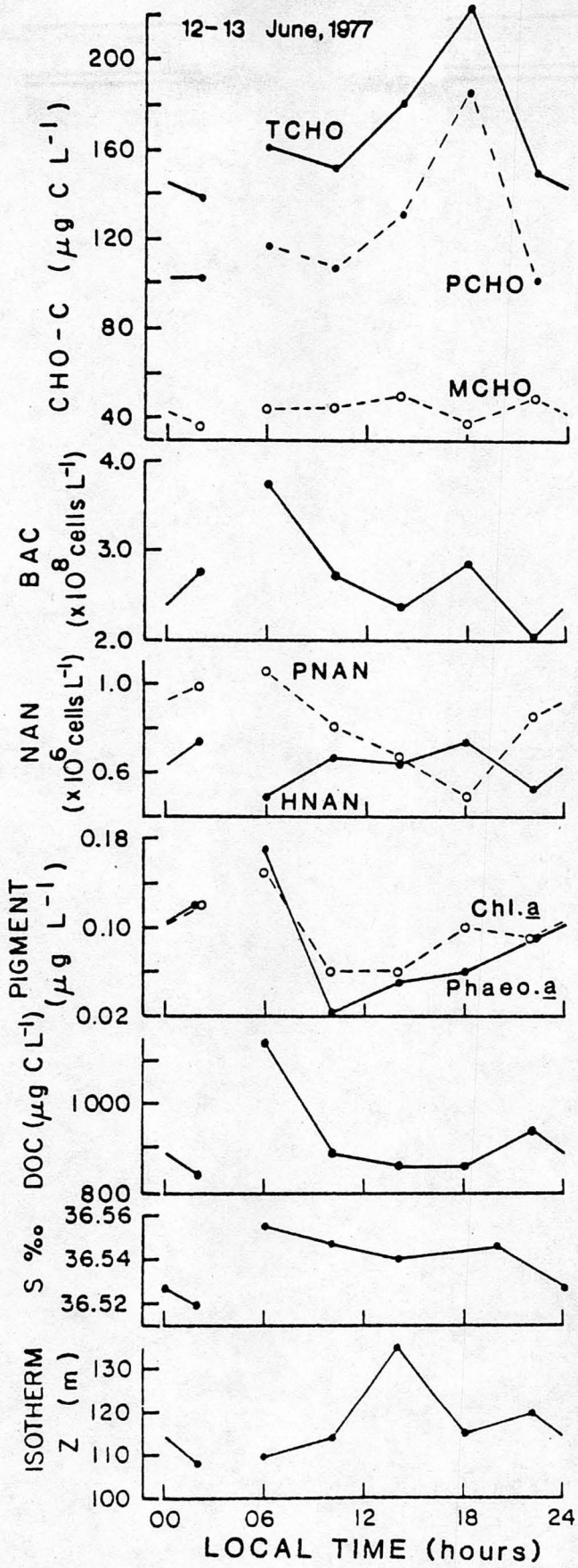
12-13 June, 1977
CHO-C (μg C L⁻¹)
200
160
120
80
40
TCHO
PCHO
MCHO
BAC (x10⁸cells L⁻¹)
4.0
3.0
2.0
NAN (x10⁶cells L⁻¹)
1.0
0.6
PNAN
HNAN
PIGMENT (μg L⁻¹)
0.18
0.10
0.02
Chl.a
Phaeo.a
DOC (μg C L⁻¹)
1000
800
S ‰
36.56
36.54
36.52
ISOTHERM Z (m)
130
120
110
100
00
06
12
18
24
LOCAL TIME (hours)

Figure 4

Figure 5: Comparison of the diel cycles of dissolved carbohydrates and organic carbon with those of the microbial plankton and pigments at station 3-S (26.5°C isotherm).

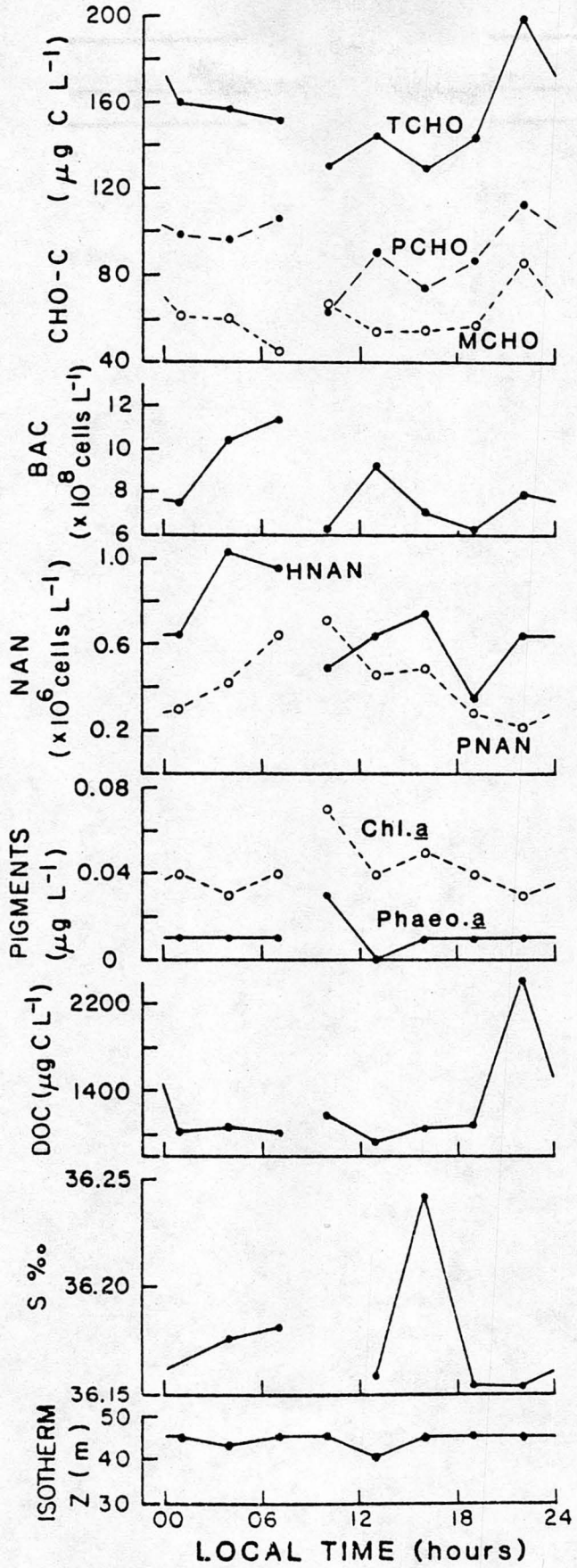
200
160
120
80
40
CHO-C (μg C L⁻¹)
TCHO
PCHO
MCHO
12
10
8
6
BAC (x 10⁸ cells L⁻¹)
1.0
0.6
0.2
NAN (x10⁶ cells L⁻¹)
HNAN
PNAN
0.08
0.04
0
PIGMENTS (μg L⁻¹)
Chl.a
Phaeo.a
2200
1400
DOC (μg C L⁻¹)
36.25
36.20
36.15
S ‰
50
40
30
ISOTHERM Z (m)
00
06
12
18
24
LOCAL TIME (hours)

Figure 5

Figure 6: Comparison of the diel cycles of dissolved carbohydrates and organic carbon with those of the microbial plankton and pigments at station 3-D (24.6°C isotherm).

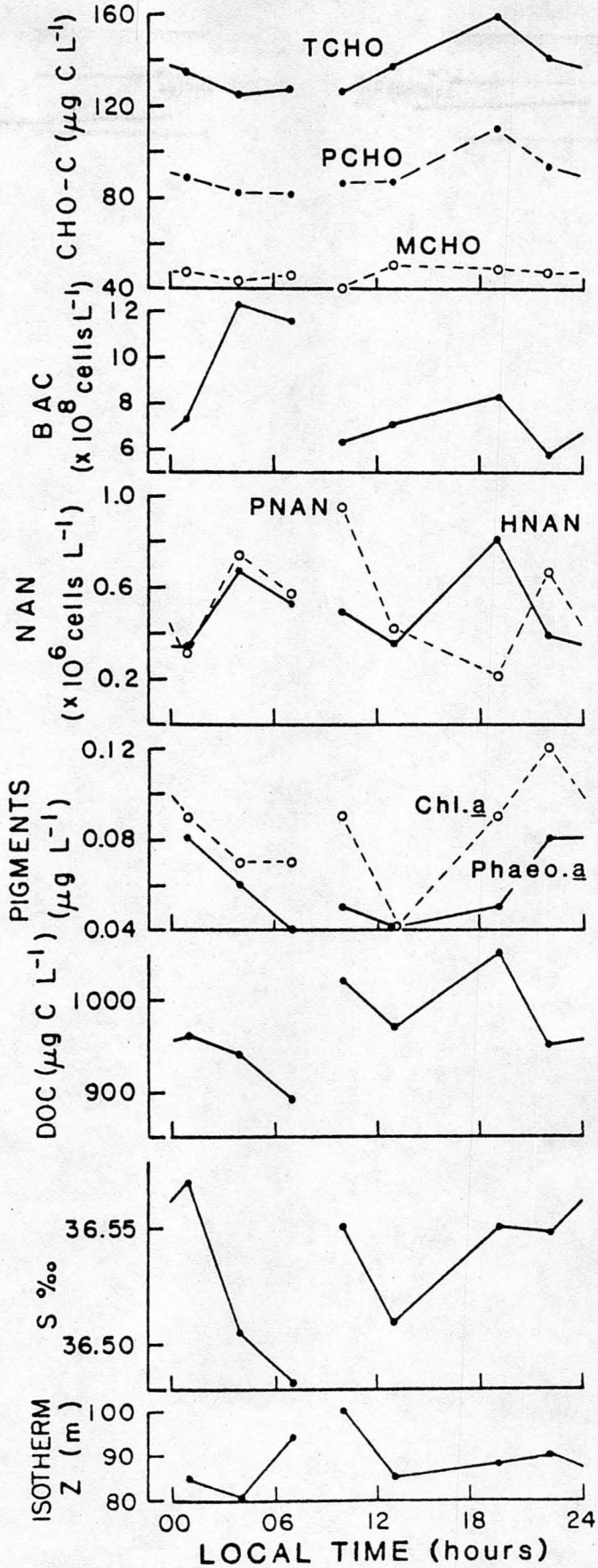
CHO-C (μg C L⁻¹)
160
120
80
40
TCHO
PCHO
MCHO
BAC (x 10⁸ cells L⁻¹)
12
10
8
6
NAN (x 10⁶ cells L⁻¹)
1.0
0.6
0.2
PNAN
HNAN
PIGMENTS (μg L⁻¹)
0.12
0.08
0.04
Chl.a
Phaeo.a
DOC (μg C L⁻¹)
1000
900
S ‰
36.55
36.50
ISOTHERM Z (m)
100
90
80
00
06
12
18
24
LOCAL TIME (hours)

Figure 6

Figure 7: The direct relationship between DOC and particulate phaeopigments (Phaeo. a) levels at station 1-S.

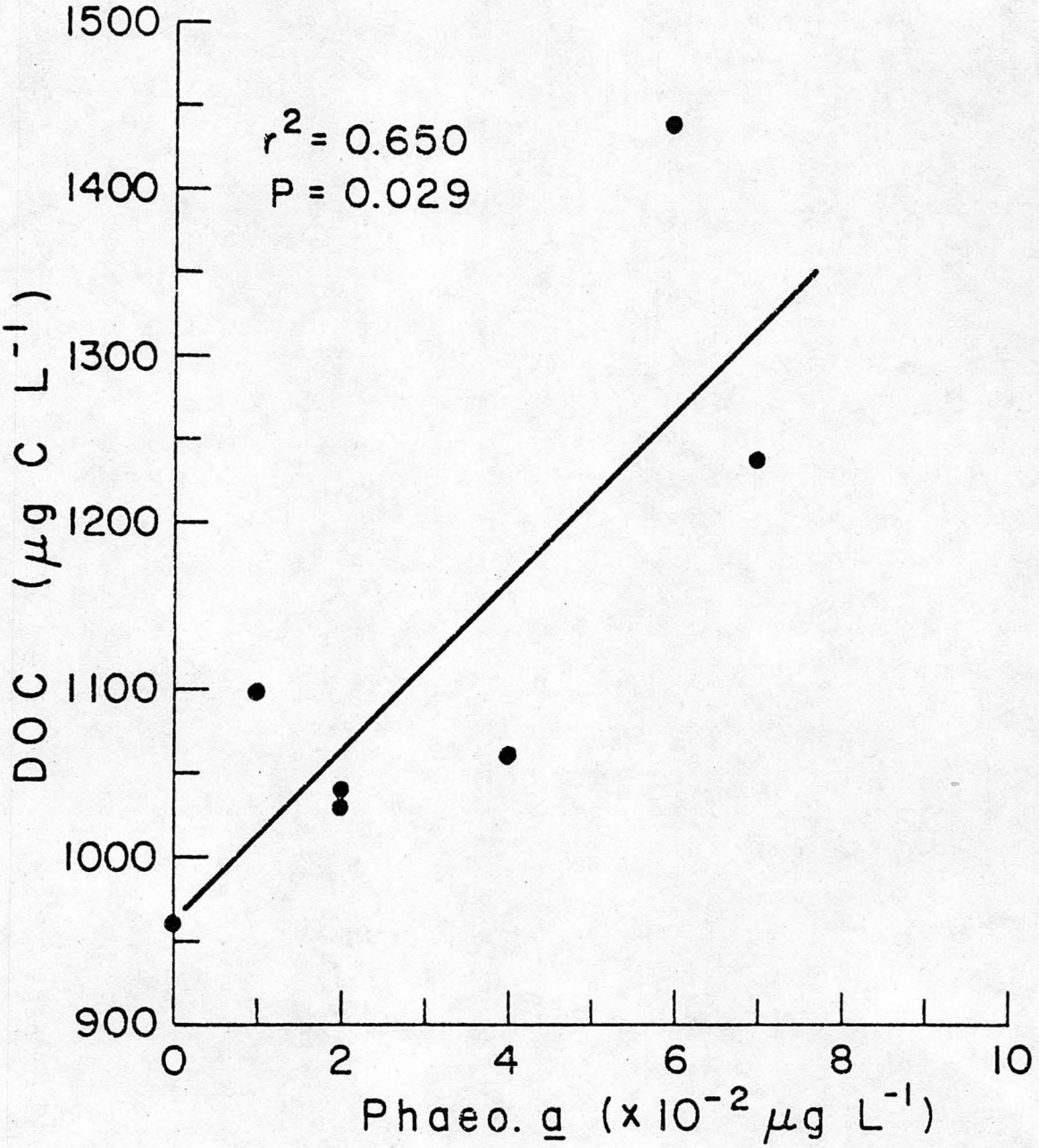
1500
1400
1300
1200
1100
1000
900
DOC (μg C L⁻¹)
r² = 0.650
P = 0.029
0
2
4
6
8
10
Phaeo. a (x 10⁻² μg L⁻¹)

Figure 7

Figure 8: The inverse relations of PNAN populations to TCHO and PCHO concentrations at station 2-S.

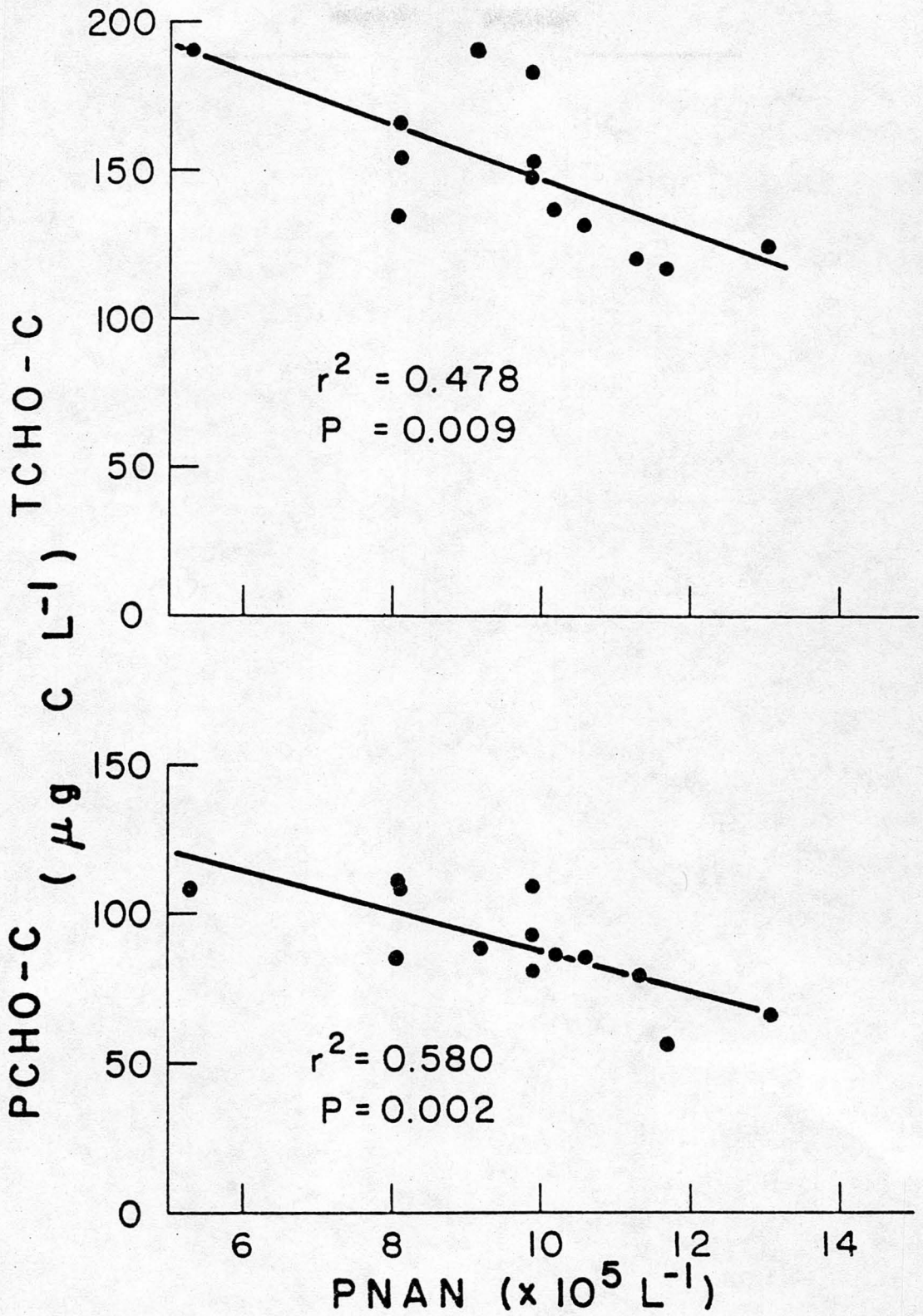
200
150
100
50
0
TCHO-C
r^2 = 0.478
P = 0.009
150
100
50
0
PCHO-C (μg C L^{-1})
r^2 = 0.580
P = 0.002
6
8
10
12
14
PNAN (x 10^5 L^{-1})

Figure 8

Figure 9: The inverse relations of PNAN populations to TCHO and PCHO concentrations at station 2-D.

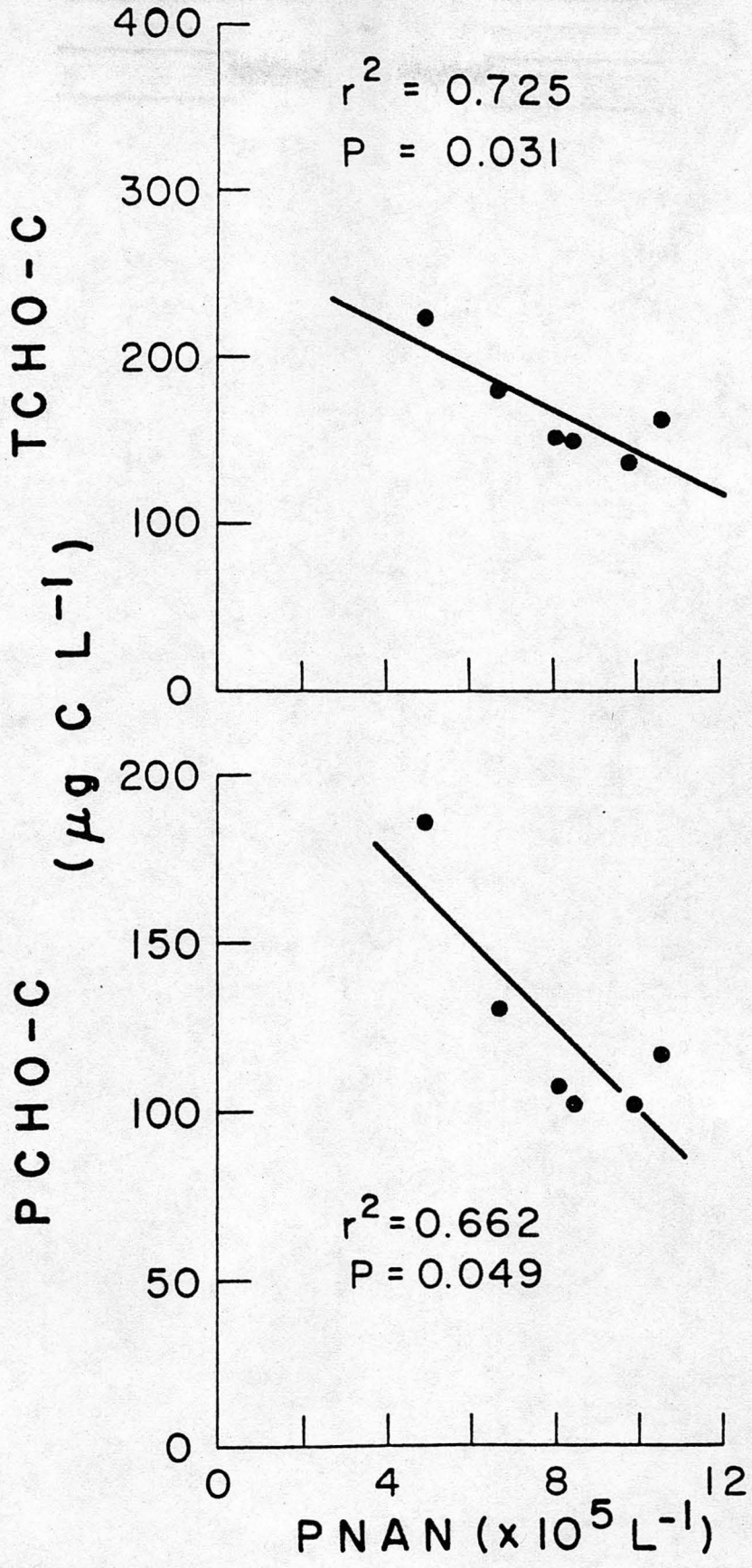
400
300
200
100
0
TCHO-C
r^2 = 0.725
P = 0.031
200
150
100
50
0
PCHO-C (μg C L^{-1})
r^2=0.662
P = 0.049
0
4
8
12
PNAN (x 10^5 L^{-1})

Figure 9

Figure 10: The relations of PCHO to PNAN and BAC populations at station 3-S.

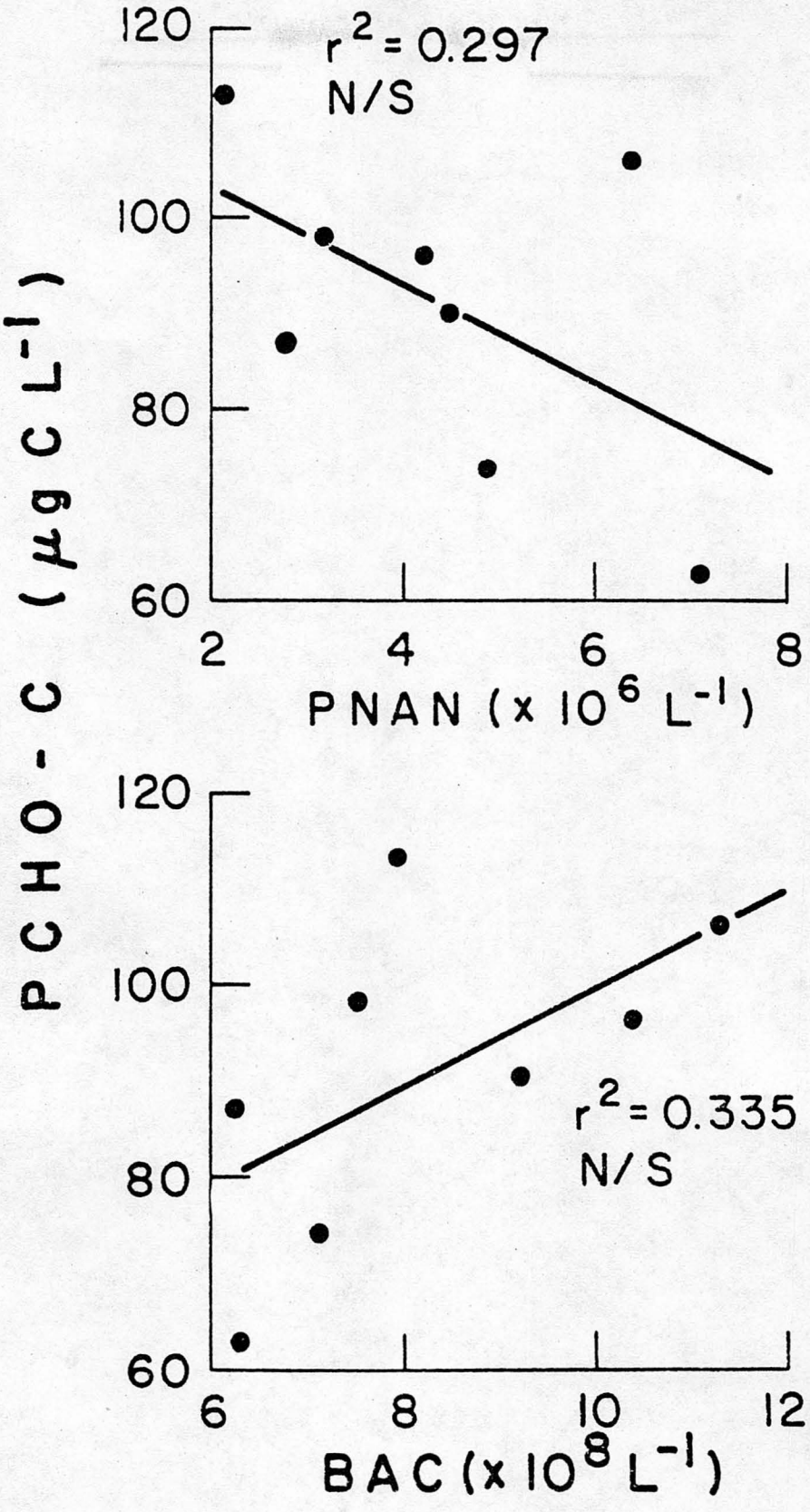
120
100
80
60
$r^2 = 0.297$
N/S
2
4
6
8
PNAN ($\times 10^6$ L^{-1})
PCHO-C (μg C L^{-1})
120
100
80
60
$r^2 = 0.335$
N/S
6
8
10
12
BAC ($\times 10^8$ L^{-1})

Figure 10

Figure 11: The relation of observed PCHO concentrations to those estimated from the multiple regression of PCHO on PNAN (L^{-1}) and BAC (L^{-1}) at station 3-S. The line represents the ideal 1:1 relationship.

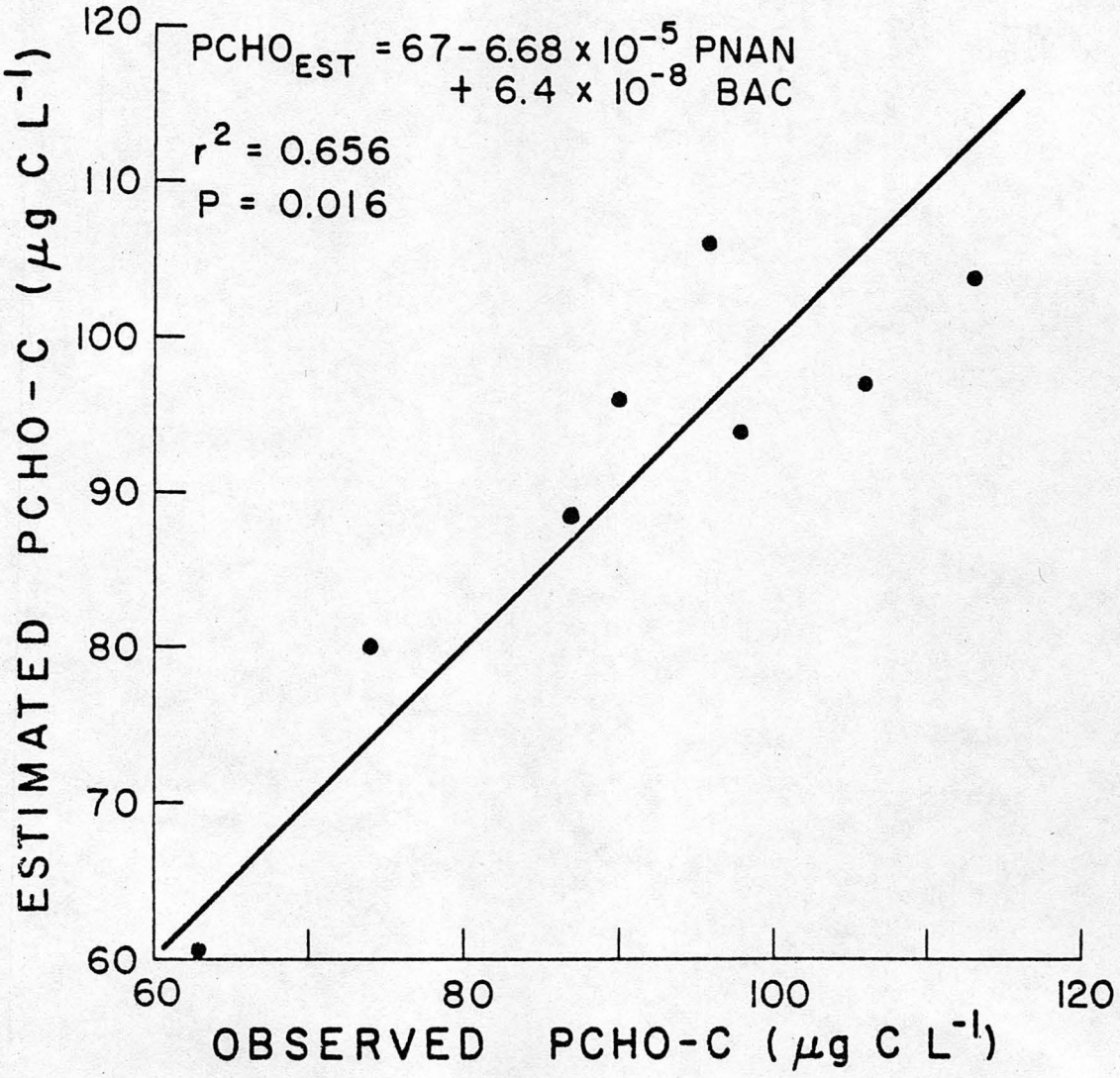
PCHO$_{EST}$ = 67 - 6.68 x 10^{-5} PNAN
+ 6.4 x 10^{-8} BAC
r^2 = 0.656
P = 0.016
ESTIMATED PCHO-C (μg C L^{-1})
OBSERVED PCHO-C (μg C L^{-1})
120
110
100
90
80
70
60
60
80
100
120

Figure 11

Figure 12: The relations of TCHO and of PCHO concentrations to PNAN , BAC and HNAN populations at station 3-D.

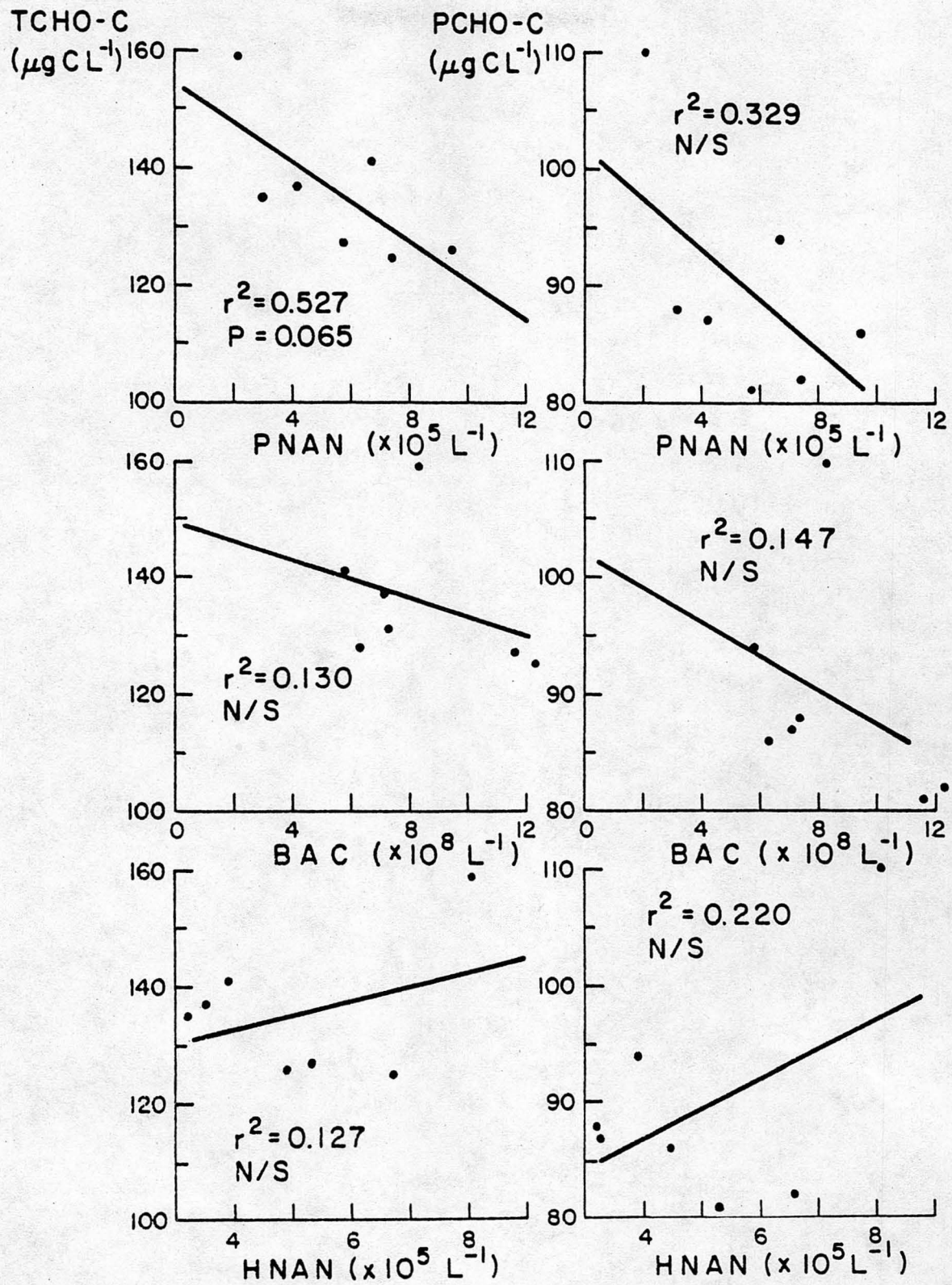
TCHO-C
(μg C L⁻¹)
PCHO-C
(μg C L⁻¹)
r²=0.527
P = 0.065
r²=0.329
N/S
PNAN (x10⁵ L⁻¹)
r²=0.130
N/S
r²=0.147
N/S
BAC (x10⁸ L⁻¹)
r²=0.127
N/S
r²=0.220
N/S
HNAN (x10⁵ L⁻¹)

Figure 12

Figure 13: The relations of observed TCHO (filled circles) and PCHO (open circles) concentrations to those estimated from PNAN (L^{-1}), BAC (L^{-1}) and HNAN (L^{-1}) populations by their respective multiple linear regression equations at station 3-D. The line represents the ideal relationship.

