

1989

Heterotrophic bacterial production: Relationships to biological and abiological factors in estuarine environments

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**Heterotrophic bacterial production: Relationships to biological
and abiological factors in estuarine environments**

Koepfler, Eric Thor, Ph.D.

The College of William and Mary, 1989

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HETEROTROPHIC BACTERIAL PRODUCTION:
RELATIONSHIPS TO BIOLOGICAL AND ABIOLOGICAL FACTORS
IN ESTUARINE ENVIRONMENTS

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

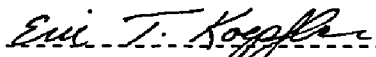
In Partial Fulfillment
Of the Requirements for the Degree of
Doctor of Philosophy

by
Eric Thor Koepfler
1989

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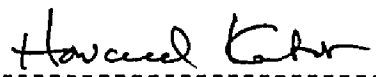
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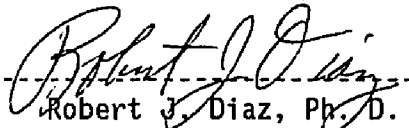
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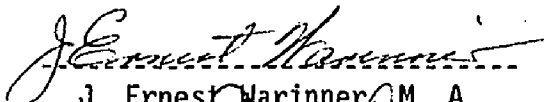
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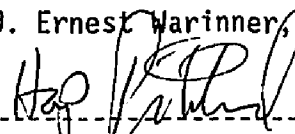
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ABSTRACT

Section 1- Ecotoxicological effects of creosote contamination on benthic bacterial communities in the Elizabeth River, Virginia were investigated using both structural and functional microbial parameters. Parameters included direct count, viable counts of heterotrophs and "cresol" utilizers, and bacterial production as measured by the tritiated thymidine uptake method. Results indicated that cell specific and total heterotrophic bacterial production were depressed in a dose dependent manner with increasing sediment PAH concentrations. Toxicity effects upon production parameters were modified by temporal trends associated with temperature as well as spatial sediment characteristics. Evidence of adaptation were equivocal, with cresol utilizer densities not significantly elevated at contaminated stations. Eh profile information suggested that creosote contaminants perturbed normal sediment redox conditions by shifting Eh towards more reduced potentials. Of the parameters employed, the tritiated thymidine production assay was found to be the most sensitive for detection of ecotoxicological effects.

Section 2- Bacterial abundance and production were examined during a destratification event at four stations in the lower James River, Virginia to determine if destratification would influence patterns of bacterial abundance and production, and relationships to other parameters. Bacterial abundance, although significantly different between stations, did not change over the study. Bacterial production (^3H -Tdr incorporation) in surface waters was significantly less during

the mixed period ($187 \mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$) compared to the most stratified state ($324 \mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$). Correlations between bacteria and chlorophyll were diminished during the mixed period. Total and flagellate specific grazing rates upon bacteria were reduced during the onset of destratification. Relationships between bacterial and nutrient parameters indicated a strong influence of destratification with greater importance of new nitrogen during the mixed period. These results indicate that destratification changes trophic interactions within the microbial loop, which are not necessarily reflected in temporal patterns of bacterial abundance.

Section 3- Bacterial thymidine incorporation (production), and ammonia assimilation and remineralization were examined monthly between April and August 1988 at three stations in the lower York River, Virginia. Size fractionation enabled estimates of bacterial contribution ($<1.6 \mu\text{m}$) relative to that of larger plankton ($>1.6 \mu\text{m}$, $>15 \mu\text{m}$) towards ammonia cycling processes. Bacterial ammonia assimilation accounted for 19-95% of total dark ammonia assimilation, with station means of 46-48%. Station means of ammonia remineralization in the $<1.6 \mu\text{m}$ treatment were 92, 48, and 38% of unfractionated values from lower to the upper river stations respectively. Regression statistics indicated that assimilation was best predicted by bacterial production, compared to bacterial abundance, chlorophyll, or PON. Remineralization was less well predicted by these variables. Bacterial biomass and production were estimated to be 15 and 70% of respective autotrophic values indicating that the importance of bacteria in ammonia cycling are greatly disproportionate to their biomass and production.

PREFACE

This dissertation presents the results of three research studies conducted between 1983 and 1989. In each study bacterial production was estimated with a radiotracer technique involving the incorporation of tritiated thymidine. Although these studies all concerned bacterial response to, or association with other factors, the hypotheses and objectives within each study were strictly independent of each other.

The dissertation is formatted as follows. First, there is a common introduction, which presents a historical overview concerning the technological developments leading to the currently used methods for measuring bacterial production. Thereafter each study is presented in separate sections which are complete with introduction, methods and materials, results, discussion (results and discussion for the first study) and conclusion subsections. Finally, literature cited within all portions of the manuscript are presented in the literature cited section which follows the third study.

INTRODUCTION

Historical impetus for the examination of bacterial production stems in part from technological developments related to their detection and enumeration. According to ZoBell (1946) the first accurately described bacterial species derived from seawater, Spirochaeta plicatilis, was isolated by Ehrenberg in 1838 (Ehrenberg, 1838). It was over fifty years later before provisions for studying the role of bacteria in the sea, as part of the Humboldt Foundation's plankton expedition, enabled Fisher (1894) to describe the spatial distribution of viable bacteria enumerated on solid media in the Atlantic Ocean. The mean abundance of bacteria reported by Fisher for all samples taken during this cruise was $1,084 \text{ cells}\cdot\text{ml}^{-1}$. Further refinements in viable counting techniques during the next century only marginally increased abundances noted for open ocean environments, but additional spatial studies indicated greatly enhanced abundances (eg. $480,000 \text{ cells}\cdot\text{ml}^{-1}$, ZoBell and Feltham, 1934) in coastal areas.

With the advent of epifluorescence microscopy in the late 1960's and early 1970's estimates of bacterial abundance markedly increased in open ocean, coastal water and sediment samples. Using epifluorescence microscopy some of the highest reported abundances of bacteria for estuarine water ($>1 \times 10^7 \text{ cells}\cdot\text{ml}^{-1}$) have recently been observed in the upper Chesapeake Bay by Malone et al. (1986) and Ducklow and Peele (1987). Bacterial abundances in sediments determined with epifluorescent microscopy typically exceed $10^9 \text{ cells}\cdot\text{gm wet sediment}^{-1}$.

In concert with the initial development and implementation of

epifluorescence microscopy, application and use of a newly developed radioisotopic technique (Parsons and Strickland, 1962) indicated that bacteria were highly efficient at the uptake of selected organic substrates. This method, which measured "relative heterotrophic potential" was later amended by William and Askew (1968) to also account for respiration of incorporated substrate. Although use of these early methods indicated that bacterial activity could be significant, production could not be accurately measured for several reasons. First, the natural concentrations of unlabeled substrate (glucose and acetate) were typically not known. Secondly, even if a "true" total uptake of a substrate were known it would not provide information on the simultaneous uptake of other organic substrates. Finally, incorporation of these organic substrates would label many macromolecular pools, not all of which are closely correlated with growth.

Brock (1967) was the first to employ ^3H -thymidine (^3H -Tdr) in estimating microbial growth rates. He found that DNA synthesis as measured by ^3H -Tdr incorporation in Leucothrix mucor on seaweeds, was directly proportional to growth rates determined by an independent method. The first reported use of ^3H -Tdr to measure bacterial production in sediments is attributed to Tobin and Anthony (1977). By the early 1980's intensive scrutiny of ^3H -Tdr incorporation methods for estimating bacterial production in water column (Fuhrman et al., 1980, Fuhrman and Azam, 1982) and sediment environments (Moriarty and Pollard, 1981; Moriarty 1981) were being pursued. Fuhrman and Azam (1982) found that several of the key requirements to effectively utilize thymidine incorporation for estimates of bacterial production were verified.

First, autoradiography indicated that << 1% of positively scored cells for ^3H -Tdr uptake were autotrophic. Second, they discovered that nearly 100% of the cells which actively took up labeled glucose and amino acids also incorporated thymidine. Finally, they found that at concentrations of ca. 5nM incorporation plateaued, indicating little isotope dilution beyond this concentration. Moriarty (1981) found similar results concerning autotrophic incorporation, and generally high specificity of uptake among heterogenous bacterial populations within sediments. However, using an isotope dilution methodology for determination of the degree of participation of exogenous substrate pools, was shown to be necessary for use in sediments.

Further studies concerned with the verification of the ^3H -Tdr method for sediments continued with Fallon et al. (1984). Fallon and co-workers compared whole system metabolism with production indicated by ^3H -Tdr incorporation and found that ^3H -Tdr uptake and oxygen consumption rates were similar. Findlay et al. (1984) reexamined the assumptions and extraction techniques of Moriarty and Pollard's (1981) isotope dilution methodology, to assess the overall suitability of the method and its specific application to blackwater river environments. They found that modifying certain steps in Moriarty and Pollard's (1981) extraction procedure could significantly increase recovery of labeled DNA. Findlay et al. (1984) further discovered significant isotope dilution in bacterioplankton production estimates, suggesting the need to employ isotope dilution techniques in certain pelagic environments. Bell (1986) found that concentrations exceeding 35 nM were required to achieve saturated uptake of ^3H -Tdr, and that uptake at 5 nM was 30-60%

of saturated rates.

Other studies during this period (Fuhrman and Azam, 1980; Kirchman et al., 1982) were devoted towards comparing ^3H -Tdr and newly developed ^3H -adenine incorporation (Karl, 1981a; 1981b) techniques for measuring bacterioplankton production. Results of these comparisons, while noting the ecological significance of RNA synthesis measurements concluded that the ^3H -Tdr method was simpler and more strongly correlated with directly observed changes in cellular abundance. For a complete review of these and earlier activity methodologies one should see Van Es and Meyer-Reil (1982).

With increasing acceptance of the ^3H -Tdr method as a sensitive measure of bacterial production, applications of the technique to other fields such as microbial toxicology began to surface in the mid-1980's. Jonas et al. (1984) were the first to use the ^3H -Tdr incorporation method in a toxicological application. They were able to demonstrate acute toxicity of organotin compounds towards microbial incorporation of ^3H -Tdr in Chesapeake Bay waters. This study was followed by Bauer and Capone (1985) and Koepfler and Kator (1986) who utilized ^3H -Tdr incorporation methodology to assess the effects of polycyclic aromatic hydrocarbons upon sediment bacterial communities in laboratory and field settings, respectively.

Several problems remain with the ^3H -Tdr incorporation method. First, contrary to the assumptions employed by many following Fuhrman and Azam's (1982) methodology for estimating bacterioplankton production, the proportion of activity in DNA versus other pools is not always ca. 80%, but can vary both temporally and spatially (McDonough et

al., 1986; Robarts et al., 1986; Hollibaugh, 1988). This problem does not appear to compromise the method however, because production can also be estimated using conversion values derived from ^3H -Tdr incorporation and new cell production over incubation intervals in the absence of grazers (Kirchman et al., 1982; Riemann et al., 1987). Conversion values calculated in this manner generally indicate values between $1.1 - 1.8 \times 10^{18}$ cells \cdot mole $^{-1}$ ^3H -Tdr incorporated. Further indications that ^3H -Tdr incorporation provides reasonable production estimates come from studies in which isotopically labeled leucine incorporation (which measures protein synthesis) have been measured concurrently with ^3H -Tdr (Kirchman et al., 1986; Kirchman and Hoch, 1988). Such studies indicate that incorporation of both labels covary rather closely. A second problem remains the potential isotope dilution effects upon perceived Tdr incorporation (Findlay et al., 1984; Bell, 1986). The degree of participation of unlabeled substrates appear to differ by environment. Determination of this dilution is considered a requirement to derive accurate production rates in sediments (Moriarty and Pollard, 1981), yet potential dilution artifacts in bacterioplankton production studies are rarely tested for, with most investigations considering ^3H -Tdr additions of 5nM as saturating for the system. Finally, recent work by Jeffrey and Paul (1988) indicate that even with an isotope dilution methodology bacterial production may be underestimated due to incorporation of thymidine bases which for some reason are unaccountable using standard isotope dilution assays. The authors who simultaneously monitored ^3H -Tdr incorporation, DNA content as measured by a Hoechst dye technique, and cellular abundance, found that ^3H -Tdr incorporation, even when

corrected for isotope dilution, nonspecific macromolecular labelling, and DNA recovery efficiency, underestimated DNA synthesized by a factor of 6-8.

Despite the aforementioned concerns, the use of $^3\text{H-Tdr}$ incorporation remains a powerful tool to assess the importance of secondary production by heterotrophic bacterial populations in marine and estuarine food webs, nutrient cycling processes, and microbial ecotoxicology. The following sections present the results of three independent studies conducted within various Chesapeake Bay subestuaries between 1983 and 1988 utilizing the $^3\text{H-Tdr}$ incorporation methodology.

SECTION 1

ECOTOXICOLOGICAL EFFECTS OF CREOSOTE CONTAMINATION
ON BENTHIC MICROBIAL POPULATIONS IN AN ESTUARINE ENVIRONMENT

INTRODUCTION

Marine and estuarine ecosystem stability is dependent on heterotrophic bacterial processes. Bacterial components mediate crucial processes such as nutrient cycling (Klump and Martens, 1981; Valiela, 1984), geochemical transformations (Sorensen et. al., 1979; Novitsky and Kepkay, 1981) and secondary production within both pelagic (Wright and Coffin, 1984) and benthic (Montagna, 1984) food webs. High specific activities, enzymatic versatility, and short generation times enable bacteria to react rapidly to environmental changes. However, much of the literature describing these characteristics concern the effects of changes in natural biogeochemical parameters. Given the ecological importance and responsiveness of bacteria, interest has recently developed towards understanding the effects of toxicants upon aquatic microbial communities.

Gustafsson and Gustafsson (1983) discussed two types of responses manifested by heterotrophic microorganisms subjected to stress. These were changes in total or specific activity and changes in biomass or composition of functional groups. Although, functional and structural parameters have been the focus of toxicity testing with bacteria, the majority of studies have dealt with the development of efficient toxicant screening bioassays and have not been directly concerned with potential effects on natural microbial populations. Further, relatively few investigators have examined the ecotoxicological effects of xenobiotics upon sediment bacterial communities, despite the commonly accepted view that sediments act as sinks for a wide variety of

toxicants (Baker, 1980).

The limited number of studies conducted with sediment bacteria have shown these groups sensitive to a variety of toxicants. Significant reductions in respiration rate (Mahaffey et al. (1982), uptake of ^{14}C labeled substrates (Barnhart and Vestal, 1983), glucose uptake V_{max} and phosphatase levels (Baker and Morita, 1983) have been reported. Additionally, depression of growth rates (Liu et al., 1977) as well as reduction in diversities (Gustafsson, 1984) of natural sediment bacteria have been demonstrated to result from toxicant exposure.