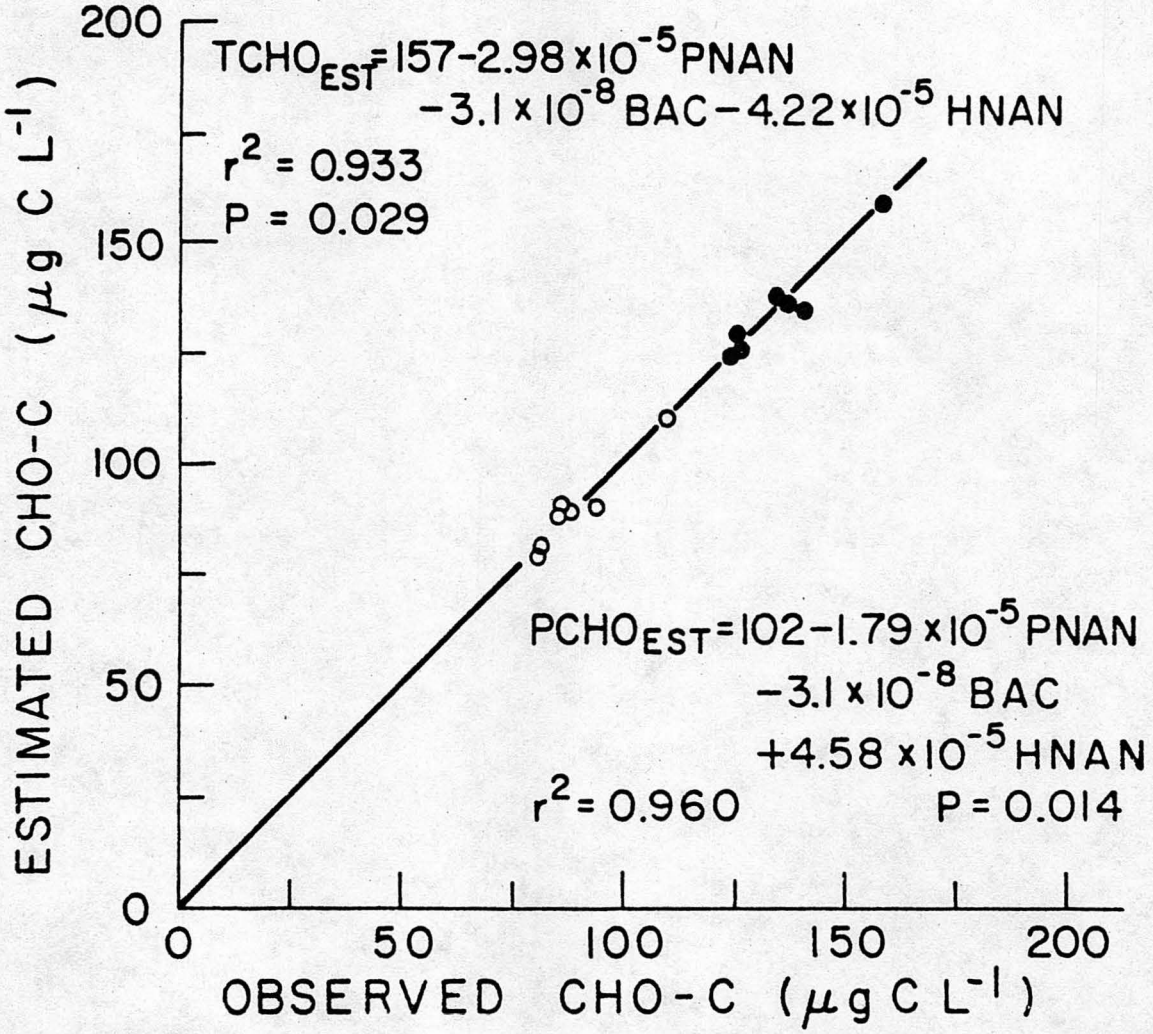
TCHO$_{EST}$=157-2.98×10^{-5}PNAN
-3.1×10^{-8}BAC-4.22×10^{-5}HNAN
r^2 = 0.933
P = 0.029
PCHO$_{EST}$=102-1.79×10^{-5}PNAN
-3.1×10^{-8}BAC
+4.58×10^{-5}HNAN
r^2= 0.960
P = 0.014
ESTIMATED CHO-C (μg C L^{-1})
OBSERVED CHO-C (μg C L^{-1})
0
50
100
150
200

Figure 13

Figure 14: The relationships of the observed TCHO (filled circles) and PCHO (open circles) concentrations to those estimated from the microbial plankton populations by their respective multiple linear regression equations for the combined EN-009 data. The dotted line represents the ideal 1:1 relationships. Solid lines are the least-squares regression fits to the data.

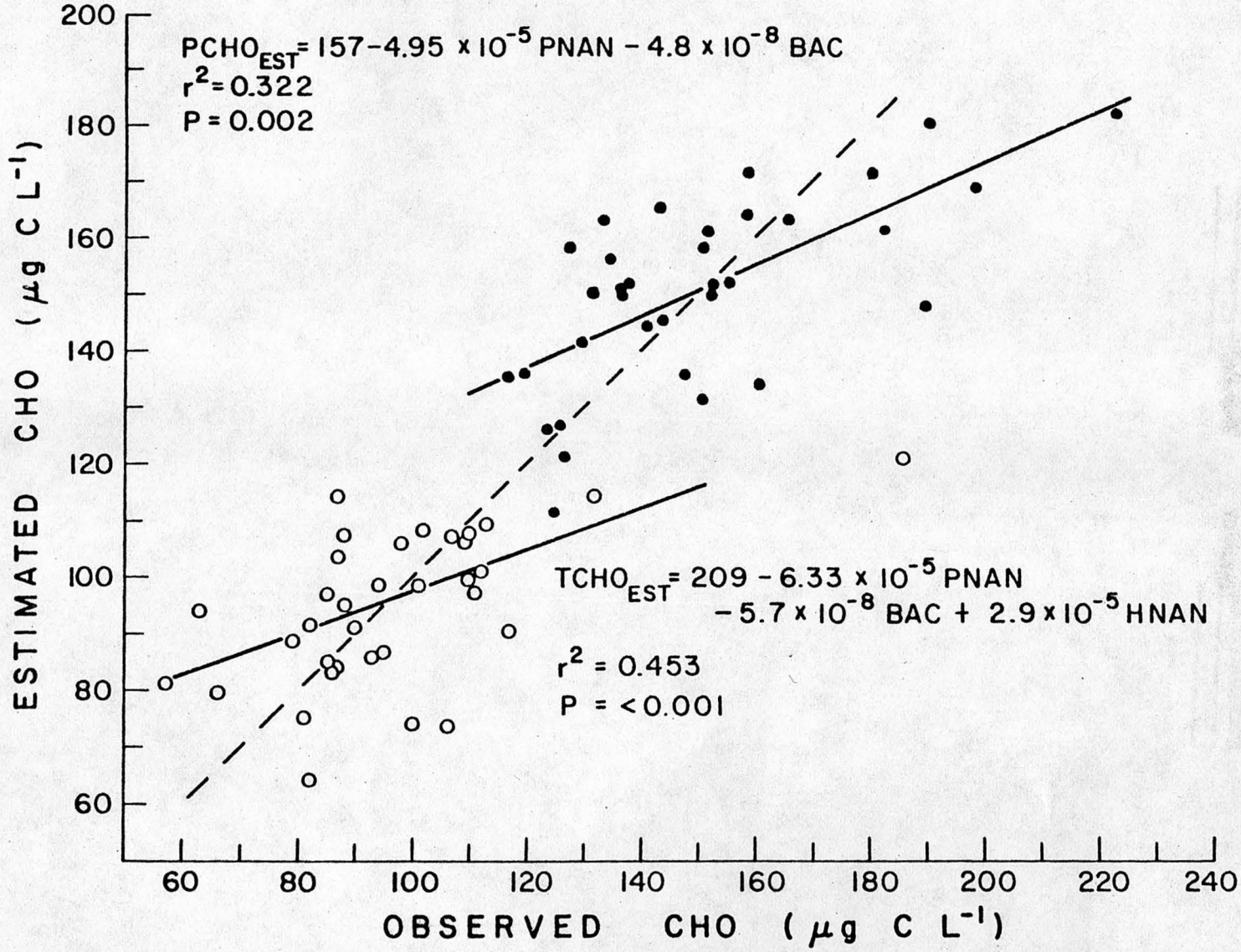
PCHO$_{EST}$ = 157 - 4.95 x 10^{-5} PNAN - 4.8 x 10^{-8} BAC
r^2 = 0.322
P = 0.002
TCHO$_{EST}$ = 209 - 6.33 x 10^{-5} PNAN
- 5.7 x 10^{-8} BAC + 2.9 x 10^{-5} HNAN
r^2 = 0.453
P = < 0.001
ESTIMATED CHO (μg C L^{-1})
OBSERVED CHO (μg C L^{-1})
60
80
100
120
140
160
180
200
220
240

Figure 14

DISCUSSION

By sampling along isopycnic surfaces (approximated by isotherms) while following the horizontal flow with a drogued buoy, it should be possible to measure purely temporal changes in dissolved CHO in a uniform water mass which would directly yield rates of microbial processes. Such temporal changes have been monitored in confined inshore systems (No. 1, this series), but even though there was some evidence of diel cycles at stations 2 and 3 (Figs. 3-6) where CHO maxima occurred only in the latter half of each day, we do not feel that the data in this study strictly warrant a temporal interpretation. Our method of vertically positioning the Niskin bottles was not precise enough to insure that the samples were actually collected from the exact isotherm depth given in Figures 2-6. Since all of these isotherms were in the thermocline, it is probable that our sampling crossed isopycnals and that the observed fluctuations primarily resulted from small scale heterogeneities maintained by vertical density gradients.

Significant salinity fluctuations also occurred which indicate that different waters were sampled, however none of the CHO patterns show strong evidence of strictly conservative behavior (except possibly Fig. 2) and their concentrations should still be related to the distributions of the microbial plankton. The relationships reported in this study probably resulted from temporal interactions of the plankton, however the data (Figs. 2-6) cannot be considered true time series, but rather snapshots of small scale spatial distributions which resulted from these interactions.

Several highly significant simple and multiple correlations

between TCHO and PCHO concentrations and microbial plankton populations were achieved at stations 2 and 3 but no combination of the NAN and BAC data produced any significant relations with any of the CHOs at station 1. This station was located in the center of a newly formed (late May) cold core Gulf Stream ring which consists of recently excised slope water with its characteristic biota (Wiebe et al., 1976). Since this study only considered the less than 20 um size plankton which dominate in the open sea (Chester, 1978), it is possible that we excluded important components of this slope community where larger organisms may be more important (Ryther, 1969). This may account for the failure of the microbial plankton data to account for a significant fraction of the CHO variance at station 1. The significant positive correlation of DOC with phaeopigments (Fig. 7) found only at station 1, is evidence for an important role of the larger zooplankton in DOC release because phaeopigments occur in their feces resulting from chlorophyll degradation during gut passage (Shuman and Lorenzen, 1975). Phaeopigment concentrations have been suggested as an index of zooplankton grazing pressure (Lorenzen, 1967). Metazooplankton also release measurable DOC during feeding and/or excretion (Conover, 1966; Webb and Johannes, 1967; Lampert, 1978).

The highly significant relationships obtained at stations 2 and 3 (Figs. 8, 9, 11, 13 and 14) strongly suggest that the less than 20 um microbial plankton actively regulate in-situ PCHO and TCHO concentrations in the western Sargasso Sea. The relationships also provide information on the function of the three trophic groups in this regulation. TCHO and PCHO were inversely related ($P<.05$) with

PNAN populations along both isotherms at station 2. Slopes of these relations were not significantly different from zero at station 3 (Figs. 10 and 12), however PNAN was a significant negative term in the multiple regression equations relating the phototrophic and heterotrophic populations to TCHO and PCHO at station 3-D (Fig. 13) and to PCHO at station 3-S (Fig. 11). TCHO and PCHO were also functions of these plankton populations in the combined data from stations 2 and 3 (Fig. 14). Such inverse or partial inverse relationships at first seem strange because PNAN represent the dominant phototrophic biomass in oligotrophic seas and must be the original source of the CHO, however there is a well known tendency for slowly growing phytoplankton in culture to produce and release much more extracellular CHO than rapidly growing cells (Guillard and Wangersky, 1958; Myklestad, 1977). Dissolved CHO declines during phytoplankton blooms and increases rapidly afterwards (Wangersky, 1959; Walsh, 1965a, 1966). The association of higher TCHO and PCHO concentrations with lower PNAN numbers in this study could result from their increased rate of excretion at lower growth rates, when cell numbers decline due to ongoing predation. The combination of decreased excretion and higher growth rates may lead to relatively higher PNAN populations and a reduction in TCHO and PCHO due to net bacterial uptake. Unlike PCHO, simple sugars do not usually seem to be major extracellular products of algae (Hellebust, 1974) which may explain the lack of significant relations between MCHO and PNAN. The magnitude of the TCHO and PCHO fluctuations relative to those of PNAN seem to preclude the possibility that these inverse relations are primarily due to spillage of PNAN cell sap due to autolysis and/or

zooplankton feeding because the maximum PNAN biomass (stations 2 and 3) calculated from cell numbers using an average cell volume of 39 um^3 (3x5 um oblate spheroid) and the carbon to volume relation of Strathmann (1967), was less than 11 ug total cell carbon per liter. Dissolved TCHO fluctuations routinely exceeded this amount by several times. The inverse relationships may be taken as evidence for an important hetrotrophic mode for PNAN (Walsh, 1965b), however over 90% of the heterotrophic potential in marine waters is due to bacterial sized organisms which pass a 1 um Nuclepore filter (Azam and Hodson, 1977).

No significant simple correlations between BAC populations and any of the CHOs were found, but the BAC data did account for a significant fraction of the PCHO and TCHO variance in the multiple regressions (Figs. 11, 13 and 14). The signs of the partial regression coefficients were variable, being positive in the PCHO relationship from station 3-S (Fig. 11) and negative in both expressions at station 3-D (Fig. 13). At stations 2 and 3 (Figs. 3-6), samples with elevated PCHO usually contained increased BAC populations (some major exceptions occur in Fig. 3), however some of the highest BAC counts were found in samples with relatively low PCHO concentrations (Figs. 4-6). This qualitative tendency for BAC to vary inversely with PCHO at some times and directly at others (which explains the lack of significant overall correlations), probably results from a lag in bacterial response to the release of PCHO. Initially, BAC divide in response to the increased substrate levels producing a direct relationship between their numbers and PCHO concentration. The BAC continue to divide and when the rate of uptake

of the population exceeds the rate of PCHO release, levels decline but the BAC population continues to increase, which produces the inverse fluctuations. The sign on the BAC terms in the regression equations depends on which of these effects dominated in the particular study. The BAC coefficients were negative in the expressions derived from the combined station 2-3 data (Fig. 14) indicating that the inverse relation dominates. Meyer-Reil et al. (1979) reported a significant inverse rank correlation between bacterial numbers and DOC concentrations during a diel drogue study in the Baltic. These relationships do not support the dormancy hypothesis for free living bacterioplankton (Stevenson, 1978).

The simple correlations between HNAN and the CHOs were also non-significant (Fig. 12), but HNAN data made a significant positive contribution to the TCHO and PCHO relationships at station 3-D (Fig. 13) and to the TCHO expression in the combined data (Fig. 14) indicating that their excretion can contribute to CHO release. Micro-zooplankton consisting primarily of protozoa (Beers and Stewart, 1969) in the HNAN size range, have been suggested as possible CHO release vectors in the North Atlantic (Burney et al., 1979).

Although the existence of significant expressions describing PCHO and TCHO as functions of the populations of the less than 20 um phototrophic and heterotrophic microbial plankton in data combined from two Sargasso Sea stations separated by 300 nautical miles (Fig. 14) suggests that the underlying interrelationships may be rather widespread, the regression equations should not be used as general predictive models. Although the regressions explain a significant fraction of the PCHO and TCHO variance, estimates from

them deviate considerably from the observed values from which the expressions were derived. Their application to other data sets would be meaningless.

ACKNOWLEDGEMENTS

Paul Davis and David Caron prepared and enumerated the nanoplankton and bacterioplankton samples, Kenneth Johnson analyzed MCHO and DOC , James Fontaine contributed most of the chlorophyll and phaeopigment data, and Richard Heffernan conducted the ATP analyses. James Hannon supplied oxygen and salinity data and, with Kenneth Hinga, conducted most of the sampling. I thank John Sieburth for consultations and for the use of his lab facilities and Scott Nixon and Michael Pilson for criticism of the manuscript.

Appreciation is also expressed to Roger Babbitt, Peter LeMay, Evelyn Lessard and Duncan Blanchard, and the officers and crew of R/V Endeavor. This work was supported by grants OCE 74-01537A02 and OCE-7681779 from the National Science Foundation Biological Oceanography Program to John Sieburth.

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THE DIEL RELATIONSHIPS OF MICROBIAL TROPHIC GROUPS AND *IN-SITU* DISSOLVED CARBOHYDRATE DYNAMICS IN THE CARIBBEAN SEA

ABSTRACT

Dissolved total carbohydrate (TCHO), polysaccharide (PCHO), monosaccharide (MCHO) and organic carbon (DOC) were determined at 3-h intervals over 5 diel cycles in the mixed layer of the northwestern Caribbean Sea while following a drogued buoy. These data have been compared to phototrophic and heterotrophic nanoplankton (2-20 um diameter) and bacterioplankton (0.2-2.0 um diameter) populations estimated by epifluorescence counts, as well as to TCO_2, ammonium, phosphate, chlorophyll a and phaeopigment data determined simultaneously.

Two apparently different types of diel CHO patterns were found. On three days when no sustained net total TCO_2 uptake was evident, TCHO and PCHO generally declined during the afternoon and early evening while MCHO tended to increase. Large evening TCHO and PCHO peaks with constant or declining MCHO levels were found on two other days when apparent sustained TCO_2 uptake occurred during the day. These TCHO and PCHO accumulations were apparently caused by the release of recently fixed PCHO from phototrophs. The cause of this day to day variation is unknown but may be related to differences in inorganic nutrient availability at dawn.

Phototrophic nanoplankton (PNAN) populations were inversely related to PCHO concentrations and MCHO was directly correlated with heterotrophic bacterioplankton (HBAC) counts. The sample to sample fluctuations in PNAN were inversely related to the apparent rates of change of TCHO and PCHO. The rate of TCHO accumulation in a log phase Isochrysis galbana culture also varied inversely with the rate of change of cell numbers over a diel cycle under natural sunlight. It

is suggested that a similar inverse association between the rates of CHO excretion and cell division of the in-situ PNAN may account for the field relationships. Fluctuations in HBAC populations were inversely correlated with PCHO dynamics and directly related to MCHO variations. The latter relationship may be caused by the hydrolysis of PCHO to MCHO by extracellular enzymes during rapid bacterial growth. The inverse relation between PCHO and HBAC dynamics appeared to be due to this hydrolysis as well as to net bacterial uptake. The combined PNAN and HNAN or HBAC fluctuations accounted for larger fractions of the variance in the apparent rates of change of TCHO and PCHO than did any single population parameter indicating that intimate interactions between the microbial plankton groups are important in the in-situ regulation of CHO dynamics. HNAN excretion probably provides a secondary source of dissolved CHO.

Since sigma-t variations in each study were small and not significantly related to either CHO or microbial plankton population fluctuations, it seems possible that the observed CHO fluctuations primarily represent true temporal dynamics in a fairly homogeneous water mass. There was no evidence of significant horizontal plankton patchiness. If temporal dynamics were observed, TCHO release and uptake rates averaged 7.4 and 5.4 ug C $L^{-1}h^{-1}$ with maximal values of 29 and 15 ug C $L^{-1}h^{-1}$, respectively. Away from the influence of shallow waters, the total net apparent TCHO flux over 3 days accounted for 33 and 48% of the total net apparent DOC release and uptake, respectively. At one 48 hr station with complete data, daily DOC release was 50% of net apparent primary production.

INTRODUCTION

To assess the importance of labile dissolved organic matter (DOM) in the food web of the open sea, it is necessary to understand the roles and in-situ interactions of the dominant plankton trophic groups in DOM release and uptake as well as the rates of these processes. Most of our information on DOM dynamics comes from studies on the extracellular release of ^{14}C labeled DOM by phytoplankton (see Sharp, 1977) and on the uptake of radioactive substrates by bacteria (summarized by Hoppe, 1978).

The ^{14}C method for measuring dissolved organic excretion by phytoplankton has been questioned on technical (Sharp, 1977) and kinetic (Wiebe and Smith, 1977) grounds. Rapid bacterial uptake of the extracellular products may lead to underestimated release rates (Nalewajko et al., 1976). Confinement of natural populations in small bottles profoundly alters the composition of the microbial plankton. Especially susceptible are the phototrophic nanoplankton (PNAN) and heterotrophic nanoplankton (HNAN) (Venrick et al., 1977), which are the main producers and nutrient regenerators in oligotrophic seas. Since these plankton communities depend upon a delicate balance between productive and regenerative processes (Wangersky, 1977), any disturbance caused by confinement will upset this balance and lead to very unnatural estimates of both primary production and organic excretion rates. The severity of these confinement effects can be reduced by using larger bottles (Gieskes et al., 1979).

Analytical and theoretical problems with the heterotrophic potential method for estimating bacterial activity (Parsons and Strickland, 1962; Wright and Hobbie, 1966) have been discussed by

Wright (1973). Especially suspect are the applicability of the Michaelis-Menten model to mixed bacterial communities (Krambeck, 1979) and the use of single substrates which may not reflect overall activities due to the presence of multiple substrate uptake systems (Wood, 1973). Total bacterial production estimated by epifluorescence microscopy in predator free diffusion chambers averaged up to 16 times the maximum net ^{14}C-glucose uptake (Delattre et al., 1979). More recent radiotracer methods which do not employ the Michaelis-Menten kinetic treatment (Dietz et al., 1976; Meyer-Reil et al., 1978, 1979) provide a more realistic index of bacterial activity, however sample handling and confinement may still disturb the system and introduce artifacts.

Information on the net flux of DOM and its biological interrelationships can be obtained from direct close interval time series sampling of the undisturbed environment. Rates of dissolved carbohydrate (CHO) release and uptake as well as some biological factors effecting in-situ CHO levels have been proposed from such studies on two inshore ecosystems (No. 1, this series). Diel drogue studies in the Sargasso Sea (No. 2, this series) have provided information on the roles of the nanoplankton and bacterioplankton in open ocean CHO regulation. Because of the possibility that this sampling was not precisely from along the intended isopycnals and that some of the observed CHO changes were due to small scale spatial inhomogeneities maintained by the vertical density structure, the data in the Sargasso Sea study were not used to calculate CHO release and uptake rates.

The purpose of this study was to ascertain if relations similar

to those found in the Sargasso Sea also occur in the northwest Caribbean Sea and, by more careful attention to hydrography, obtain diel CHO data which are more likely to represent true temporal fluctuations occurring in a homogeneous water mass. Data are presented which show that all three trophic groups of microorganisms are apparently involved in the regulation of CHO dynamics which can be sizeable.

MATERIALS AND METHODS

Five stations were occupied in the northwest Caribbean Sea (Fig. 1) during R/V Endeavor Cruise 033 (22 Feb. to 19 Mar. 1979). Niskin bottle samples from 15 m (stations 1-3) or 70 m (stations 4-5) depths were taken at 3 h intervals over 24 to 48 h periods while following a window shade drogued buoy. The depth of the mixed layer, which always exceeded 70 m, was determined at least every 6 h by Sippican (Marion, MA) model T-11 expendable bathythermographs (XBT) during stations 4 and 5 (less frequently for stations 1-3). Temperature was determined either by XBT or by mercury thermometer on water pumped from sampling depth.

Samples for carbohydrate and DOC analyses were filtered by gravity through precombusted (450°C, 12 h) Gelman type A/E glass fiber filters into precombusted glass bottles. Quadruplicate DOC aliquots were immediately drawn, sealed in ampules, and oxidized for analysis ashore by the method of Menzel and Vaccaro (1964) as modified by Kerr and Quinn (1975) using an Oceanography International (College Station, TX) total carbon system, model 0524B. Carbohydrate samples were frozen at -30°C until the end of each station when aliquots were analyzed in quadruplicate for MCHO and in hextuplicate for TCHO by the methods of Johnson and Sieburth (1977) and Burney and Sieburth (1977), respectively, except that 13 mm polypropylene linerless caps (Nalge, Rochester, NY) were used on the reaction tubes, replacing the previous teflon-lined, black phenolic plastic caps which caused gross contamination when the liners did not seal perfectly. PCHO was estimated as the difference in TCHO and MCHO concentrations, calculated as hexose. Water for TCO_2 analysis was immediately

drawn from the Niskin samplers by overflowing glass bottles by several volumes and stoppering without bubbles. TCO_2 was measured at sea by infrared analysis of the CO_2 purged by nitrogen from acidified samples, using the same equipment as for DOC.

Samples for bacterioplankton and nanoplankton estimation were screened through 20 um Nytex mesh (Sterling Marine Products, Montclair NJ) and stored in 2% formalin at 5°C. At the end of each station aliquots were stained with 0.1% (W:V) acridine orange solution (Hobbie, Daley and Jaspers, 1977). Preparations for the enumeration of total nanoplankton (TNAN) and heterotrophic bacteria (HBAC) were filtered onto 1.0 and 0.2 um Nuclepore filters prestained with irgalan black respectively, and counted using an Olympus (Tokyo) Vanox epifluorescence microscope at 1000x for HBAC and 400x for TNAN. Phototrophic nanoplankton (PNAN) and phototrophic bacterioplankton (PBAC) were enumerated by filtering unstained sample aliquots as above and counting the cells made visible by the autofluorescence of their photosynthetic pigments. At least 300 cells were counted for each determination. Numbers of heterotrophic nanoplankton (HNAN) were estimated from the difference in TNAN and PNAN counts. HBAC data were not corrected for PBAC because HBAC exceeded PBAC by two orders of magnitude.

Samples for ammonia and phosphate determination were frozen at -30°C and analyzed ashore on a Technicon (Tarrytown, NY) AutoAnalyzer II. Chlorophyll *a* and phaeopigment extracts were analyzed fluorometrically (Strickland and Parsons, 1968) at sea, using an Aminco (Silver Spring, MD) fluorocolorimeter. Salinity was determined by induction salinometer (Plessy model 6230N, San Diego,

CA). Incident photosynthetically active radiation (PAR) was recorded using a Lambda Instruments (Lincoln, NB) model 185 quantum sensor mounted on the ship in a position as free from shadows as possible.

Stepwise regression analysis was used to relate the PNAN, HNAN, PBAC, HBAC and sigma-t data to each of the carbohydrates and DOC. Possible net apparent hourly rates of change for the carbohydrates and DOC were calculated from the time series data assuming that all fluctuations were purely temporal. Net daily fluxes were estimated using the single diurnal curve method of Odum and Hoskins (1958) by integration of the area under hourly rate of change curves. Only hourly rates calculated from analytically significant fluctuations (t test, $P<.05$) or significant overall trends were used. Non-significant hourly rates were replaced by zeros in the daily rate calculations.

In order to determine if the observed relationships were experimentally verifiable, a series of observations were made with a phototroph and a phagotroph in non-axenic laboratory culture. Dissolved TCHO and cell numbers were followed in an early log phase culture of the prymnesiophyte Isochrysis galbana at 3 h intervals over a diel cycle. The culture was grown under natural sunlight in an east facing window in Guillard's (1963) f media (no TRIS) substituting 100 uM NH_4Cl for $NaNO_3$ and omitting silicate. The effect of feeding by the marine scuticociliate Uronema marina on Isochrysis galbana in regard to CHO release and particulate phaeopigment production was also studied. Isochrysis was grown in f/2 media modified as above. When log phase commenced, the culture was divided into two 500 ml aliquots. One culture, and another bottle containing 500 ml of seawater were inoculated with Uronema marina. All three

bottles received 20 uE $m^{-2}s^{-1}$ continuous incandescent illumination and were bubbled with sterile air stripped of ammonia by passing through a saturated zinc chloride solution.

Figure 1: Locations of the drifting drogued buoy stations occupied during R/V Endeavor cruise EN-033, February-March, 1979. Station 1: start 24 Feb.,1630, end 26 Feb.,0730. Station 2: start 1 Mar.,0630, end 3 Mar.,0620. Station 3: start 6 Mar.,0600, end 7 Mar.,0700. Station 4: start 9 Mar.,0600, end 11 Mar.,0600. Station 5: start 14 Mar.,0600, end 16 Mar.,0600.

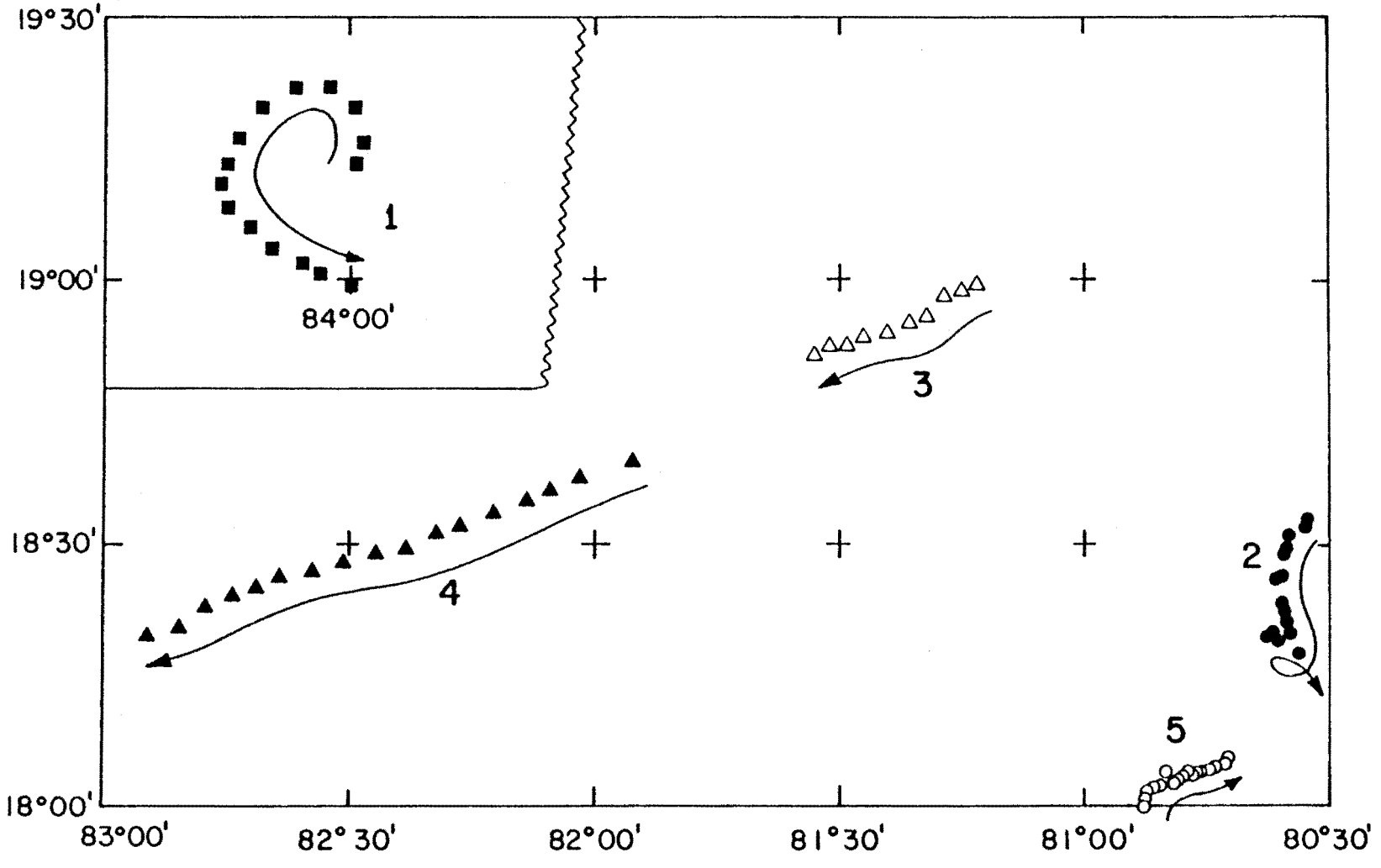

Figure 1

between the direction and magnitude of the sample to sample fluctuations rather than overall daily trends and are more meaningful in illustrating short term interactions. Significant inverse overall relationships were found between the rates of change of TCHO and PNAN (Fig. 7) and of PCHO and PNAN (Fig. 8). The points tend to cluster around the origins in these figures and the significance of the relationships depends on the more extreme values, however these points represent the largest and most significant fluctuations in the raw data (Figs. 2-4). Figures 9 and 10 show significant overall relationships of HBAC changes to those of PCHO and MCHO, respectively. HBAC varied inversely with PCHO fluctuations and directly with MCHO changes. Figure 11 presents the observed hourly TCHO fluctuations compared to those estimated from a multiple linear regression equation expressing TCHO dynamics as a function of the changes in PNAN and HNAN numbers. Partial regression coefficients for PNAN and HNAN were negative and positive respectively, and both were significantly different from zero ($P<.05$). Figure 12 illustrates the multiple correlation of PCHO fluctuations with those of PNAN and HBAC. Both partial regression coefficients are negative and significant ($P<.05$). Solid lines in Figures 11 and 12 are the least squares regression fits to the data while the dotted lines represent the ideal 1:1 relations between observed and estimated values.

Table 1 lists the possible values for daily net TCHO and DOC flux as well as net system production calculated from the significant downward TCO_2 trends, assuming that all of the observed variations (Figs. 2-4) represent purely temporal dynamics.

The relationship of Isochrysis cell counts and TCHO

concentrations in culture during a diel cycle under natural sunlight is shown in Fig. 13. Figure 14 illustrates the inverse correlation between the rates of change of TCHO and cell numbers in the same experiment.

Data from the Uronema feeding experiment are shown in Fig. 15. Uronema grew logarithmically until day 8, reaching 10^7 cells per liter. The Isochrysis control culture remained in log phase until day 12 and attained 7.25×10^9 cells per liter. TCHO concentrations were significantly ($P << .01$) higher in the experimental (Uronema + Isochrysis) culture than in the Isochrysis control, in every sample following inoculation. TCHO remained consistently low in the Uronema control. The ciliates fed voraciously and decimated the Isochrysis culture, sharply reducing the chlorophyll a concentrations but without producing particulate phaeopigments significantly in excess of Isochrysis control levels.

Figure 2: Comparison of the diel cycles of dissolved carbohydrates and organic carbon with those of microbial plankton, pigments and environmental variables for Station 1. Curves break at 2400 and are continued from 0000 hrs. Sampling commenced at 1630 hrs and ceased 39 hrs later at 0730.