The goal of this investigation was to examine the effects of known creosote contamination on the structural and functional characteristics of bacterial communities in estuarine sediments. Creosote is a complex mixture of pyrogenic oxidation products dominated by polycyclic aromatic hydrocarbons (PAHs), but also containing lesser amounts of cresols and other substituted aromatic compounds (U.S. Forest Products Laboratory, 1974). Evidence of the in vitro toxicity of PAHs and substituted PAHs toward water column bacteria have been demonstrated (Calder and Lader, 1976; Blakemore, 1978; Sayler et al., 1982a). However, extrapolation of this information for predicting the responses of natural sediment bacterial communities to similar toxicants is questionable. Therefore, the hypothesis being tested in this study was that the presence of creosote would alter structural and functional characteristics of sediment bacterial communities.

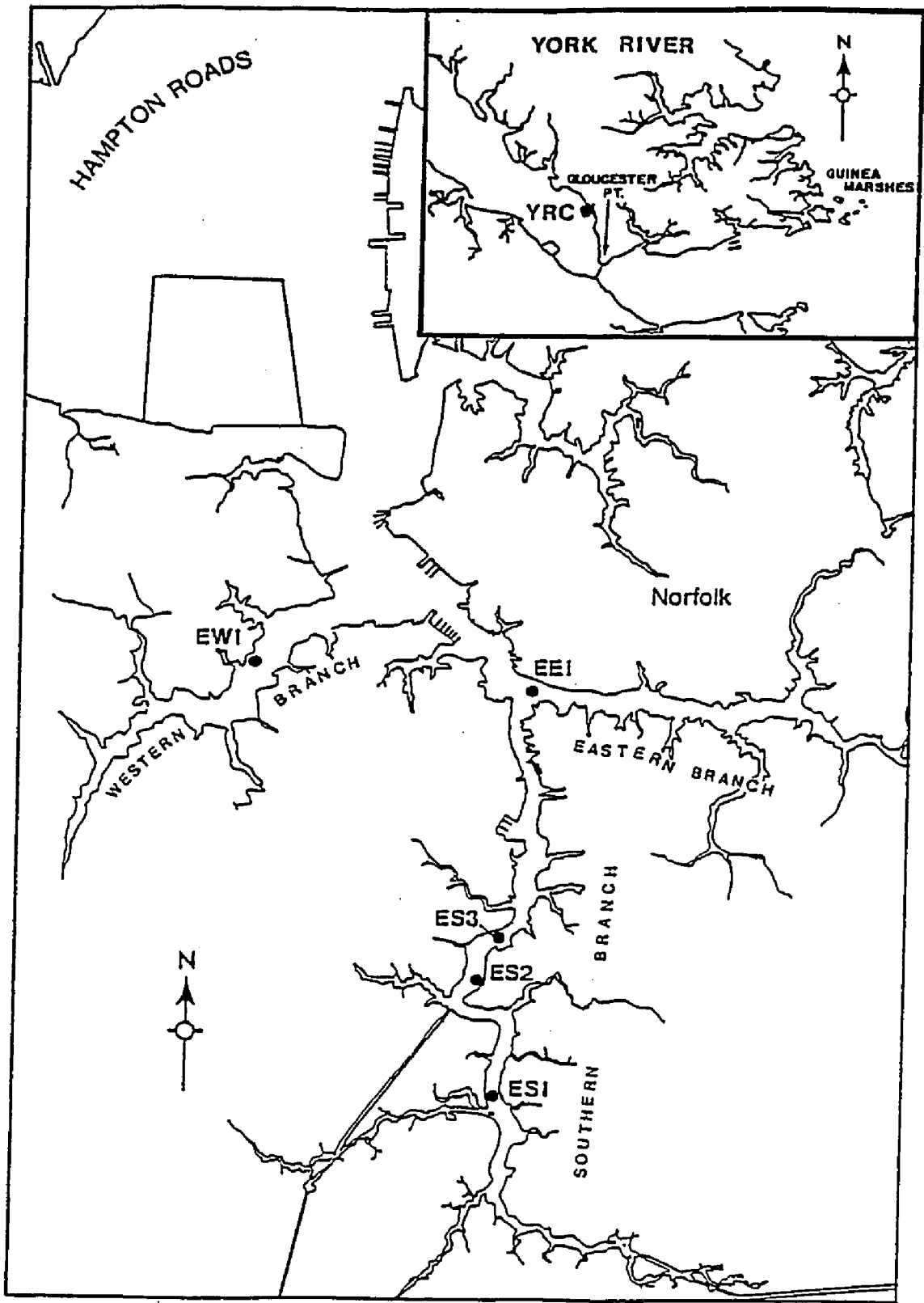
METHODS AND MATERIALS

Study area and station locations. The Elizabeth River, located in southeastern (Tidewater) Virginia, is composed of four tributaries which include the Lafayette River and the Eastern, Southern and Western branches. The system is characterized by low topographic relief, high suspended particulate loading, and poor flushing mediated by tidal mixing. The river drains approximately 300 square miles of highly industrialized and urbanized shoreline. Historically, at least three major spills from wood-treatment plants utilizing creosote have occurred in the Southern branch (Lu, College of William and Mary, Dissertation, 1983).

Station locations for all cruises are shown in Figure 1. All stations were shoal stations, i.e., the depth sampled was generally less than 4m. Station EW1 located in the Western branch and YRC located in the York River, were sampled to obtain data in environments considered comparatively unimpacted relative to the Southern Branch of the Elizabeth River.

Sediment collection. Sediments were collected using a Smith-MacIntyre grab (sample area = 0.1 m^2). Four subsamples were taken from each grab using (5 cm I.D.) acrylic cores. Appropriate sediment strata were then extruded and combined to yield a composite sample. Subsequent enumeration and production estimates were performed upon these composite samples. Separate intact cores were also taken for E_n and chemical analysis. On the first sampling date (November 1983), only one grab was sampled from each station, from which both 0-3 and 3-6 cm strata

Figure 1 Station locations in the Elizabeth and York
(inset, upper right) Rivers



composites were examined. The following two sample dates (June 1984, November 1984) three replicate grabs were taken from each station, from which the 0-3 cm strata composites were examined from each grab, while the 3-6 cm strata composite was examined from only the third grab.

Physical and sedimentological parameters. Physical parameters assessed during sampling included bottom water salinity and temperature and sediment temperature. Salinity was determined by refractometer and temperature was measured using an electronic thermometer.

Sedimentological parameters examined included percent sand, silt, clay and total organic carbon. Percent sand, silt, and clay were determined by pipet analysis (Folk, 1980). Total organic carbon was determined by the change in weight of a dried sample (100 °C, 24 hrs) after 1 h combustion at 550 °C.

E_h measurements. Acrylic core tubes (5 cm I.D.), penetrated with a series of holes spaced 0.5 cm apart on the vertical axis and sealed with silicone sealer, were used for E_h profile determinations. A platinum microelectrode (ca. 0.05 mm diam.) was used to puncture the sealer at successive depths. E_h values were calculated from the resultant potentials observed against a saturated KCl reference electrode, which was placed in the overlaying water within the core tube. Between stations the platinum microelectrode tip was cleaned with Bon-Ami® abrasive, soaked for approximately 5 min. in 1N HCL, and checked for calibration with a ferric-ferrous cyanate standard solution (454 mV).

Chemical analysis. In the absence of an analytical technique for the direct determination of sediment creosote concentration, the concentrations of total resolved polycyclic aromatic hydrocarbons

(TRPAH) were measured. Sediment samples for analysis of PAH content were collected in 5 cm acrylic cores, transferred to solvent-cleaned glass jars and stored frozen until processing by the Department of Chemical Oceanography, Virginia Institute of Marine Science. Sediments were dried with sodium sulfate, soxhlet extracted with dichloromethane, the concentrated extract fractionated and analyzed using capillary gas chromatography. A complete description of the procedure can be found in Bieri et al. (1981).

Viable counts. Each composite was sampled using sterile plastic syringe "mini-corers" to remove 20 ml of sediment. Sediment inoculum weight was then determined and the sediment extruded into a blender jar containing 180 ml of sterile estuarine water. The sediment was blended for 2 minutes at high speed, a homogenate sample removed and serially diluted by factors of 10. Selected dilutions were used to inoculate three-tube most probable number (MPN) series of two liquid media. The first, heterotrophic medium (HM) was for estimation of densities of mesophilic heterotrophic bacteria (Anderson et al., 1983). The second medium consisted of mineral salts (1 g/l $(\text{NH}_4)_2\text{SO}_4$ and 0.1 g/l K_2HPO_4) enriched estuarine water to which cresol (Mallinckrodt, U.S.P.) was introduced as the sole added carbon source for estimating the densities of cresol-utilizers. A final cresol concentration of 100 ppm (v/v) was chosen on the basis of toxicity screening tests. Ordinary commercial creosote and purified creosote were not used as growth substrates because they produced turbid dispersions which rendered MPN determinations impossible.

Direct counts. Bacterial standing crop or biomass was estimated

by a direct microscopic counting procedure. Eighteen ml of each composite homogenate was added to 2 ml of a 5% glutaraldehyde solution (v/v in distilled water and stored in the dark at 4°C until further processing. Appropriate dilutions of each sample were processed as described by Rhodes and Kator (1983), modified to allow for staining before filtration. Filter preparations were examined with a Zeiss microscope equipped for epifluorescence as previously described (Rhodes and Kator, 1983). Fields on each filter were randomly chosen and the total number of cells in a calibrated area counted. Counts were expressed as cells·g dry sediment⁻¹.

Benthic heterotrophic secondary production. Benthic heterotrophic bacterial production was measured following the basic thymidine uptake /recovery procedure of Tobin and Anthony (1978). Two ml of sediment (from each grab composite) was dispensed into 4 replicate tubes in groups of 5 treatments. Treatments (including killed controls) were expressed in terms of added cold thymidine concentrations (isotope dilution technique, Moriarity and Pollard, 1981). Controls were prepared in advance by addition of 0.5 ml of 100% formalin prior to label addition. All tubes received 1.0 ml of 20-25 μCi of tritiated thymidine (³H-Tdr) in 0.2 μm membrane-filtered seawater. ³H-Tdr specific activity was 60-80 Ci·mmole⁻¹. Tubes were incubated in the dark at in situ temperature over an interval chosen beforehand on the basis of a time-course uptake experiment. This interval was equal to or less than 1 h. Incubations were terminated by addition of 0.5 ml formalin solution and the samples stored at 4° C until further processing. Processed samples were counted using a Beckman® LS-150

scintillation counter (1% counting error). Counting efficiency was calculated using an external standard ratio to internal standard determined counting efficiency calibration curve. DNA recovery efficiency was estimated by the method of Fallon et. al. (1982) and production rates, calculated using the graphical method of Moriarity and Pollard (1981).

Data analysis. Elizabeth River data were examined using analysis of variance (ANOVA), and multiple classification analysis (MCA). One-way ANOVA and Duncan's a posteriori range contrast tests were employed to examine the June and November 1984 replicated data sets which included all stations. Linear regression analysis was utilized to examine relationships of microbial parameters to TRPAH concentrations for the entire data set and for individual sampling dates. Calculations were performed using the Statistical Package for Social Sciences (SPSS) on a Prime 850 computer.

RESULTS AND DISCUSSION

Chemical, sedimentological and physical data for all sample dates are shown in Table 1. Salinity and temperature regimes were relatively uniform between stations during each sampling date. Mean sediment data indicated that stations ES1, ES3, and YRC possessed similar grain size distribution characteristics (ca. 50% sand). Stations ES2, EE1 and EW1 were comparatively finer grained (ca. 25% sand). Mean total resolved polynuclear aromatic hydrocarbon (TRPAH) concentrations ranged from 1.45 to 259.43 ppm for YRC and ES3, respectively. Sedimentological and chemical characteristics were most variable at stations in the Southern branch. This may have been a reflection of known physical disturbance and redistribution of the sediment due to shipping activities and the physical heterogeneous nature of the creosote pollutant. "Control" stations EW1 and YRC exhibited the lowest TRPAH concentrations and significantly less variability in sedimentological and chemical parameters.

Viable count data are shown in Figure 2. ANOVA results (Table 2) indicated that significant differences in the means of total heterotroph and cresol-utilizer densities existed in date, depth and station groupings. Multiple classification analysis (MCA) (Table 3), which describes the deviations of microbial parameters based upon date, station and depth groupings from the zero standardized grand mean, indicated the following tendencies for total heterotrophs. Mean heterotroph counts were higher in November samples than in the June sample, were significantly less in subsurface sediments, and tended to

Table 1. Physical, sedimentological, and chemical parameter data

Station	Strata	Physical			Sedimentological							Chemical
		Temp °C	Sal ‰	Temp °C	Temp °C	Sal ‰	Temp °C	Sal ‰	% Sand ^a	% Silt ^a	% Clay ^a	
R81	0-3 cm	18.0	18.0	24.0	10.0	22.0	16.0	50.26 ± 27.35	18.74 ± 10.57	30.99 ± 16.89	6.47 ± 4.00	10.90 ± 4.36
	3-6 cm	18.0	18.0	24.0	10.0	22.0	16.0	26.30 ± 13.41	26.67 ± 4.44	47.03 ± 13.50	10.12 ± 2.31	
R82	0-3 cm	16.0	18.0	22.5	12.0	21.0	16.0	23.41 ± 5.75	24.47 ± 6.53	37.33 ± 15.34	11.41 ± 0.99	112.87 ± 80.60
	3-6 cm	16.0	18.0	22.5	12.0	21.0	16.0	25.82 ± 19.42	28.05 ± 4.86	46.14 ± 14.62	11.21 ± 0.35	
R93	0-3 cm	16.0	18.0	23.5	12.0	21.0	18.0	49.68 ± 28.62	18.37 ± 8.05	31.94 ± 21.85	6.49 ± 3.10	259.43 ± 205.10
	3-6 cm	18.0	18.0	23.5	12.0	21.0	18.0	52.68 ± 37.49	17.63 ± 13.53	29.91 ± 24.69	6.48 ± 4.92	
R21	0-3 cm	16.0	20.0	22.0	12.0	20.0	18.0	22.35 ± 6.98	20.12 ± 6.24	47.53 ± 4.45	7.71 ± 0.55	16.97 ± 4.28
	3-6 cm	16.0	20.0	22.0	12.0	20.0	18.0	25.24 ± 18.94	23.37 ± 4.21	51.39 ± 15.68	7.23 ± 1.02	
R11	0-3 cm	15.0	20.0	22.0	14.0	19.0	18.0	12.79 ± 4.66	34.57 ± 5.04	53.15 ± 1.86	0.45 ± 0.36	3.33 ± 0.74
	3-6 cm	15.0	20.0	22.0	14.0	19.0	18.0	9.39 ± 7.10	37.55 ± 5.44	52.86 ± 1.67	7.88 ± 0.76	
TNC	0-3 cm	-----	-----	24.0	17.0	21.0	20.0	31.46 ± 13.19	29.15 ± 6.19	19.39 ± 7.00	3.91 ± 0.90	1.45 ± 0.15
	3-6 cm	-----	-----	24.0	17.0	21.0	20.0	60.65 ± 17.85	24.35 ± 11.35	15.10 ± 6.50	3.15 ± 0.68	

^a - values for sediment characteristics are means for the three sample dates ± one standard deviation.

^b - values for chemical analysis are mean total resolved polynuclear aromatic hydrocarbon (TPAH) concentrations in ppm (dry sediment) of the 0-6 cm strata, for the three sample dates ± one standard deviation.

Figure 2 Mean viable counts of total heterotrophs and cresol utilizers with date, station and depth in sediment

VIALE COUNT (MPN)
(density . g dry sed⁻¹)

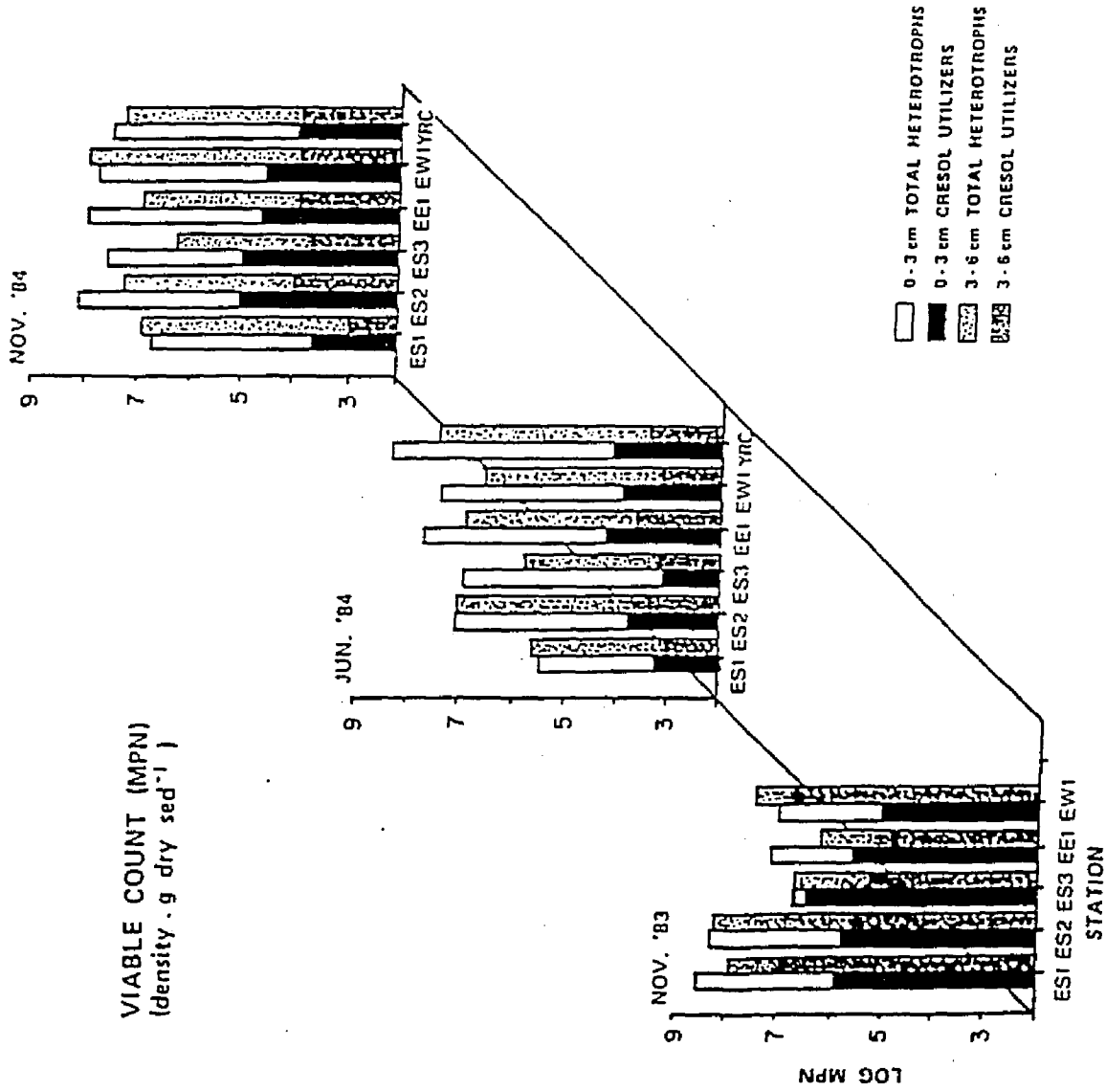


Table 2. Analysis of Variance of log transformed microbiological parameters by data set groupings. Variables include; log of total heterotroph density (LTH, cells·g dry sed.⁻¹), log of cresol utilizer density (LCU, cells·g dry sed.⁻¹), log ratio of cresol utilizers to total heterotroph density (LCUTH, unitless), log of total heterotrophic bacterial production (LPROD, g C·h⁻¹·g dry sed.⁻¹), and log of cell specific bacterial production (LCSP, g C·h⁻¹·cell⁻¹)

	DATE	Data Set Grouping STATION	DEPTH
Microbiological Parameter			
1) LTH	***	***	***
2) LCU	***	***	**
3) LCUTH	***	NS	NS
4) LPROD	***	***	***
5) LCSP	NS	***	NS

* = $p < 0.10 > 0.05$

** = $p < 0.05 > 0.01$

*** = $p < 0.01$

NS = not significant

Table 3. Multiple classification analysis^a (MCA) of microbiological parameters by data set groupings

		LPROD		LCU		LIR		LCSP		LCUTH	
		ETA ^c	BETA ^b	ETA	BETA	ETA	BETA	ETA	BETA	ETA	BETA
DATE	Nov 83	-0.05	-0.08	1.65	1.70	0.18	0.27	-0.19	-0.20	1.46	1.43
	June 84	-0.45	-0.38	-0.80	-0.82	-0.38	-0.41	-0.04	0.01	-0.47	-0.41
	Nov 84	0.44	-0.03	-0.04	-0.10	0.32	0.30	0.19	0.13	-0.35	-0.34
STATION	ES1	-0.10	-0.09	-0.42	-0.42	-0.60	-0.61	0.54	0.56	0.18	0.19
	ES2	0.45	0.41	0.20	0.20	0.46	0.44	0.03	0.02	-0.26	-0.24
	ES3	-1.06	-0.93	-0.17	-0.17	-0.28	-0.25	-0.70	-0.67	0.11	0.08
	EE1	-0.13	-0.12	0.30	0.30	0.07	0.10	-0.16	-0.17	0.23	0.20
	EW1	0.54	0.46	0.11	0.10	0.33	0.31	0.25	0.23	-0.23	-0.21
DEPTH	0-3	0.13	0.13	-0.03	0.10	0.12	0.15	0.00	-0.02	-0.15	-0.05
	3-6	-0.28	-0.28	0.06	-0.22	-0.27	-0.33	-0.00	0.04	0.34	0.10
Multiple R ² =		0.743		0.785		0.416		0.251		0.536	

^aMCA indicates deviations of mean microbiological parameters based on data set groupings, from respective zero standardized mean values derived from all observations.

^cETA = mean microbiological parameter deviations calculated including data set grouping interactions.

^bBETA = mean microbiological parameter deviations calculated excluding data set grouping interactions.

be lower at ES1 and ES3 versus the remaining Elizabeth River stations. Heterotroph counts were lower in subsurface sediments at the stations with the highest TRPAH concentrations (ES3, ES2 and EE1) on all sampling occasions (Figure 2). Seasonal mean counts at stations ES1 and ES3 were lower than those at other Elizabeth River stations, but were not always significantly different than counts at stations EW1 and YRC (Table 4). Sayler et al. (1982b) found that total heterotroph density was a strong discriminating variable when multiple discriminant analysis was employed to examine the effects of coal-coking effluent upon microbial sediment communities. However, the lower total heterotroph densities at stations ES1 and ES3 may in part be explained by the generally observed inverse relationship between bacterial densities and sediment grain size (Dale, 1974). Interestingly, regression analysis failed to reveal significant relationships between TRPAH and total heterotroph MPN (Table 5) for any sampling date or the entire data set.

Although direct counts were determined for sediment samples from the June 1984 and November 1984 samples, we minimized use of these data for interpretative purposes. Difficulties with counting cells in samples from contaminated stations were encountered due to high background fluorescence, presumably related to the presence of elevated concentrations of sediment absorbed PAHs. However, direct counts were significantly related to total heterotroph MPN (regression, $P < 0.001$).

Cresol-utilizer densities manifested the same seasonal trends as the total heterotroph population (Figure 2, Table 3). Highest densities were observed during November 1983 when the lowest sediment temperatures were recorded. The densities and distribution by depth of cresol-

Table 4. One-way ANOVA and Duncan's a posteriori range contrast of microbiological parameters for A. June 1984 and B. November 1984 samples

Parameter	One Way ANOVA F-probability	Duncans a-posteriori range contrast ^a					
		ES1	ES3	ES2	EW1	EE1	YRC
LTH	0.0000	ES1	ES3	ES2	EW1	EE1	YRC
LCU.	0.0024	ES3	ES1	ES2	EW1	YRC	EE1
A. LCUTH	0.0060	YRC	ES3	EW1	EE1	ES2	ES1
LPROD	0.0067	ES3	EE1	ES1	ES2	YRC	EW1
LCSP	0.0001	ES3	YRC	EE1	ES2	EW1	ES1
Parameter	One Way ANOVA F-probability	Duncans a-posteriori range contrast					
		ES1	ES3	YRC	EE1	EW1	ES2
LTH	0.0339	ES1	ES3	YRC	EE1	EW1	ES2
LCU	0.0252	ES1	YRC	EW1	EE1	ES3	ES2
B. LCUTH	0.1072	YRC	EW1	ES1	EE1	ES2	ES3
LPROD	0.0013	ES1	ES3	EE1	YRC	EW1	ES2
LCSP	0.0138	ES3	EE1	ES1	EW1	ES2	YRC

^aStations overscored by the same line are not significantly different at alpha = 0.05.

Table 5. Regression analysis of microbiological parameters versus Log TPAH

Data Grouping	Parameter	r ²	Significance	Formula
Total data set (all cruises)	LTH	---	NS	
	LDC	0.24	**	$y = -0.30(x) + 10.07$
	LCU	0.07	**	$y = 0.40(x) + 3.81$
	LCUTH	0.06	**	$y = 0.36(x) - 3.39$
	LPROD	0.11	**	$y = -0.34(x) - 5.57$
	LCSP1	0.09	**	$y = -0.32(x) - 12.81$
	LCSP2	---	NS	
	Eh	0.36	***	$y = -89.64(x) + 404.83$
Cruise #1, November 1983	LTH	---	NS	
	LDC	---	NA	
	LCU	---	NS	
	LCUTH	---	NS	
	LPROD	0.31	**	$y = -0.38(x) - 5.49$
	LCSP1	0.24	*	$y = -0.49(x) - 12.60$
	LCSP2	---	NA	
	Eh	---	NA	
Cruise #2, June 1984	LTH	---	NS	
	LDC	---	NS	
	LCU	---	NS	
	LCUTH	---	NS	
	LPROD	0.38	***	$y = -0.66(x) - 5.69$
	LCSP1	---	NS	
	LCSP2	0.34	**	$y = -0.59(x) - 15.62$
	Eh	0.52	***	$y = -102.36(x) + 422.56$
Cruise #3, November 1984	LTH	---	NS	
	LDC	0.37	**	$y = -0.33(x) + 10.11$
	LCU	0.20	**	$y = 0.30(x) + 3.92$
	LCUTH	0.32	***	$y = 0.38(x) - 3.64$
	LPROD	---	NS	
	LCSP1	---	NS	
	LCSP2	---	NS	
	Eh	0.42	***	$y = -104.26(x) + 430.28$

Parameters; LTH = Log MPN of total heterotrophs
 LDC = Log direct count of heterotrophs
 LCU = Log MPN of cresol utilizers
 LCUTH = Log of ratio of cresol utilizers to total heterotrophs
 LPROD = Log of production measured by thymidine uptake
 LCSP1 = Log of cell specific production (Total Production/TH)
 LCSP2 = Log of cell specific production (Total Production/direct count)
 Eh = Mean value of Eh for sediment strata (examined for 0-3 cm sediment only)

* $p < 0.10 > 0.05$; ** $p < 0.05 > 0.01$; *** $p < 0.01$; NS=not significant; NA=not available

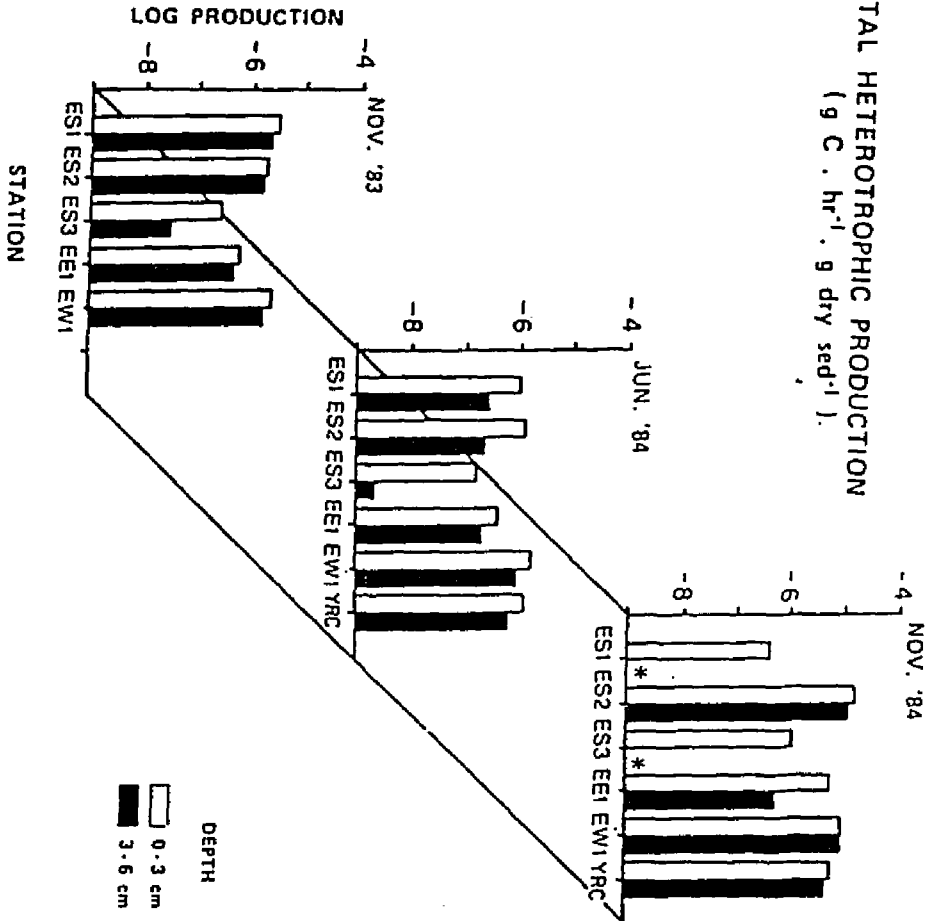
utilizers appeared related to prevailing seasonal sediment E_h . Densities were greater in surface versus subsurface sediments during the warmer sampling dates (June 1984, November 1984) when subsurface sediments were more highly reduced. This tendency was not observed in November 1983 when sediment temperatures were lowest and E_h values in subsurface sediments were presumably higher. This is consistent with observations that mineralization of hydrocarbons are inhibited under reducing conditions (Hambrick, et al., 1980).