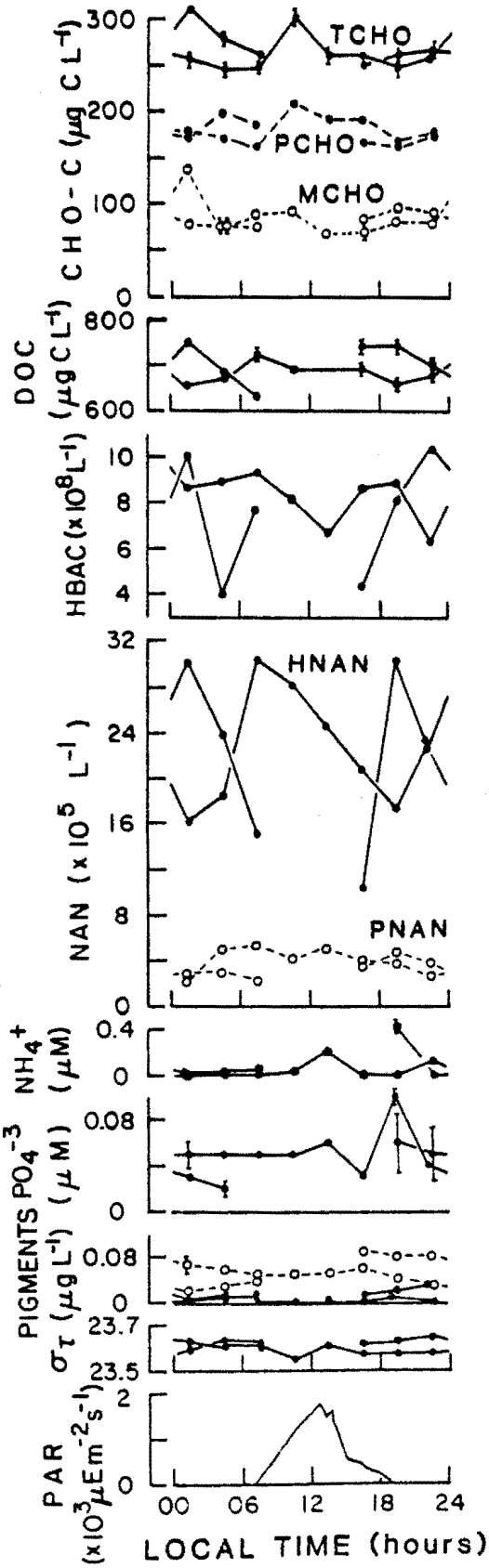
TCHO
PCHO
MCHO
CHO-C (μg C L⁻¹)
300
200
100
0
DOC (μg C L⁻¹)
800
600
HBAC (x10⁸ L⁻¹)
10
8
6
4
HNAN
PNAN
NAN (x10⁵ L⁻¹)
32
24
16
8
0
NH4+ (μM)
0.4
0
PO4-3 (μM)
0.08
0
PIGMENTS (μg L⁻¹)
0.08
0
σt
23.7
23.5
PAR (x10³ μE m⁻² s⁻¹)
2
0
00
06
12
18
24
LOCAL TIME (hours)

Figure 2

Figure 3: Comparison of the diel cycles of dissolved carbohydrates and organic carbon with those of microbial plankton, pigments and environmental variables for Station 4. A= Day 1, 9 Mar.,0600 to 10 Mar.,0600. B= Day 2, 10 Mar.,0600 to 11 Mar.,0600.

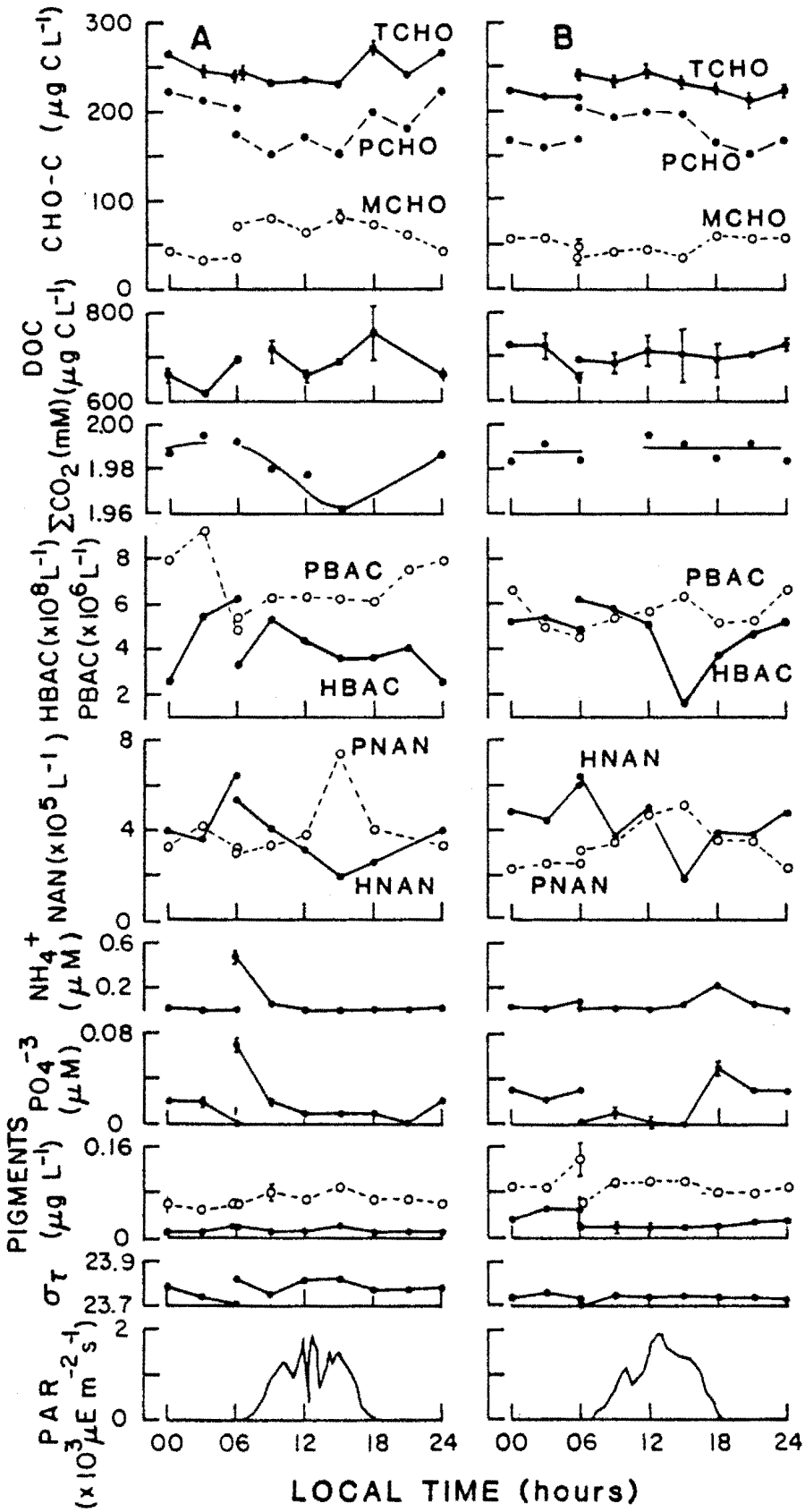
A
B
TCHO
PCHO
MCHO
CHO-C (μg C L⁻¹)
300
200
100
0
DOC (μg C L⁻¹)
800
600
ΣCO2 (mM)
2.00
1.98
1.96
PBAC (x10⁶ L⁻¹)
HBAC (x10⁸ L⁻¹)
8
6
4
2
PBAC
HBAC
NAN (x10⁵ L⁻¹)
PNAN
HNAN
NH4+ (μM)
0.6
0.2
PO4-3 (μM)
0.08
0
PIGMENTS (μg L⁻¹)
0.16
0
σT
23.9
23.7
PAR (x10³ μE m⁻² s⁻¹)
2
0
00
06
12
18
24
LOCAL TIME (hours)

Figure 3

Figure 4: Comparison of the diel cycles of dissolved carbohydrates and organic carbon with those of microbial plankton, pigments and environmental variables at station 5. A= Day 1, 14 Mar.,0600 to 15 Mar.,0600. B= Day 2, 15 Mar.,0600 to 16 Mar.,0600.

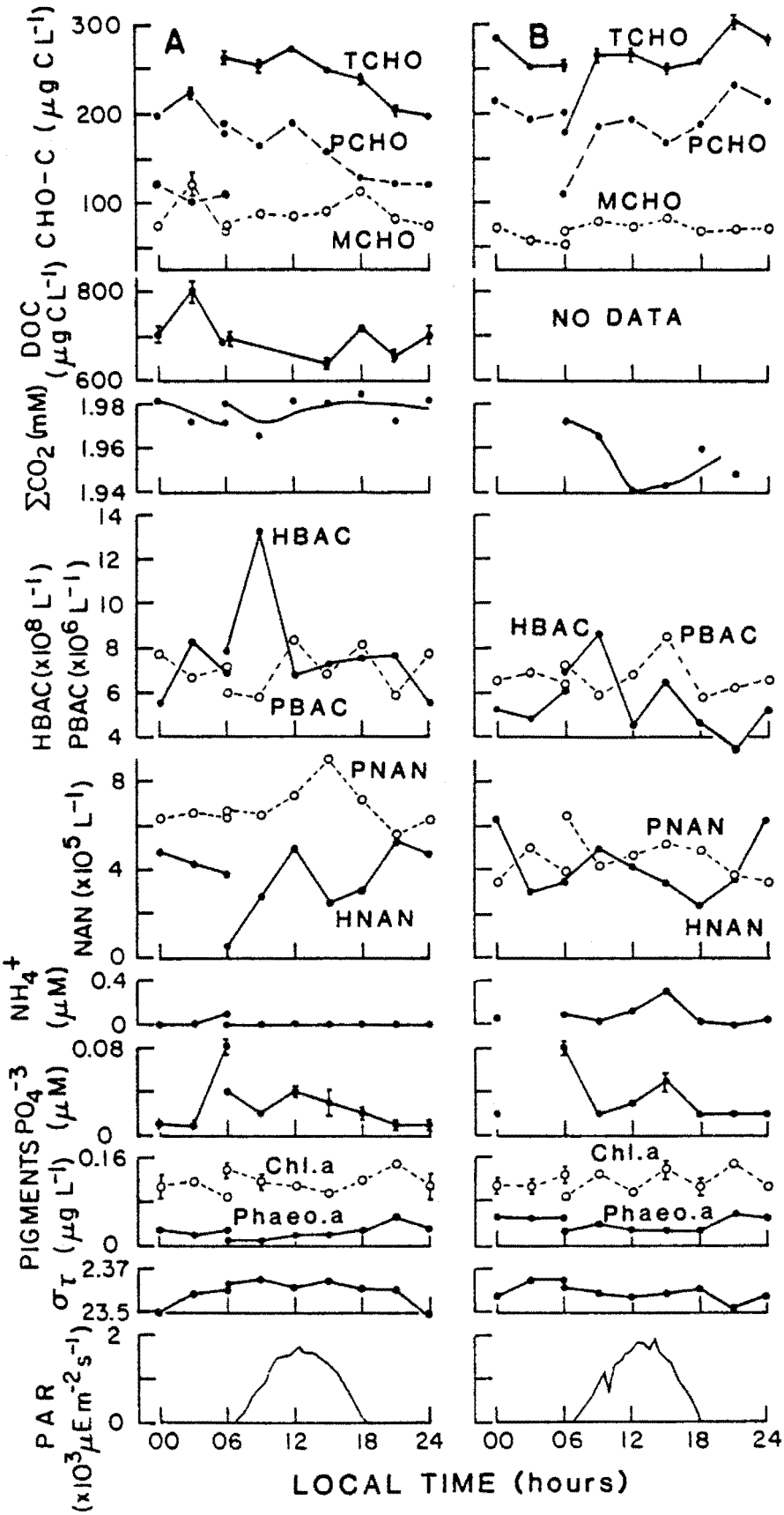
A
B
TCHO
PCHO
MCHO
CHO-C (μg C L⁻¹)
300
200
100
DOC (μg C L⁻¹)
800
600
NO DATA
ΣCO2 (mM)
1.98
1.96
1.94
HBAC
PBAC
HBAC (x10⁸ L⁻¹)
PBAC (x10⁶ L⁻¹)
14
12
10
8
6
4
PNAN
HNAN
NAN (x10⁵ L⁻¹)
8
4
0
NH4+ (μM)
0.4
0
PO4-3 (μM)
0.08
0
PIGMENTS (μg L⁻¹)
Chl.a
Phaeo.a
0.16
0
σT
2.37
23.5
PAR (x10³ μE m⁻² s⁻¹)
2
0
00
06
12
18
24
LOCAL TIME (hours)

Figure 4

Figure 5: The inverse relationship of PCHO concentrations to PNAN populations in the combined data from stations 1, 4 and 5.

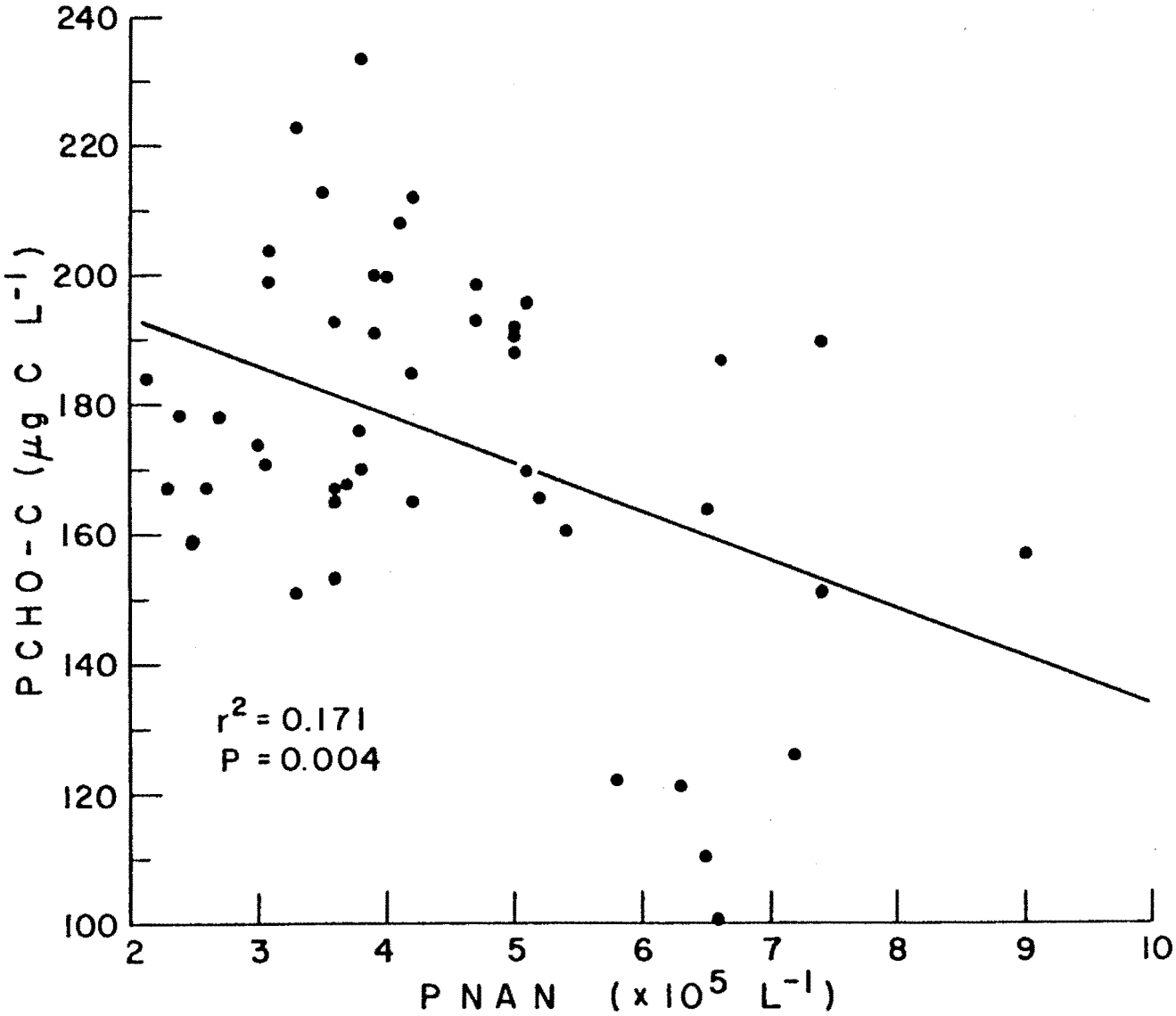
240
220
200
180
160
140
120
100
PCHO-C (μg C L⁻¹)
r² = 0.171
P = 0.004
2
3
4
5
6
7
8
9
10
PNAN (x 10⁵ L⁻¹)

Figure 5

Figure 6: The direct relationship of MCHO concentrations to HBAC populations in the combined data from stations 1, 4 and 5.

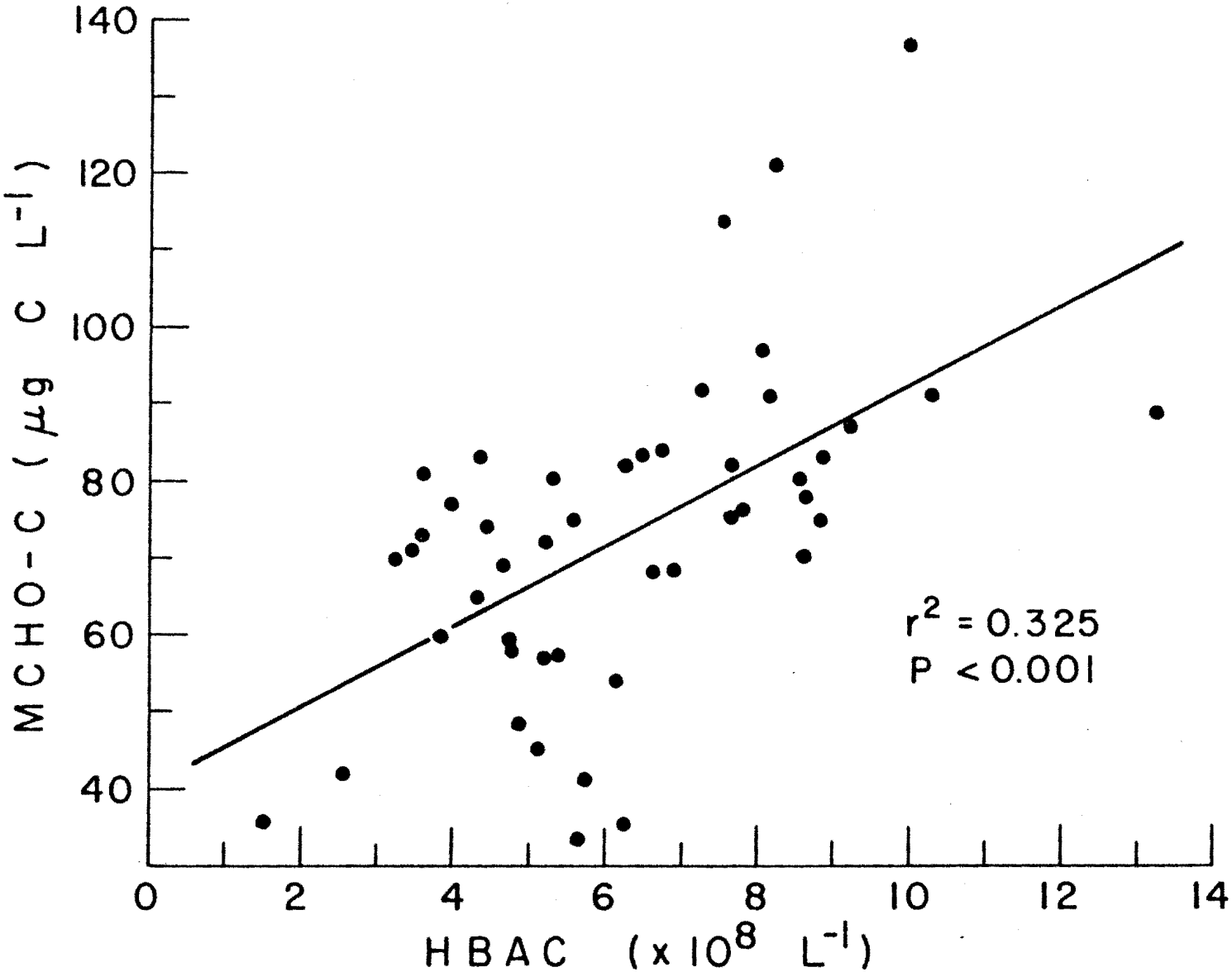
140
120
100
80
60
40
MCHO-C (μg C L⁻¹)
0
2
4
6
8
10
12
14
HBAC (x 10⁸ L⁻¹)
r² = 0.325
P < 0.001

Figure 6

Figure 7: The inverse relationship between the sample to sample fluctuations of TCHO and PNAN. Combined data, stations 1, 4 and 5.

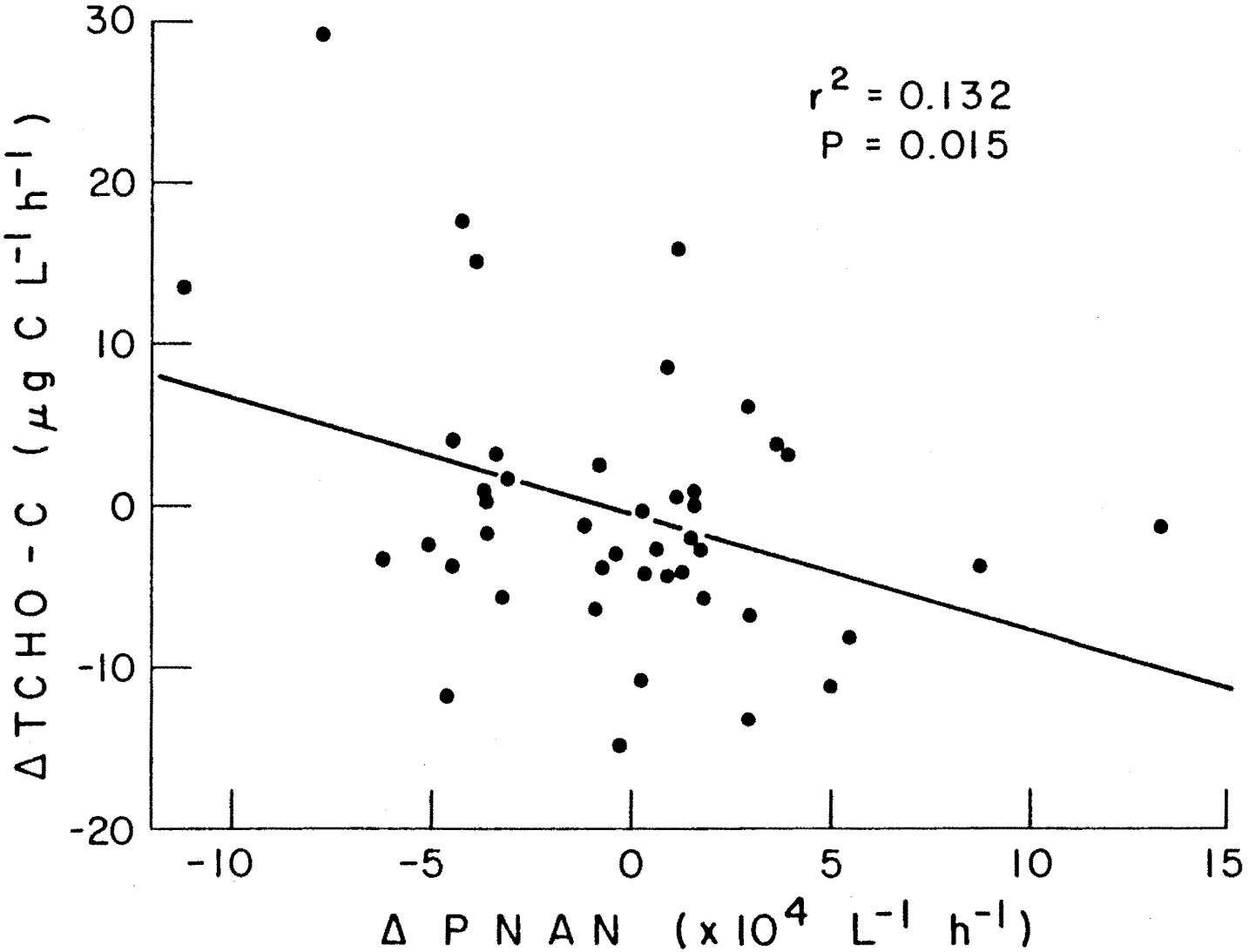
30
20
10
0
-10
-20
-10
-5
0
5
10
15
$r^2 = 0.132$
P = 0.015
ΔTCHO-C (μg C L^{-1} h^{-1})
Δ P N A N (x 10^4 L^{-1} h^{-1})

Figure 7

Figure 8: The inverse relationship between PCHO and PNAN fluctuations. Combined data, stations 1, 4 and 5.

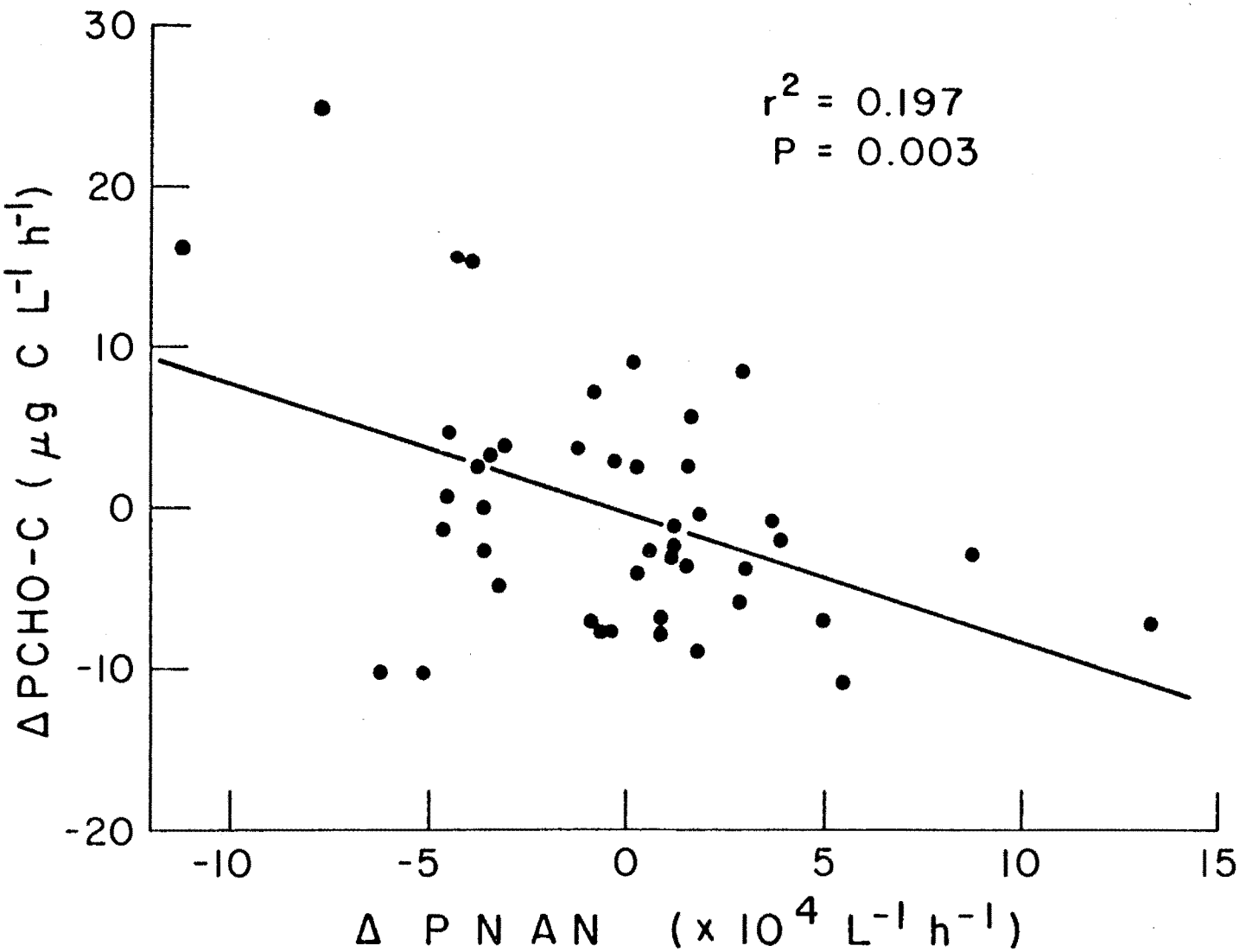
30
20
10
0
-10
-20
-10
-5
0
5
10
15
r^2 = 0.197
P = 0.003
ΔPCHO-C (μg C L^{-1} h^{-1})
Δ P N A N (x 10^4 L^{-1} h^{-1})

Figure 8

Figure 9: The inverse relationship between PCHO and HBAC fluctuations. Combined data, stations 1, 4 and 5.

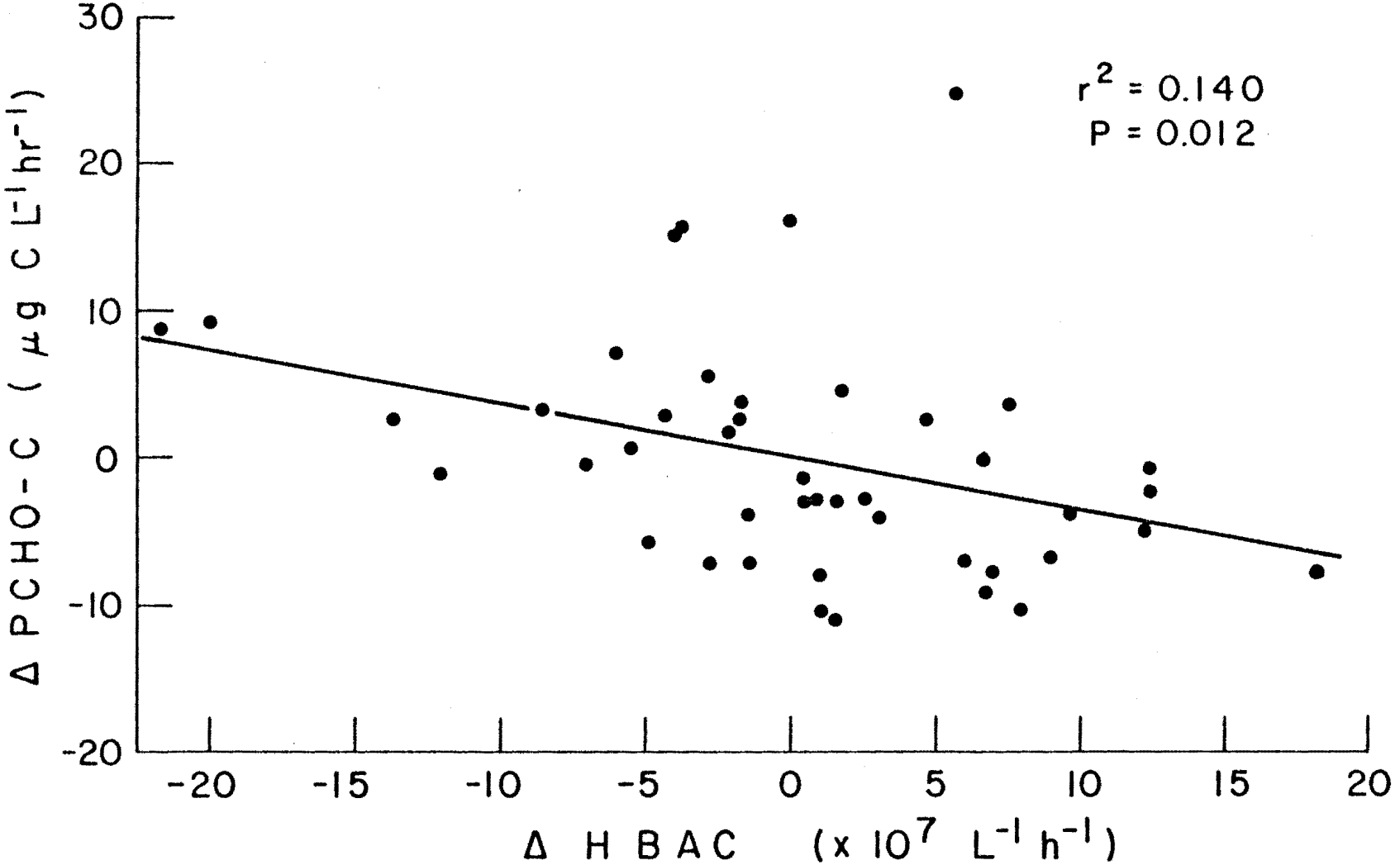
30
20
10
0
-10
-20
-20
-15
-10
-5
0
5
10
15
20
Δ PCHO-C (μg C L⁻¹ hr⁻¹)
Δ H B A C (x 10⁷ L⁻¹ h⁻¹)
r² = 0.140
P = 0.012

Figure 9

Figure 10: The direct relationship between MCHO and HBAC fluctuations. Combined data, stations 1, 4 and 5.

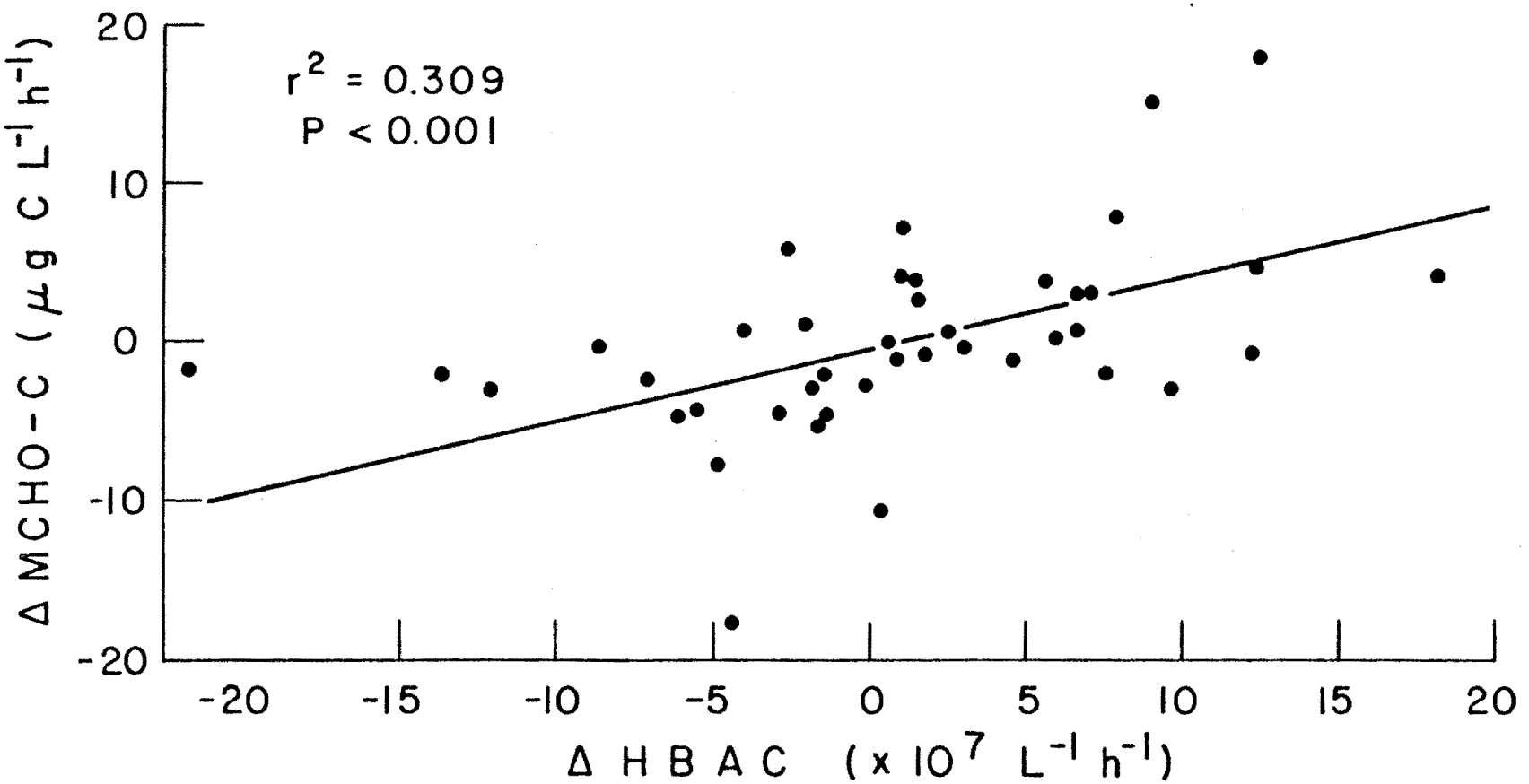
$r^2 = 0.309$
$P < 0.001$
Δ MCHO-C (μg C L^{-1} h^{-1})
Δ HBAC (x 10^7 L^{-1} h^{-1})
20
10
0
-10
-20
-20
-15
-10
-5
0
5
10
15
20

Figure 10

Figure 11: Observed apparent hourly TCHO fluctuations compaired to those estimated (EST) from an empirical multiple linear regression equation expressing TCHO dynamics as a function of the hourly changes in PNAN and HNAN numbers L^{-1}.