Evidence of acclimation, i.e., increased densities of cresol-utilizers at contaminated stations, was equivocal. Although regression analysis indicated a significant positive relationship between cresol-utilizers and TRPAH (Table 5), station ES3 was shown by MCA to possess relatively lower counts of cresol-utilizers compared to other Elizabeth River stations. Because it appeared that cresol-utilizer densities were positively correlated with total heterotrophs, the value of the ratios of these two groups were also examined. ANOVA indicated that the mean ratios were significantly different only by date. Although YRC displayed the lowest ratios of all stations, the means were not statistically different from the means of most Elizabeth River stations (Table 4). The ability to utilize cresol as a sole carbon source was apparently commonplace among estuarine sediment bacteria. Whether this is simply a reflection of the enzymatic versatility of these bacteria or indicates acclimation to cresol or similar naturally occurring compounds in the environment, requires further study.

Bacterial production data based on the uptake of tritiated thymidine are shown in Figure 3. Carbon production values ranged from

Figure 3 Mean total heterotrophic bacterial production
with date, station and depth in sediment

TOTAL HETEROTROPHIC PRODUCTION
 (g C · hr⁻¹ · g dry sed⁻¹)



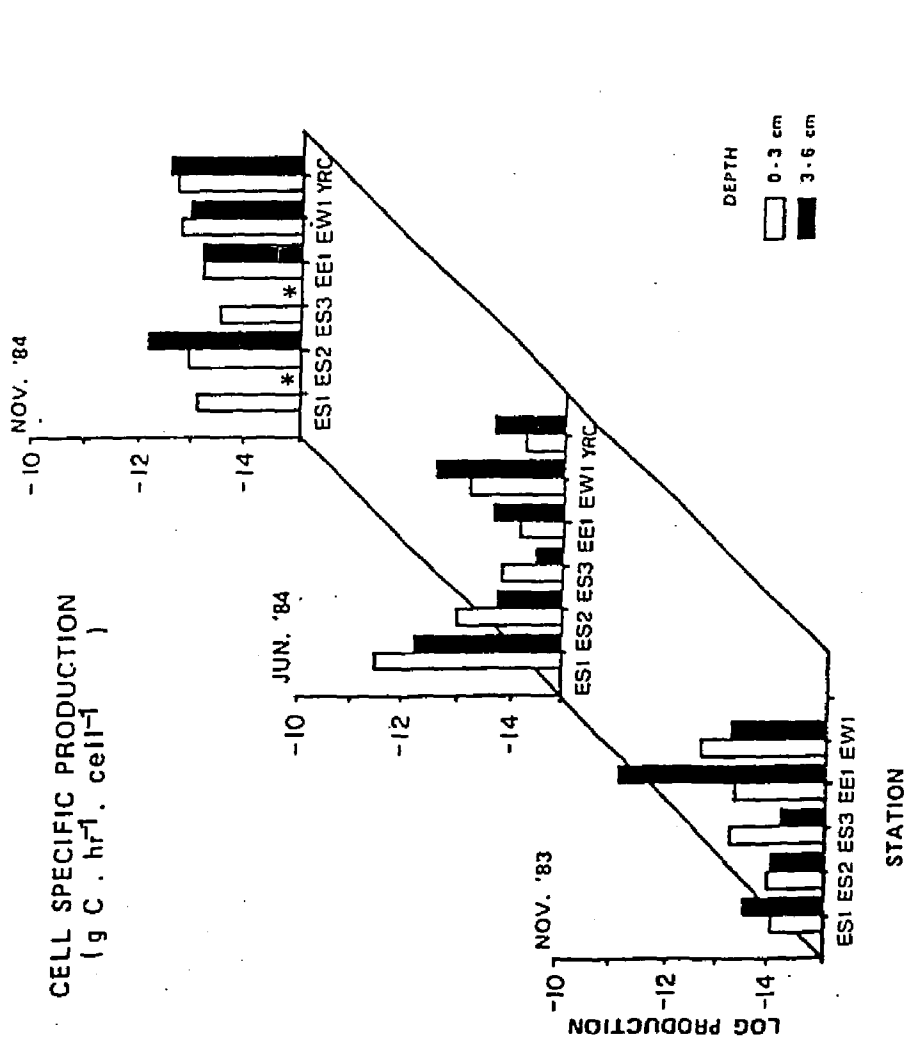
2.09×10^{-9} to 2.11×10^{-5} g C·g dry sediment⁻¹·h⁻¹. These values encompassed a range greater and less than values previously reported for bacterial production in marine sediments as estimated using the H-Tdr uptake method (Moriarity and Pollard, 1981; Fallon et al., 1983). ANOVA indicated that there were highly significant ($P < 0.01$) differences in production as a function of date, depth and station. Production tended to exhibit a seasonal fluctuation similar to that observed for viable counts (Table 3). Although production in subsurface sediments was always less than that observed in surface sediments, this differential was most pronounced at Station ES3 (Figure 2). Mean production was lower at ES3 compared to the remaining Elizabeth River stations (Table 3). In addition, production at ES3 was significantly less than all other stations during the June 1984 sampling (Table 4).

Linear regression analysis of bacterial production data versus TRPAH indicated a significant inverse relationship existed between these variables, suggesting a dose-response effect on production (Table 5). Comparison of regression formulae for total bacterial production between November 1983 and June 1984 revealed that during this period the negative value of the slope increased and the y-intercept remained essentially unchanged. This observation suggests that toxicity at contaminated stations was enhanced by increasing temperature. Whitehouse (1984) has shown that absolute temperature increases similar to those which occurred between the sampling dates may almost double the solubilities of certain aromatic hydrocarbons. Resulting elevated concentrations of PAHs in sediment interstitial water could increase toxicity and lead to depressed production. Invoking a temperature

relationship on toxicity may in fact account for the more highly significant differences observed between stations (with regards to microbiological parameters) in June 1984 versus November 1984 samplings (Table 4). Production measured in the subsurface sediment at ES3 in June 1984 was the lowest value measured during this study, approaching several orders of magnitude less than estimates for the remaining stations during this season. Recent chemical analyses have shown that TRPAH concentrations tend to increase with depth in contaminated sediments at ES2 and ES3 (Lu, Dissertation, College of William and Mary, 1984; R. J. Huggett et al., unpublished results). Since chemical analyses for our study were based on 0-6 cm samples, it is probable that the actual concentrations in the 3-6 cm profile were higher than the values indicated in Table 1. Therefore, we infer that depressed production in subsurface sediments at ES3 was due to greater toxicant loading.

Decreased production of station ES3 appeared to be the combined result of both lower heterotrophic bacterial densities and lower cell specific production rates (Figure 4, Table 3). Cell specific production, based upon all sampling dates, exhibited a significant negative relationship with TRPAH (Table 5). Station ES1, most similar to station ES3 in physical and sedimentological characteristics but significantly less contaminated with PAHs (Table 1), exhibited the highest rates of cell specific production among Elizabeth River stations. Fallon et al. (1982) reported higher cell specific production rates in sandy compared to muddy sediments. Therefore, decreased cell specific production at ES3 suggested a toxic response. Balboni (Thesis,

Figure 4 Mean cell specific bacterial production with date, station and depth in sediment



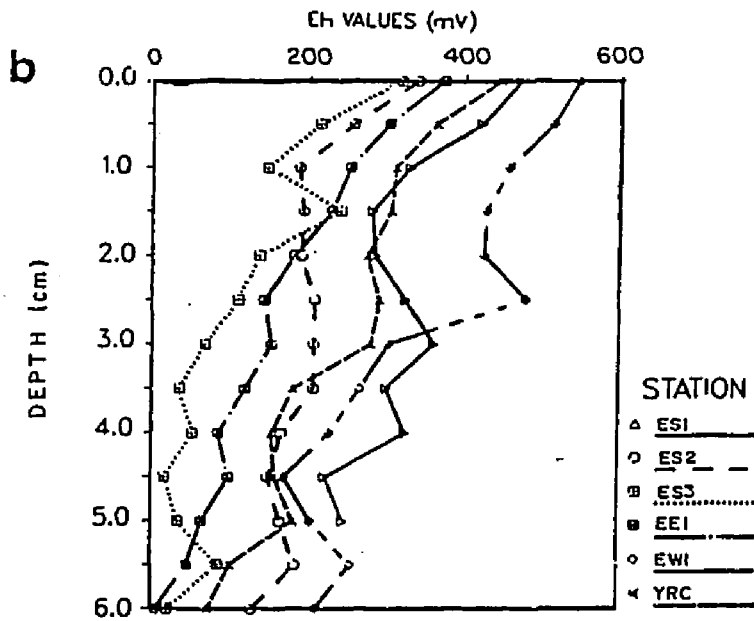
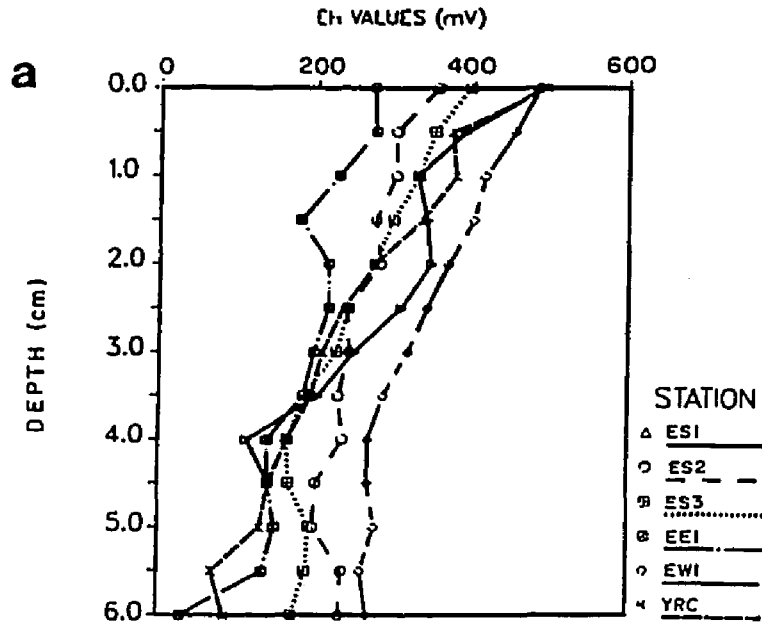
Univ. of Rhode Island, 1984) observed similar toxicity effects on cell specific production of estuarine sediment bacteria in polluted versus control microcosm systems.

Toxicity effects on benthic bacterial production may ultimately effect the bioenergetics of other trophic levels. Calculations of annual heterotrophic bacterial production based upon our estimates indicated that productions at stations EW1 and YRC were between five to eight times greater than that observed at ES3 (136-176 g C·m⁻²·yr⁻¹ versus 23 g C·m⁻²·yr⁻¹). Benthic field studies of the Elizabeth river (R. J. Diaz, unpublished results) as well as microcosm experiments utilizing creosote contaminated sediments as dosed toxicants (Tagetz et al., 1983; S. K. Thornton and R. J. Diaz, unpublished results), have indicated that benthic macrofaunal biomass and production were significantly reduced in the presence of these contaminants. Since many macrofauna species have been shown to ingest and assimilate bacteria (Yingst, 1976; Lopez et al., 1977; Montagna, 1984), depressed production and biomass of creosote exposed macrofauna may be a reflection of both reduced bacterial food sources and creosote toxicity.

Mean E_h profiles for the June and November 1984 cruises are shown in Figure 5. E_h profiles reflect the oxidation-reduction conditions which prevail with depth, and are a function of a number of interacting variables including microbial activities, bioturbation, sediment characteristics and hydrographic conditions. Profiles from the most contaminated stations (ES2, ES3, EE1) displayed lower mean E_h values than either EW1 or YRC. There was a highly significant ($P < 0.01$)

Figure 5 Mean sediment E_h profiles for Elizabeth and York River stations on a.) June 1984 and b.) November 1984 samples

SEDIMENT Eh PROFILES



negative relationship of mean E_h with TRPAH (Table 6). Similar depressions of E_h profile values in benthic microcosms exposed to creosote contamination have been observed (Tagetz et al., 1983; E. T. Koepfler, unpublished results). Despite the absence of an unequivocal explanation for this phenomenon, depression of E_h by creosote contaminants occurs over a variety of sediment types at contaminated stations in the Elizabeth River. Regardless of the mechanism, depression of E_h in sediments may subsequently alter microbial community structure (Baas Becking et al., 1960), affect nutrient regeneration and carbon cycling processes (Pritchard and Bourquin, 1984), and alter biodegradation rates of remaining toxicants (Hambrick et al., 1980; Gambrell et al., 1984).

Toxic effects of creosote contamination upon both structural and functional community characteristics were observed at station ES3. However, microbial parameters measured at station ES2 were essentially similar to EW1 and YRC, despite the fact that ES2 was characterized as having the second highest concentration of TRPAHs. Sedimentary characteristics at station ES2 such as relatively fine grain size and high TOC concentrations may account for this buffering effect. Weber and Rosenberg (1984) suggest that increased concentrations of organic matter may exert a protective effect on bacterial communities exposed to toxic compounds. Adsorption phenomena undoubtedly contribute to the frequently observed reduction in toxicity of many xenobiotics in sediment versus water column microbial communities (Barnhart and Vestal, 1983).

Bacterial production as measured by the H-Tdr uptake method was

found to be more sensitive to the presence of creosote contaminants than the other microbial parameters employed in this study. To the authors knowledge, this study is the first to employ the H-Tdr uptake method in conjunction with an ecotoxicological assessment of benthic bacterial communities. Jonas et al. (1984) utilized the H-Tdr uptake method in an examination of metal effects on Chesapeake bay water column bacteria. They found the method to be more sensitive in detecting metal toxicity than other parameters which included, viable plate counts and ^{14}C -labeled glutamate uptake measurements. Although further studies will be required to properly assess the utility of this method in microbial toxicity testing, we feel it offers promise as a sensitive and meaningful integrator of toxicity damage upon natural heterotrophic bacterial communities.

CONCLUSION

High concentrations of creosote contaminants in sediments of the Elizabeth River exerted toxic effects on benthic bacterial populations. Functional parameters such as heterotrophic bacterial production and cell specific production were more sensitive indicators of this toxicity than are structural parameters. However, the potential for creosote toxicity towards benthic bacterial communities was a function of not only contaminant concentration, but also environmental parameters such as temperature and sediment characteristics. Increasing temperatures may have enhanced the solubilities of many creosote toxicants in sediment interstitial water such that the realized dose to the community was greater than that experienced during lower temperatures. Sediment characteristics such as fine particle size and high organic content may act to adsorb and or chelate these toxicants and therefore lower the realized dose. In contaminated sediments, reduced bacterial production combined with comparatively depressed EH profiles indicated a poor state of "benthic health". Such systems may be characterized by aberrant carbon and nutrient recycling, energy flow imbalance within benthic food webs, and reduced toxicant biodegradation efficiency.