The solid line is the least squares regression fit to the data points. The dotted line represents the ideal 1:1 relationship between observed and estimated values.

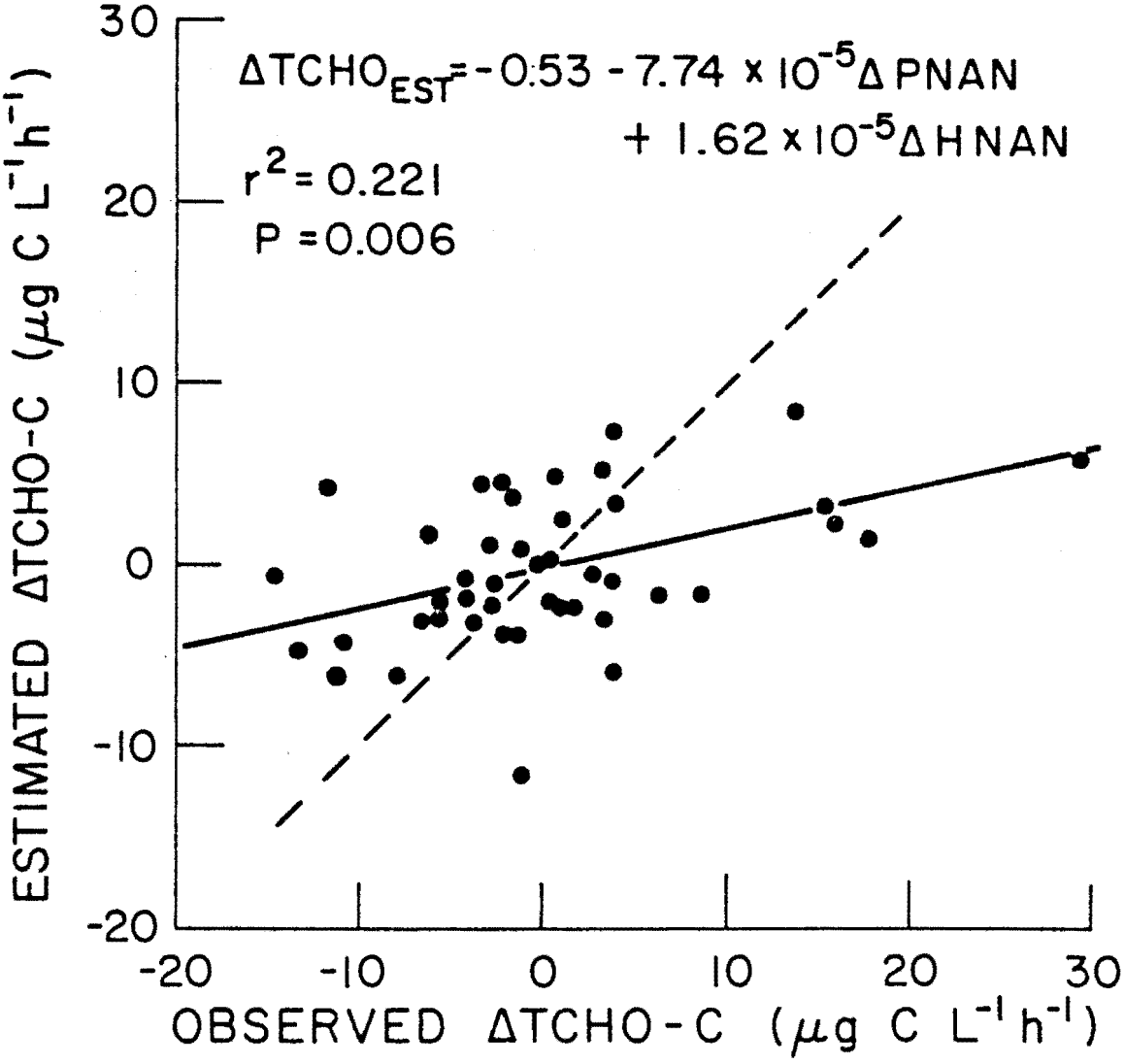
ΔTCHO$_{EST}$ = -0.53 - 7.74 x 10^{-5} Δ PNAN
+ 1.62 x 10^{-5} Δ HNAN
r^2 = 0.221
P = 0.006
ESTIMATED ΔTCHO-C (μg C L^{-1} h^{-1})
OBSERVED ΔTCHO-C (μg C L^{-1} h^{-1})
30
20
10
0
-10
-20
-20
-10
0
10
20
30

Figure 11

Figure 12: Observed apparent hourly PCHO fluctuations compaired to those estimated (EST) from an empirical multiple linear regression equation expressing PCHO dynamics as a function of the hourly changes in PNAN and HBAC numbers L^{-1}. The solid line is the least squares regression fit to the data points. The dotted line represents the ideal 1:1 relationship between observed and estimated values.

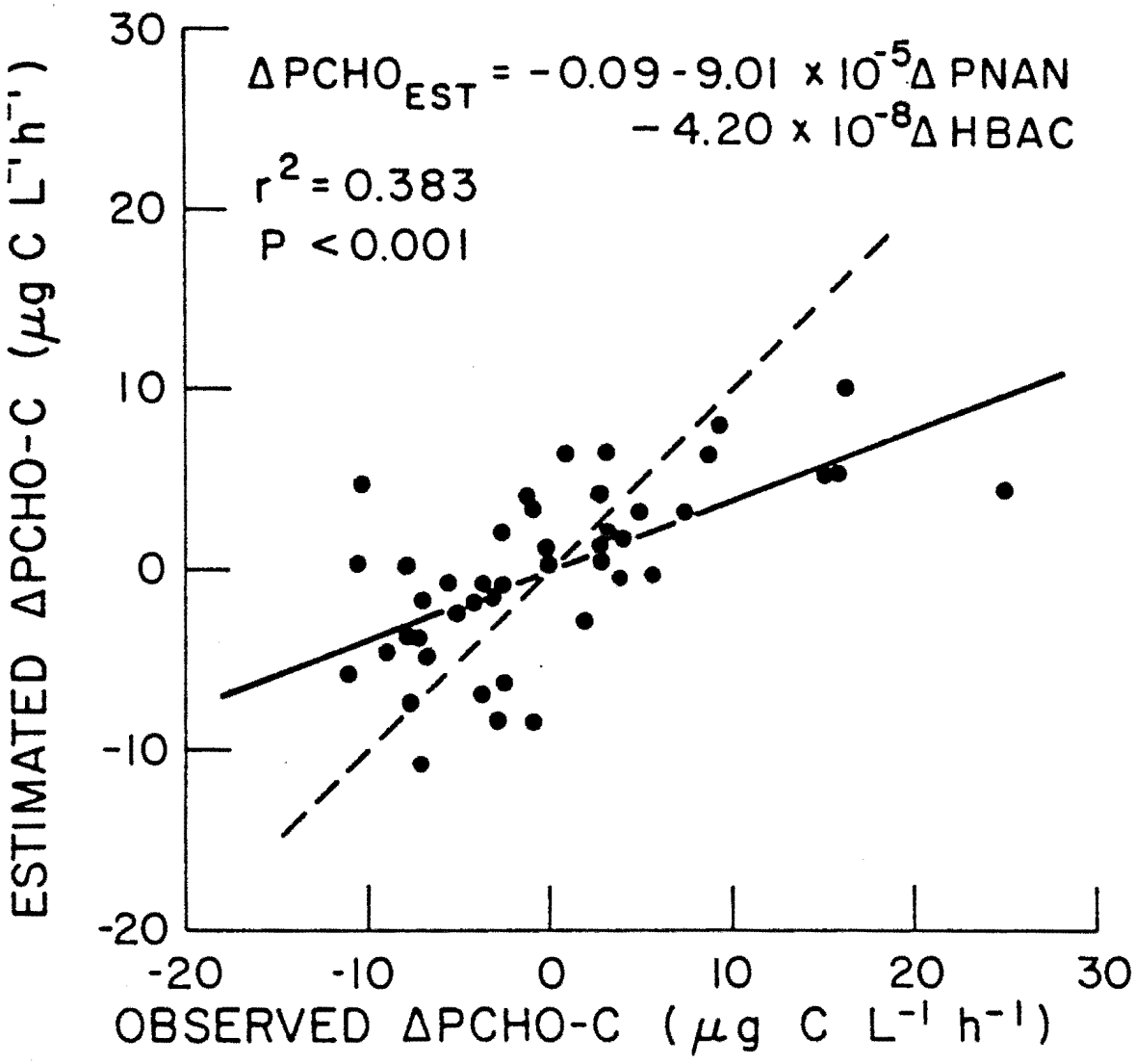
$\Delta PCHO_{EST} = -0.09 - 9.01 \times 10^{-5} \Delta PNAN$
$- 4.20 \times 10^{-8} \Delta HBAC$
$r^2 = 0.383$
$P < 0.001$
ESTIMATED ΔPCHO-C (μg C L⁻¹ h⁻¹)
OBSERVED ΔPCHO-C (μg C L⁻¹ h⁻¹)
30
20
10
0
-10
-20
-20
-10
0
10
20
30

Figure 12

Table 1. Net daily flux of TCHO, DOC and TCO_2 ($\mu g\ C\ L^{-1}d^{-1}$) assuming purely temporal dynamics.

Station:	1	4		5	
Day:	1st	1st	2nd	1st	2nd
TCHO Release	47	53	9	61	112
TCHO Uptake	51	30	29	85	71
DOC Release	43	149	36	187	n[1]
DOC Uptake	115	104	36	163	n[1]
TCO_2 Production	n	372	nt[2]	nt	372

[1]No data available.

[2]No consistent trend in the data.

Figure 13: The diel patterns of TCHO concentration (open circles) and cell numbers (ISO, closed circles) observed in a non-axenic log phase Isochrysis galbana culture compaired to the cycle of photosynthetically active solar radiation (PAR) recieved in an east facing window.

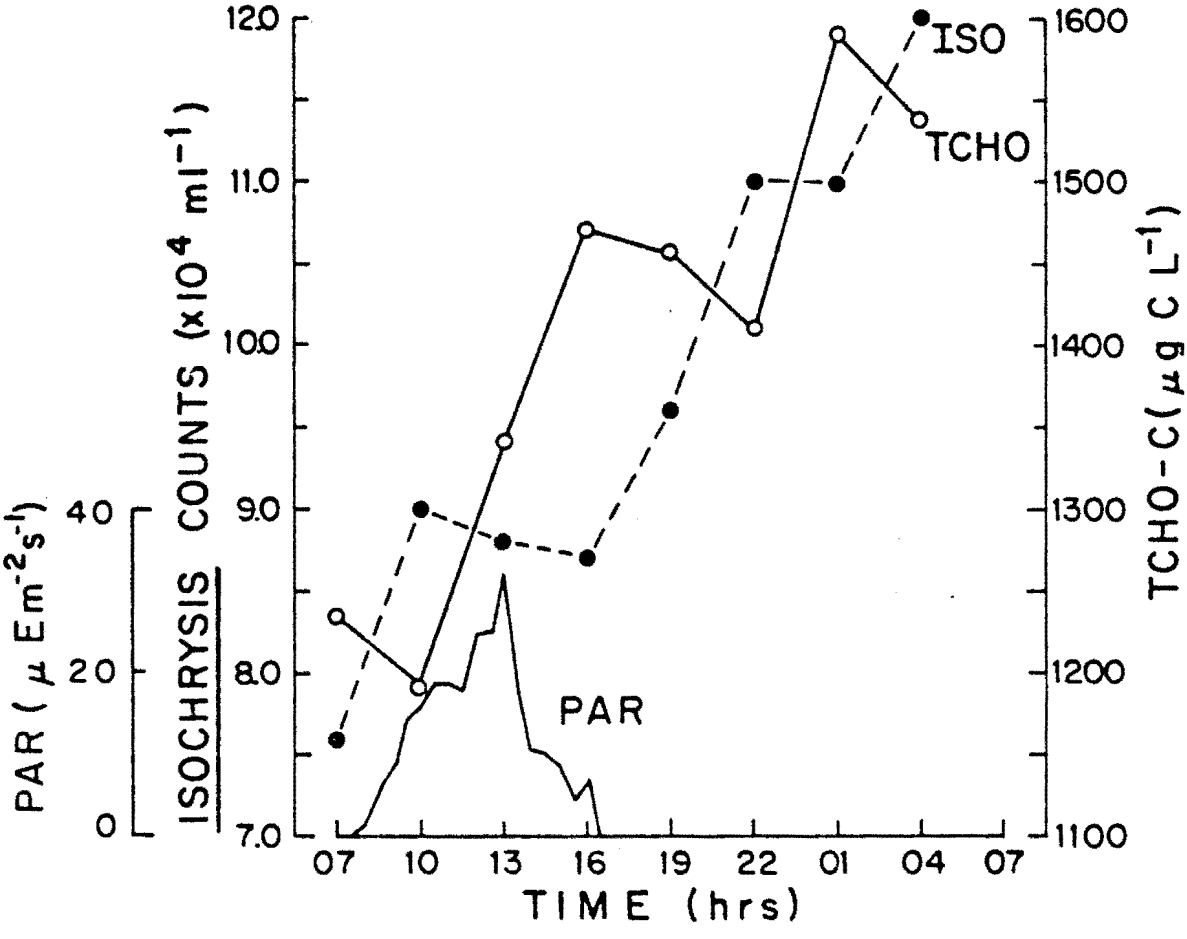
PAR (μ Em^{-2}s^{-1})
ISOCHRYSIS COUNTS (x10^4 ml^{-1})
TCHO-C(μ g C L^{-1})
TIME (hrs)
ISO
TCHO
PAR
0
20
40
7.0
8.0
9.0
10.0
11.0
12.0
1100
1200
1300
1400
1500
1600
07
10
13
16
19
22
01
04
07

Figure 13

Figure 14: The inverse relation between the rates of change of TCHO concentrations and cell numbers in a non-axenic log phase Isochrysis galbana culture grown in an east facing window and sampled at 3 hr intervals over a diel cycle.

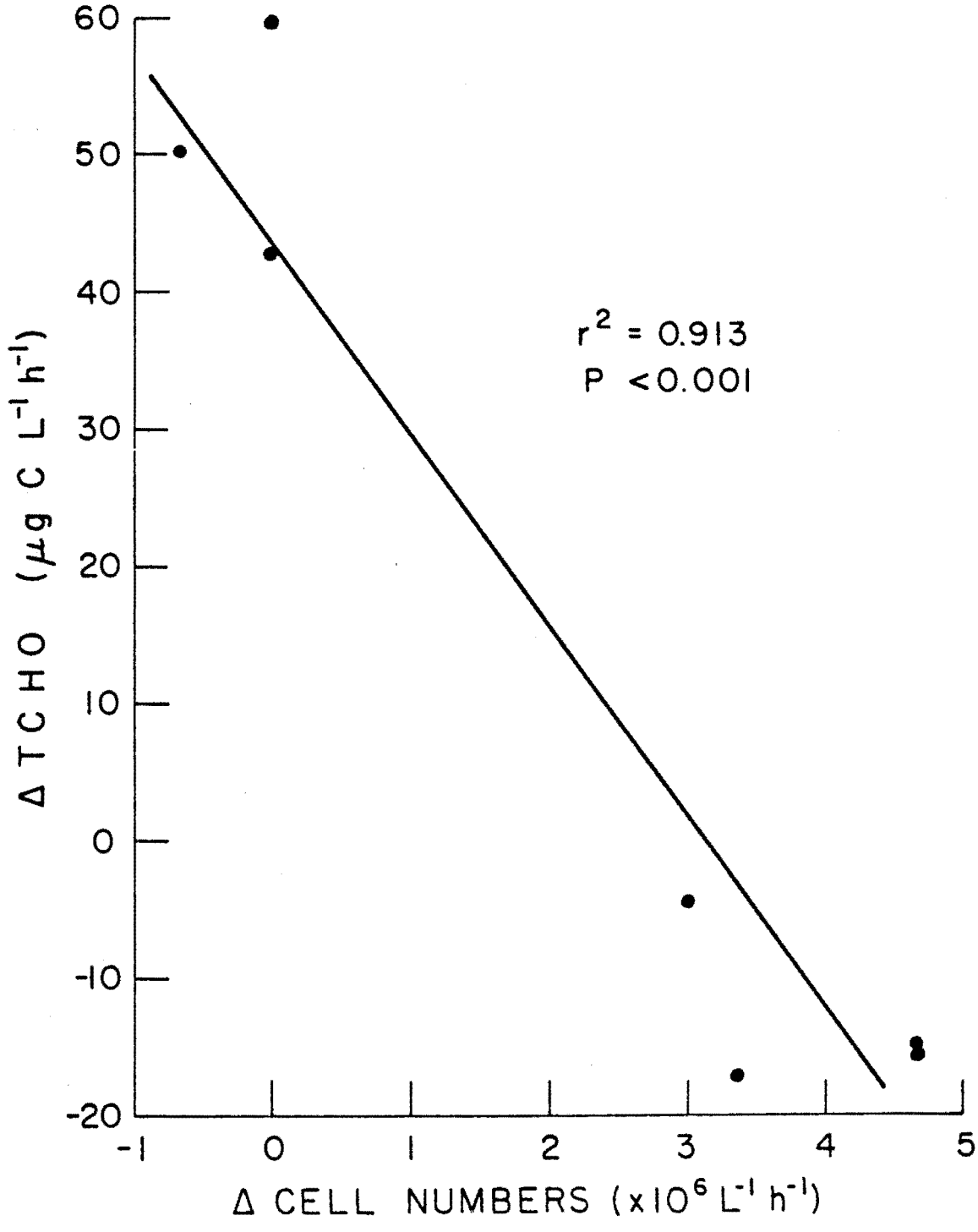
60
50
40
30
20
10
0
-10
-20
-1
0
1
2
3
4
5
Δ TCHO (μg C L⁻¹ h⁻¹)
Δ CELL NUMBERS (x10⁶ L⁻¹ h⁻¹)
r² = 0.913
P <0.001

Figure 14

Figure 15: The influence of Uronema marina eating Isochrysis galbana on TCHO, chlorophyll a and phaeopigment concentrations, as shown by observations on cultures of Isochrysis alone (ISO), Uronema alone (URO) and the two combined (EXP).

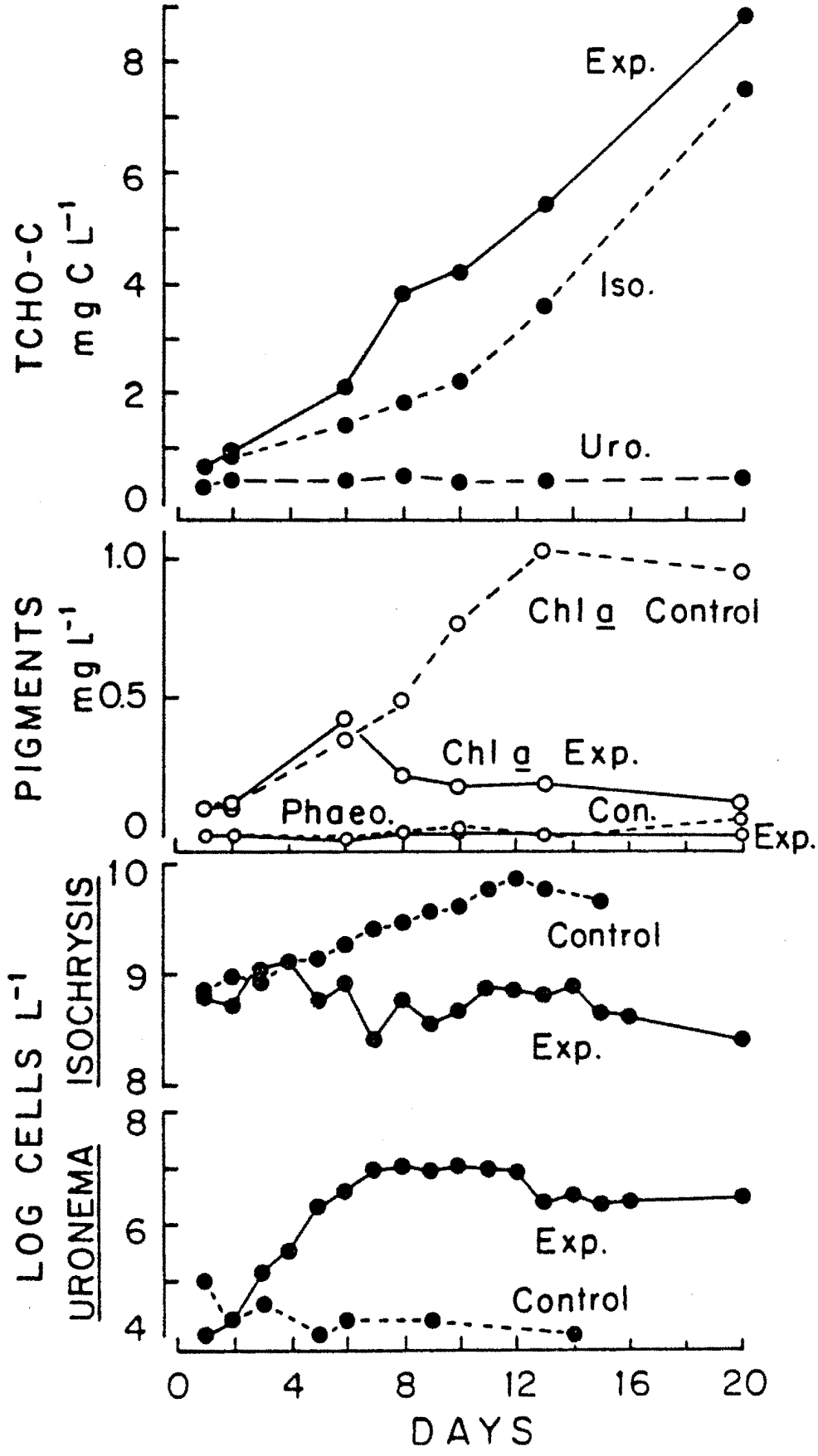
TCHO-C
mg C L⁻¹
8
6
4
2
0
Exp.
Iso.
Uro.
PIGMENTS
mg L⁻¹
1.0
0.5
0
Chl a Control
Chl a Exp.
Phaeo.
Con.
Exp.
LOG CELLS L⁻¹
ISOCHRYSIS
10
9
8
Control
Exp.
URONEMA
8
6
4
Exp.
Control
0
4
8
12
16
20
DAYS

Figure 15

DISCUSSION

Highly significant CHO and DOC fluctuations were observed at all stations (Figs. 2-4). DOC patterns appeared to be unrelated to those of PCHO but were more similar to the MCHO curves in some cases (Figs. 2 and 4-A). Populations of PNAN and HNAN were not significantly different at Stations 4 and 5, averaging between 3.7 and 5.7 x 10^5 cells L^{-1}. Similar PNAN levels occurred at station 1, but HNAN averaged over five times higher than at Stations 4 or 5, possibly due to the location of the first station just to the north of the shallow Mysteriosa Banks. This station also had significantly higher overall TCHO concentrations than the other areas and a nearly significant (P=.053) direct correlation between TCHO and HNAN. While this may indicate a direct influence of HNAN excretion on TCHO levels, it is possible that both parameters were elevated at station 1, due to the proximity of shallow waters. Populations of HBAC were generally on the order of 10^8 cells L^{-1} but were significantly lower at station 4 (Fig. 3). PBAC numbers were two orders of magnitude lower than HBAC and were not related to any of the CHO concentrations. Ammonia was usually undetectable except for a few significant peaks. Phosphate was generally low but detectable and showed patterns similar to those of ammonia except at station 1. Overall, chlorophyll *a* concentrations averaged .09, .08 and.12 ug L^{-1} at stations 1, 4 and 5, respectively, and was significantly higher at station 5. Chlorophyll *a* was never significantly related to PNAN counts. Daily phaeopigment levels averaged between .01 and.03 ug L^{-1} and usually showed very small fluctuations.

The small sigma-t fluctuations shown in Figures 2-4, which were

primarily due to small temperature variations probably caused by slight calibration differences in the XBT probes, indicate that essentially isopycnic water masses were sampled. None of the stations showed any significant ($P<.05$) relationships between sigma-t fluctuations and variations in any of the CHO concentrations or microbial plankton populations. This nonconservative behavior alone does not completely justify a temporal interpretation of these variations because phytoplankton can maintain distinct patches (Steele, 1976) which may sustain spatial CHO inhomogeneities, even in thoroughly mixed waters. However, there is no strong evidence that such patches were encountered. Patchiness need not be invoked to explain the PNAN fluctuations because the fastest apparent rates of increase in PNAN numbers correspond to one population doubling in three hours which is not unprecedented in oligotrophic seas (Sheldon and Sutcliffe, 1978). PNAN patches capable of maintaining horizontal gradients sufficient to account for the observed CHO data, should contain a distinctly higher biomass than the surrounding waters. Neither PNAN numbers or chlorophyll a concentrations alone are good biomass estimators, however they should vary directly with each other if large variations in PNAN biomass were experienced. The lack of a direct relationship between these factors probably reflects the independent temporal patterns of chlorophyll synthesis and cell division in the PNAN (Sournia, 1974) rather than patchiness. The diameter of a phytoplankton patch necessary to maintain its integrity against turbulent diffusion is usually placed in the 10-100 km range (Steele, 1974, 1976). The total linear distance covered by the drogued buoy during stations 1, 4 and 5 was just over 50, 100 and 25

km, respectively (Fig. 1). Therefore, if horizontal patches were encountered, their boundaries would have been crossed infrequently if at all. Such patches should also have been advected along with the drogued buoy. CHO pulses occurring on smaller spatial scales would be dissipated rapidly by turbulent diffusion. Calculations from Okubo (1962) indicate that the peak concentration in a patch of a conservative tracer measured 5 min after a point source addition, would be reduced by three orders of magnitude during the next hour. In the absence of evidence suggesting that the data (Figs. 2-4) resulted from the sampling of spatial inhomogeneities, it seems possible that a substantial portion of the observed variation represents true temporal dynamics occurring in substantially uniform water masses.

No single consistent diel CHO cycle was evident, however there are indications of two distinctly different patterns. Three days, shown in Figures 2, 3-B and 4-A (which will be referred to as Type I days) show generally declining PCHO and TCHO levels from 1200 to about 2100 hours. MCHO tended to increase during these periods and showed distinct peaks (Figs. 2 and 4-A) or higher than average concentrations (Fig. 3-B) in the early morning. TCO_2 fluctuated on Type I days (Figs. 3-B and 4-A) but showed no consistent trends. Figures 3-A and 4-B (referred to as Type II days) were characterized by significant PCHO and TCHO accumulations in the afternoon and evening (Fig. 4-B also shows substantial morning accumulation) and extremely large net apparent reductions in TCO_2 during the daylight hours. MCHO either remained substantially constant or declined while PCHO was accumulating.

The origin of these two apparent types of CHO patterns is uncertain, however it is probable that the net PCHO accumulation on the Type II days was associated with the periods of net production indicated by the apparent TCO_2 uptake. The lack of coincident MCHO accumulations is not surprising since unlike PCHO, simple sugars are not usually major extracellular products of unicellular algae (Hellebust, 1974). Periods of PCHO accumulation through the afternoon and into the early evening were observed in five diel studies conducted during all seasons in a New England salt marsh. These were probably related to the rate of net primary production because net PCHO release was a significant function of total daily solar radiation and mean daily temperature (No. 1, this series). These two variables cannot explain the difference in CHO patterns on Type I and Type II days, nor are there consistent differences in the microbial plankton patterns. However, significant ($P<.01$) net apparent ammonium and phosphate uptake occurred between 0600 and 0900 only on the Type II days which may have triggered the increased net primary production leading to the release and net accumulation of PCHO. One Type I day (Fig. 4-A) shows apparent phosphate uptake during this time, but ammonium remained undetectable. On Type I days, bacterial uptake seemed to dominate the TCHO and PCHO patterns after 1200 hrs when no detectable net release occurred. The MCHO peaks on Type I days may have resulted from extracellular enzymatic hydrolysis of PCHO by bacteria (Khailov, 1968). This is discussed further below.

The PCHO and TCHO peaks occurring after 1200 hrs in the Type II days are reminiscent of similar features observed in five of the six diel studies in the Sargasso Sea (No. 2, this series). There are also

other similarities between the results of this earlier open ocean study and the present data. The inverse correlation of PCHO concentrations with PNAN numbers shown in Fig. 5 is similar to the relationships found in two data sets from the Sargasso Sea off the Carolina coast. These relations were not significant at a second location off central Florida, however, significant ($P<.05$) inverse partial correlations relating PCHO to PNAN while holding HBAC (and in one case HNAN) constant, were found. The existence of inverse relationships between PCHO and PNAN from two separate studies in the Sargasso and Caribbean Seas suggests that these associations may result from general processes in these oligotrophic areas rather than from local hydrographic conditions or the precise species composition of the plankton.

The strong direct relationship between MCHO concentrations and HBAC populations in this study (Fig. 6) was not found in the Sargasso Sea (No. 2, this series) nor were the concentrations of dissolved glucose and fructose related to planktonic bacterial numbers or biomass in a diel study in the Baltic (Meyer-Reil et al., 1979). Also unlike the Sargasso Sea study, no combination of PNAN, HNAN and HBAC population data from the Caribbean produced multiple correlations with any of the CHO fractions which were more significant than the simple correlations (Figs. 5-6).

Additional significant simple and multiple correlations were uncovered when the sample to sample fluctuations of the CHOs were related to the variation in the plankton populations (Figs. 7-12). Values are plotted on a per hour basis for convenience. If the CHO fluctuations primarily represent temporal dynamics, the values on the

ordinates of Figures 7, 8 and 10 give the net apparent hourly flux of TCHO, PCHO and MCHO, respectively, however these relationships do not strictly depend on this interpretation as discussed earlier (No. 2, this series).

Figures 7-12 indicate that a close association between dissolved CHO and the microbial plankton occurs in situ over short intervals. Figure 8 shows that the relation of PCHO to PNAN (Fig. 5) was primarily due to the between sample fluctuation of these parameters. A significant inverse correlation between TCHO and PNAN variations (Fig. 7) was found even though one did not exist when measured TCHO and PNAN levels were compared. These inverse relationships probably did not result from heterotrophic uptake of CHO by PNAN because over 90% of the heterotrophic activity in marine waters has been attributed to free bacteria <1 um in diameter (Azam and Hodson, 1977). The nanoplankton observed in this study averaged 3x5 um. They also could not be primarily due to loss of cellular CHO during destruction of PNAN by grazing or autolysis because net TCHO accumulation far exceeds the net biomass reductions indicated by the PNAN counts. The maximum observed PNAN population ($9x10^5$ cells per liter) accounts for less than 8 ug cell C per liter using a mean cell volume of 39 um^3 (3x5 um oblate spheroid) and the cell volume to carbon relation of Strathmann (1967) for phytoplankton other than diatoms.

Burney (No. 2, this series) suggested that the inverse (or partial inverse) correlations of TCHO and of PCHO with PNAN in that study could be primarily due to an inverse relation between the rates of cell division and CHO excretion by PNAN. There is usually a much smaller accumulation of extracellular CHO in logarithmic phase,

relative to stationary phase phytoplankton cultures (Guillard and Wangersky, 1958; Myklestad, 1974, 1977). Dissolved CHO usually remains low during natural phytoplankton blooms and increases rapidly thereafter (Wangersky, 1959; Walsh, 1965, 1966). Figure 13 indicates that a similiar relation between the rates of net TCHO release and cell division can also occur in a nutrient replete Isochrysis culture grown under natural sunlight and sampled at a much shorter interval than employed in these previous studies. TCHO concentrations declined slightly due to bacterial uptake during periods of rapid Isochrysis reproduction and accumulated only when the cell division rate slowed. Comparison of the rates of change of TCHO and cell numbers (Fig. 14) produced a highly significant inverse, and clearly temporal, relationship similar to the field results (Figs. 7-8), suggesting that the latter may also represent time varying processes.

Two additional processes should accentuate the inverse variation of PNAN and CHO in nature. When the rate of PNAN reproduction slows and the increased rate of CHO release causes its accumulation, ongoing consumption by phagotrophs should cause a net reduction in PNAN numbers which would accent the inverse variation between CHO and PNAN. Likewise, when PNAN are accumulating due to reproduction in excess of consumption, and conserving cellular CHO, net bacterial uptake would cause dissolved CHO to decline, producing the opposite inverse fluctuation.

If the relations in Figures 7 and 8 actually resulted from this mechanism, the growth and division cycles of the dominant PNAN species must be roughly synchronous. Otherwise the inverse variation would be obscured by out of phase processes. Some sort of synchrony (except in

areas where tides prevail) seems to be rather common in nature (Sournia, 1974). At stations 4 and 5 (Figs. 3-4) PNAN populations seemed to increase from dawn, or the next sampling thereafter, until mid-afternoon. Smaller indications of possible PNAN reproduction also occurred in the early morning during day 1 of station 4 (Fig. 3-A) and day 2 at station 5 (Fig. 4-B). PNAN populations increased from 0130 to 0730 hrs at station 1 (Fig. 2) on one day, but this was not repeated 24 hrs later.

PNAN appear to play an important role, through their daily growth and division cycles, in the control of in-situ CHO dynamics, however they are clearly not the only important group assisting in this regulation. Strong relationships between the apparent rates of change of PCHO and of MCHO with HBAC fluctuations were also found (Figs. 9-10). The inverse correlation of PCHO and HBAC dynamics (Fig. 9) is not an unexpected relationship between a consumer population and a labile substrate. A similar partial inverse correlation between PCHO and HBAC (holding PNAN constant) was found in the combined data from two Sargasso Sea stations (No. 2, this series).

The reasons for the direct relations between MCHO and HBAC concentrations (Fig. 6) and fluctuations (Fig. 10) are more elusive. It is possible that HBAC populations followed MCHO levels or that MCHO limited HBAC numbers, but this does not seem reasonable because of their inverse relation to PCHO, whose concentrations averaged over twice those of MCHO. The inverse relation of PCHO and HBAC variations (Fig. 9) could be a reflection of the direct association of MCHO and HBAC changes (Fig. 10) caused by the inverse fluctuation of MCHO and PCHO. Overall rates of change of MCHO and PCHO were inversely related

(P=.044), however the relation between PCHO and HBAC fluctuations (Fig. 9) remained significantly inverse (P=.046) when their common associations with MCHO dynamics were removed.

Both of the relationships in Figures 9 and 10 could be caused by extracellular enzymatic hydrolysis of PCHO by HBAC (Khailov, 1968) during their growth periods. Glucose accumulation during rapid growth of bacteria in batch cultures supplied with laminarin, indicating extracellular hydrolysis, have been observed (M. Baxter, personal communication). The times of PCHO reduction during the afternoon or evening on the Type I days (Figs. 2, 3-B, 4-A) show smaller coincident MCHO increases as well as HBAC accumulations. Declining TCHO concentrations during these times (especially in Figures 3-B and 4-A) show that actual net bacterial uptake of PCHO also occurred in addition to their enzymatic conversion of PCHO to MCHO.

As was sometimes the case in the Sargasso Sea (No. 2, this series), the fluctuations of TCHO and PCHO appeared to be more dependent on the interactions of the microbial plankton than on any single group. The rates of change of PNAN and HNAN combined to account for a significantly larger fraction of the variance in TCHO dynamics (Fig. 11) than did PNAN alone (Fig. 7). No significant simple correlation existed between TCHO and HNAN fluctuations, however HNAN made a significant positive contribution to the multiple relationship (Fig. 11) indicating that their excretion may be a source of in-situ TCHO. This has been suggested previously (Burney et al., 1979; No. 2, this series).

The HNAN are a heterogeneous group consisting of phagotrophic dinoflagellates, choanoflagellates, small bactivorous flagellates

amoebae and small ciliates (Paul Johnson, personal communication). Figure 15 shows that a common marine ciliate (Uronema marina) can definitely accelerate TCHO release in an Isochrysis culture. Phaeopigments have been suggested as an index of metazooplankton feeding and defecation (Lorenzen, 1967; Shuman and Lorenzen, 1975) because they occur in fecal pellets due to chlorophyll degradation during gut passage. Direct relationships of the rates of change of TCHO and of PCHO with phaeopigment fluctuations in a large simulated estuarine ecosystem (No. 1, this series) suggested that zooplankton excretion was an important source of dissolved CHO. The lack of similar field relations in the present study does not necessarily suggest that excretion by the protist dominated consumer populations (HNAN) may not contribute significantly to CHO levels, because Figure 15 also shows that, in addition to accelerating TCHO release, a ciliate in culture can consume large quantities of chlorophyll a without producing particulate phaeopigments in excess of the control. The fluctuations of HNAN populations do seem to be of lesser importance in the regulation of CHO dynamics than those of PNAN and HBAC, however, the rates of HNAN excretion and reproduction may not be as closely coupled as the rates of CHO release and uptake appear to be related to the division rates of PNAN and HBAC. If this is true, the importance of HNAN excretion in this study may be underestimated.

The fluctuations of PNAN and HNAN combined to account for a very significant fraction of the rate of change of PCHO (Fig. 12). The partial regression coefficients were both negative, as were the simple regression slopes, however the multiple relationship was more significant than either of the simple correlations. Although the

relationships in Figures 11 and 12 are significant, they are not good predictors of TCHO or PCHO rates since they tend to underestimate both the positive and negative fluctuations. This suggests that there are other factors which contribute to the control of CHO dynamics in addition to those considered here.

Calculation of biological release and uptake rates from free water measurements is always tenuous because one is never certain whether the observed dynamics result from true temporal changes occurring in a uniform water mass or from the sampling of waters with different biological histories. The precautions of sampling from the mixed layer while following a drogued buoy were designed to minimize the latter possibility. The small sigma-t variation of the samples which were unrelated to CHO dynamics at all stations, and the apparent lack of interference by plankton patchiness suggests that homogeneous water masses may have actually been sampled. Assuming this to be true, net hourly TCHO release and uptake rates (represented on the ordinate of Fig. 7) averaged 7.4 and 5.4 ug C $L^{-1}h^{-1}$, respectively. Most values were less than $\pm$10 ug C $L^{-1}h^{-1}$, however 9 release and uptake rates in the 10 to 20 ug C $L^{-1}h^{-1}$ range were observed. Positive and negative PCHO and MCHO rates of change probably do not entirely represent true microbial release and uptake because of the possible extracellular bacterial hydrolysis of PCHO to MCHO. Bacteria can also excrete PCHO composed of recently assimilated MCHO (Nalewajko and Lean, 1972; Dunstall and Nalewajko, 1975). Because of the possibility of this interconversion of MCHO and PCHO which complicates the interpretation of their rates (No. 1, this seres), they will not be discussed further. The net TCHO rates,

however, are not affected by this ambiguity and should represent microbial release and uptake, if the assumption of temporal variation is valid.

The average rates of net TCHO uptake in this study exceed maximum open ocean heterotrophic glucose uptake velocities (Hoppe, 1978) measured by the heterotrophic potential method of Wright and Hobbie (1966), by at least two orders of magnitude and the total hourly organic carbon excretion rates (^{14}C method) of offshore phytoplankton (summarized by Sharp, 1977) by at least one order of magnitude. The heterotrophic potential method suffers from several analytical and theoretical problems (Wood, 1973; Wright, 1973; Krambeck, 1979). This method was origionally intended to indicate only relative heterotrophic potential rather than actual bacterial uptake rates (Parsons and Strickland, 1962). Newer methods, which do not assume Michaelis-Menten kinetics, indicate higher bacterial uptake rates. Glucose uptake of up to 5.2 ug C $L^{-1}h^{-1}$ from nearshore waters in British Columbia (Dietz et al., 1976), 7.6 ug C $L^{-1}h^{-1}$ from waters overlying a sandy beach (Meyer-Reil et al., 1978) and 0.65 ug C $L^{-1}h^{-1}$ from the open Baltic Sea (Meyer-Reil et al., 1979) have been reported. These values may still underestimate true bacterial activities due to the system disturbance produced by sample handling and confinement.

Shortcomings of the ^{14}C method for measuring dissolved organic excretion by phytoplankton have been discussed (Nalewajko et al., 1976; Sharp, 1977; Wiebe and Smith, 1977). Severe alterations in the natural species composition of the plankton due to sample confinement during incubation (Venrick et al., 1977) may lead to very

artificial rate determinations, especially in oligotrophic areas.

Time series analyses of in-situ DOM typically indicate much larger fluctuations than do the bottle methods. Barber (1967) measured a 2.1 mg L^{-1} DOC reduction over a five-day period while following a patch of recently upwelled water in the Peru Current which suggests an overall net heterotrophic uptake of 420 ug C $L^{-1}d^{-1}$ if this occurred in a homogeneous water mass. Data given by Brockmann et al. (1979) from daily analyses of 3 m^3 tanks of North Sea water indicate maximum TCHO release and uptake rates of about 70 ug C $L^{-1}d^{-1}$ which are similar to those reported in Table 1. Meyer-Reil et al. (1979) followed a homogeneous water mass in the Baltic Sea and reported diel changes in dissolved glucose and fructose suggesting uptake rates of up to 2.4 and 8.3 ug C $L^{-1}h^{-1}$, respectively.

Daily rates of TCHO release and uptake calculated from the analytically significant hourly fluctuations (Table 1) averaged 56 and 53 ug C $L^{-1}d^{-1}$, respectively, and exceed ^{14}C primary production values by at least an order of magnitude. Evidence from a number of diverse studies indicate that accepted open ocean production rates have been seriously underestimated. The standard ^{14}C method for primary production (Steeman-Nielsen, 1952) has been recently challenged on the basis of detrimental small bottle effects (Gieskes et al., 1979) as well as in-situ diel oxygen (Tijssen, 1979) and particulate organic carbon (Postma and Rommets, 1979) dynamics. Bacterial production estimates from diffusion culture (Sieburth et al., 1977) and total system respiration (Joiris, 1977) exceed ^{14}C production values in the North Atlantic and North Sea, respectively.

Production calculated from the underlying exponential growth rates of Sargasso Sea phytoplankton observed when grazing pressure is removed, is an order of magnitude greater than is indicated by the ^{14}C method (Sheldon et al., 1973; Sheldon and Sutcliffe, 1978). Apparent CO_2 uptake on the Type II days in this study (Figs. 3-A and 4-B) both indicate a net system production of 372 mg C $m^{-3}d^{-1}$ which exceeds the total daily ^{14}C production of the water column (per m^2) of the area (Koblentz-Mishke et al., 1970).

While this value appears impossibly high compared to accepted rates, the data in Table 1 show a degree of internal consistency. For station 4, where complete data are available (assuming zero net system production on day 2), mean daily DOC release accounted for 50% of net system primary production. This result is in the high end of the range of literature values (summarized by Sharp, 1977) for percent extracellular release (PER) determined with the ^{14}C method. Higher PER values are characteristic of oligotrophic areas (Thomas, 1971; Ignatiades, 1973).

Two days (station 4, day 2, and station 5, day 1) showed apparent net DOC release and uptake with no sure indications of net primary production. The relatively small DOC release rate on the second day of station 4 (Table 1) is based on only one significant ($P<.05$) incremental DOC increase which may be erroneous or could represent release of DOC synthesized on (or before) the previous day. Net apparent DOC excretion during the first day of station 5 (Table 1), however was the highest observed. There were no indications of sustained TCO_2 uptake on this day, however some of its fluctuations were analytically significant. If the apparent TCO_2

reduction between 0600 and 0900 (Fig. 5-A) was due to primary production, it would represent fixation of 168 ug C $L^{-1}d^{-1}$ which would bring net production within 19 ug C $L^{-1}d^{-1}$ of net DOC release which could easily be accounted for by excretion of previously fixed carbon or analytical error. This would still result in a very high PER, however it should be possible for such plankton populations to release (or lose) virtually all of their net daily community production without ill effects because longer term energy storage is not important to these ephemeral organisms (Parsons, 1976).

At station 1, net daily TCHO release appeared to slightly exceed that of DOC, but the discrepancy is well within methodological error. The large apparent proportion of the daily DOC release accounted for by TCHO may be due to benthic processes on the nearby Mysteriosa Banks. Away from the Banks (station 4 and the first day of station 5), the combined net TCHO flux accounted for 33% of the combined net apparent DOC release and 48% of net apparent DOC uptake. Antia et al. (1963) reported that dissolved carbohydrate accounted for an identical 33% of apparent extracellular DOC release from phytoplankton in a large volume plastic sphere. TCHO flux accounted for an average of 30% of DOC release and 47% of DOC uptake on four summer days in a New England salt marsh (No. 1, this series). Maximum TCHO fluctuations accounted for a mean 49% of the apparent DOC reductions observed between diurnal and nocturnal hydrocasts in the North Atlantic (Sieburth et al., 1977).

Results of this study indicate an intimate and dynamic interrelationship between open ocean microbial plankton and dissolved carbohydrate. The occurrence of relationships similar to those found

earlier in the Sargasso Sea suggests that they represent general processes of oligotrophic plankton communities rather than fortuitous local conditions or chance. It appears that in general the rates of DOC and TCHO flux based on direct in-situ measurements make sense when compared to each other and to similarly estimated primary production. While such high values must be viewed with due caution, they should not be rejected out of hand on the basis of massive data derived from studies employing the same basic methodologies (radio-tracers and bottle incubations) which may themselves by faulty. If anything approaching the rates suggested here actually occur in the open sea, a total reevaluation of microbial plankton dynamics is critically needed.

ACKNOWLEDGEMENT

I wish to thank Paul Davis, Michael Sieracki, Lorraine Cocozza and Germaine Reusch for providing the plankton counts. Kenneth Johnson assisted by Janice Miller analyzed DOC and TCO_2. Joyce Burney assisted with the CHO and pigment determinations. Melissa Hutchins provided inorganic nutrient data. Roger Roussell supplied salinity data and assisted with sampling along with Richard Heffernan, Molly Baxter, Brian O'Halloran and Steve Zeeman. Paul Hargraves and Anthony Soldo supplied the Isochrysis and Uronema cultures, respectively. I thank John Sieburth for financial support and the use of his facilities and Scott Nixon and Michael Pilson for criticism of the manuscript as well as the Officers and Crew ofR/V Endeavaor. This work was supported by grants OCE-7826388 and OCE-7681779 from the Biological Oceanography Program of the National Science Foundation to John Sieburth.

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APPENDIX I:

DETAILS OF THE

CARBOHYDRATE ANALYSES

ERRATA: Page 143, 6th line from the bottom should read .57 g vice 57 g.

Marine Chemistry, 5(1977) 1—13

Research Papers

DISSOLVED CARBOHYDRATES IN SEAWATER. I, A PRECISE SPECTROPHOTOMETRIC ANALYSIS FOR MONOSACCHARIDES

KENNETH M. JOHNSON and JOHN McN. SIEBURTH
Graduate School of Oceanography, University of Rhode Island, Kingston, R.I. 02881 (U.S.A.)

(Received March 23, 1976; revision accepted June 29, 1976)

ABSTRACT

Johnson, K. M. and Sieburth, J. McN., 1977. Dissolved carbohydrates in seawater. I, A precise spectrophotometric analysis for monosaccharides. Mar. Chem., 5: 1—13.

A relatively precise and rapid method for the analysis of total dissolved monosaccharides at the concentrations that occur in seawater is described which uses 1-ml quantities for each analytical and control replicate. The alditols remain unchanged while the pentoses and hexoses are reduced to their alditol form by borohydride. The total alditols are then oxidized with periodate to form two moles of formaldehyde per mole of monosaccharide and the formaldehyde is determined spectrophotometrically with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH). Unlike other spectrophotometric methods, similar curves are obtained for equimolar concentrations of different carbohydrates while the differences on a weight basis are only due to the small difference in molecular weight between pentoses and hexoses. Winter—Spring samples from surface waters of lower Narragansett Bay ranged from 122 to 226 $\mu g\ l^{-1}$ with a mean of 159 $\mu g\ l^{-1}$.

INTRODUCTION

Primary producers synthesize carbohydrates as energy, storage and structural materials, which are released during cell metabolism and disintegration as fecal remnants. In order to study the distribution of these compounds in the sea and to get an idea of their flux, a good starting point would be a precise and manageable procedure for total dissolved monosaccharides which would include the sugar alcohols and exclude the polysaccharides.

The isolation of the trace amounts of carbohydrate from the salts in seawater as well as other problems such as multiple peaks and time required make gas chromatography with both trimethylsilyl (Ludlow et al., 1966) and acetylated (Sawardeker et al., 1965) derivatives as well as electrodialysis and liquid-liquid chromatography (Josefsson, 1970) impractical for routine observations. Existing spectrophotometric methods have three fundamental problems: the calibration curves vary with each carbohydrate (Schaefer,

1965); they do not detect sugar alcohols; and the use of strong acids (Lewis and Rakestraw, 1955; Antia and Lee, 1963; Handa, 1966; Josefsson et al., 1972) includes the polysaccharides as well as interfering substances.

A more precise spectrophotometric analysis of just the monosaccharides can be made by incorporating a series of well-established chemical procedures. Pentoses and hexoses dissolved in aqueous solution can be easily reduced to sugar alcohols with borohydride (Abdel-Akher et al., 1951). Periodate oxidation of the naturally occurring and newly derived sugar alcohols yields two moles of formaldehyde per mole of carbohydrate (Sawicki et al., 1967) while 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) can be used to determine low levels of formaldehyde in aqueous solution (Sawicki et al., 1961). Levels of 10^{-5} moles of mannitol in serum were reported by Pays and Beljean (1970). The purpose of this paper is to show how the above chemical reactions have been developed into a total dissolved monosaccharide assay and to report values obtained from Narragansett Bay, Rhode Island, and adjacent waters.

METHODS AND MATERIALS

Surface samples of seawater used to develop the test and for the Narragansett Bay survey were collected in alkali-cleaned bottles, filtered with Gelman Type A glass fiber filters with no vacuum or less than 10 cm of vacuum and used immediately or held at −45°C. Both the sample bottles and filters were combusted at 490°C for 12 h before use. Water from the Narragansett Marine Laboratory Pier used to develop the test has a salinity of 28—31‰ and a total DOC content of 1.6—3.1 mg l^{-1}. Stock carbohydrate solutions of 250 mg l^{-1} in distilled water containing 20 mg l^{-1} of mercuric bichloride as a preservative, were held at 5°C, and for use were diluted with seawater or distilled water and used in the range of 125—2500 μg l^{-1}. Some carbohydrates were from Sigma Chemical (St. Louis, Mo.) and Pfanstiehl Labs (Waukegan, Ill.) but most of the final work was with products from Eastman Organic Chemicals (Rochester, N.Y.) and Schwarz/Mann (Orangeburg, N.Y.). The melting points of all carbohydrates and the specific rotation of mannitol were checked.

All reagents were of certified grade from Fisher Scientific (Boston, Mass.) unless otherwise specified. Potassium borohydride (K&K Fine Chemicals, Plainview, N.Y.) solutions of 10—100 mg per 5 ml of cold distilled water were freshly prepared for each batch of samples. Hydrochloric acid was used at 0.1, 0.36 and 2.0 *N* concentrations to destroy unreacted borohydride. Periodic acid (Matheson Coleman & Bell, Norwood, Ohio) of 0.025 *M* was prepared with 57 g dissolved in 100 ml of distilled water and stored away from light at room temperature (rt). Sodium arsenite 0.25 *M* prepared with 3.247 g dissolved in 100 ml of distilled water was used to destroy the unreacted periodate. The 5% solution of ferric chloride was filtered and stored at 5°C. The 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) reagent (Aldrich Chemical, Milwaukee, Wis.) was prepared by dissolving 276 mg in

10 ml of 0.1 *N* HCl with slight heating, filtered and stored in an amber bottle at rt for a week or until discoloration. MBTH from several other sources proved unsatisfactory. Combusted glass filters were used for all filtrations. All samples and reagents were dispensed with automatic pipettes (Biopipet, Schwarz/Mann) with disposable tips. Analytical determinations were made with an Hitachi Perkin-Elmer Coleman 111 Spectrophotometer with Model 68 and 80 digital concentration readout and printer (A. C. Thomas, Philadelphia, Pa.). Absorption spectra were determined on a Model 17 Cary recording spectrophotometer. Dissolved organic carbon determinations were made with a carbonaceous analyzer (Beckman Instruments, Fullerton, Cal.) modified for use with seawater.

RESULTS

MBTH determination of formaldehyde liberated by periodate oxidation of monosaccharides

The starting point for this work was the MBTH procedure of Pays and Beljean (1970). One-ml portions of seawater supplemented with 10^{-5} to 10^{-7} *M* concentrations of carbohydrate in triplicate were oxidized with periodate and the formaldehyde produced was determined with MBTH according to their procedures except that higher absorption was measured at 635 nm (rather than 620 nm) and the 3-min heating step after MBTH addition, of Sawicki et al. (1967), was used to improve reproducibility. The oxidation of mannitol in seawater was essentially complete after 5 min as shown by Table I, although the 10 min oxidation time was retained throughout the work to insure completion. Regression lines for absorbance against concentration for three trials were: $y = +0.003 + 0.227x$, $y = +0.001 + 0.240x$, and $y = +0.003 + 0.229x$. The regression coefficients (r) ranged from 0.997—0.999 for seven concentrations between 0.25 and 2.5 mg l^{-1}. These quantitative results were encouraging and the capability of the procedure to

TABLE I

Rate of periodate oxidation of mannitol in natural seawater, as indicated by the MBTH detection of formaldehyde

Time of oxidation (min)	% absorbance of 10-min values at 635 nm	
	2.5 mg l^{-1}	5.0 mg l^{-1}
1	91	87
2	98	98
5	99	99
10	100	100

4

TABLE II

Millimolar absorptivity of the MBTH reaction to formaldehyde formed by the periodate oxidation of alditols, hexoses and pentoses in seawater*

Class compound	No. of determinations	Absorbance** (635 nm)	$s^{\pm}$	coefficient of variation (%)	$m(\sum cm^{-1})$
Alditol					
mannitol	6	0.568	0.014	2.4	115
sorbitol $\frac{1}{2}$ H_2O	6	0.525	0.015	2.8	113
dulcitol	6	0.515	0.018	3.4	105
xylitol	6	0.673	0.020	2.9	114
arabitol	6	0.655	0.019	2.9	111
Ketohexose					
fructose	6	0.504	0.043	8.5	101
sorbose	6	0.321	0.013	4.0	64
Aldohexose					
galactose	6	0.152	0.085	55.9	30
mannose	6	0.083	0.023	27.7	16
glucose	6	0.072	0.016	22.2	14
Pentose					
arabinose	3	0.133	0.008	6.0	20
xylose	3	0.083	0.008	9.6	14

* Samples contained 2.5 mg l^{-1} (approx. $5 \cdot 10^{-6} M$) of test compound in seawater with a salinity of 29—31 o/oo.
** Corrected for control.

determine other alditols as well as pentoses and hexoses was tested and the results are shown in Table II. The results for the alditols were quantitative but the pentoses and hexoses were incompletely oxidized as one might predict.

The ability of other compounds to interfere with the reaction was tested at a concentration of 2.5 mg l^{-1} in seawater. Compounds which yielded insignificant or no color with MBTH after periodate oxidation were formic acid, acetic acid, glycolic acid, glycine, alanine, phenylalanine, glutamic acid, aspartic acid, lysine, sucrose, trehalose, and alginic acid. Only serine showed a reaction, but at concentrations far in excess of concentrations to be expected in seawater.

Effect of borohydride reduction on the MBTH test

In order to reduce simple sugars to sugar alcohols, an excessive amount of 1 mg of potassium borohydride (in 0.05 ml of distilled water) was added to

the 1.0 ml portions of seawater containing carbohydrate. This amount of borohydride raised the pH of seawater from 8.0 to 9.6 but produced no visible precipitation. Reduction was carried out in an 18°C incubator and was complete within four hours. Before the next step, periodate oxidation, excess borohydride must be destroyed with 0.18 meq of HCl (0.05 ml of 0.36 N HCl) ml^{-1} of sample which yields a pH of 3.3—3.4 upon the addition of periodate, virtually identical to the pH obtained when periodate alone is added to seawater. The results of the MBTH procedure, with and without prior reduction with borohydride, for a number of monosaccharides and other possibly interfering compounds, is given in Table III. Data from Sawicki et al. (1967)

TABLE III

Comparison of the millimolar absorptivities of the MBTH reaction to formaldehyde formed by the periodate oxidation of a variety of monosaccharides and other compounds with and without prior borohydride reduction

Class compound*	Millimolar absorptivity			
	prior reduction (635 nm)		without prior reduction	
			(635 nm)	613—670 nm (from Sawicki, 1967)
Alditol				
mannitol	110	(10)**	115	95
dulcitol	94	(2)***	105	99
sorbitol	103	(2)	113	106
xylitol	111	(3)	114	101
arabitol	113	(3)	111	110
Hexose				
fructose	109	(2)	101	68
sorbose	105	(1)	64	—
galactose	96	(2)***	30	55
mannose	103	(2)	16	8
glucose	106	(5)	14	21
Pentose				
arabinose	106	(1)	20	44
xylose	107	(1)	14	35
ribose	90	(2)***	11	43
Deoxy sugars				
2 deoxy galactose	64	(2)	44	—
2 deoxy glucose	62	(2)	32	37
fucose	61	(3)	14	44
rhamnose	65	(1)	14	67
Sugar acids				
gluconic acid	106	(5)	75	—
glucuronic acid	91	(4)	10	24

(cont. next page)

TABLE III (*cont.*)

Class compound*	Millimolar absorptivity		
	prior reduction (635 nm)	without prior reduction	
		(635 nm)	613—670 nm (from Sawicki, 1967)
Amino acids			
glycine	0	<1	—
alanine	0	<1	—
aspartic acid	0	<1	—
glutamic acid	0	<1	—
phenyl alanine	0	<1	—
lysine	0	<1	—
serine	41	25	0
Misc. water soluble comp.			
glycolic acid	<1	<1	<1
methyl-D-glycoside	0	0	—
inositol	—	—	1
sucrose	3	3	8
trehalose	1	1	—
maltose	79	—	8
ethyl alcohol	0	—	0
lactic acid	—	—	0
formaldehyde	—	—	48, 56
Combined results			
hexitols	102		100
hexoses	103		
pentoses	101		
pentitols	112		
deoxy sugars	63		
monocarboxylic sugar acids	99		

* All test compounds made up to 2.5 mg l^{-1} in natural seawater. Final concentrations about 10^{-6} *M* depending upon the molecular weight of the substance.
** Figures in parentheses represent the number of times analysis was repeated.
*** Same results obtained with compounds from 2 different sources.

for the same compounds without prior reduction are given for comparison with our data. Borohydride reduction prior to periodate oxidation permitted the quantitative determination of pentoses, hexoses, and sugar acids as well as the sugar alcohols. With the exception of serine there was negligible interference from other compounds. Regressions of absorbance against concentration in both $\mu M\ l^{-1}$ and mg l^{-1} for each sugar and sugar alcohol of the pentoses, hexoses, and deoxy sugars were made and the results for these standard curves are given in Table IV. Fig. 1 is a plot of the composite regression lines derived from Table IV showing the range of data points used for the calculation. The borohydride reduction step has the dramatic effect of increasing the precision

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TABLE IV

Characteristics of the analysis of monosaccharides in natural seawater by the MBTH detection of formaldehyde released from the periodate oxidation of alditols and the alditol derivatives of pentoses and hexoses after prior borohydride reduction

Compound	n*	r	Intercept	Slope abs/mg l^{-1}	Slope abs/μM l^{-1}
Hexitol					
mannitol	7	0.999	+0.002	0.214	0.0392
sorbitol	7	0.999	+0.001	0.199	0.0365
dulcitol	7	0.999	+0.001	0.190	0.0347
Hexose					
glucose	7	0.998	−0.002	0.209	0.0377
glucose	7	0.999	−0.002	0.218	0.0393
galactose	7	0.999	+0.007	0.191	0.0344
sorbose	7	0.998	+0.002	0.208	0.0376
mannose	7	0.999	+0.001	0.204	0.0368
fructose	7	0.999	−0.005	0.221	0.0398
Pentitol					
xylitol	7	0.999	+0.002	0.244	0.0389
arabitol	7	0.999	+0.003	0.256	0.0391
Pentoses					
ribose	7	0.999	+0.0004	0.228	0.0347
xylose	7	0.999	−0.004	0.265	0.0405
arabinose	7	0.999	−0.0008	0.254	0.0388
Values for composite lines in Fig.1					
(1) Pentoses and pentitols	35	0.996	−0.0005	0.252	0.0384
(2) Hexoses and hexitols	62	0.996	+0.001	0.206	0.0371
(3) Pentoses and hexoses	97	0.996	−0.0002	—	0.0378
(4) Galactose and dulcitol	14	0.999	+0.004	0.190	0.0343
(5) Deoxy sugars	21	0.997	+0.005	0.137	0.0226

* The concentrations of standards were: 0.125, 0.25, 0.50, 1.0, 1.5, 2.0, and 2.5 mg l^{-1}

of the determinations of total monosaccharides in natural seawater. Results for determinations with and without prior borohydride reduction are given in Table V. The values are not only higher, showing a more complete oxidation, but the precision of the measurements increased sharply as shown by the decrease in the mean coefficient of variation from 8.7 to 1.5%.

Analytical procedure for total dissolved monosaccharides

To reduce aldehyde groups to alcohols, 0.05 ml of freshly made cold potassium borohydride (100 mg/5 ml) in distilled water is added to 1.0 ml of the

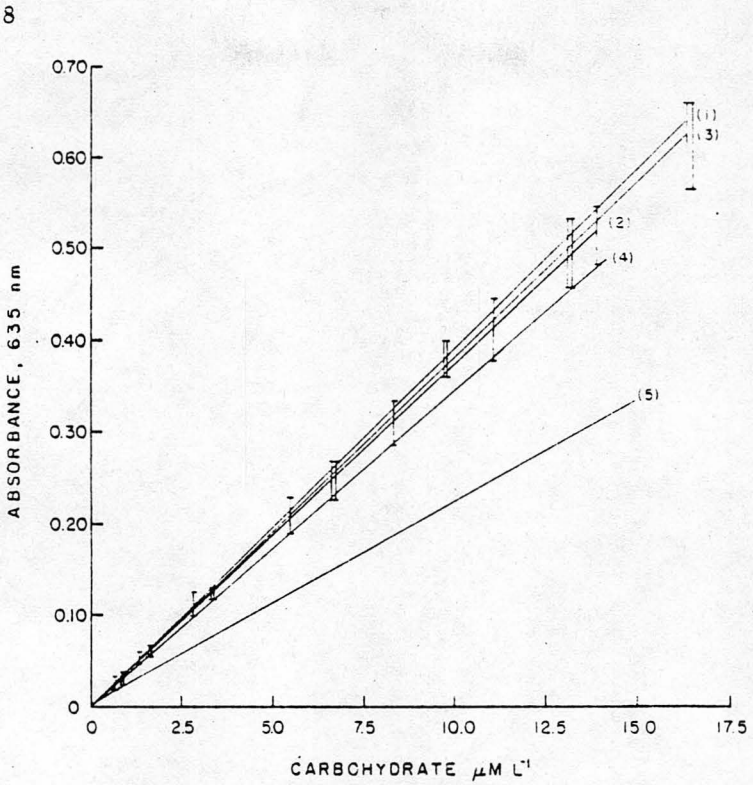

Fig. 1. Plot of the composite regression lines for the sugars examined (see Table IV). Bars I and II show range of data points for hexoses (I) and pentoses (II).

sample and incubated for four hours at 17—18°C in the absence of light. The excess borohydride is destroyed with 0.05 ml of 0.36 *N* HCl and left for 10 min to permit hydrogen gas to evolve and the pH to stabilize to neutrality. The samples are then oxidized for 10 min with 0.1 ml of periodic acid before

TABLE V

Intrasample and intersample variation of the MBTH reaction in unsupplemented natural seawater samples with and without borohydride reduction prior to periodate oxidation

Date (1975)	No reduction				Borohydride reduction			
	n	abs.	*s*	V(%)	*n*	abs.	*s*	V(%)
1/6	3	0.100	±0.011	11.0				
1/8	3	0.124	±0.010	8.0				
1/13					3	0.109	±0.0015	1.3
1/14					3	0.097	±0.0017	1.7
1/15	3	0.104	±0.011	10.5	3	0.136	±0.003	2.2
1/15					3	0.134	±0.0011	0.8
1/16	3	0.078	±0.004	5.1	3	0.086	±0.0015	1.7
1/18	3	0.089	±0.008	8.9				
mean			±0.009	8.7%			±0.002	1.5%

stopping the reaction with 0.1 ml of sodium arsenite. Both the periodate oxidation and sodium arsenite addition are carried out at rt in foil-covered racks to shield the tubes from excess light. After at least a 10 min wait, 0.2 ml of 2 *N* HCl is added and the amber color evolved is allowed to disappear before the formaldehyde produced from the sugar alcohol groups is detected with the MBTH reagent. A 0.2-ml portion of the MBTH reagent is added, the tubes tightly capped, heated for 3 min in a boiling water bath, and cooled in an rt water bath. After cooling, 0.2 ml of the 5% ferric chloride solution is added, and the color allowed to develop at 18°C in darkness for 30 min, whereupon 1.0 ml of acetone is added. The tubes are then mixed and the samples read at 635 nm as soon as possible in 1.0-cm glass cells. All steps with the exception of the borohydride neutralization should be done in tightly capped tubes.

For analytical controls 1.0-ml portions (in triplicate) of the sample are reduced with borohydride and neutralized as described for the analysis. However, in order to prevent periodate oxidation, 0.2 ml of a premixed solution (1:1)of sodium arsenite and periodic acid that is allowed to react at least 10 min prior to the addition is used. From this point on the control is treated exactly the same as the sample. The absorbances of both the samples and the controls are read against a distilled water—acetone (1.8:1) reference, and the absorbance of the control is subtracted from that of the sample. The difference is presumed to be due to formaldehyde liberated by the periodate oxidation of the alditols present in the original sample or produced during borohydride reduction. These controls or blanks must be run on each sample as they correct for turbidity and free aldehydes, as well as the reagents themselves. The usual practice is to draw six 1.0-ml portions from each sample and treat three as controls and three as samples.

The amounts of borohydride and periodate are adequate for the reduction and oxidation of carbohydrates to concentrations of 2.5 mg l^{-1}. Salinity has no effect on the test and results obtained with distilled water and seawater controls are virtually identical.

Occurrence and distribution of total dissolved monosaccharides in Narragansett Bay, Rhode Island

Samples taken to determine the concentration of total dissolved monosaccharides in lower Narragansett Bay R.I. were obtained at the Graduate School of Oceanography pier during the Winter and Spring of 1975. The results shown in Table VI are expressed both as μM l^{-1} and as μg l^{-1} of hexose. Values ranged from 122 to 226 μg l^{-1} with a mean of 159 μg l^{-1} which accounts for 2.2% of the DOC using a mean value of 3.1 mg Cl^{-1} obtained with a modified Beckman Carbonaceous Analyzer. However, if the more precise but lower mean value of 1.6 mg C l^{-1} obtained with the persulfate method is used, 159 μg l^{-1} accounts for 3.9% of the DOC. The assumption of homogeneous variance was made for the number of samples (K) in Table VI where three determinations were made. According to Youden (1951), for a series of

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TABLE VI

The occurrence of total dissolved monosaccharides in surface seawater from lower Narragansett Bay as estimated by the MBTH reaction of borohydride-reduced, periodate-oxidized samples

Date (1975)	n	$\mu M\ l^{-1}$ carbohydrate	$\mu g\ l^{-1}$ as hexose*	s	Est. % DOC**
1/13	3	0.91	163	±7	2.1
1/16	2	1.11	199	—	2.5
1/23	2	1.08	194	—	2.5
1/24	3	1.11	200	±13	2.5
1/28	1	0.74	133	—	1.7
1/29	2	0.68	122	—	1.6
1/30	2	0.68	122	—	1.6
1/31	2	1.26	226	—	2.9
2/4	2	1.03	185	—	2.3
2/13	3	1.24	224	±14	2.8
2/14	3	0.83	149	±14	1.9
2/17	2	0.98	177	—	2.2
2/18	3	0.86	154	±26	1.9
2/19	3	1.23	222	±27	2.8
2/24	3	0.98	175	±2	2.2
4/21	3	0.79	143	±7	1.8
mean			159		2.2%

* Standard curve based on results from all monosaccharides tested and expressed as $\mu M\ l^{-1}$; for conversion to $\mu g\ l^{-1}$, values expressed as hexose.
** Assumes the mean value of DOC for sampling area is 3.1 $mg\ l^{-1}$, as determined by the Beckman Carbonaceous Analyzer.

determinations:

$$s = \sqrt{\frac{\sum_{i=1}^{k}\sum_{j=1}^{n_i} X_{ij}^2 - \sum\left[\left(\sum_{j=1}^{n_i} X_{ij}\right)^2 / n_i\right]}{\sum_{i=1}^{k} n_i - k}}$$

and for these data: precision = $15/\sqrt{n}\ \mu g\ l^{-1}$.

DISCUSSION

A sensitive and precise procedure for the spectrophometric determination of total monosaccharides dissolved in seawater has been developed by utilizing a sequence of reliable carbohydrate reactions. The reduction of naturally occurring amounts of monosaccharides in natural seawater to alditols was

quantitative for glucose, galactose, mannose, fructose, sorbose, xylose, arabinose and ribose. The naturally occurring alditols and the alditols derived from the above pentoses and hexoses formed two moles of formaldehyde per mole of alditol during periodate oxidation. The millimolar absorptivity of formaldehyde with the MBTH reagent of 48—56 (Sawicki et al., 1967) should be double for the alditols and be in the range 96—112. This is precisely the range of values that were obtained for the alditols shown in Table II. These uniform values for pentoses and hexoses, shown in Table IV, illustrate the obvious advantages over the other spectrophotometric procedures. The similar curves obtained with most of the monosaccharides allow glucose to be used as a single standard. Only galactose and its alditol derivative dulcitol showed a slightly lower extinction which is consistent with the slower rate of periodate oxidation observed for dulcitol than for mannitol (O'Dea and Gibbons, 1953). The sugar acids gluconic and glucuronic gave results similar to the hexoses. The three carbon sugar alcohol glycerol gives two moles of formaldehyde per mole and is analysed along with the other carbohydrates giving the same millimolar absorptivity but overestimates by 50% on a weight basis. Although the deoxy sugars give quantitative results, they yield only one mole of formaldehyde per mole of sugar as one would expect. However for the major monosaccharides that occur in seawater, the procedure gives quantitative results at very low environmental concentrations and with good precision, without the elaborate and time consuming preparation required for chromatographic and other analytical techniques.