SECTION 2

EXAMINATION OF SPATIAL AND TEMPORAL BACTERIOPLANKTON DYNAMICS
DURING DESTRATIFICATION IN THE JAMES RIVER SUBESTUARY, VIRGINIA

INTRODUCTION

Knowledge concerning the importance of bacterioplankton in microbial food webs has accrued rapidly since the seminal works of Williams (1981) and Azam et al. (1983). Studies of bacterioplankton production and distribution in estuaries and coastal areas indicate that bacteria greatly influence carbon cycling processes by assimilating photosynthetically produced dissolved organic matter (Chrost and Faust, 1983; Iturriaga and Zsolnay, 1983; Ward, 1984), and serving as an important food resource for heterotrophic flagellates and ciliates (Haas and Webb, 1979; Fenchel, 1982a-d; Wright and Coffin, 1983, 1984a,b; Coffin and Sharp, 1987; Sherr and Sherr, 1977; McManus and Fuhrman, 1988). Work by Ducklow and Peele (1988) further emphasize the importance of bacteria in estuaries, indicating that bacterial production and biomass often exceeded that of autotrophic assemblages for extended periods in the upper Chesapeake Bay. Although it is widely accepted that substrate uptake and removal by grazing control bacterioplankton populations, little is known regarding the influence of estuarine hydrography upon these processes.

Estuarine stratification and destratification events provide an opportunity to examine the influence of hydrographic changes upon bacterioplankton dynamics. Haas (1977) demonstrated that portions of lower Chesapeake Bay subestuaries oscillate between moderately stratified and vertically homogenous conditions on time scales as brief as days. Destratification in the York River estuary has been shown to redistribute ammonium and phosphate rich bottom water as well as more

highly oxygenated surface water throughout the water column (Webb and D'Elia, 1980). Such mixing processes may also result from less extreme hydrographic changes such as wind driven pycnocline oscillations (Malone et al., 1986). The immediate effects of water column mixing upon various microbial components are not well known or predictable. Although phytoplankton blooms (Haas et al., 1981) and the stimulation of bacterial production (Ducklow, 1982) have been reported coincident with destratification, other studies have indicated that these processes are depressed under destratified conditions and are enhanced only when stratification reoccurs (Malone et al., 1986; Ray et al., 1988).

The major hypothesis of this study was that bacterial production and abundance would not change significantly over the course of a destratification event in the lower James River. However, information collected was also examined to determine the degree of spatial and temporal variability of all study parameters, whether relationships between bacterioplankton and other parameters changed over temporal and spatial scales, and the relative importance of various biological and abiological parameters in accounting for the variance of bacterial abundance and production estimates.

METHODS AND MATERIALS

Study area and sample acquisition

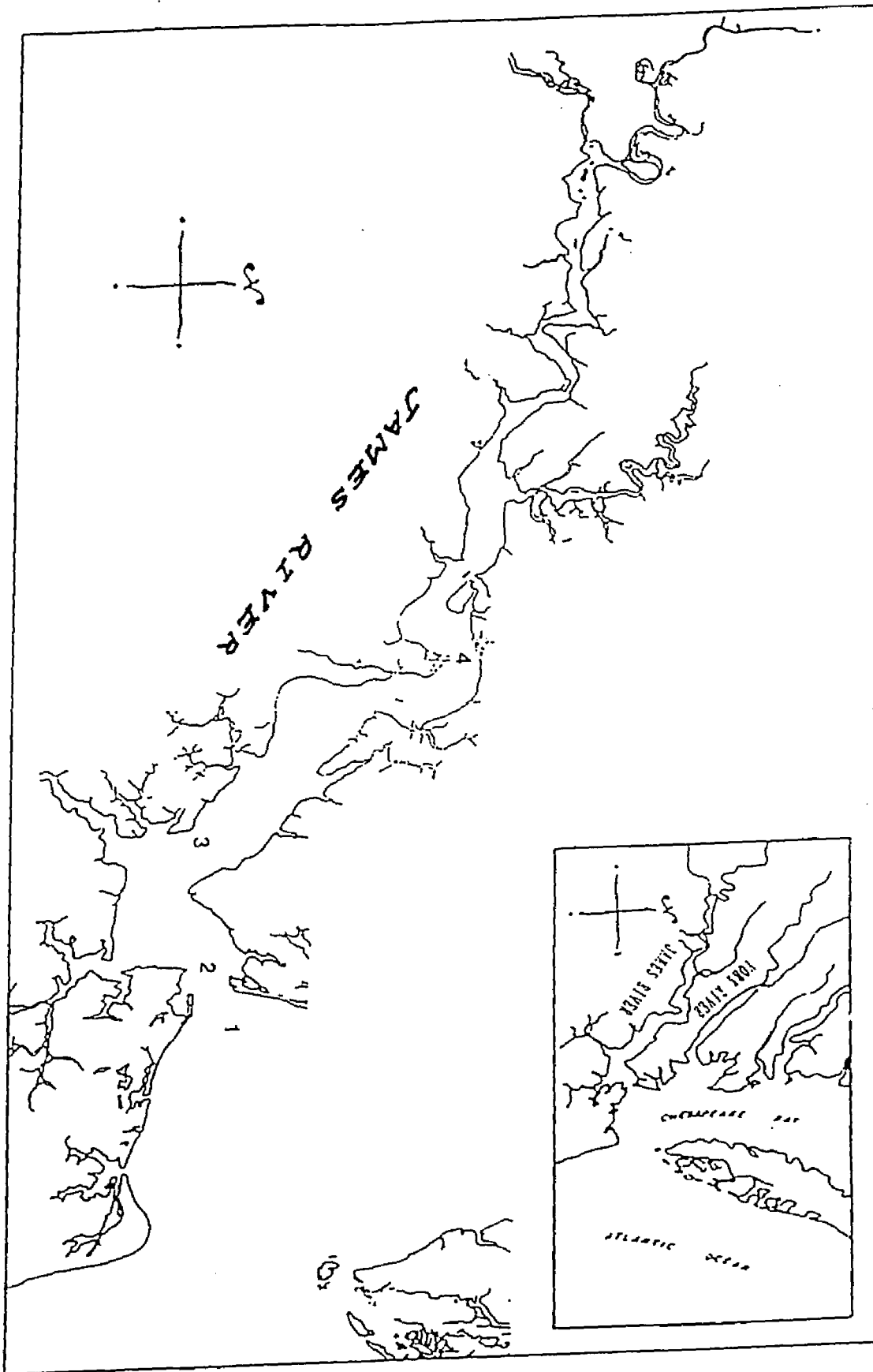
Samples were collected at four stations over six dates using the R.V. Ridgely Warfield (Johns Hopkins University). Station locations (Figure 6) ranged from station 1 which was 4 km outside the mouth of the James River, to station 4 which was 54 km upriver from the river mouth. Sampling of surface, pycnocline, and bottom waters was performed on August 6th, 9th, and 12th of 1983. Additional surface water samples were taken on August 8th, 10th, and 13th of 1983. On dates in which depth profile samples were taken, preliminary hydrographic information was obtained using a Neil-Brown CTD. Results of each cast were immediately analyzed in order to determine the appropriate depth for sampling of the pycnocline. Surface (0.5m) and bottom water (ca. 1m above sediment-water interface) samples were collected with a 5 l Niskin bottle which was washed with 95% EtOH and rinsed with distilled water between casts. Samples at each station were obtained as close as possible to low slack water.

Analytical Methods

Bacterioplankton Abundance and Production

Bacterial abundance was estimated via an epifluorescence direct counting technique using the dye proflavin as described by Haas (1982). Counts were made using a Zeiss standard microscope equipped with a 50 W high pressure mercury lamp, 10x calibrated ocular, 100x Neofluar® or 63x Plan apo® (Carl Zeiss; New York, NY) objectives and two epifluorescence

Figure 6 **Location of stations in the James River and
inset showing James River location in the
Chesapeake Bay**



filter sets. Blue excitation (450-490 nm; Zeiss #487709) was used to observe and differentiate heterotrophic from autotrophic cells and to count phycoerythrin-rich (PE) cyanobacteria. Green excitation (510-560nm; Zeiss # 487714) was used to count both PE and phycocyanin-rich (PC) cyanobacteria since PC cyanobacteria were not readily visible under blue light excitation. Heterotrophic bacterial biomass was estimated using a 20×10^{-15} g C·cell⁻¹ conversion value (Lee and Fuhrman, 1987).

Bacterial production was estimated using the tritiated thymidine (3H-Tdr) incorporation method (Fuhrman and Azam, 1980, 1982). Triplicate 10 ml water samples were incubated with 5 nM of 62 Ci·mmole⁻¹ of 3H-Tdr for 30 minutes in a dark water bath at in situ water temperatures. Triplicate 10 ml abiotic control samples (pre-incubation killed with 300 ul of buffered formalin) were treated in a similar manner. Sample incubations were terminated with 300 ul of buffered formalin and samples were stored at 4° C prior to further processing. Samples were further processed and prepared for counting by extracting with ice cold 5% TCA, filtering onto 0.45µm Gelman GN-6 membrane filters and washing with 5-6 ml of ice cold 5% TCA. Filters were then transferred to scintillation vials and acid hydrolyzed for 30 minutes in a boiling water bath with 0.5ml of 0.5N HCL. After cooling, filters were dissolved with 1 ml of ethyl acetate and 10 ml of Aquasol® scintillation cocktail. Disintegrations per minute were determined using a Beckman® scintillation counter, correcting for counting efficiency using an external standard channels and standard quench curve. Bacterial cell production was calculated using the conversion

factor of 1.7×10^{18} cells·mol⁻¹ of 3H-Tdr (Fuhrman and Azam 1982). Carbon production was estimated assuming 20×10^{-15} g C·cell⁻¹ (Lee and Fuhrman, 1987).

Ancillary parameters

Ancillary parameters were measured using the methods described in Table 6. Multiple citations are listed for a parameter where methodological refinements described in later citation(s) were also employed. Autotrophic biomass was estimated from chlorophyll a concentration using $70 \mu\text{g C} \cdot \mu\text{g Chl a}^{-1}$ (Malone et al., 1988).

Statistical analysis

Statistical analysis performed using the Statistical Package for Social Sciences (SPSS), included descriptive statistics of mean and standard deviations used to calculate coefficients of variation (CVs), Spearman rank correlation coefficients and multiple discriminant analysis (MDA). Analysis for the statistical difference in means of depth integrated bacterial parameters, and of grazing rate information employed the general linear model (GLM) routine of SAS Institute Inc. software.

Table 6. Methodologies utilized for determination of ancillary parameters.

PARAMETER(S)	REFERENCE(S)
1) Whole and <15 μm chlorophyll a, and phaeopigment ratio.	Holm-Hansen et al. 1965 Ray et al. 1988
2) Abundances of cyanobacteria, microflagellates, dinoflagellates, diatoms, and heterotrophic flagellates.	Haas 1982
3) Nutrients; ammonium, nitrite, nitrate, silicate, and phosphate.	U. S. Environmental Protection Agency 1979
4) Particulate organic carbon and nitrogen.	Patterson 1973
5) Relative dissolved organic matter.	Foster and Morris 1974
6) Salinity, temperature, and conductivity.	Ruzecki and Welch 1979
7) Dissolved oxygen.	Wetzel 1983

RESULTS

Hydrographic and physical data (Table 7) indicated that destratification occurred on or about August 9th, the date of maximum spring tides. Differences in surface to bottom salinities, conductivities and temperatures were considerably greater on August 6th compared with August 9th. By August 12th the water column at stations 2, 3, and 4 could be characterized as reapproaching a stratified state, whereas station 1 remained in a more stratified condition throughout the duration of the study.

Mean coefficients of variation (CVs) of bacterial and ancillary data grouped by date, station, and depth (Table 8) indicated that variability of most parameters was greater on spatial than on temporal scales. Bacterial abundance was much less variable, regardless of grouping, relative to other plankton components. Bacterial abundance and production were most variable over depth as was found for diatoms, chlorophyll a, and phaeopigment ratio. Nutrients and abundance of autotrophic taxa varied most when grouped by station. Only dissolved oxygen and cyanobacterial abundances displayed greater temporal than spatial variability.

Bacterial abundance and production rates (Figures 7a-d) tended to be greater in surface waters than in pycnocline and bottom waters. Spatial trends in surface and pycnocline waters suggested that abundance and production were higher in the lower estuary (stations 1 and 2) compared to the upper estuary (stations 3 and 4). Spatial distributions in bottom waters were more variable and displayed no distinct trend.

Table 7. Absolute values of surface minus bottom physical data obtained on depth profile sampling dates. Values include; Sal. (salinity; ppt), Con. (conductivity; $\text{mmho}\cdot\text{cm}^{-1}$), Tem. (temperature; $^{\circ}\text{C}$), and Oxy. (dissolved oxygen; ppm).

STATION	DATE			
	Aug06	Aug09	Aug12	
1	Sal.	7.65	4.72	3.47
	Con.	7.48	3.47	3.39
	Tem.	6.21	3.97	3.09
	Oxy.	1.20	1.06	1.20
2	Sal.	3.61	0.06	0.15
	Con.	3.48	0.08	0.27
	Tem.	3.17	0.15	0.52
	Oxy.	1.60	0.50	0.70
3	Sal.	3.13	0.81	0.99
	Con.	2.74	0.87	0.80
	Tem.	1.22	0.81	0.19
	Oxy.	0.08	1.36	0.70
4	Sal.	1.06	0.33	0.91
	Con.	0.73	ns	0.66
	Tem.	0.25	0.02	0.33
	Oxy.	0.94	1.20	0.20

ns - not sampled

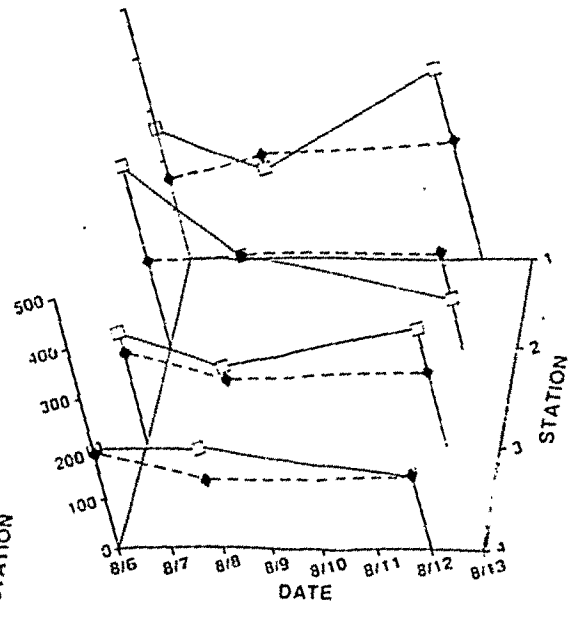
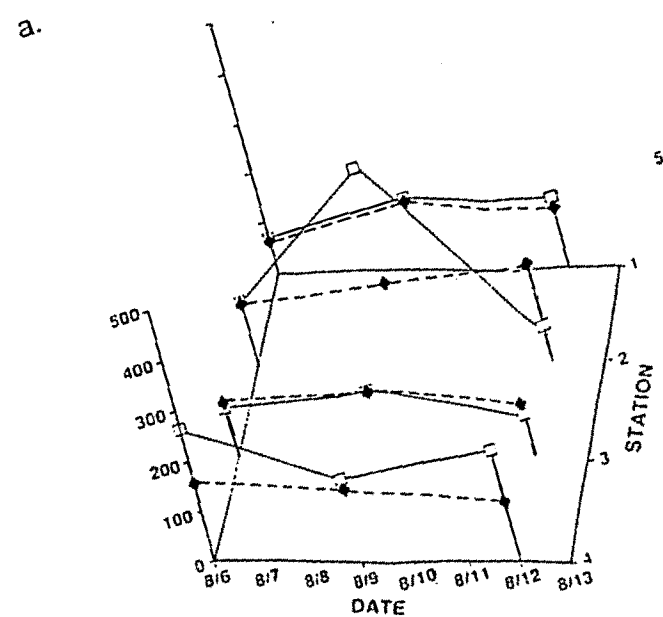
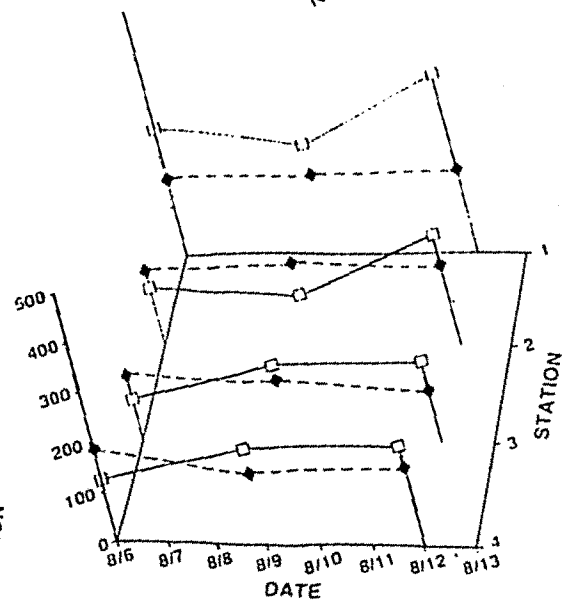
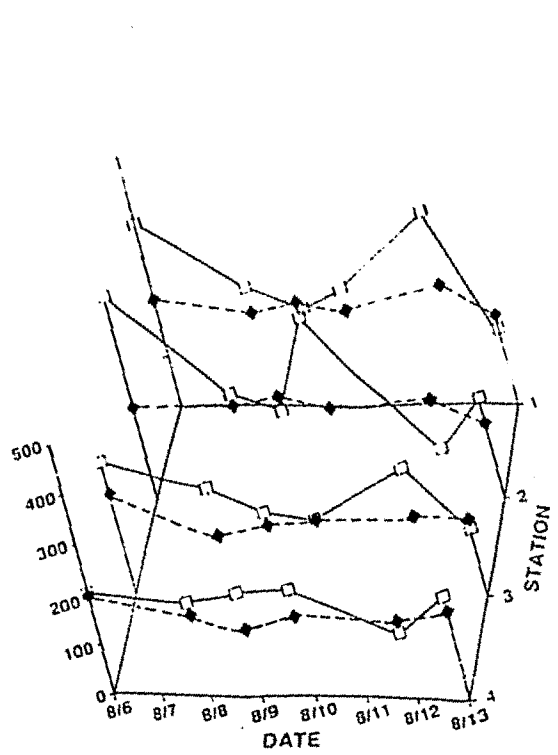
Table 8. Descriptive statistics of study parameters. Values include range, mean, and mean coefficient of variation (CV') based upon data groupings of the entire data set and date, station and depth categories. CV' was calculated as $\sum_{k,i} (CV_{k,i}) / (i*j)$ where k is the CV grouping of concern (i.e. depth), and i and j are alternative groupings (i.e. station, date) such that CV' would be the mean of $i=(4) \times j=(3) = 12$ station-date combinations. CV' for entire category indicates coefficient of variation for the entire data set.

PARAMETER	UNITS	RANGE	MEAN	COEFFICIENT OF VARIATION (CV')		
				ENTIRE	DATE	STATION DEPTH
Bacterial production (PD)	$\mu\text{gC} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$	74-426	205	45.6	38.1	44.5
Log bacterial abundance (LBA)	$\text{cells} \cdot \text{ml}^{-1}$	6.50-7.10	6.90	1.52	0.90	1.26*
Log flagellate abundance (LHMAN)	$\text{cells} \cdot \text{ml}^{-1}$	2.33-3.81	3.52	7.53	3.36*	4.89*
Log cyanobacteria abundance (LCY)	$\text{cells} \cdot \text{ml}^{-1}$	4.32-6.11	5.48	7.23	6.21*	4.62
Log diatom abundance (LDI)	$\text{cells} \cdot \text{ml}^{-1}$	2.82-3.98	3.49	9.17	4.42	7.67*
Log dinoflagellate abundance (LDN)	$\text{cells} \cdot \text{ml}^{-1}$	0.00-3.17	1.80	61.4	75.4	90.9*
Log microflagellate abundance (LUF)	$\text{cells} \cdot \text{ml}^{-1}$	2.80-3.82	3.32	8.80	6.68	8.40*
Total chlorophyll a (TCH)	$\mu\text{g} \cdot \text{l}^{-1}$	5.86-17.4	11.3	27.8	19.0	18.2
Less than $15\mu\text{m}$ chlorophyll (SCHL)	$\mu\text{g} \cdot \text{l}^{-1}$	4.35-14.2	8.30	29.9	19.7	26.7
Total phaeopigment ratio (PHA)	---	1.26-1.67	1.50	7.27	4.66	6.11
Less than $15\mu\text{m}$ phaeopigment ratio (SPHA)	---	1.28-1.68	1.49	5.84	2.94	3.96*
Ammonium concentration (NH4)	$\mu\text{mol} \cdot \text{l}^{-1}$	0.00-7.68	2.40	84.3	57.3	71.1*
Nitrite concentration (NO2)	$\mu\text{mol} \cdot \text{l}^{-1}$	0.18-3.80	1.09	84.8	36.0	77.8*
Nitrate concentration (NO3)	$\mu\text{mol} \cdot \text{l}^{-1}$	0.03-3.71	1.16	91.8	47.1	97.1*
Phosphate concentration (PO4)	$\mu\text{mol} \cdot \text{l}^{-1}$	0.48-3.02	1.47	41.7	27.6	37.5*
Silica concentration (SI)	$\mu\text{mol} \cdot \text{l}^{-1}$	17.1-57.2	37.6	30.4	11.1	32.6*
Particulate organic carbon (POC)	$\mu\text{g} \cdot \text{l}^{-1}$	326-5556	1465	61.8	39.7	54.6*
Particulate organic nitrogen (PON)	$\mu\text{g} \cdot \text{l}^{-1}$	82-424	189	34.6	11.8	37.0*
DOM-salinity residual (SR)	ppt	4.76-26.9	15.0	44.9	6.84*	50.8*
Dissolved oxygen (DO)	$\text{mg} \cdot \text{l}^{-1}$	5.36-8.46	6.83	11.9	12.3	6.72

* most variable parameter grouping of date, station and depth categories
 ** coded by addition of 15

Figure 7 Bacterial production and abundance by station and date for surface (a), pycnocline (b), bottom (c), and depth and hypsometrically integrated (d) water columns

□ Bacterial production
 (μg C · l⁻¹ · d⁻¹)
 ◆ Bacterial abundance
 (cells · ml⁻¹ × 2E⁻⁵)



c.

d.

Temporal trends in bacterial production varied depending upon the depth examined, and suggested mixing of surface and bottom waters. Production decreased with destratification in surface waters while increasing in pycnocline and bottom waters.

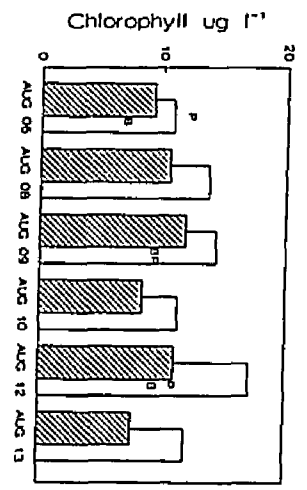
Means of bacterial parameters by station (Figure 7d) were calculated from depth integrated cross-sectional areas. Means calculated in this manner were considered more accurate than simply averaging values from surface, pycnocline and bottom waters; especially since volumes of water at these depths were markedly different. Means of bacterial abundance (Figure 7d) initially displayed a gradation from low values at station 1 to higher values up-estuary. By August 12th this pattern had reversed with low bacterial abundance observed at station 4 grading to higher abundance down-estuary.

Mean bacterial production (Figure 7d) was more variable than bacterial abundance. Temporal variability of production was greater at stations 1 and 2 which were characterized by deeper and initially more strongly stratified water columns as compared to stations 3 and 4. Rates of production decreased between August 6th and August 9th at stations 1 through 3, but remained unchanged at station 4.

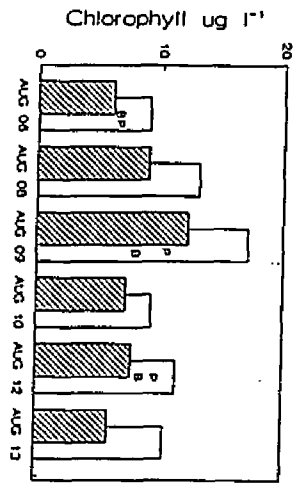
Total chlorophyll a and <15 μm chlorophyll a also displayed considerable spatial variation and generally increased at all stations between August 6th and August 12th (Figure 8). Total chlorophyll at station 3 doubled between August 6th and 9. Means of total phaeopigment ratio, <15 μm phaeopigment ratio, particulate organic carbon and C to N ratios of particulate organic matter (data not shown) all exhibited maximum values August 9th.

Figure 8 Total and $<15 \mu\text{m}$ chlorophyll *a* concentrations by station and date of surface water (bars), with markers indicating pycnocline (P) and bottom water (B) concentrations of total chlorophyll *a*

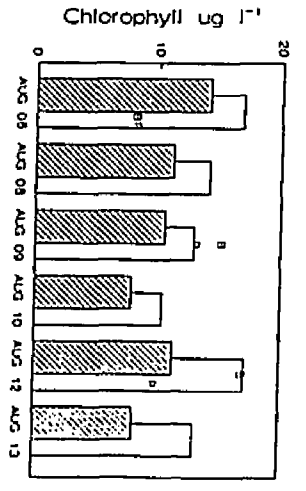
TEMPORAL PATTERNS OF SURFACE WATER CHLOROPHYLL BY STATION
 STATION 1



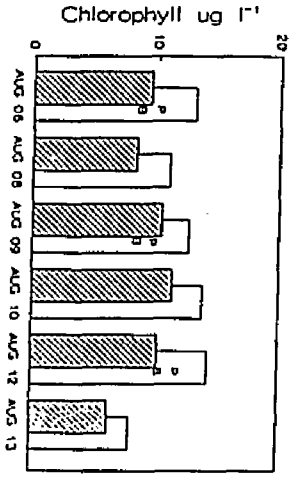
STATION 3



STATION 2

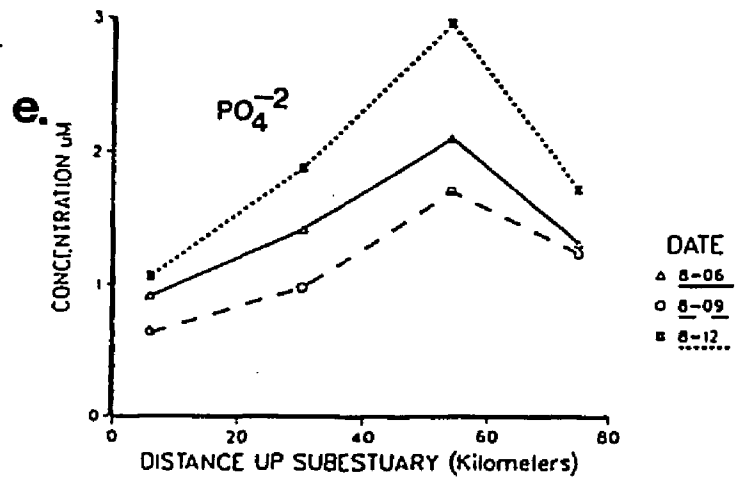
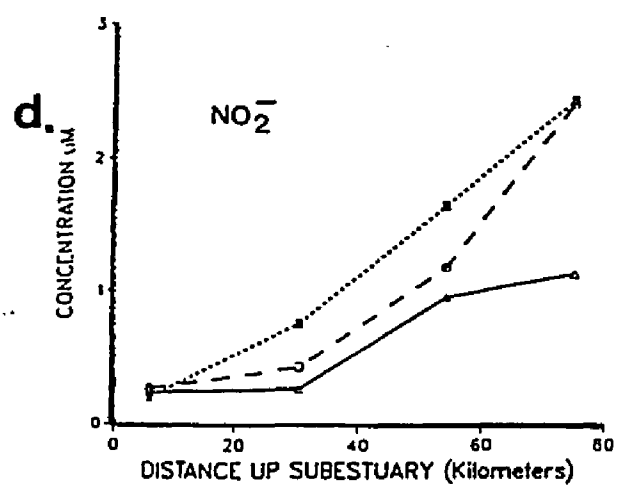
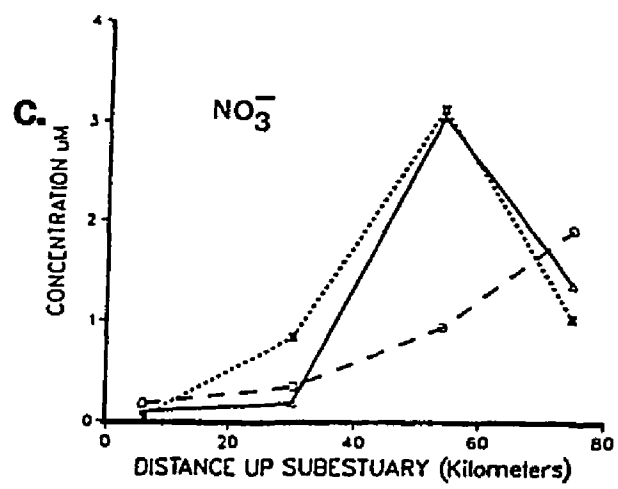
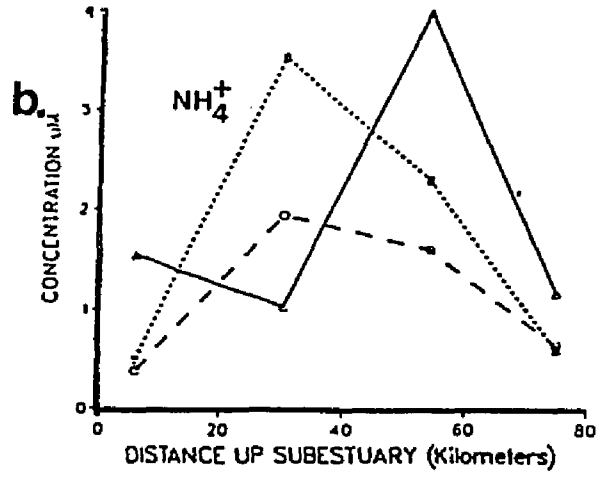
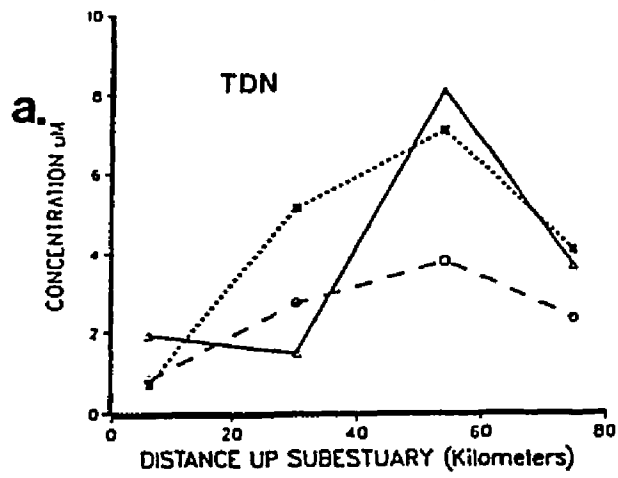


STATION 4



Total chlorophyll
 < 15 μm chlorophyll
 0.4 to 0.6 μm & below for particulate chlorophyll

Figure 9 Depth and hypsometrically integrated mean nutrient concentrations by station and date of total dissolved inorganic nitrogen (a.), ammonium (b.), nitrate (c.), nitrite (d.), and phosphate (e.)