The procedure for the controls appears to be responsible for much of the reproducibility and specificity of the method. Any substance reacting directly with the MBTH reagent is apparently corrected for. Deliberate attempts to contaminate the samples with aldehydes caused elevated extinctions but they were corrected for by the controls. However, cigarette smoke and the use of formalin in the analytical area, which can add aldehydes, should be discouraged in the interest of reproducible and optimal results. Only serine of the amino acids tested gave color with the test. This is very important since serine is the most abundant amino acid on human skin (Bohling, 1970). Caution must be exercised in the cleaning and handling of all glassware used in sampling and analysis. The MBTH method does not work in polystyrene plastic test tubes. Precautions should be used in the analysis of culture media, as some of the phytoplankton media contain ingredients such as TRIS buffer which interfere. Glycolic acid, an important product of phytoplankton photosynthesis and excretion, does not interfere. Several polysaccharides (alginic acid, laminarin, fucoidin, and soluble starch) were tested for interference. The most reactive of these was soluble starch. However, the method is about ten times less sensitive for soluble starch than glucose on a weight basis. Apparently hydrolysis and subsequent reduction and oxidation do not occur during the short time and mild conditions encountered in the procedure.

The fact that this method does not detect significant amounts of polysaccharide makes it unique among the tests for carbohydrates in seawater. With the addition of a hydrolysis step before analysis (Burney and Sieburth,

1977; this issue, pp. 15—28) which yields total carbohydrate values, polysaccharide can be estimated by difference from the monosaccharide value.

The analytical results for inshore waters given in Table VI are not unreasonable when compared with the meager data available for dissolved monosaccharides in seawater. Much more is known about total carbohydrate levels. However, Josefsson (1970) examined surface samples from the Gullmarfjord of Sweden and reported values for the following monosaccharides which in the order of their abundance were glucose, galactose, mannose, rhamnose, fructose, arabinose, ribose and xylose. The hexoses predominated and the total amount of monosaccharides was 156 $\mu g\ l^{-1}$. Vaccaro et al. (1968) measured glucose directly in seawater for surface waters between Woods Hole and Bermuda and reported values between 70 and 195 $\mu g\ l^{-1}$.

ACKNOWLEDGEMENTS

We acknowledge the valuable discussions and encouragement from Michael E. Q. Pilson and our colleagues at the Institute of Marine Biochemistry of the Technical University of Norway. Special appreciation is given to our co-worker Curtis M. Burney for his assistance and cooperation as well as his energetic conduct of the accompanying study which helped catalyse the final stages of this one. Dana R. Kester kindly determined the absorption spectra. This study has been supported by the Biological Oceanography Program of the National Science Foundation through Grants Nos. GB-18000, GA-41501, and DES-74-01537-A01.

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DISSOLVED CARBOHYDRATES IN SEAWATER. II, A SPECTROPHOTOMETRIC PROCEDURE FOR TOTAL CARBOHYDRATE ANALYSIS AND POLYSACCHARIDE ESTIMATION

CURTIS M. BURNEY and JOHN McN. SIEBURTH
Graduate School of Oceanography, University of Rhode Island, Kingston, R.I. 02881 (U.S.A.)
(Received March 23, 1976; revision accepted June 29, 1976)

ABSTRACT

Burney, C. M. and Sieburth, J. McN., 1977. Dissolved carbohydrates in seawater. II, A spectrophotometric procedure for total carbohydrate analysis and polysaccharide estimation. Mar. Chem., 5:15—28

A procedure is described which adds a hydrolysis step to the Johnson and Sieburth 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) assay for total dissolved monosaccharides. The advantages of the monosaccharide test are retained in the total carbohydrate test, giving nearly equivalent responses for a variety of combined carbohydrates. A total sample of 50 ml is adequate for both total carbohydrate and monosaccharide assays which permit the estimation of polysaccharide by difference. Values for Narragansett Bay and adjacent waters ranged from 452 to 2023 $\mu g\ l^{-1}$ for total dissolved carbohydrate, 272 to 1353 $\mu g\ l^{-1}$ for polysaccharide, and 153 to 814 $\mu g\ l^{-1}$ for monosaccharide, which accounted for 6—18%, 4—13%, and 2—5% of the total dissolved carbon, respectively. We suggest that this is a sensitive and precise procedure which will be useful for investigating the distribution of dissolved carbohydrates in seawater and factors which affect its production, distribution and utilization.

INTRODUCTION

Many workers have reported the colorimetric analysis of total dissolved carbohydrate (TCHO) in seawater (summarized in Table I). With the exception of a chromatographic technique (Starikova and Yablokova, 1972) and a fluorometric procedure (Hirayama, 1974), three general methods have been employed. Most of the earlier work used the N-ethylcarbazol method of Erdman and Little (1950) or its modification (Zein-Eldin and May, 1958). This method has fallen from favor since McLaughlin et al. (1960) reported interference from noncarbohydrate substances and has been largely replaced by the anthrone (Dreywood, 1946) and the phenol sulfuric acid (Dubois et al., 1956) tests. The latter method has gained ascendency since the work of Handa (1966a) and its subsequent adoption by Strickland and Parsons (1968) as

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TABLE I

Summary of published total carbohydrate measurements for seawater

Reference	Location	Method	Standard	Total range CHO concentration ($mg\ l^{-1}$)	Remarks
Collier et al. (1950)	Florida waters	N-ethyl--carbazole	arabinose	2.0—25	—
Collier et al. (1953)	Gulf of Mexico	N-ethyl--carbazole	arabinose	0—20	—
Lewis and Rakestraw (1955)	off southern California	anthrone and N-ethyl--carbazole	sucrose	0.14—0.45	up to 7.9 $mg\ l^{-1}$ in coastal lagoons
Anderson and Gehringer (1958a)	South Atlantic coast, U.S.	N-ethyl--carbazole	arabinose	0—12.0	most values $<3.0\ mg\ l^{-1}$
Anderson and Gehringer (1958b)	South Atlantic coast, U.S.	N-ethyl--carbazole	arabinose	0—35.8	most values $<3.0\ mg\ l^{-1}$
Collier (1958a)	shelf, Gulf of Mexico	N-ethyl--carbazole	arabinose	0—3.0	up to 19.4 $mg\ l^{-1}$ during red tide
Collier (1958b)	western shore, Gulf of Mexico	N-ethyl--carbazole	arabinose	0—2.6	—
Wangersky (1959)	Long Island Sound	N-ethyl--carbazole	glucose	0—2.5	most values $<1.5\ mg\ l^{-1}$
Dragovich (1961)	Pier at Naples, Florida	N-ethyl--carbazole	arabinose	0.2—4.1	annual range most $<2.0\ mg\ l^{-1}$
Koyama (1962)	northwest Pacific	N-ethyl--carbazole	sucrose	0.24—1.68	2 deep water profiles
Antia and Lee (1963)	northeast Pacific	anthrone	glucose	<0.10—0.58 0.00—0.40	(coastal) (open ocean)
Walsh (1965)	Cape Cod waters	anthrone	sucrose	1.16—3.17 0.40—1.00	(productive estuar.) (other waters)
Walsh and Douglass (1966)	Sargasso Sea off Bermuda	anthrone	sucrose	0.18—0.75	single deep profile
Handa (1966b)	Indian Ocean	phenol-sulfuric acid	glucose	0.19—0.66	several deep profiles
Handa (1967)	northwest Pacific offshore	phenol-sulfuric acid	glucose	0.09—0.46 0.17—0.42	(total range) (surface)
Kawahara et al. (1967)	off southern Hokkaido, Japan	anthrone	sucrose	0—1.3	most values $<0.5\ mg\ l^{-1}$
Handa (1970)	off Honsyu, Japan	phenol-sulfuric acid	glucose	0.24—0.37	single deep profile
Erokhin (1972)	eastern Murman, USSR (littoral)	phenol-sulfuric acid	glucose	0.5—3.0 2.7—7.0	(surface) (bottom in macrophy.)
Starikova and Yablokova (1972)	Black Sea	paper chromatography		0.93—2.99	hydrolyzed samples
Hirayama (1974)	Kojima and Osaka, Japan	fluorimetric method	xylose and glucose	0.480—0.523 0.983—1.131	Kojima Misaki
Cedeño Fermin (1972)	Gulf of Cariaco, Venezuela	phenol—sulfuric acid	glucose	0.35—3.25	

their standard method of seawater analysis. These methods produce strikingly dissimilar absorbances with equal concentrations of different sugars. This, coupled with the use of different standards (see Table I) has made the comparison of results from different workers quite difficult.

The colorimetric methods currently used in seawater employ concentrated sulfuric acid which hydrolyzes polysaccharides and produces mostly furfural from pentoses and hydroxymethylfurfural from hexoses which combine with the various reagents to yield the colored products. The strong acid can produce other degradation products (Josefsson et al., 1972) which will lead to underestimation of the TCHO. The addition of concentrated sulfuric acid to seawater also increases the absorbance of yellow organic materials which can interfere with the phenol sulfuric acid test (Bikbulatov and Skopintsev, 1974).

The 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) total dissolved monosaccharide assay of Johnson and Sieburth (1977; this issue, pp. 1—13) which does not employ concentrated mineral acid and does not hydrolyze polymeric carbohydrates (PCHO), has been shown to produce nearly equivalent color with several sugar alcohols, hexoses, pentoses and sugar acids. To realize the advantages of this method for dissolved TCHO, it must be coupled to a quantitative hydrolysis procedure. Its use with and without hydrolysis gives direct determination of TCHO and monomeric carbohydrate (MCHO) respectively. PCHO can be estimated by the difference between these values. The development, evaluation and application of a method for analysis of TCHO and estimation of PCHO form the basis of a Master of Science thesis (Burney, 1976) and are the subjects of this report.

MATERIAL AND METHODS

Sample collection and filtration

Surface samples were taken from the pier at Narragansett Marine Laboratory, Narragansett, R.I., and from 12 other stations in and around Narragansett Bay (Fig. 1) between 22 January and 25 July 1975. Pier samples were collected in a polyethylene bucket. The Bay and adjacent samples were obtained in chemically cleaned glass bottles or 5-l Niskin samplers. The Rhode Island Sound sample was collected with an acid-rinsed Niskin bottle during Cruise 170 of R/V "Trident". Samples were filtered immediately through precombusted (450° C) Gelman type A glass fiber filters using less than 10 cm of Hg vacuum. Analysis was commenced immediately on all samples except those taken 27 February and 17 March which were frozen at —45° C for up to 3 days after filtration. All filter setups and other glassware used throughout the analysis were carefully chemically cleaned.

Analysis

Initially, a range of hydrolysis times and HCl concentrations were tested. Hydrolysis was conducted at approximately pH 2.3, 1.1, and 0.1 for up to

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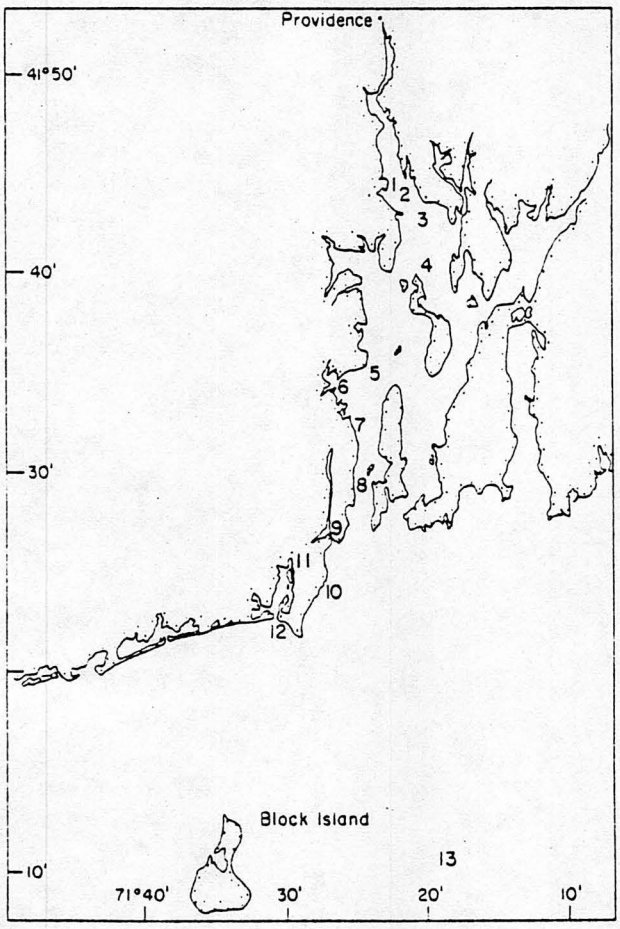

Fig. 1. Sampling stations for Narragansett Bay, Rhode Island, and adjacent waters. 1, Pawtuxet River; 2, Sabine Point; 3, Rocky Point; 4, Providence Point; 5, Quonset Pier; 6, Wickford Marina; 7, Jamestown Bridge; 8, Marine Lab Pier; 9, Narrow River Bridge; 10, Narragansett State Pier; 11, Saugatucket River; 12, Pt. Judith Breakwater; 13, Rhode Island Sound.

48 h at 100°C. The following procedure employs the empirically derived optimal hydrolysis conditions for winter samples from Narragansett Bay. Ten ml seawater samples with 1 ml of 1 *N* HCl (pH 1.1) were hydrolyzed in sealed ampules at 100°C for 20 h. After cooling, the hydrolysis tubes were opened and 10 ml from each was transferred to 16 x 125 mm screw topped test tubes equipped with teflon-lined caps. The acid was neutralized with 2 ml of 0.45 *N* NaOH. To each tube containing 12 ml of neutralized hydrolysate, 0.1 ml of 10% KBH_4 (250 mg in 2.5 ml distilled water) was added. The borohydride solution was made with cold (4°C) water immediately before use. The tubes were tightly capped and held for 4 h at 18°C before oxidizing the excess KBH_4 with 0.1 ml of 2 *N* HCl. When visible gas evolution ceased, duplicate 1 ml aliquots were drawn from each tube and dispensed into

separate 13 x 100 mm screw capped test tubes with teflon-lined screw caps. One tube of each pair was designated the control for the other called the analytical sample. Periodate oxidation and spectrophotometric analysis of the resulting formaldehyde with MBTH was according to Johnson and Sieburth (1977; this issue, pp. 1—13). The absorbance of each control sample was subtracted from that of the corresponding analytical sample.

Standards

Samples of soluble starch, arabic acid, melezitose and raffinose were obtained from Pfanstiehl Laboratories (Waukegan, Ill.). Samples of xylan and sodium alginate were purchased from Sigma Biochemicals (St. Louis, Mo.). Laminarin (Nutritional Biochemicals, Cleveland, Ohio), purified agar (Difco, Detroit, Mich.), fucoidin (Pfaltz and Bauer, Flushing, N.Y.), and glucose (Eastman Organic Chemicals, Rochester, N.Y.) were also used. Concentrated stock solutions (100—250 mg l^{-1}) were prepared in distilled water containing 20 mg l^{-1} $HgCl_2$. To correct for inorganic impurities, the total dissolved organic carbon (DOC) of each solution was determined with a Beckman Carbonaceous Analyzer (Beckman, Fullerton, Cal.) and its actual carbohydrate concentration calculated assuming that the specified compound comprised all the DOC. When needed, the stock solutions were diluted with filtered seawater and analyzed in the 10^{-6}—10^{-5} *M* range. Molarity of polysaccharide solutions refers to that of their glycosidically linked residues. Glucose standards were analyzed both with and without the hydrolysis procedure to check for artifacts. For comparison, eight of the compounds (excluding soluble starch and fucoidin) were also analyzed in the same concentration range using a slight modification of the phenol sulfuric acid test as described by Strickland and Parsons (1968) using twice the specified reagent and sample volumes in order to fill large 10-cm cells.

Analysis of natural waters

Molar concentrations of TCHO and MCHO were determined from the control corrected absorbance data using a glucose regression standard (n = 49, r = 0.997). PCHO in $\mu g\ l^{-1}$ was computed using (μM TCHO—μM MCHO) x 162, and MCHO using μM MCHO x 180. TCHO in $\mu g\ l^{-1}$ was obtained by adding MCHO and PCHO values. In order to use the glucose standard for both MCHO and TCHO, absorbances from hydrolyzed samples were multiplied by a factor of 1.32 to correct for dilution of the samples by the hydrolysis and neutralization reagents.

The validity of the PCHO calculation was tested with two dialysis experiments. PCHO was directly determined on dialyzed seawater (Dialya-Por C tubing, M.W. cutoff 3500, National Scientific, Cleveland, Ohio) using the method for TCHO. An equal length of tubing filled with distilled water was used as a control for membrane bleed. In the first experiment, 100-ml samples were dialyzed against large volumes of distilled water containing

20 mg l^{-1} $HgCl_2$ for 48 h in a refrigerator, after which the $HgCl_2$ inside the sacs was diluted by a ½-h dialysis against distilled water alone. The final volume in the sacs was measured in order to correct for PCHO concentration changes due to the gain or loss of water through the membranes. After correcting for bleed, the PCHO results were compared to those determined by the difference method on the same water.

In the second experiment, 50 ml seawater and distilled water samples were dialyzed at room temperature on a magnetic mixer for 48 h after which a 1½-h dialysis was used to remove the $HgCl_2$.

Interference studies

Since MBTH has been shown to react with a large number of aromatic amines (Sawicki et al., 1961b, c), a seawater solution of glucose (1.25 mg l^{-1}) was assayed with and without 2.0 mg l^{-1} of N,N-dimethyl-*p*-aminobenzaldehyde (DMAB) which produces an identical absorbance maximum as formaldehyde with MBTH (Sawicki et al., 1961b). The effect of nitrate ($NaNO_3$, 150 mg l^{-1}), phosphate ($NaH_2PO_4.H_2O$, 10 mg l^{-1}), and silicate ($Na_2SiO_4.9H_2O$, 30 mg l^{-1}) on analysis was studied in a similar manner. Spectra of the MBTH chromophores in pier seawater, Saugatucket River water (Station 11) and distilled water solutions of laminarin and fucoidin (0.75 mg l^{-1}) were run on a Cary 17 recording spectrophotometer.

RESULTS AND DISCUSSION

Evaluation of the method

Slope-intercept data, regression coefficients, millimolar absorbance and % recoveries as glucose for the tested carbohydrates are given in Table II. With the exception of sodium alginate (calculated as alginic acid) and agar, all slopes vary less than 16% from their mean. Glucose analyzed with and without "hydrolysis" produced essentially equal slopes, indicating that no artifacts are introduced by the hydrolysis.

Fig. 2 presents a comparison of the standard curves produced with the MBTH and phenol sulfuric acid methods. MBTH curves are corrected for the dilution of the samples by the hydrolysis and neutralization reagents. The MBTH method offers considerably improved sensitivity relative to phenol sulfuric acid even when a ten-fold advantage is given the latter method by the use of 10-cm cells. Unlike the MBTH method, the phenol sulfuric acid test shows evidence of nonlinearity in four of the curves, casting doubt on its accuracy at the low concentrations which occur in natural waters.

Table III shows that the differences in the PCHO values obtained directly using dialysis and calculated from the difference method were well within the standard deviation of a single measurement. The two dialysis experiments

TABLE II

The regression of absorbance vs. concentration for standard solutions of carbohydrates estimated with MBTH after reduction and periodate oxidation

Compound	conc. range (mg l^{-1})	n	r	y intercept*[1]	Slope*[2]		Milli-molar absorp-tivity*[3]	% as glucose*[4]
					(abs/mg l^{-1})	(abs/ mM l^{-1})		
Na-Alginate*[5]	0.390–1.56	10	0.995	−0.004	0.117	20.7	58.0	52.7
Soluble starch	0.160–1.14	10	0.999	0.011	0.247	39.9	112.0	101.0
Laminarin	0.093–1.86	21	0.997	0.001	0.242	39.2	110.0	100.0
Melezitose	0.193–1.93	12	0.998	−0.010	0.219	36.9	103.0	93.6
Raffinose	0.163–1.63	11	0.999	0.000	0.202	34.0	95.2	86.5
Arabic acid	0.089–1.79	12	0.999	0.002	0.237	36.8	103.0	93.6
Fucoidin	0.210–0.750	5	0.995	−0.020	0.205	30.4	85.1	77.4
Xylan	0.220–1.55	9	0.997	0.001	0.249	32.9	92.1	83.7
Agar	0.211–1.40	11	0.992	−0.005	0.124	19.0	53.2	48.4
Glucose unhydrolyzed	0.125–2.50	33	0.998	0.006	0.219	39.5	111.0	101.0
Glucose "hydrolyzed"	0.250–2.50	16	0.996	0.001	0.218	39.3	110.0	100.0
Glucose combined	0.125–2.50	49	0.997	0.005	0.218	39.4	110.0	100.0

*[1] After correction for empirically determined blank.
*[2] Corrected for dilution of sample by hydrolysis reagents.
*[3] abs/mM l^{-1} x final volume (2.8 ml) (after Sawicki et al., 1967).
*[4] Molar basis.
*[5] Calculated as alginic acid.

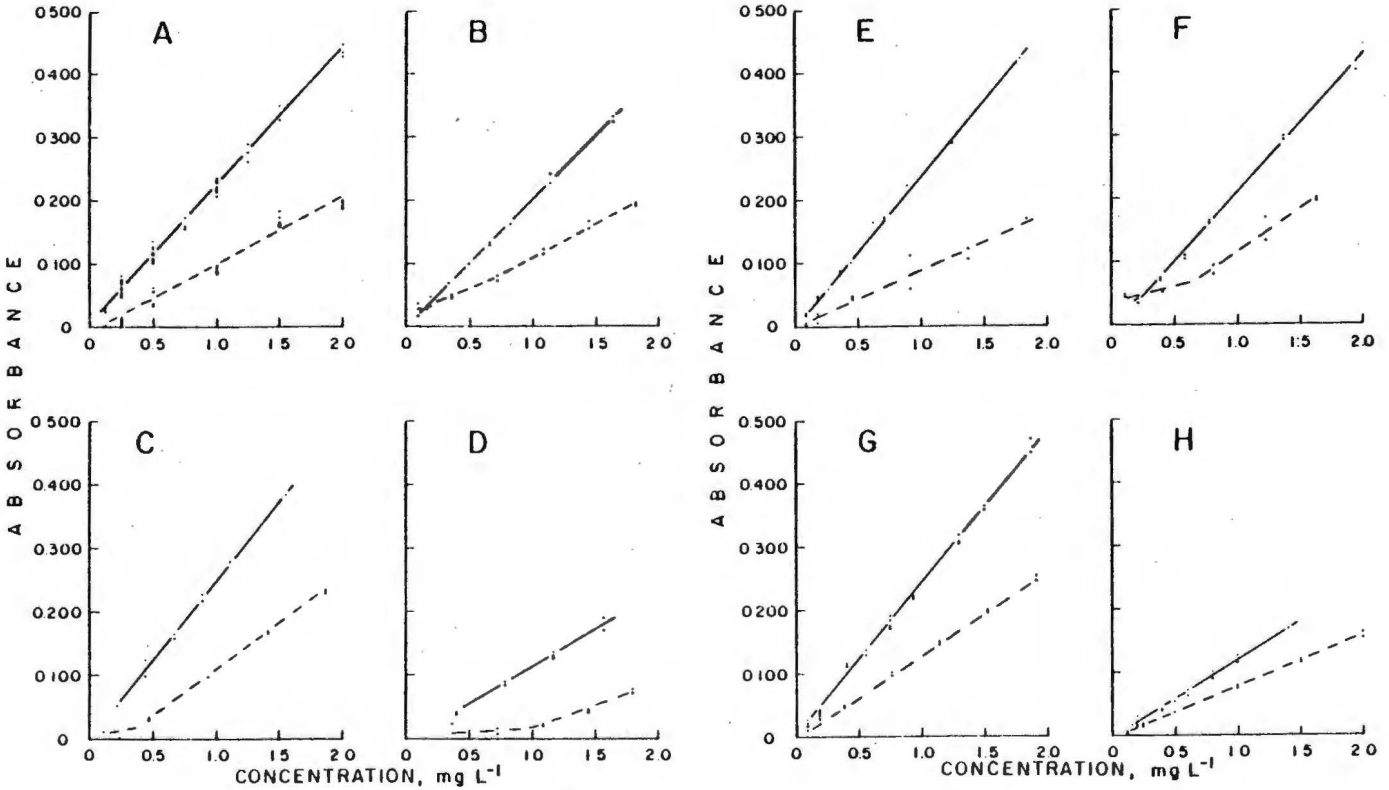

Fig. 2. Comparison of standard curves with the MBTH (——) and the phenol sulfuric acid (- - -) methods. A, glucose; B, raffinose; C, xylan; D, sodium alginate calculated as alginic acid; E, arabic acid; F, melezitose; G, laminarin; H, agar.

gave identical corrected PCHO results in spite of large differences in contamination due to membrane bleed. The difference method seems to provide a simple technique for PCHO determination which appears to be valid in practice as well as in theory.

Analysis of natural waters

Table IV gives concentrations of TCHO, MCHO, PCHO and DOC determined in a survey of Narragansett Bay, R.I., and adjacent waters. Carbohydrate concentrations ranged from 452 to 2023 $\mu g\ l^{-1}$ for TCHO, 272 to 1353 $\mu g\ l^{-1}$ for PCHO, and 153 to 814 $\mu g\ l^{-1}$ for MCHO. PCHO/MCHO ratios varied from 1.15 to 2.80. Samples from or near polluted tributaries (Stations 1—4 and 11) generally contained considerably higher amounts of all fractions. The TCHO concentrations are in good agreement with most published values (Table I)

TABLE III

Comparison of the MBTH difference method with dialysis for the determination of polysaccharide in natural seawater

	Date	
	21 April	30 April
Dialysis method[*1]		
Seawater		
measured PCHO ± S.D. ($\mu g\ l^{-1}$)	1044 ± 101	624 ± 62
volume correction[*2]	1.1	1.2
actual PCHO ($\mu g\ l^{-1}$)	1148	749
Distilled water control		
measured PCHO ± S.D. ($\mu g\ l^{-1}$)	923 ± 87	480 ± 31
volume correction	0.9	0.9
actual PCHO ($\mu g\ l^{-1}$)	831	432
Corrected seawater PCHO ($\mu g\ l^{-1}$)	317	317
Difference method[*3]		
MCHO ± S.D. (μM)	0.84 ± 0.02	0.90 ± 0.13
TCHO ± S.D. (μM)	2.68 ± 0.17	2.91 ± 0.18
PCHO (μM)	1.84	2.01
PCHO ($\mu g\ l^{-1}$)	298	326
Difference between methods ($\mu g\ l^{-1}$)	19	9
% Difference	6.0	2.8

[*1] Triplicate determinations.
[*2] Final vol./initial vol. in dialysis sacs.
[*3] Quadruplicate determinations

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TABLE IV

Total, free and combined carbohydrate of surface samples from Narragansett Bay and Rhode Island Sound

Station *[1]	Date	DOC*[2] (mg l^{-1})	Total CHO					Monomeric CHO	
			n	(μM)	($\mu g\ l^{-1}$)	S.D. ($\mu g\ l^{-1}$)	%DOC	*n*	(μM)
1	27 Feb	6.7	4	9.59	1627	7.9	10.3	3	4.08
2	27 Feb	6.1	4	7.38	1252	75.7	8.7	3	3.18
3	27 Feb	4.6	4	7.81	1327	19.5	12.2	3	3.42
1	17 Mar	7.0	2	11.98	2023	24.3	12.3	3	4.52
2	17 Mar	6.8	3	7.91	1328	127.0	8.3	3	2.63
3	17 Mar	4.8	3	3.97	667	17.2	6.0	2	1.31
4	17 Mar	3.9	2	4.36	733	21.1	8.0	2	1.47
7	21 Mar	3.2	3	2.98	503	19.8	6.6	3	1.13
8	24 Mar	3.3	3	2.68	452	47.0	5.8	4	1.00
6	01 Apr	3.8	3	3.61	603	21.8	6.8	3	1.01
7	01 Apr	2.7	3	3.24	541	38.5	8.5	3	0.91
8	01 Apr	3.5	2	2.90	488	30.2	6.0	2	1.00
10	01 Apr	3.0	4	2.82	474	31.2	6.7	3	0.96
5	07 May	2.9	3	3.51	588	26.0	8.6	3	1.06
7	07 May	3.0	3	4.90	820	17.0	11.7	3	1.46
9	07 May	3.0	3	5.40	899	53.7	12.9	3	1.34
10	07 May	3.2	2	3.60	601	9.0	8.0	3	0.99
11	07 May	5.7	3	12.06	2021	46.0	15.1	3	3.71
12	07 May	3.2	3	4.32	719	25.8	9.7	3	1.05
13	25 Jul	1.2*[3]	3	3.01	503	38.6	17.9	3	0.85
Mean		4.1	—	5.40	908	34.9	9.5	—	1.85

*[1] See Fig.1 for location.
*[2] Beckman Carbonaceous Analyzer
*[3] Method of Menzel and Vaccaro (1964).

including those from methods other than colorimetric techniques (Starikova and Yablokova, 1972; Hirayama, 1974).

The mean coefficient of variation for the TCHO determinations (4.5%) is much lower than the 30 and 23.7% with anthrone and 38% with N-ethylcarbazol calculated for the data of Lewis and Rakestraw (1955). Antia and Lee (1963) reported a coefficient of variation of 11.3% for six 1.2-mg l^{-1} glucose standards analyzed on different days with anthrone, while Zein-Eldin and May (1958) achieved 5.5% for six 1-mg l^{-1} standards using an improved N-ethylcarbazol method. The calculated precision of the mean for the MBTH method (from eight pier samples taken in April) is 66 . $n^{-1/2}\ \mu g\ l^{-1}$ (95% confidence) which compares favorably with the 60 . $n^{-1/2}\ \mu g\ l^{-1}$ figure given by Strickland and Parsons (1968) for the phenol sulfuric acid method.

			Polymeric CHO			Coeff. of variation		
($\mu g\ l^{-1}$)	S.D.	%DOC	(μM)	($\mu g\ l^{-1}$)	%DOC	TCHO	MCHO	PCHO/MCHO
734	53.5	4.4	5.51	893	5.9	0.4	7.3	1.22
572	36.9	3.8	4.20	680	4.9	6.0	6.5	1.19
616	34.5	5.4	4.39	711	6.8	1.5	5.6	1.15
814	35.9	4.7	7.46	1209	7.6	1.2	4.4	1.49
473	57.9	2.8	5.28	855	5.5	9.6	12.2	1.81
236	2.0	2.0	2.66	431	4.0	2.6	0.8	1.83
265	0.0	2.7	2.89	468	5.3	2.9	0.0	1.77
203	7.6	2.5	1.85	300	4.1	3.9	3.7	1.48
180	15.9	2.2	1.68	272	3.6	10.4	8.8	1.51
182	13.7	1.9	2.60	421	4.9	3.6	7.5	2.31
164	10.4	2.4	2.33	377	6.1	7.1	6.3	2.30
180	6.4	2.1	1.90	308	3.9	6.2	3.6	1.71
173	3.5	2.3	1.86	301	4.4	6.6	2.0	1.74
191	15.9	2.6	2.45	397	6.0	4.4	8.3	2.08
263	14.6	3.5	3.44	557	8.2	2.1	5.6	2.12
241	23.2	3.2	4.06	658	9.7	6.0	9.6	2.73
178	24.1	2.2	2.61	423	5.8	1.5	13.5	2.38
668	18.7	4.7	8.35	1353	10.4	2.3	2.8	2.03
189	9.3	2.4	3.27	530	7.3	3.6	4.9	2.80
153	4.6	5.1	2.16	350	12.8	7.7	3.0	2.28
334	19.4	3.1	3.55	575	6.4	4.5	5.8	1.90

Interference studies

Addition of nitrate, phosphate and silicate had no effect on analysis. Samples with added DMAB produced elevated absorbances in accordance with Sawicki et al. (1961b) but the increase was equal in analytical and control samples and was eliminated by subtraction. This supports the theory that the controls will correct for compounds reacting directly with MBTH.

The absorbance spectra were very similar to that of the formaldehyde-MBTH chromogen given by Sawicki et al. (1961a) with maxima at 635 and 670 nm. MBTH forms chromogens with a number of aliphatic aldehydes which produce slightly different absorbance maxima (Sawicki et al., 1961a) but no evidence of interference from these could be detected in the spectra. Interference from noncarbohydrate compounds which produce formaldehyde during periodate oxidation would not be detected by this technique. Johnson and Sieburth (1977; this issue, pp. 1—13) have found serine to be in this category, producing 40% of the color of an equal molar amount of hexose. TRIS buffer used in some culture media drastically interferes in a similar manner.

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CONCLUSIONS

The extension of the MCHO test of Johnson and Sieburth (1977; this issue, pp. 1—13) for TCHO analysis seems to retain the advantages of the parent method and gives good yields of several combined carbohydrates relative to glucose. The method is based on quantitative and reliable carbohydrate reactions and produces a nearly equivalent response with a large array of carbohydrates. It produces reasonable results with greater sensitivity when applied to natural waters. No significant interfering substances occurring in seawater other than serine have been found and there is no apparent salinity dependence. When the TCHO procedure is coupled to its MCHO version, the technique can be used to estimate PCHO as well. This unique property should make it a valuable tool for establishing the distribution and flux of dissolved carbohydrates in the sea. Its improved sensitivity, linearity, and versatility more than offset its time-consuming nature.

ACKNOWLEDGEMENTS

The authors thank Kenneth M. Johnson for valuable cooperation, discussion and technical assistance in analysis of several DOC samples. We thank Dana Kester for the use of the recording spectrophotometer. One of us (C.M.B.) is grateful for a traineeship from the Environmental Protection Agency. The work was primarily supported by the Biological Oceanography Program of the National Science Foundation through Grant No. DES-74-01537-A01.

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APPENDIX II:

DISSOLVED CARBOHYDRATE AND MICROBIAL ATP IN THE NORTH ATLANTIC:

CONCENTRATIONS AND INTERACTIONS

Deep-Sea Research, Vol. 26A, pp. 1267 to 1290
0011-7471/79/1101-1267 $02.00/0

Dissolved carbohydrate and microbial ATP in the North Atlantic: concentrations and interactions

CURTIS M. BURNEY,* KENNETH M. JOHNSON,* DENNIS M. LAVOIE† and JOHN MCN. SIEBURTH*

(*Received* 1 *May* 1978, *in final revised form* 20 *June* 1979; *accepted* 25 *June* 1979)

Abstract—A selective and sensitive spectrophotometric assay for monosaccharide (MCHO) before and after a hydrolysis step has permitted the estimation of total carbohydrate (TCHO) and of polysaccharide (PCHO) by difference. The concentrations and diel variations of MCHO, TCHO, PCHO, and dissolved organic carbon (DOC) were estimated on some 90 samples obtained at 15 stations between Rhode Island and Spain. DOC ranged from 570 to 1330 μg C l^{-1} with a mean of 940 μg C l^{-1}. MCHO, calculated as hexose, ranged from 65 to 356 μg l^{-1} (mean 163 μg l^{-1}), accounting for 3.5 to 13.2% of the DOC. TCHO varied from 175 to 583 μg l^{-1} (mean 348 μg l^{-1}), 8.0 to 24.5% of the DOC. PCHO ranged from 0 to 379 μg l^{-1} (mean 184 μg l^{-1}), up to 16.0% of the DOC. MCHO, TCHO, and PCHO were positively correlated with DOC at the 0.01 level. PCHO as a % of TCHO decreased significantly from 69% in coastal to 37% in mid-ocean samples.

The vertical distribution of the microbial plankton smaller and larger than 3 μm was determined at 12 of the stations by ATP assay on samples sequentially filtered through Nuclepore membranes. Living biomass for the bacterioplankton (0.2 to 3.0 μm) calculated from the ATP concentration ranged from 1 to 55 μg C l^{-1} (approx 10^{4} to 10^{6} bacterial cells ml^{-1}) and accounted for 3 to 80% of the total living biomass in the microbial plankton, averaging 30% in the photic and 40% in the aphotic zone. Vertical profiles of bacterial and protist ATP at six stations are compared with those for dissolved carbohydrates, DOC, chlorophyll *a*, phaeopigments, dissolved oxygen, and temperature. These include four diel drift stations in which a daylight sampling was followed by a predawn resampling on the following day. Carbohydrate peaks were often associated with accumulations of organisms in the >3-μm size fraction, which were low in chlorophyll *a*, possibly indicative of collections of protozooplankton. During the day there was evidence of net release of carbohydrate at the depth of the chlorophyll *a* maxima at oceanic stations but not at neritic stations. Appreciable bacterioplankton maxima (0.2 to 3.0 μm ATP) occurred most often with carbohydrate minima.

INTRODUCTION

THERE IS A considerable literature reporting total dissolved carbohydrate (TCHO) measurements in the sea (summarized by BURNEY and SIEBURTH, 1977), with most open ocean values obtained during the past 10 years falling in the 0 to 750 μg l^{-1} range. TCHO can comprise a significant fraction of the biologically labile dissolved organic carbon (DOC) and often exceeds the total quantity of particulate organic matter in suspension (MENZEL and RYTHER, 1970).

Dissolved carbohydrate in the sea exists in both monomeric (MCHO) and polymeric (PCHO) forms. Little is known about their distribution and cycling because the spectrophotometric methods used did not distinguish MCHO from PCHO without laborious physical separation by dialysis, gel filtration, or ultrafiltration (WHEELER, 1976).

* Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881, U.S.A.
† NORDA, NSTL Station, Mississippi 39526, U.S.A.

Chromatographic methods have been used to determine MCHO (DEGENS, EGON, REUTER and SHAW, 1964; JOSEFSSON, 1970; MOPPER, 1977) and, with hydrolysis, TCHO. Although these methods provide more information by identifying the sugars, they take hours to analyze single samples and require desalting which may introduce contamination or cause MCHO loss. So far, chromatographic methods have not been used for large-scale studies of the kind we report here.

MCHO and PCHO distributions could have significant biological implications because both are known to be released by phytoplankton (GUILLARD and WANGERSKY, 1958; MARKER, 1965; HELLEBUST, 1965, 1974; MYKLESTAD and HAUG, 1972; MYKLESTAD, 1974) and may also be released by the protozoa and metazoa. MCHO and PCHO would be expected to cycle at different rates as, unlike MCHO, PCHO must first be depolymerized before bacterial uptake. To study such phenomena, a spectrophotometric method to estimate MCHO (JOHNSON and SIEBURTH, 1977) requiring no physical separation (or desalting) has been developed. A separate hydrolysis step (BURNEY and SIEBURTH, 1977) that allows measurement of TCHO has been added. PCHO can then be estimated by difference. The mechanism of this method differs from previous carbohydrate assays and uses 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) to measure the formaldehyde produced by a periodate oxidation of monosaccharides that have been previously reduced to sugar alcohols. Unlike older spectrophotometric methods, it gives a nearly equivalent response to equal molar amounts of a wide variety of hexoses and pentoses and includes other important carbohydrate classes such as sugar alcohols, deoxysugars, and uronic acids. The method is precise and about 20 times more sensitive than the phenol sulfuric acid test (DUBOIS, GILLES, HAMILTON, REBERS and SMITH, 1956), making it useful in the study of *in situ* biological processes, relationships, and rates.

A complete understanding of the marine occurrence and cycling of labile dissolved organic substances such as MCHO and PCHO requires a knowledge of the biomass and activities of the phototrophic producers, the phagotrophic releasers, and the osmotrophic consumers (SIEBURTH, 1976, 1977). The adenosine triphosphate (ATP) method for estimating the biomass of microbial plankton has become widely used in ecological studies since its introduction by HOLM-HANSEN and BOOTH (1966). The method allows the estimation of living biomass with an accuracy comparable or superior to the classical counting and cultural techniques (HOLM-HANSEN, 1969, 1970, 1973). Its chief limitation is the variability in the ATP content of living cells (HAMILTON and HOLM-HANSEN, 1967). ATP assays of the total microbial plankton cannot distinguish between bacterioplankton and protist plankton (HOLM-HANSEN, 1970). JASSBY (1975) attempted to estimate the bacterioplankton from the difference between total biomass calculated from ATP and the phytoplankton biomass calculated from chlorophyll *a* concentration and showed that the large inherent variation in the chlorophyll-to-carbon ratio precluded statistically reliable data. The presence of a significant protozooplankton biomass would result in the overestimation of the bacterioplankton biomass. A simple physical separation of the small procaryote cells in the bacterioplankton from the larger protist cells in the phytoplankton and protozooplankton is necessary to obtain reliable and meaningful ATP biomass estimates of both the bacterioplankton and the protist plankton.

Epifluorescence microscopy has demonstrated a large population of free cells in the bacterioplankton compared to a minor population of bacteria attached to the particles of organic debris in the suspended seston (WIEBE and POMEROY, 1972; FERGUSON and RUBLEE, 1976; HOPPE, 1976; JOHNSON, CARON and SIEBURTH, unpublished data). The small size of

these bacteria (< 1 μm diameter) has been shown by HOPPE (1976) and WATSON, NOVITSKY, QUINBY and VALOIS (1977). Even 3-μm porosity cellulose ester membranes, which are much more retentive than their stated pore size, permit the passage of microorganisms responsible for 70 to 80% of the heterotrophic uptake of [^{14}C]labeled organic compounds (WILLIAMS, 1970; SOROKIN, 1971b; DERENBACH and WILLIAMS, 1974). SHELDON (1972) reported that perforated membranes such as Nuclepore®, unlike cellulose ester and fiberglass filters, both passed and retained particles according to their stated pore size. SALONEN (1974) used this sieve-like property of Nuclepore membranes and found that 2- to 5-μm porosities separated bacteria from the phytoplankton in samples of lake water. Some 90% of the bacteria active in seawater pass through a 1-μm Nuclepore® filter (HOPPE, 1976; AZAM and HODSON, 1977). This simple physical separation of the small-celled bacterioplankton from the larger-celled protist plankton before ATP analysis is practical and has been used in our laboratory since 1974 (LAVOIE, 1975).

This report presents the first data comparing the neritic and oceanic occurrence of MCHO and PCHO in relation to DOC and documents the role of the various trophic groups in controlling the distributions of these bacterial substrates. Data on the viable biomass of bacterioplankton from the same areas, estimated from the ATP content of selectively filtered natural populations, are presented for the first time. A preliminary report presented the rationale of this approach for studying the cycling of dissolved organic matter in the sea (SIEBURTH, JOHNSON, BURNEY and LAVOIE, 1977).

MATERIALS AND METHODS

Detailed profiles were obtained from 15 Niskin bottle casts in the North Atlantic during R.V. *Trident* Cruise 170 in July and August 1975. Samples from 5 to 4000 m were taken, but most were from the upper 300 m. Four of the casts were predawn resamplings of the same depths as in the previous daylight casts (Fig. 1, Tables 1, 2, 4, and 5). The Niskin bottles (5 or 30 l) were scrubbed in N/10 HCl before each cast (SOROKIN, 1971b) and rinsed

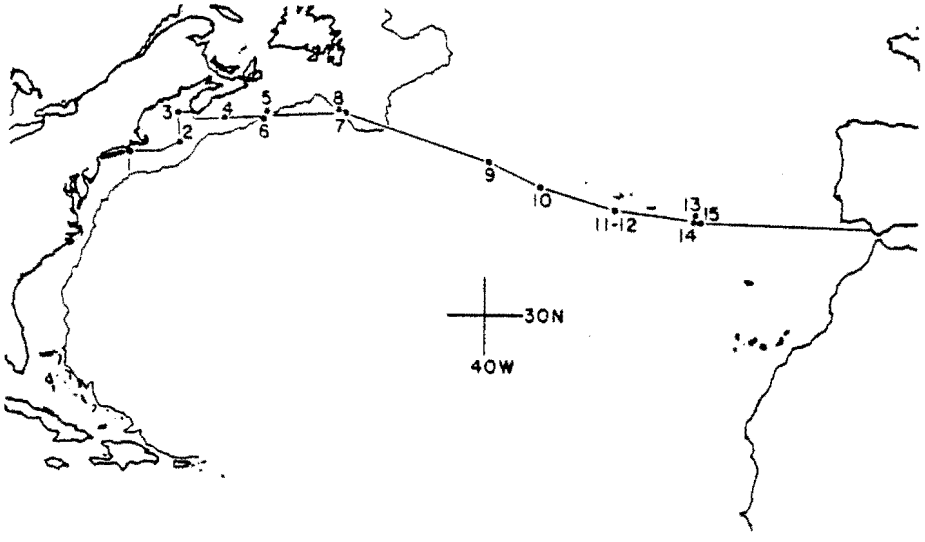

Fig. 1. The positions of the stations occupied during R.V. *Trident* Cruise TR-170 between Narragansett, Rhode Island, and Cadiz, Spain. See Tables 1 and 2 for exact locations.

Table 1. Distribution of dissolved organic carbon (μg C l^{-1}) and carbohydrate (μg hexose l^{-1}) at 8 stations over the continental shelf of North America. The % DOC represented by each carbohydrate fraction is given in parentheses

Station description	Analyses	Depths sampled (m) 5	10	20	30	40	45
1	DOC		1200	1170	1100		
1400 July 25*	MCHO(%DOC)‡		153 (5.1)	133 (4.5)	103 (3.7)		
41°12'N, 71°17'W	PCHO(%DOC)§		350(12.8)	275(10.3)	217 (8.7)		
40 m†	TCHO(%DOC)¶		503(17.9)	409(14.9)	320(12.4)		
2	DOC		1080	1180	—	960	
1850 July 26	MCHO(%DOC)		126 (4.7)	189 (6.4)	139(—)	124 (5.2)	
41°35'N, 67°05'W	PCHO(%DOC)		261(10.6)	293(10.9)	238(—)	233(10.7)	
50 m	TCHO(%DOC)		387(15.3)	482(17.3)	377(—)	357(15.8)	
3	DOC		1120		1140		
1315 July 27	MCHO(%DOC)		157 (5.6)		127 (4.5)		
43°41'N, 67°08'W	PCHO(%DOC)		293(11.5)		233 (9.0)		
140 m	TCHO(%DOC)		450(17.1)		361(13.4)		
4	DOC		1140	1050	950		
1300 July 31	MCHO(%DOC)		212 (7.4)	173 (6.6)	157 (6.6)		
43°24'N, 63°09'W	PCHO(%DOC)		206 (8.0)	249(10.4)	125 (5.8)		
175 m	TCHO(%DOC)		418(15.4)	422(17.0)	281(12.4)		
5	DOC			1330		900	
1300 August 1	MCHO(%DOC)			185 (5.6)		130 (5.8)	
43°31'N, 59°29'W	PCHO(%DOC)			379(12.5)		191 (9.3)	
1794 m	TCHO(%DOC)			564(18.1)		321(15.1)	
6	DOC			1090		950	
0430 August 2	MCHO(%DOC)			198 (7.3)		167 (7.0)	
43°19'N, 59°41'W	PCHO(%DOC)			222 (9.0)		199 (9.2)	
1794 m	TCHO(%DOC)			420(16.2)		367(16.2)	
7	DOC	1270	1250	1140	1200		1120
0835 August 4	MCHO(%DOC)	356(11.2)	292 (9.3)	162 (5.7)	239 (8.0)		166 (5.9)
43°40'N, 52°40'W	PCHO(%DOC)	188 (6.5)	292(10.3)	296(11.4)	314(11.5)		142 (5.6)
296 m	TCHO (%DOC)	544(17.7)	583(19.6)	458(17.1)	554(19.5)		308(11.5)
8	DOC	1230	1150	1180	1230		1190
0410 August 5	MCHO(%DOC)	218 (7.1)	194 (6.7)	167 (5.7)	153 (5.0)		148 (5.0)
43°50'N, 52°54'W	PCHO(%DOC)	284(10.2)	238 (9.1)	245 (9.1)	271 (9.7)		267 (9.9)
296 m	TCHO(%DOC)	501(17.2)	432(15.9)	412(14.8)	424(14.7)		415(14.8)

* Local time and date (1975). † Sonic depth. ‡ Assuming 40% carbon. § Assuming 44% carbon. ¶ TCHO carbon = MCHO carbon + PCHO carbon. ‖ For samples with no MCHO data, TCHO is assumed to be 42% carbon. ** Pooled standard deviation for all samples assuming homogeneous variance.

at sampling depth for 20 min prior to closing. For carbohydrate and DOC analysis, 100 ml aliquots were immediately filtered by gravity into alkali-cleaned glass bottles. The filters (Gelman A/E) and sample bottles were precombusted at 480°C for 12 h and kept foil-covered until use. The glass filter units were soaked in N/10 HCl between casts and thoroughly rinsed with distilled water and portions of the next sample before reuse. For ATP analysis, sample water was drained through a 1-mm mesh nylon screen into calibrated 145-ml bottles and stored at 4°C. Analysis usually commenced within the hour. All glassware used in this and subsequent operations was cleaned in N/10 HCl and rinsed with deionized water.

Triplicate MCHO and TCHO analyses were begun immediately after filtration using the MBTH methods of JOHNSON and SIEBURTH (1977) and BURNEY and SIEBURTH (1977).

Depths sampled (m)									Precision
50	60	90	100	135	150	290	500	1000	**
									26
									14.0
									25.8
1080									56
315(11.7)									11.0
254(10.3)									
569(22.0)									34.8
890			900	—					64
83 (3.7)			—	—					19.0
309(15.3)			—	—					
392(19.0)			294(13.7)*	254(—)					30.0
910			1040		870				60
130 (5.7)			196 (7.5)		124 (5.7)				18.5
117 (5.7)			187 (7.9)		154 (7.8)				
246(11.4)			383(15.4)		278(13.5)				29.3
	920	840		840			640	690	74
	115 (5.0)	194 (9.2)		162 (7.8)			—	—	27.3
	138 (6.6)	100 (5.2)		183 (9.6)			—	—	
	253(11.6)	295(14.5)		345(17.3)			254(16.7)	197(12.0)	27.2
	950	770		790					45
	94 (4.0)	115 (6.0)		—					21.8
	220(10.2)	133 (7.6)		—					
	314(14.1)	248(13.6)		—					21.7
					1030	960			51
					130 (5.0)	254(10.6)			26.0
					125 (5.3)	117 (5.4)			
					254(10.4)	370(15.9)			37.3
				1080		890			40
				139 (5.1)		130 (5.8)			22.8
				183 (7.5)		79 (3.9)			
				322(12.6)		209 (9.7)			36.0

PCHO was then calculated by difference according to the latter reference. Standards of highest purity mannitol and glucose (Schwarz-Mann, Orangeburg, NY) in filtered seawater at concentrations of 125 to 1000 $\mu g\, l^{-1}$ were analyzed at sea. The data were combined to generate a standard curve ($n = 44$). All absorbance readings were made on a Beckman DU spectrophotometer. DOC was determined by the method of MENZEL and VACCARO (1964) as modified by KERR and QUINN (1975). Triplicate samples were sparged and sealed in ampules immediately after filtration and oxidized within 24 h. Determinations were made ashore on an integrator-equipped Oceanography International Total Carbon Analyzer (Model 0524) using potassium hydrogen phthalate as a standard and correcting for reagent carbon.

Samples for ATP analysis were drawn through 25-mm diameter 3-μm Nuclepore® filters (Nuclepore Corp., Pleasanton, CA) by a gentle vacuum (100 Torr); the filtrate was caught in a clean flask. The filter was immediately immersed in 5 ml of boiling 0.05 M Tris buffer (ICN Pharmaceuticals, Cleveland, OH) at pH 9.0. [This pH appears to give better

Table 2. Distribution of dissolved organic carbon (μg C l^{-1}) and carbohydrate (μg hexose l^{-1}) at 7 stations in the open North Atlantic

Station description	Analyses	Depths sampled (m) 5	10	20	30	40	60	80
9	DOC		1120	1100	1130	1140		950
1040 August 8	MCHO(%DOC)		185 (6.6)	157 (5.7)	166 (5.9)	180 (6.3)		153 (6.4)
40 36'N, 39 48'W	PCHO(%DOC)		99 (3.9)	176 (7.0)	121 (4.7)	97 (3.7)		34 (1.6)
4972 m	TCHO(%DOC)		284(10.5)	333(12.7)	287(10.6)	277(10.1)		187 (8.0)
10	DOC	870					820	
1430 August 9	MCHO(%DOC)	194 (8.9)					203 (9.9)	
39 01'N, 34 59'W	PCHO(%DOC)	188 (9.5)					130 (7.0)	
3655 m	TCHO(%DOC)	382(18.4)					333(16.9)	
11	DOC	940		900		1060	840	820
1145 August 11	MCHO(%DOC)	194 (8.3)		124 (5.5)		351(13.2)	203 (9.7)	153 (7.5)
37 28'N, 28 43'W	PCHO(%DOC)	213(10.0)		212(10.4)		193 (8.0)	133 (7.0)	183 (9.8)
2000 m	TCHO(%DOC)	407(18.2)		336(15.9)		544(21.3)	336(16.6)	336(17.3)
12	DOC	990		870		900	920	1040
0516 August 12	MCHO(%DOC)	194 (7.8)		139 (6.4)		162 (7.2)	148 (6.4)	218 (8.4)
37 28'N, 28 44'W	PCHO(%DOC)	159 (7.1)		257(13.0)		154 (7.5)	100 (4.8)	138 (5.8)
1880 m	TCHO(%DOC)	353(14.9)		396(19.4)		316(14.7)	248(11.2)	356(14.2)
13	DOC	1040		920		1040	900	—
0939 August 14	MCHO(%DOC)	207 (8.0)		142 (6.2)		254 (9.8)	115 (5.1)	209(—)
36 59'N, 21 22'W	PCHO(%DOC)	206 (8.7)		305(14.6)		184 (7.8)	172 (8.4)	199(—)
3803 m	TCHO(%DOC)	413(16.7)		447(20.8)		438(17.6)	287(13.5)	408(—)
14	DOC	1070		930		900	940	820
0345 August 15	MCHO(%DOC)	185 (6.9)		157 (6.8)		148 (6.6)	139 (5.9)	130 (6.3)
36 59'N, 21 20'W	PCHO(%DOC)	242(10.0)		280(13.2)		272(13.3)	200 (9.4)	132 (7.1)
4517 m	TCHO(%DOC)	427(16.9)		437(20.0)		420(19.9)	339(15.3)	262(13.4)
15	DOC							
1710 August 15	MCHO(%DOC)							
36 43'N, 21 15'W	PCHO(%DOC)							
4486 m	TCHO(%DOC)							

recoveries than the lower pH buffer used by HOLM-HANSEN and BOOTH (1966) and CHEER, GENTILE and HEGRE (1974), probably due to a downward shift in pH that this buffer exhibits at higher temperature, which may result in partial hydrolysis of the ATP.] After 2 min the extract (3- to 1000-μm fraction) was quickly cooled in a water bath and then frozen at −10°C until it could be assayed. The filtrate was then drawn through a 25-mm dia. 0.2-μm Nuclepore® filter, extracted, and frozen as above to obtain the ATP of the 0.2- to 3-μm fraction. ATP was assayed on board ship at the end of the station, using a DuPont Model 760 luminescent biometer and enzyme system (ALLEN, 1972). Duplicate samples were drawn and extracted for each depth and at least three determinations made on each extract. The values thus obtained were pooled and mean ATP concentration and 95% confidence limits were calculated according to YOUDEN's (1951) methods for small sets of data.

Larger metazooplankton could have been included in the 3- to 1000-μm fraction, but this would have increased our analytical variability as they are distributed too sparsely to have been subsampled reproducibly. The good precision of the analysis indicates that this ATP fraction was dominated by the more uniformly distributed protists. The 0.2- to 3-μm fraction is dominated by bacterioplankton even though an occasional eucaryotic flagellate

Depths sampled (m)									
100	150	250	500	1000	2000	3000	3500	4000	Precision
910									42
—									15.3
—									
176 (8.1)									23.2
		730	660	570	580	630	730		30
		207(11.3)	212(12.8)	121 (8.5)	124 (8.6)	—	148 (8.1)		22.0
		76 (4.6)	0 (0.0)	—	—	—	100 (6.0)		
		283(15.9)	212(12.8)	—	—	215(14.3)	248(14.1)		26.3
860	710								38
162 (7.5)	180(10.1)								20.3
108 (5.5)	68 (4.2)								
270(13.1)	248(14.4)								19.5
800	750								29
135 (6.8)	167 (8.9)								22.0
141 (7.8)	62 (3.6)								
276(14.5)	229(12.5)								33.7
850		850							52
139 (6.5)		147 (6.9)							14.3
116 (6.0)		83 (4.3)							
255(12.5)		230(11.2)							22.3
960		750							75
126 (6.3)		65 (3.5)							11.0
131 (6.0)		—							
257(11.3)		—							27.6
		910	660	660	630	650		650	15
		195 (8.6)	115 (7.0)	106 (6.4)	133 (8.4)	97 (6.0)		83 (5.1)	17.8
		330(16.0)	156(10.1)	151(10.1)	60 (4.2)	123 (8.3)		92 (6.2)	
		525(24.5)	271(17.4)	257(16.5)	193(12.6)	219(14.2)		175(11.3)	29.4

is sometimes included (SIEBURTH, SMETACEK and LENZ, 1978; JOHNSON and SIEBURTH, unpublished).

Standard hydrographic observations including dissolved oxygen (Winkler), temperature (expendable bathythermograph and reversing thermometers), salinity (induction salinometer), and light profiles (Model 185 quantum sensor, Lambda Instruments, Lincoln, NB, U.S.A.), were also made. Chlorophyll *a* and phaeopigments (acetone extracts) were determined fluorometrically using a Turner 110 fluorometer according to HOLM-HANSEN, LORENZEN, HOLMES and STRICKLAND (1965) except that the residue after extraction was removed by filtration rather than centrifugation (SHAH and SAMUEL, 1972).

RESULTS

Tables 1 and 2 summarize the DOC and carbohydrate data. Although the results are usually the average of three determinations, in a few cases when the coefficient of variation of the three replicates exceeded 20%, the highest value was dropped and the average of the remaining replicates was reported only if the recalculated coefficient of variation was less

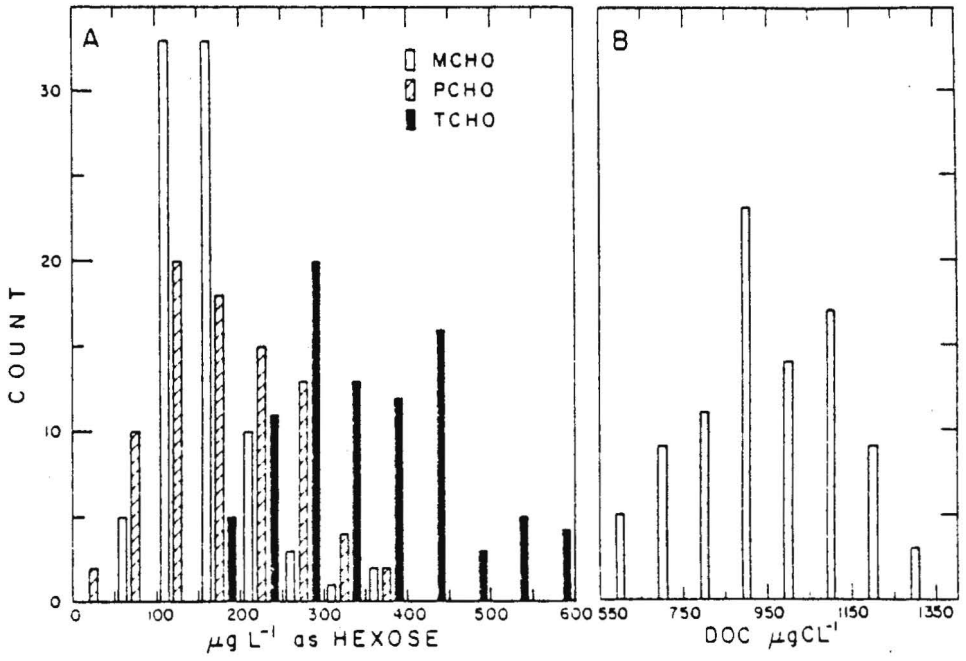

Fig. 2. The distribution patterns for (A) monosaccharide (MCHO), polysaccharide (PCHO) and total dissolved carbohydrate (TCHO), and (B) dissolved organic carbon for all analyzed samples from TR-170.

than 20%. None of the values is based on a single determination. The estimate of analytical precision for each hydrocast is a pooled standard deviation assuming that the variance of the method was homogeneous (YOUDEN, 1951) in each cast. Figure 2 shows the distribution of all the carbohydrate and DOC data. Table 3 compares the mean PCHO/TCHO ratios for samples taken from general regions along the Atlantic transect at depths to 300 m. Data for the two particulate size fractions expressed as ATP and

Table 3. Polysaccharide as a % of the total carbohydrate and a comparison of the difference of means between adjacent type locations

Type location	Stations	n*	$\frac{\text{PCHO}}{\text{TCHO}} \times 100$	d.f.†	t	Significance level
Coastal	1 and 3	6	69			
				15	3.57	0.01
Shelf	2 and 4	11	55			
				32	0.48	NS‡
Slope	5–8	23	53			
				30	3.98	0.01
Mid-ocean	9–10	9	37			
				21	1.71	NS
Azores Plateau	11–12	14	45			
				25	2.08	0.05
Iberian Basin	13–14	13	53			

* Samples from < 300 m only. † Degrees of freedom.
‡ Not significantly different above the 0.05 level.

computed cellular carbon as well as the per cent of the total ATP represented by the <3-μm fraction for 6 stations over the continental shelf are given in Table 4 and similar data for 6 oceanic stations are given in Table 5.

Depth profiles of carbohydrate, ATP, pigments, dissolved oxygen, DOC, and temperature are shown in Figs 3 to 8. Figures 3 and 4 are from single daylight casts made over Georges' Bank and the Nova Scotia shelf. Profiles from the paired hydrocasts during the morning or early afternoon and again just before dawn of the following day (at approximately the same location) during the four diel stations are given in Figs 5 to 8, representing the Sable Island shelf [Fig. 5(A, B)], Grand Banks [Fig. 6(A, B)], Azores Plateau [Fig. 7(A, B)], and the Iberian Basin [Fig. 8(A, B)]. The daylight and predawn casts in each pair are designated A and B. Decimal numbers on the >3-μm ATP profiles are chlorophyll *a*/>3-μm ATP ratios (w/w). The stations shown in Figs 3 to 6 are considered neritic and those in Figs 7 and 8 oceanic, based on location and depth differences. Chlorophyll *a* concentrations at oceanic stations were less than 25% of those from neritic areas.

DISCUSSION

The DOC levels reported in Tables 1 and 2 (570 to 1330, $\bar{x} = 940\ \mu g\ C\ l^{-1}$) are consistent with other reports (WAGNER, 1969; MENZEL and RYTHER, 1970; SKOPINTSEV, 1972; SHARP, 1973). Analytical precision was always well within $\pm 100\ \mu g\ C\ l^{-1}$. DOC showed a highly significant ($P \ll 0.01$) negative correlation with depth, consistent with the aforementioned literature. TCHO concentrations (175 to 583; $\bar{x} = 348\ \mu g\ l^{-1}$) are also in good agreement with previously published open ocean data (summarized by BURNEY and SIEBURTH, 1977) and accounted for 8.0 to 24.5% of the DOC.

MCHO (Tables 1 and 2) ranged from 65 to 356 $\mu g\ l^{-1}$ ($\bar{x} = 163\ \mu g\ l^{-1}$) and comprised 3.5 to 13.2% of the DOC. There are fewer reports of total MCHO determinations in seawater than of TCHO determinations. Smaller amounts (15 to 38 $\mu g\ l^{-1}$) were reported by DEGENS *et al.* (1964) and DEGENS (1970) using paper chromatography; however, their extraction and ion exchange desalting procedure may have resulted in MCHO loss. MOPPER (1977), using electrodialysis and automatic liquid chromatography, reported MCHO equivalent to 105 and 57 $\mu g\ l^{-1}$ in surface samples from the Black Sea and the North Sea, respectively. Using partition chromatography after desalting by electrodialysis, JOSEFSSON (1970) estimated 34 to 157 $\mu g\ l^{-1}$ of total MCHO from the Gullmarfjord of Sweden, with glucose representing 27% of the total. The enzymatic determination of glucose in the North Atlantic by VACCARO, HICKS, JANNASCH and CAREY (1968) yielded a range of 5 to 195 $\mu g\ l^{-1}$, with the lowest levels in mid-ocean. MEYER-REIL, DAWSON, LIEBEZEIT and TIEDGE (1978) reported glucose concentrations ranging from 15.8 to 80.4 $\mu g\ l^{-1}$ from water overlying sandy beach sediments during June and July. The total MCHO concentrations in these studies would be higher, and if the relation of glucose to the total shown by JOSEFSSON (1970) held, the range of total MCHO would be similar to our findings.

The estimates of PCHO in this study varied from 0 to 379 $\mu g\ l^{-1}$ ($\bar{x} = 184\ \mu g\ l^{-1}$) and accounted for up to 16% of the DOC. This report presents the first extensive attempt to determine PCHO in the open sea. The results agree with WHEELER's (1976) calculated mean of 364 $\mu g\ l^{-1}$ (assuming 44% carbon) in the >1000 molecular weight carbohydrate

Table 4. Distribution of two size fractions of particulate ATP and cell carbon at 6 stations along the outer continental shelf of North America

Station Description	Analysis	Depths sampled (m)				
2		5	10	20	30	40
1850 July 26*	<3 μm ATP‡		62±3.2	43±1.9	65±0.4	123±2.9
41°35'N, 67°05'W	<3 μm C§		15±0.8	11±0.5	16±0.1	31±0.7
Georges Bank	>3 μm ATP		140±4.6	132±2.3	143±2.3	186±1.6
50 m†	>3 μm C		35±1.2	33±0.6	36±0.6	47±0.4
	<3 μm % total		31	25	31	40
3			10	20	30	40
1315 July 27	<3 μm ATP		29±3.6	76±3.3	63±2.6	39±0.9
43°41'N, 67°08'W	<3 μm C		7±0.9	19±0.8	16±0.7	10±0.2
Bay of Fundy	>3 μm ATP		123±4.5	56±5.2	952±27.0	1186±36.9
140 m	>3 μm C		31±1.1	14±1.3	238±6.8	296±9.2
	<3 μm % total		19	57	06	03
5				20		40
1300 August 1	<3 μm ATP			33±0.7		37±0.4
43°31'N, 59°29'W	<3 μm C			8±0.2		9±0.1
Sable Island	>3 μm ATP			135±4.0		167±5.2
1794 m	>3 μm C			34±1.0		42±1.3
	<3 μm % total			20		18
6				20		40
0430 August 2	<3 μm ATP			26±1.0		44±2.4
43°19'N, 59°41'W	<3 μm C			7±0.3		11±0.6
Sable Island	>3 μm ATP			59±3.7		88±1.1
1794 m	>3 μm C			15±0.9		22±0.3
	<3 μm % total			31		33
7		5	10	20	30	
0835 August 4	<3 μm ATP	59±6.7	65±3.2	70±2.9	100±2.1	
43°40'N, 52°40'W	<3 μm C	15±1.7	16±0.8	17±0.7	25±0.5	
Georges Bank	>3 μm ATP	74±3.1	96±4.6	108±2.5	322±14.7	
296 m	>3 μm C	19±0.8	24±1.2	27±0.6	80±3.7	
	<3 μm % total	44	40	39	24	
8		5	10	20	30	
0410 August 5	<3 μm ATP	45±2.2	27±0.3	89±1.7	54±1.5	
43°50'N, 52°54'W	<3 μm C	11±0.6	7±0.1	22±0.4	14±0.4	
Georges Bank	>3 μm ATP	79±2.0	48±0.9	198±20.7	502±33.9	
296 m	>3 μm C	17±0.5	12±0.2	49±5.2	125±8.5	
	<3 μm % total	40	36	31	10	

* Local time and date. † Sonic depth. ‡ ATP in pg/cm^3. § Carbon in mg/m^3 as cellular carbon computed from ATP values (HAMILTON and HOLM-HANSEN, 1967). Values ±95% confidence limits. <3 μm Fraction: plankton between 0.2 μm and 3.0 μm in size. >3 μm Fractions: plankton between 3.0 μm and 1000 μm in size.

fractions of four surface samples from 15 and 23 km off the Georgia coast that were separated by ultrafiltration and estimated with the phenol sulfuric acid test. Our findings are also consistent with the 115 to 250 μg l^{-1} range reported by MAURER (1971) in similar size fractions from the Gulf of Mexico. Calculations from the total and monomeric carbohydrate data given by MOPPER (1977) indicate a PCHO concentration of 645 and 186 μg l^{-1} for North Sea and Black Sea samples, respectively.