DATE

- △ 8-06
- 8-09
- 8-12

Means of dissolved nutrients by station displayed strong spatial and temporal patterns (Figure 9). Concentrations of ammonium, nitrate, total dissolved nitrogen and phosphate all exhibited high values at station 3, and generally lowest values at station 1. Nitrite concentrations ranged from low values at station 1 to higher values up the estuary. Temporal changes in nitrogen nutrients varied by station and nutrient species.

Correlations of bacterial parameters upon environmental variables (Tables 9a-b) indicated a greater number of significant relationships occurred between bacterial abundance and environmental variables than between bacterial production and environmental variables. Cyanobacterial abundance, concentrations of ammonium and total dissolved inorganic nitrogen exhibited highly significant ($P < 0.005$) correlations with both bacterial parameters, when comparisons were made using the entire data set. Other parameters which demonstrated highly significant relationships with bacterial abundance included total chlorophyll a, $< 15 \mu\text{m}$ chlorophyll a, diatom and heterotrophic flagellate abundance, and concentrations of nitrate, nitrite, and phosphate.

Variables which displayed highly significant correlations with bacterial parameters based upon the entire data set generally demonstrated complex patterns of correlation when examined by spatial and temporal groupings. Variables not significantly correlated with bacterial parameters using the entire data set, generally failed to exhibit highly significant correlations when viewed by spatial or temporal groupings. Exceptions to this generalization were observed in certain comparisons of bacterial abundance versus phaeopigment ratio,

Table 9a. Significant Spearman Rank correlation coefficients between bacterial abundance and other variables. Variables include salinity (SA), temperature (TM), and others as in Table 8.

VARIABLE	DATA GROUPING									
	ENTIRE		DATE		DEPTH		STATION			
	Aug06	Aug09	Aug12	Surface	Pycnocline	Bottom	1	2	3	4
PD	.45 ^a	--	--	.40 ^c	--	--	.73 ^a	--	--	--
CHL	.54 ^a	--	.79 ^a	--	.56 ^c	--	.77 ^a	.57 ^c	--	--
SCHL	.56 ^a	.59 ^c	.58 ^c	--	.58 ^c	.63 ^c	.81 ^a	--	.61 ^c	--
PHA	--	--	--	--	--	--	.69 ^b	--	--	--
SPHA	.33 ^c	--	--	--	--	--	.75 ^a	--	--	--
LCY	.60 ^a	.81 ^a	.67 ^b	.60 ^a	--	.60 ^c	.85 ^a	.64 ^c	--	--
LUF	--	--	--	--	--	--	--	--	.80 ^a	--
LDN	.26 ^c	--	--	--	--	--	--	.62 ^c	--	--
LDI	.51 ^a	.75 ^a	--	-.45 ^c	--	--	--	.54 ^c	--	--
LHWAN	.43 ^a	.62 ^c	.53 ^c	.51 ^a	--	--	.78 ^a	--	--	--
NH4	-.46 ^a	--	--	-.36 ^c	--	--	--	-.61 ^c	--	-.57 ^c
NO2	-.47 ^a	--	-.56 ^c	-.74 ^a	--	--	-.88 ^a	--	--	--
NO3	-.47 ^a	-.64 ^c	-.63 ^c	-.65 ^a	-.56 ^c	--	-.73 ^a	--	--	--
SI	--	--	-.57 ^c	-.41 ^c	--	--	-.65 ^c	--	--	--
PO4	-.50 ^a	-.75 ^a	--	-.52 ^b	--	--	--	.54 ^c	--	-.67 ^c
POC	.29 ^c	--	.59 ^c	.37 ^c	.53 ^c	--	--	--	--	--
PON	.30 ^c	.57 ^c	.50 ^c	.46 ^c	--	--	--	--	--	--
SR	--	--	-.43 ^c	--	--	--	--	--	--	--
SA	--	--	--	.42 ^c	--	--	.80 ^a	.81 ^a	.55 ^c	--
TM	--	--	--	-.54 ^a	--	--	.90 ^a	--	-.56 ^c	--
O2	.26 ^c	--	--	--	--	--	.88 ^a	.51 ^c	--	--

Superscript denotes significance value of (a) ≤ 0.005 , (b) $\leq 0.01 > 0.005$, and (c) $\leq 0.05 > 0.01$.

Table 9b. Significant Spearman Rank correlation coefficients between bacterial production and other variables. Variables include salinity (SA), temperature (TM), and others as in Table 8.

VARIABLE	DATA GROUPING									
	ENTIRE		DATE		DEPTH		STATION			
	Aug06	Aug09	Aug12	Surface	Pycnocline	Bottom	1	2	3	4
LBA	.45 ^a	--	--	.40 ^c	--	--	.73 ^a	--	--	--
CHL	.29 ^c	--	--	--	--	--	.57 ^c	--	--	--
SCHL	.38 ^b	--	--	--	--	--	.63 ^c	--	.70 ^b	--
PHA	.28 ^c	--	--	--	--	--	.68 ^b	--	.72 ^b	--
SPHA	.34 ^c	.58 ^c	--	--	--	.65 ^c	--	--	--	.54 ^c
LCY	.45 ^a	--	--	--	.78 ^a	--	.75 ^a	--	--	--
LUF	--	--	--	--	--	--	--	--	.63 ^c	--
LDN	.28 ^c	.64 ^c	--	--	--	.65 ^c	--	--	.92 ^a	--
LDI	.36 ^b	--	--	--	--	--	--	--	.83 ^a	--
LHMAN	.25 ^c	--	--	--	.66 ^c	--	.69 ^b	--	--	.17 ^c
NH4	-.52 ^a	--	-.51 ^c	--	--	-.73 ^a	-.56 ^c	--	--	--
NO2	--	--	--	-.44 ^c	--	--	--	-.52 ^c	--	--
NO3	-.26 ^c	--	--	--	-.61 ^c	--	--	-.50 ^c	--	--
SI	--	--	--	--	--	--	.56 ^c	--	--	.25 ^c
P04	-.35 ^c	--	--	--	--	-.56 ^c	--	--	--	--
POC	--	--	--	--	--	--	--	--	--	--
PON	--	--	--	--	--	--	--	--	--	--
SR	--	--	--	--	-.68 ^c	--	--	--	.76 ^a	--
SA	--	--	--	--	--	--	-.63 ^c	--	-.83 ^a	--
TM	--	--	--	--	--	--	.54 ^c	--	.57 ^c	--
O2	.24 ^c	--	--	--	--	.54 ^c	--	--	--	--

Superscript denotes significance value of (a) ≤ 0.005 , (b) ≤ 0.01 > 0.005, and (c) ≤ 0.05 > 0.01.

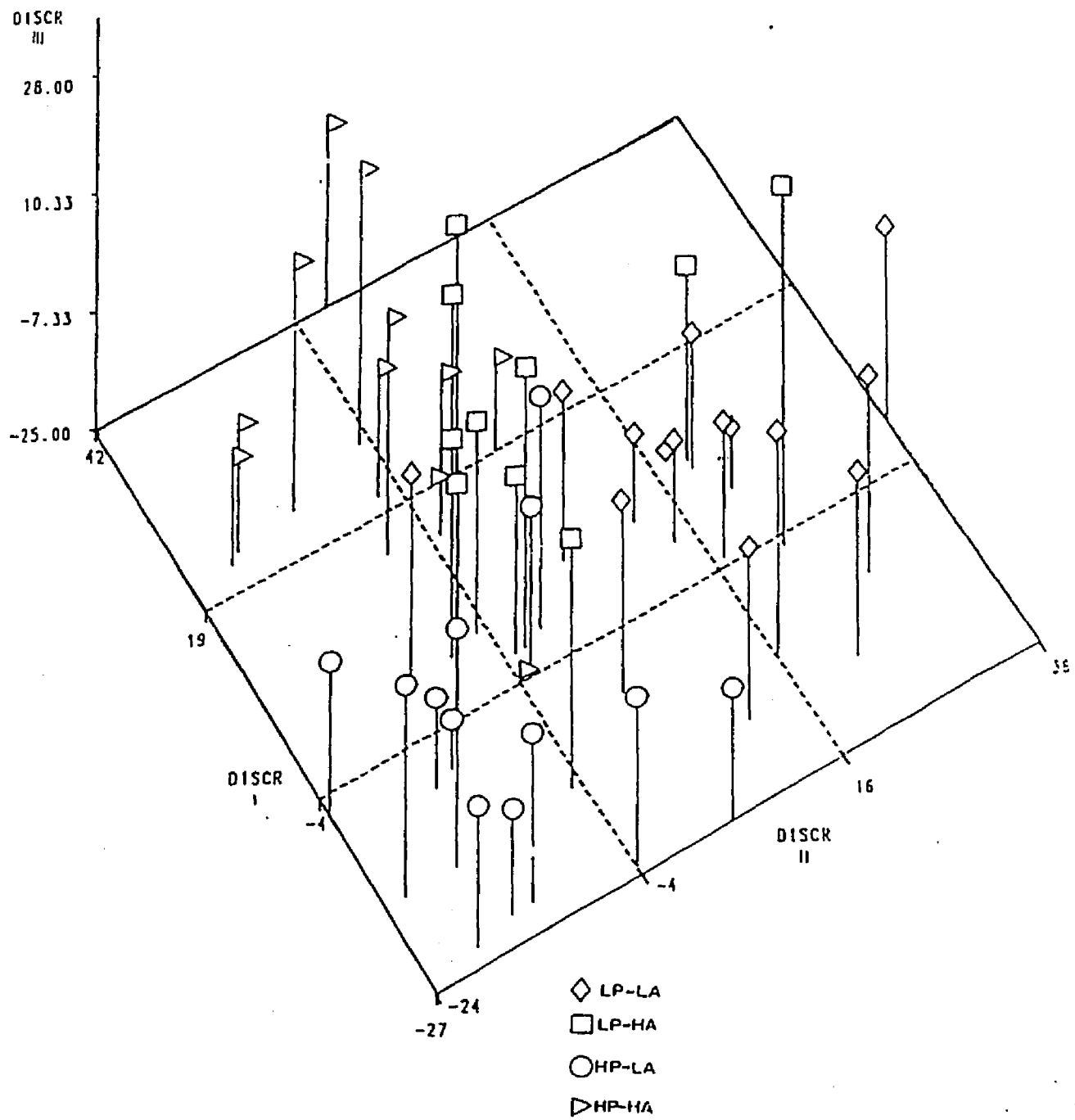
microflagellate abundance, salinity residuals (dissolved organic matter), salinity and temperature.

Multiple discriminant analysis (MDA) of the data yielded separation of combination abundance-production groups as shown in Figure 10. Table 11 presents information on the percent variance described by the discriminant functions, as well as standardized discriminant function coefficients, and correlation coefficients. Discriminant function I (dfI), which tended to effectively separate groups based upon abundance, accounted for 52% of sample variance but was weakly loaded (low values of correlation coefficients) with many variables. The most important variables on this axis were measures of phaeopigment ratio and dissolved inorganic nutrient concentration. Discriminant function II (dfII) tended to separate groups based upon production characteristics. This axis accounted for 36% of sample variance and was more highly loaded by a few variables including dinoflagellate abundance, ammonium, and measures of chlorophyll *a*.

Table 10. Multiple discriminant analysis of bacterial abundance-production groupings employing a direct analysis procedure with a tolerance of 0.01. Results include percent variance described by discriminant functions, correlations between variables and discriminant function (α), and standardized correlation coefficients (β).

DISCRIMINANT FUNCTION	I		II		III	
% VARIANCE	51.5		35.8		12.7	
VARIABLE	α	β	α	β	α	β
NO2	-.27	-.20	-.04	-.46	-.30	.63
BA	.26	1.01	.22	-.23	-.22	-.66
SBA	.26	-.07	-.25	-.13	.08	.25
LDI	.25	.67	-.23	-.38	.24	-.46
PO4	-.25	-.48	.35	.72	-.37	-.53
NO3	-.23	-.22	.18	.12	-.21	.23
LCY	.22	.22	-.33	.79	.11	.07
NH3	-.22	-.30	.50	.09	-.04	.38
SCHL	.14	-.77	-.51	-.62	.35	-.47
CHL	.13	1.03	-.37	.80	.39	.93
SA	.12	8.05	.14	11.4	.09	8.33
SR	-.11	7.57	-.12	12.3	-.06	8.43
TM	-.08	.72	-.19	-.36	.04	.80
LDN	-.06	-1.11	-.64	-.83	.32	.24
LUF	-.06	-.86	.04	.49	.27	.52
LHNAN	-.03	-.39	-.01	-.32	.09	-.17

Figure 10 Multiple Discriminant Analysis separation of samples within bacterial production-abundance groupings. Groups include; (\diamond) low production-low abundance (LP-LA, $<190\mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, $<7.32\times 10^6$ cells $\cdot\text{ml}^{-1}$), (\square) low production-high abundance (LP-HA, $<190\mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, $>7.32\times 10^6$ cells $\cdot\text{ml}^{-1}$), (\circ) high production-low abundance (HP-LA, $>190\mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, $<8.51\times 10^6$ cells $\cdot\text{ml}^{-1}$), (\triangleright) high production-high abundance (HP-HA, $>190\mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, $>8.51\times 10^6$ cells $\cdot\text{ml}^{-1}$)



DISCUSSION

Bacterioplankton Distribution and Production

Bacterial abundance and production rates observed in this study were similar to values reported from several other studies conducted within estuaries. Bacterial abundance ranged from $0.31 - 1.26 \times 10^7$ cells·ml⁻¹, similar to values reported by Malone et al. (1986) for a study site in the upper Chesapeake Bay, Maryland, and slightly higher than the range of values reported by Ducklow (1982) for the York River ($0.1 - 0.8 \times 10^7$ cells·ml⁻¹). Bacterial production ranged from $73.8 - 426 \mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$, which is similar to values reported by Malone et al. (1986), and somewhat higher than values reported for the Delaware Bay (Wright and Coffin, 1984; Coffin and Sharp, 1987).