Depths sampled (m)									
45	50	60	75	90	100	135	290	500	1000
	44 ± 1.8								
	11 ± 0.4								
	197 ± 1.5								
	49 ± 0.5								
	18								
	50		75		100				
	25 ± 0.8		19 ± 0.6		84 ± 2.9				
	6 ± 0.2		5 ± 0.2		21 ± 0.7				
	61 ± 1.4		24 ± 1.1		24 ± 1.0				
	15 ± 0.4		6 ± 0.3		6 ± 0.2				
	29		45		78				
		60		90		135		500	1000
		219 ± 10.3		12 ± 0.4		23 ± 0.6		10 ± 0.3	12 ± 0.7
		55 ± 2.6		3 ± 0.1		6 ± 0.1		3 ± 0.1	3 ± 0.2
		155 ± 5.2		32 ± 0.9		16 ± 0.6		16 ± 0.5	12 ± 0.5
		39 ± 1.3		8 ± 0.2		4 ± 0.2		4 ± 0.1	3 ± 0.1
		59		27		59		40	49
		60		90		135			
		28 ± 0.7		13 ± 0.2		12 ± 5.6			
		7 ± 0.2		3 ± 0.1		3 ± 1.4			
		163 ± 8.4		16 ± 0.8		11 ± 0.8			
		41 ± 2.1		4 ± 0.2		3 ± 0.3			
		15		44		51			
45		60				135	290		
42 ± 0.9		74 ± 2.8				58 ± 2.5	47 ± 1.5		
10 ± 0.2		18 ± 0.7				14 ± 0.6	12 ± 0.4		
347 ± 12.9		368 ± 14.4				34 ± 1.3	24 ± 0.6		
87 ± 3.2		92 ± 3.6				9 ± 0.3	6 ± 0.2		
11		17				63	66		
45		60				135	290		
44 ± 1.7		27 ± 0.9				21 ± 1.0	35 ± 1.1		
11 ± 0.4		7 ± 0.2				5 ± 0.3	9 ± 0.3		
325 ± 8.2		53 ± 2.8				17 ± 0.5	13 ± 0.6		
81 ± 2.0		13 ± 0.7				4 ± 0.1	3 ± 0.2		
12		34				55	74		

Each of our carbohydrate types (MCHO, PCHO, and TCHO) showed significant positive correlations ($P < 0.01$) with DOC and negative correlations ($P \leq 0.02$) with depth. The presence of measurable MCHO in all samples and its skewed distribution (Fig. 2) suggest an uptake threshold for natural populations of planktonic bacteria (Sieburth, 1979) between 100 and 150 $\mu g\, l^{-1}$. A threshold effect has been observed by Jannasch (1970) in chemostat experiments, but at much higher substrate concentrations for the cultures of epibacteria (Sieburth, 1979) capable of growth on enriched seawater agar media. Twelve PCHO values fell well below the MCHO threshold in the open sea (to zero in one case). This could be explained by extracellular microbial hydrolysis, adsorption, or precipitation of high molecular weight material (Khailov, 1968; Khailov and Finenko, 1970). Unlike

Table 5. Distribution of two size fractions of particulate ATP and cell carbon at 6 stations across the open North Atlantic

Description	Analysis	Depths sampled (m)					
Station 9		5	10	20	30	40	50
1040 August 8*	<3 μm ATP‡		43±1.0	55±4.6	41±1.6	64±2.1	
40°36'N, 39°48'W	<3 μm C§		11±0.3	14±1.2	10±0.4	16±0.5	
Continental Slope	>3 μm ATP		84±1.8	84±4.2	108±2.6	133±5.8	
4972 m†	>3 μm C		21±0.4	21±1.0	27±0.7	33±1.5	
	<3 μm % total		34	40	28	32	
10		5					
1430 August 9	<3 μm ATP	18±0.3					
39°01'N, 34°59'W	<3 μm C	4±0.03					
Mid-ocean	>3 μm ATP	35±0.8					
3655 m	>3 μm C	9±0.2					
	<3 μm % total	34					
11		5		20		40	50
1145 August 11	<3 μm ATP	27±0.7		8±0.1		8±0.2	7±0.2
37°28'N, 28°43'W	<3 μm C	7±0.2		2±0.03		2±0.04	2±0.04
Azores Plateau	>3 μm ATP	19±0.3		11±0.4		49±1.7	27±0.1
2000 m	>3 μm C	5±0.08		3±0.1		12±0.4	7±0.3
	<3 m % total	59		41		13	21
12		5		20		40	50
0516 August 12	<3 μm ATP	17±0.3		16±0.4		12±0.1	11±0.2
37°28'N, 28°44'W	<3 μm C	4±0.06		4±0.1		3±0.03	3±0.04
Azores Plateau	>3 μm ATP	16±0.9		69±2.4		15±0.4	22±0.5
1880 m	>3 μm C	4±0.2		17±0.6		4±0.09	5±0.1
	<3 μm % total	51		19		44	35
13		5		20	30	40	50
0939 August 14	<3 μm ATP	17±0.09		18±0.2	17±0.3	17±0.2	26±0.6
36°59'N, 21°22'W	<3 μm C	4±0.02		5±0.06	4±0.06	4±0.05	7±0.1
Off Eur. shelf	>3 μm ATP	57±2.1		142±2.0	193±1.7	108±2.3	81±0.9
3803 m	>3 μm C	14±0.5		35±0.5	48±0.4	27±0.6	20±0.2
	<3 μm % total	23		12	8	13	24
14		5		20	30	40	50
0345 August 15	<3 μm ATP	13±0.3		24±5.1	32±1.7	29±0.5	26±0.3
36°47'N, 21°20'W	<3 μm C	5±0.07		6±1.3	8±0.4	7±0.1	7±0.09
Off Eur. shelf	>3 μm ATP	12±1.5		119±1.2	164±1.9	123±0.8	49±1.1
4517 m	>3 μm C	3±0.4		30±0.3	41±0.5	31±0.2	12±0.3
	<3 μm % total	60		17	16	19	19

* Local time and date. † Sonic depth. ‡ ATP in pg/cm³. § Carbon in mg/m³ as cellular carbon computed from ATP values (HAMILTON and HOLM-HANSEN, 1967). Values ±95% confidence limits. <3 μm Fraction: plankton between 0.2 μm and 3.0 μm in size. >3 μm Fractions: plankton between 3.0 μm and 1000 μm in size.

MCHO, both DOC and TCHO show bimodal distributions due to higher neritic and lower oceanic levels. The mean concentrations of DOC, TCHO, and PCHO in the eight neritic casts (Table 1) were significantly greater (*t*-test, $P < 0.01$) than those for the oceanic samples (Table 2) even when samples from below 300 m were eliminated to avoid the bias of the lower deep water concentrations. This was not true for MCHO, which had virtually identical means (169 and 170 $\mu g\, l^{-1}$, respectively) in this comparison. PCHO accounted for a smaller fraction of the TCHO in oceanic than in coastal waters. For samples from the

Depths sampled (m)											
60	70	80	100	150	250	500	1000				
73±2.7		51±2.2	42±0.1	32±0.1							
19±0.7		13±0.6	11±0.03	8±0.03							
296±7.0		60±8.3	42±1.0	41±1.3							
74±1.8		15±2.1	11±0.3	10±0.3							
20		46	50	44							
60					250	500	1000	2000	3000	3500	
19±0.5					13±0.3	10±0.3	16±4.9	4±0.2	7±0.1	17±1.3	
5±0.1					3±0.07	2±0.08	4±1.2	1±0.06	2±0.04	4±0.3	
146±3.4					17±1.0	7±(—)	4±1.0	3±0.3	4±0.1	4±0.07	
37±0.8					4±0.3	2±(—)	1±0.3	1±0.07	1±0.03	1±0.02	
11					43	57	78	55	64	80	
60	70	80	100	150							
9±0.3	6±0.6	14±0.2	11±0.1	10±0.3							
2±0.07	1±0.2	3±0.04	3±0.03	3±0.07							
24±0.8	24±3.0	23±0.7	12±0.3	24±0.6							
6±0.2	6±0.7	6±0.2	3±0.07	6±0.2							
27	19	37	48	30							
60											
13±0.2											
3±0.05											
24±0.3											
6±0.07											
35											
60	70	80	100		250						
15±0.2	23±0.1	24±0.2	16±0.2		6±0.2						
4±0.06	5±0.03	6±0.04	4±0.1		2±0.04						
50±1.0	32±0.4	90±0.3	43±0.5		11±0.3						
13±0.2	8±0.1	23±0.07	11±0.1		3±0.07						
23	41	21	27		36						
60	70	80	100								
24±1.0	19±0.2	13±0.1	7±0.1								
6±0.3	5±0.04	3±0.04	2±0.03								
31±0.3	6±1.3	18±0.1	19±(—)								
8±0.08	15±0.3	5±0.03	5±(—)								
44	25	42	26								

upper 300 m, Table 3 shows a significant decline in this fraction from 69% nearshore to 37% in mid-ocean, with an increase upon approaching the European coast. This trend receives some support from WHEELER (1976), who reported a decrease in high molecular weight carbohydrates with increasing distance from shore. The coastal values we observed were consistent with values of 66% from Narragansett Bay, Rhode Island (BURNEY and SIEBURTH, 1977) and 64% from a New England salt marsh in summer (BURNEY, unpublished data).

An appreciable amount of living bacterioplankton biomass occurs throughout oceanic waters (Tables 4 and 5), even at abyssal depths. Bacterioplankton ATP ranged from 4 to 219 pg ml^{-1}. This is equivalent to 1 to 55 μg $C\ l^{-1}$ or approximately 10^4 to 10^6 bacterial

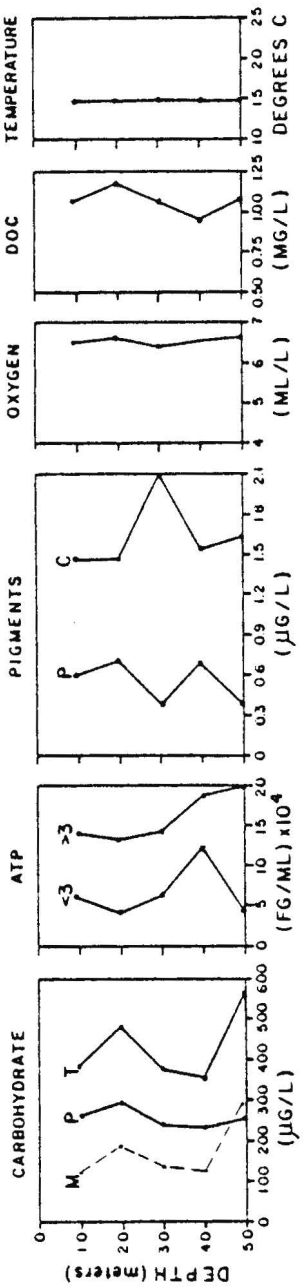

Fig. 3. Station 2, 1850 h local time, 26 July 1975, Georges Bank. Monosaccharide, M; polysaccharide, P; total carbohydrate, T; 0.2–3.0 μm particulate ATP, <3; 3–1000 μm ATP, >3; chlorophyll *a*, C; phaeopigments, P.

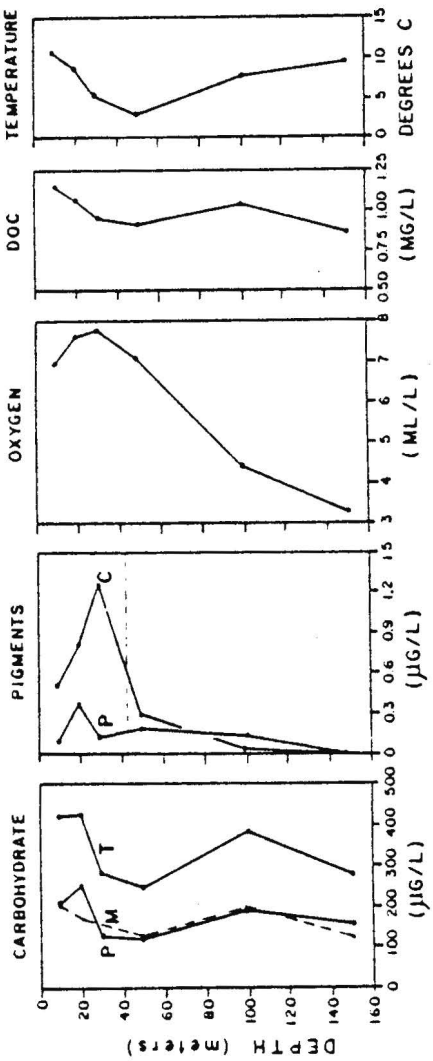

Fig. 4. Station 4, 1300 h, 31 July 1975, Nova Scotia Shelf. Symbols as in Fig. 1. Horizontal line at depth of the 1% light level.

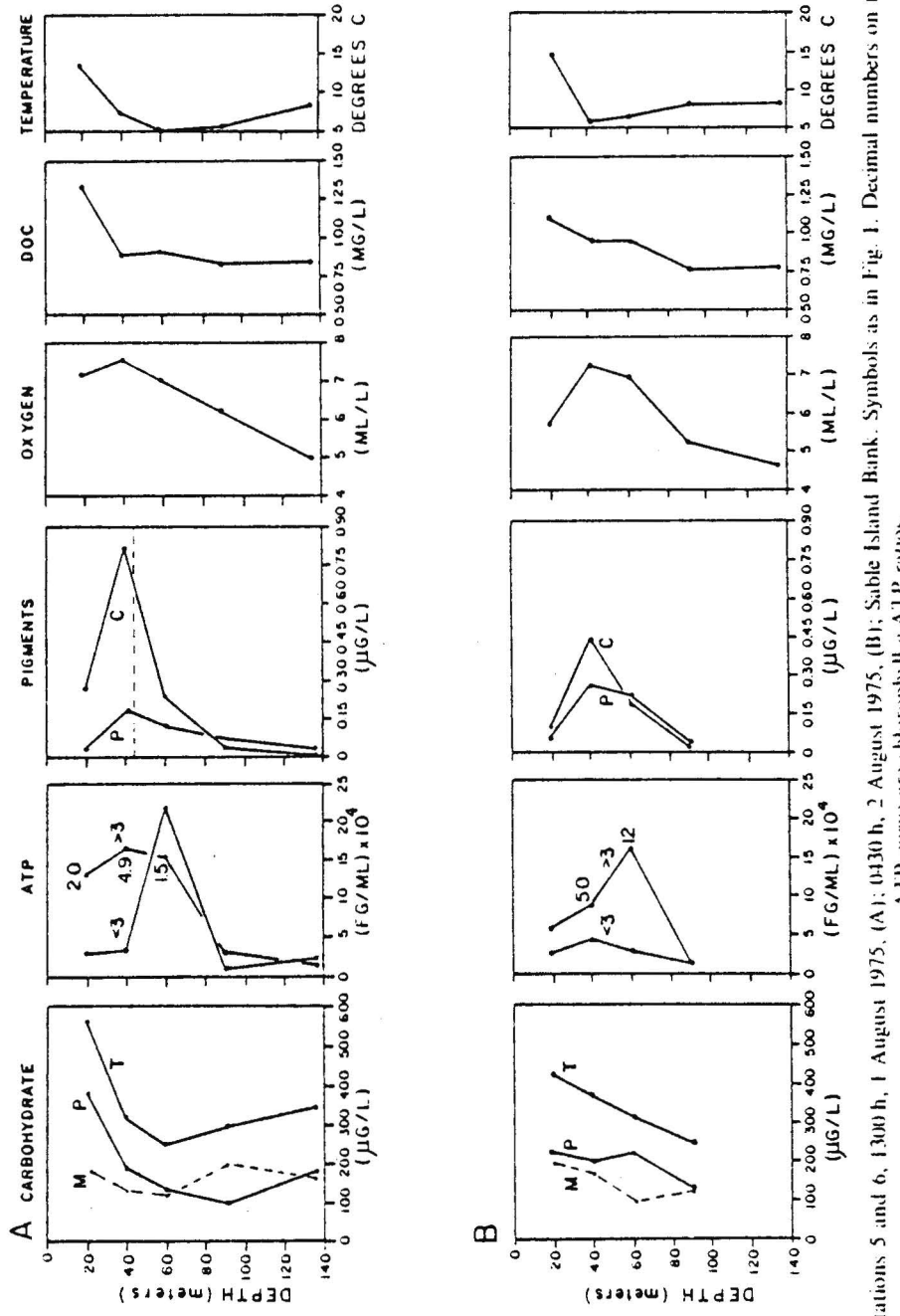

Fig. 5. Stations 5 and 6, 1300 h, 1 August 1975. (A); 0430 h, 2 August 1975, (B); Sable Island Bank. Symbols as in Fig. 1. Decimal numbers on the >3 μm ATP curve are chlorophyll *a* ATP ratios.

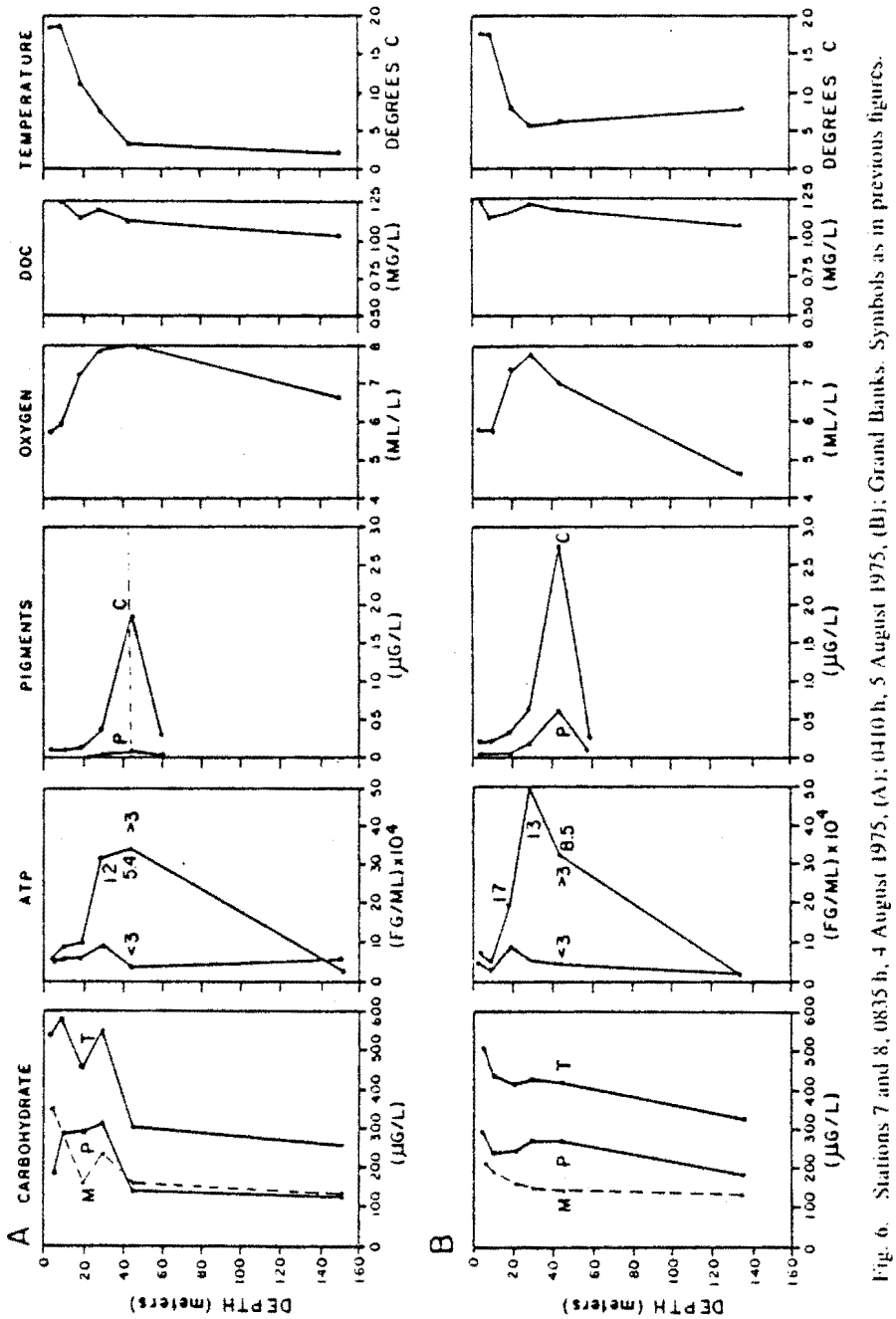

Fig. 6. Stations 7 and 8, 0835 h, 4 August 1975, (A); 0410 h, 5 August 1975, (B); Grand Banks. Symbols as in previous figures.

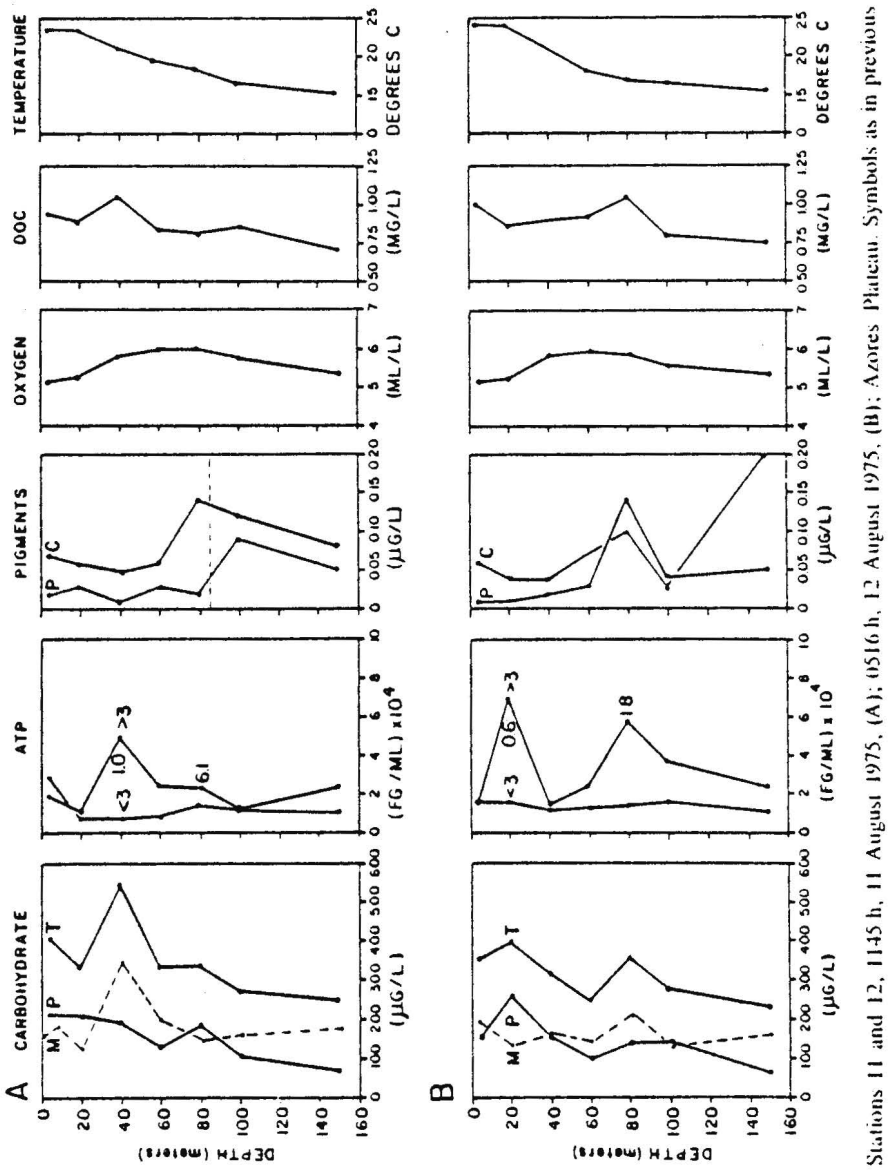

Fig. 7. Stations 11 and 12, 1145 h, 11 August 1975, (A); 0516 h, 12 August 1975, (B); Azores Plateau. Symbols as in previous figures.

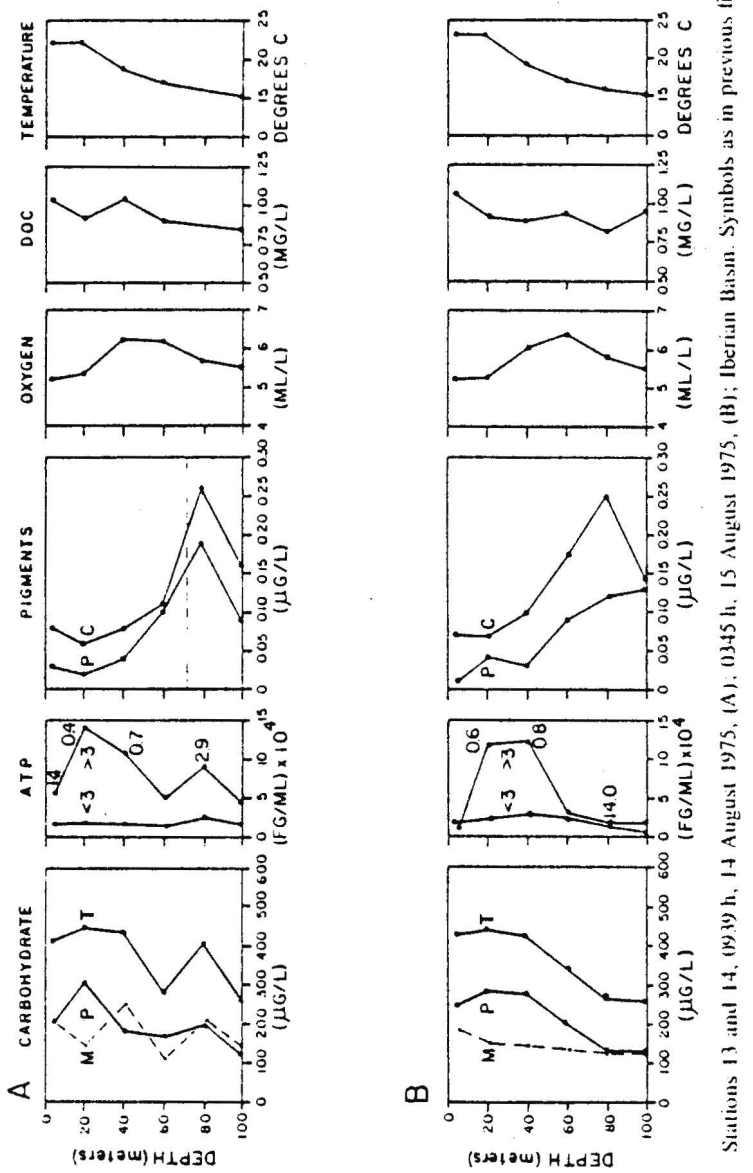

Fig. 8. Stations 13 and 14, 0939 h, 14 August 1975, (A); 0345 h, 15 August 1975, (B); Iberian Basin. Symbols as in previous figures.

cells ml^{-1} based upon the ATP : cell carbon : cell number ratios reported by HAMILTON and HOLM-HANSEN (1967), but corrected for the order of magnitude smaller cell volume of the dominant minibacteria (HOPPE, 1976; WATSON *et al.*, 1977). Bacterioplankton ATP in this study is in the same range as the total particulate ATP of the aphotic zone observed by HOLM-HANSEN (1973) and KARL, LAROCK, MORSE and STURGES (1976). Our estimates also agree with those based on microscopic counts (VINOGRADOV, GITELZON and SOROKIN, 1970; SOROKIN, 1971a, b, c; HOBBIE, HOLM-HANSEN, PACKARD, POMEROY, SHELDON, THOMAS and WIEBE, 1972; HOBBIE, DALEY and JASPER, 1977; ZIMMERMAN and MEYER-REIL, 1974; WATSON *et al.*, 1977) and the *Limulus* amoebocyte lysate titration for lipopolysaccharide by the latter investigators. The slight but statistically significant vertical variations in the living bacterioplankton biomass are consistent with those observed in the studies cited above.

Bacterioplankton ATP accounted for 3 to 80% of the total microbial ATP, averaging 30% for the photic zone and 40% for the aphotic zone. The only similar estimates of the bacterioplankton as a fraction of the total microbial plankton were reported by FERGUSON and RUBLEE (1976) for the shallow coastal waters of North Carolina; they ranged from 4 to 25% with a mean of 9%. Their values are based on fluorescent direct counts of acridine orange stained preparations to estimate bacterial carbon and ATP values to estimate total plankton carbon. The value probably underestimates the bacterioplankton as the 0.45-μm Sartorius membranes they used would lose a significant portion of the smaller cells (WATSON *et al.*, 1977).

The carbohydrate and DOC profiles (Figs 3–8) are apparently governed by the activities of the planktonic communities. Peaks in one or more of the carbohydrate fractions occurred at depths characterized by elevated >3-μm (protist) ATP and a chlorophyll *a*/protist ATP ratio of less than 2.5 [Figs 5(A), 20 m; 5(B), 60 m; 6(A), 30 m; 7(A), 40 m; 7(B), 20 m, 80 m; 8(A), 20 m, 40 m; 8(B), 20 m, 40 m]. The two exceptions [Figs 5(A), 60 m, and 6(B), 20 m] have bacterioplankton maxima that could have prevented the accumulation of carbohydrates. ATP peaks with chlorophyll *a*/>3-μm ATP ratios less than 2.5 may indicate accumulations of microzooplankton. This number was calculated from a carbon/chlorophyll *a* ratio of 100, which usually is not exceeded in phytoplankton (STRICKLAND and PARSONS, 1968; EPPLEY, HARRISON, CHISHOLM and STEWART, 1977), and a carbon/ATP ratio of 250 (HAMILTON and HOLM-HANSEN, 1967). Ratios less than 2.5 would indicate an accumulation of microzooplankton because there would be insufficient chlorophyll *a* to account for the >3-μm ATP if the population were primarily phytoplankton. FALKOWSKI and STONE (1975) obtained chlorophyll *a*/ATP ratios between 7 and 20 (w/w) from cultures of *Skeletonema costatum* grown under varying temperature and light regimes. Similar observations by SAKSHAUG (1977) and SAKSHAUG and HOLM-HANSEN (1977) yielded ratios greater than 2.5 for *Asterionella japonica* and *Pavlova* (*Monochrysis*) *lutheri* (except during a period of possible cell fusion in *Pavlova*). *Skeletonema* cultures also gave values greater than 2.5 except during periods of sexual reproduction, growth at low temperatures, and extreme nitrogen starvation.

Several of the carbohydrate peaks also occurred with phaeopigment maxima or low chlorophyll *a*/phaeopigment ratios [Figs 3, 20 m; 4, 20 m; 5(B), 60 m; 7(B), 80 m] indicative of chlorophyll degradation by zooplankton (LORENZEN, 1967) and providing further evidence for the association of feeding with carbohydrate accumulation. The fact that this association does not occur in every case is not necessarily damaging to the hypothesis because preliminary results of BURNEY (unpublished data) indicate that a marine ciliate (*Uronema*) can feed voraciously on *Isochrysis*, releasing carbohydrates and

decimating the culture without forming particulate phaeopigments in excess of the control. These possible microzooplankton accumulations occurred most often at 20 to 40 m and are reminiscent of the bioluminescence profiles reported by VINOGRADOV *et al.* (1970), which also showed peaks at these depths that did not correspond to the chlorophyll distribution. Our qualitative microscopic observations made both at sea and on shore coupled with the reproducibility of replicate ATP analyses on 145-ml unconcentrated samples indicated that microzooplankton, which consists primarily of protozoa (BEERS and STEWART, 1971; SOROKIN, 1974; VINOGRADOV, SHUSHKINA and KUKINA, 1976), were responsible for these ATP peaks. A variety of oligotrichous ciliates including tintinnids were present in detectable numbers. Net carbohydrate accumulations, possibly associated with protozooplankton, were most often in the form of PCHO although notable MCHO releases [especially Fig. 8(A), 40 m] did occur. Many of the depths apparently dominated by protozooplankton also showed large DOC accumulations, indicating the release of other soluble organic substances in addition to carbohydrates. BARBER (1967) determined extremely high DOC concentrations (5.4 mg C l^{-1}) in a bloom of the ciliate *Cyclotrichium meunieri* off the coast of Peru.

Even though there appears to be considerable evidence associating protozooplankton activities with carbohydrate release, a second possible explanation of the data exists. SAKSHAUG and HOLM-HANSEN (1977) have shown that nitrogen limitation leads to low chlorophyll/ATP ratios (approximately unity) in *Skeletonema* cultures. Although this was not found with the flagellate *Pavlova*, if this phenomenon were a characteristic of the phytoplankton in the present study, it could explain the occurrence of carbohydrate peaks with low chlorophyll *a*/ATP ratios, because nitrogen depletion is known to lead to a rapid production of carbohydrates by phytoplankton (MYKLESTAD and HAUG, 1972; SAKSHAUG, MYKLESTAD, KROGH and WESTIN, 1973). This explanation cannot be ruled out but is considered unlikely, especially where large DOC and/or phaeopigment accumulations occurred.

The relationship between the phytoplankton maxima (as indicated by the chlorophyll *a* profiles) and the carbohydrates is not so clear-cut as desired because the presence of microzooplankton cannot be excluded. However, the depth of the chlorophyll maximum at the neritic stations was not marked by carbohydrate accumulations in the daylight casts [Figs 3, 4, 5(A), 6(A)]. In some cases the lowest carbohydrate values in the euphotic zone occurred at the chlorophyll *a* and O_2 maxima [Figs 4 and 6(A)]. This may be related to the finding that excretion of organic matter by phytoplankton as a per cent of fixation is inversely related to chlorophyll *a* concentration (SAMUEL, SHAH and FOGG, 1971; THOMAS, 1971; IGNATIADES, 1973). The possibility that release did occur but not in excess of a closely coupled bacterial uptake cannot be excluded but appears unlikely because the distribution of bacterioplankton (<3-μm ATP) does not follow that of chlorophyll. The predawn carbohydrate profiles from the neritic stations lack significant features at the depth of the chlorophyll *a* maxima. In contrast to the neritic areas, the open ocean profiles (Figs 7 and 8) show evidence of net carbohydrate accumulation during the day at the chlorophyll *a* maximum occurring as PCHO in Fig. 7(A) (80 m) and a mixture in Fig. 8(A) (80 m). There is also a slight but significant increase in bacterioplankton biomass at these depths, unlike the neritic stations. This may be indicative of senescent phytoplankton because it is known that senescence leads to greater extracellular release of organic substances (IGNATIADES and FOGG, 1973) and a decreased inhibitory effect upon bacteria (DROOP and ELSON, 1966).

Although a bacterioplankton maximum occurred once with a carbohydrate peak during

the day [Fig. 6(A), 30 m], this was not usually the case. Conversely, maxima in bacterial biomass occurred several times with carbohydrate minima [Figs 3, 40 m; 5(A), 60 m; 6(B), 20 m] indicating that uptake and growth have taken place. Evidence of bacterial influence on the carbohydrate distributions is also obtained from a comparison of their diurnal and nocturnal profiles. In Figs 6, 7, and 8, the MCHO profiles in each pair are almost identical except for the large and statistically significant ($P \leqslant 0.05$) peaks [Figs 6(A), 5 m, 10 m, 30 m; 7(A), 40 m; 8(A), 40 m, 80 m] that occurred during the day and seem to be reduced to a threshold concentration at night when the profiles smoothly decrease with depth. This does not appear to be due to sampling of different water masses or to patchiness because these would introduce randomness irrespective of time of day. Nocturnal uptake by bacteria of the diurnal carbohydrate accumulations seems a more reasonable explanation. Even the occasional nocturnal peak [Fig. 7(B), 80 m] seems to be related to other biological parameters, as already discussed. No basis exists for attributing these peaks to contamination. The Niskin bottles were rotated between casts except when diel comparisons were made. In these cases, the same bottles were used at the same depths in both casts. It is improbable that the bottles would be contaminated only during the day. The same conclusions can be reached from an examination of the diel changes in the PCHO and TCHO profiles; however, they are more complex and often show large nocturnal increases possibly due to PCHO release. Still, the paired casts in Figs 7 and 8 are very similar except at the depths that show increased biological activity.

If one calculates apparent heterotrophic uptake rates from the diel changes where large daytime carbohydrate accumulations disappear at night, values up to 5 $\mu g\ C\ l^{-1}\ h^{-1}$ are obtained. These are one to two orders of magnitude greater than traditionally accepted open oceanic rates (VACCARO *et al.*, 1968), but they may be closer to the actual *in situ* activities than estimates obtained in small volume, non-diffusion, batch cultures isolated from the environment (SIEBURTH, 1977). More reliable estimates must await the results of close-interval sampling and diffusion culture studies of both the phototrophic and heterotrophic components of the picoplankton and nanoplankton now in progress.

Acknowledgements—Appreciation is expressed to JAMES M. HANNON for the standard oceanographic data as well as help in profiling and sampling; to FREDERICK W. FRENCH III for the pigment analyses; KENNETH R. HINGA and PAULA WILLIS THERRIEN for their help in ATP analyses; PAUL G. DAVIS and DAVID A. CARON for preparation and examination of the reverse flow concentrates; and BLAINE MCBURNEY and SCOTT MCN. SIEBURTH for assistance in the analysis of carbohydrates. JAMES P. QUINN is thanked for the use of a carbon analyzer, and SCOTT NIXON for the loan of a Turner fluorometer. This work was supported by the Biological Oceanography Program of the National Science Foundation through Grants GA-41501X, OCE-74-01537, and OCE-76-81779 and presents part of the research to be used by CMB in partial fulfilment of the requirements for the Ph.D. degree.

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APPENDIX III:

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