The distribution of bacterial abundance in surface waters (figure 7a) generally revealed higher values in the lower estuary. Similar patterns have been observed in the Delaware estuary (Coffin and Sharp, 1987) and in the Rhode River estuary, Maryland (Ruble et al. 1984). In other estuarine systems, bacterial abundance maxima have been observed in mid-estuary (Wright and Coffin, 1983a) or further up-estuary (Palumbo and Ferguson, 1978; Bell and Albright, 1981; Albright, 1983). The location of bacterial abundance maxima within estuarine systems is thought to be a function of substrate availability (Coffin and Sharp, 1987). In the present study, patterns of correlation between bacterial abundance and relative concentrations of DOM as indicated by salinity residuals suggested that bacterial abundance was enhanced in the DOM rich surface waters of stations in the lower estuary. Since chlorophyll

a concentrations tended to be greater in the lower estuary, it is likely that autochthonous DOM from photosynthesis was an important source of substrate for bacterial production at this location.

Although pycnocline and bottom waters (Figures 7b-c) exhibited less distinctive longitudinal gradients than surface waters, there was a strong gradient in bacterial abundance over the vertical plane with values significantly higher in surface waters than in bottom waters (one tailed t-test, $P \leq 0.0005$). Bacteria abundance maxima in surface waters have also been observed by Malone et al. (1986) in the upper Chesapeake Bay, and by Ducklow (1982) in the York River, Virginia. Data from the present study indicating bacterial abundance, measures of chlorophyll and phaeopigment ratios were most variable over depth (Table 8), provide additional indications of close relationships between bacteria and autotrophic communities within euphotic waters of the Chesapeake Bay and its subestuaries.

Temporal changes in bacterioplankton abundance displayed different patterns in the lower estuary versus the upper estuary. Abundance increased at all depths between August 6th and 12th at stations 1 and 2 whereas abundance generally decreased at stations 3 and 4. Increases in depth integrated abundances at stations 1 and 2 were 41% and 7%, respectively, of initial values on August 6th. Increases in bacterial abundance at stations 1 and 2 were significantly less than the doubling of bacterial abundance observed by Ducklow (1982) during destratification in the York River.

Distribution patterns of bacterial production (Figures 7a-c) revealed several trends. Although surface water production rates

exhibited a pattern similar to bacterial abundance (increasing down-estuary) production data were more variable. Pycnocline waters exhibited a similar pattern, yet production rates were less variable, and clearly increased down-estuary. Bottom waters displayed no clear trends and were highly variable. As observed for bacterial abundance, bacterial production tended to decrease with depth, from a mean of $245 \mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ in surface waters to $165 \mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ for bottom waters. However, this difference was not as highly significant (one tailed t-test, $P=0.033$) as that observed for differences in surface and bottom water bacterioplankton abundance. Temporal patterns of bacterial production indicated that destratification resulted in decreased integrated production rates. This decrease was due to a marked depression of surface water rates, as subsurface water rates increased with destratification.

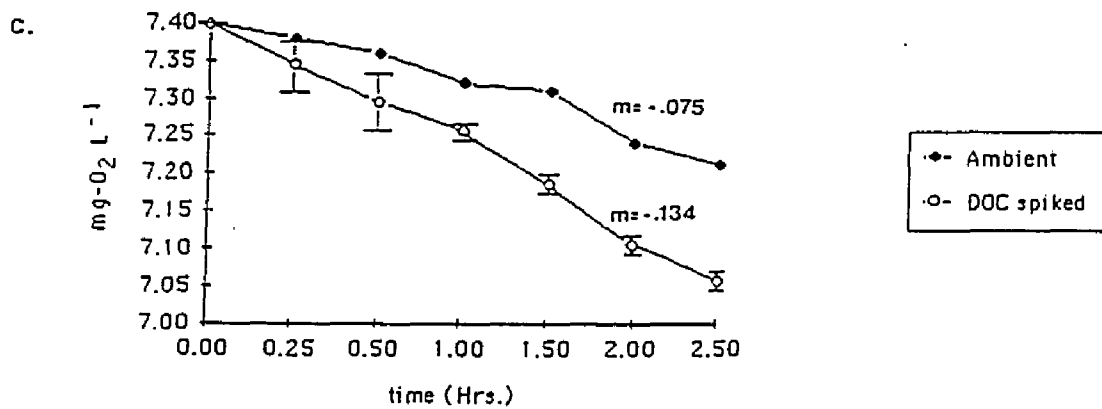
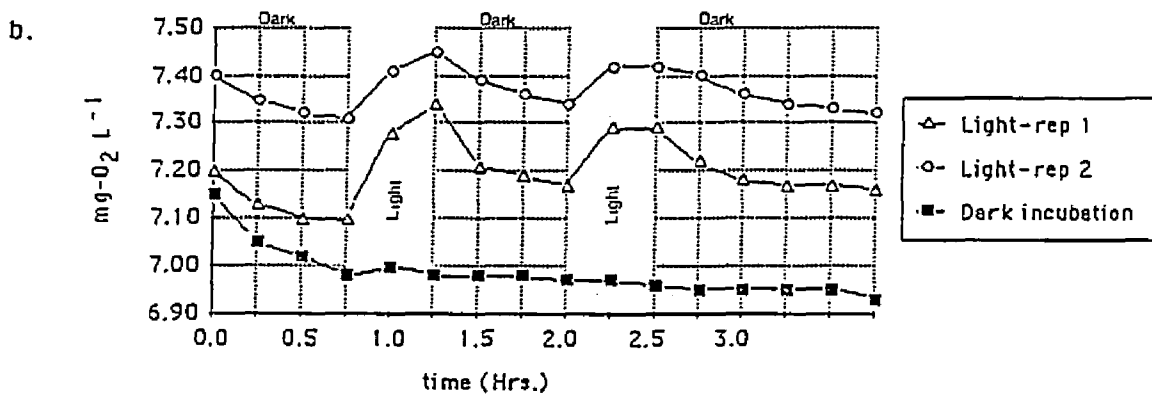
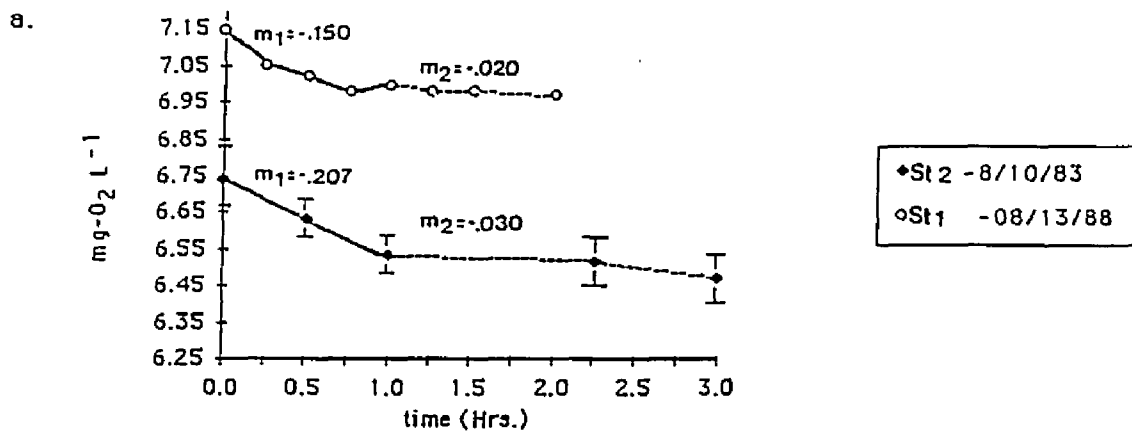
Comparison of CVs of all plankton components indicated that variability of heterotrophic bacterioplankton was considerably less than that of other autotrophic and heterotrophic groups. Although bacterial abundance and production were highly correlated based upon the entire data set, bacterial abundance varied by a factor of four whereas bacterial production varied by a factor of seven. Similar findings concerning the relative invariable nature of bacterial densities have been discussed by Larsson and Hagstrom (1982), Wright and Coffin (1983b), Ducklow (1983), Malone et al. (1986) and Gocke et al. (1987). Given the known potential for rapid bacterial growth in coastal and estuarine systems (Kirchman et al., 1982; Wright and Coffin 1983b), very close temporal controls through top-down (grazing) and bottom-up

(substrate) influences apparently confine bacterial abundance to narrow bounds in the natural environment.

Trophic Relationships

Oxygen metabolism experiments conducted during the study (Figures 11a-c) provided information regarding the nature and mechanism of substrate utilization by heterotrophic bacteria. Continuous dark incubations of surface water samples taken during the day (Figure 11a) displayed biphasic respiration curves, with slopes indicating a metabolic down-shift after incubation periods of one hour. These data can be interpreted either as a switching in bacterial substrate utilization from a rapidly utilized labile substrate to one more recalcitrant in nature, or secondly, gradually reduced metabolism due to the exhaustion of a single substrate. Light pulsing of samples (Figure 11b) resulted in the reestablishment of the steeper slope characteristic of the initial incubation period suggesting that phytoplankton was the source of a labile substrate. Incubation of a sample taken from station 1 in the early evening (Figure 11c) did not result in a biphasic respiration curve, however amendment of this sample with $10 \text{ mg} \cdot \text{l}^{-1}$ of glucose resulted in significantly higher respiration rates suggesting substrate limitation. Taken together this information suggested that heterotrophic bacterial metabolism in surface waters of the James River was tightly coupled to production of photosynthetically derived dissolved organic carbon and that in certain cases bacterial production may have been limited by lack of substrate. Lack of correlations between bacterial production and POC or PON provided additional evidence

Figure 11 Results of oxygen metabolism experiments; biphasic metabolic slopes of surface water from stations 2 and 3 (a.), light pulsing of surface water from station 3 (b.), glucose amendment of a surface water sample taken during the evening from station 3 (c.)



that the major metabolic substrates were dissolved. Photosynthetically derived dissolved organic substrates would be in much lower concentrations in pycnocline and bottom waters during stratified conditions, but would be mixed into deeper waters during destratification. Mixing of substrate may partially explain the observed increase in bacterial production rates in pycnocline and bottom waters as destratification progressed in the estuary.

Comparison of bacterial production based upon oxygen metabolism (assuming all metabolism was due to heterotrophic bacteria, and using the highest rate of $207 \mu\text{g O}_2 \cdot \text{l}^{-1} \cdot \text{h}^{-1}$) with that determined using thymidine incorporation (assuming a 33% growth efficiency, and using the highest rate of $426 \mu\text{g C} \cdot \text{l}^{-1} \cdot \text{d}^{-1}$) indicated a thymidine incorporation value only 20% of the value determined for oxygen metabolism. Such a discrepancy in rates may arise for several reasons; 1) bacterial growth efficiency was much lower than the assumed 33%, 2) a large proportion of metabolism was due to non-bacterial components, or 3) conversion values used in estimates of bacterial production were too conservative.

Correlations between heterotrophic bacterial abundance and autotrophic community parameters revealed highly significant ($p < 0.005$) positive relationships to cyanobacteria and diatom abundance as well as to both measures of chlorophyll (Table 9a). These data augment the numerous studies reporting positive correlations between bacterial abundance and chlorophyll (Fuhrman et al., 1980; Griffiths et al., 1982; Linley et al., 1983; Lancelot and Billen, 1984; Bird and Kalff, 1984; Laanbroek et al., 1985; Fuhrman et al., 1985; and Malone et al., 1986). The overall regression relationship between direct counts (DC) of

heterotrophic bacteria and total chlorophyll a (TCH) found in this study was; $\log DC = 6.45 + 0.43 \log TCH$ ($n=47, r=0.51$). This relationship was remarkably similar to that observed by Malone et al. (1986) for stratified water at a series of stations transecting the normal axis of the upper Chesapeake Bay ($\log DC = 6.52 + 0.45 TCH$ ($n=160 r=0.91$)). Both regression relationships differ markedly from regressions derived from literature reviews by Bird and Kalff (1984) and Cole et al. (1988), and display bacterial abundance values one-half log unit greater at the zero chlorophyll a intercept and lower slope values. In fact, abundance values observed by Malone et al. (1986) and in the present study are among the highest reported for aquatic environments. Ducklow and Peele (1987) found that during certain seasons bacterial biomass may exceed phytoplankton biomass by a factor of three. In the present study bacterial biomass was at most 33% of autotrophic biomass and averaged 20%, however bacterial production averaged 80% of areal primary production (data not shown). The existence of high bacterial biomass and production rates in the Chesapeake Bay and subestuaries may be due to high inputs of allochthonous carbon and nutrients and/or more efficient energy transfers and nutrient recycling between autotrophic and heterotrophic components in the microbial loop.

Correlative relationships between bacteria and chlorophyll a at stations in the lower estuary were stronger than in the upper estuary. This feature may have been partially due to deeper water column depths at lower estuary stations, promoting a greater range of parameter values between surface and bottom waters. Similar relationships observed between bacterial abundance and salinity residuals (DOM) suggested

greater vertical gradients in DOM and bacterial abundances at lower estuary stations. Although an association between bacterioplankton abundance and chlorophyll *a* concentration in the euphotic zone was anticipated it was not observed. Relationships between bacterial abundance and production with chlorophyll *a* in the euphotic zone may be obscured by grazing activities of flagellates, which were highly correlated ($P < 0.001$) with bacterial abundance in surface waters. In pycnocline and bottom waters, where no relationships between bacteria and flagellates were found, weak relationships ($0.05 \geq P \geq 0.01$) to total and $< 15 \mu\text{m}$ chlorophyll *a* were observed.

Relationships of $< 15 \mu\text{m}$ chlorophyll *a* and cyanobacterial abundance with bacterial abundance suggested that heterotrophic bacteria were more closely coupled to smaller autotrophic assemblages. Correlation of bacterial production with these parameters supported this premise. Multiple discriminant analysis indicated that loadings of $< 15 \mu\text{m}$ chlorophyll *a* and cyanobacteria on *dfI* and *dfII* (Table 10) were higher than that indicated for total chlorophyll *a*. $< 15 \mu\text{m}$ chlorophyll *a* was highly loaded on *dfII* which discriminated between bacterial production groupings. Such findings would be reasonable if these smaller forms comprised a major portion of the biomass or were important in the production of the autotrophic community. In this study $< 15 \mu\text{m}$ chlorophyll averaged 75% of total chlorophyll. Similar proportions (88%) of $< 15 \mu\text{m}$ chlorophyll *a* have been observed in the York River during the fall by Ray et al. (1988). The overall relationship between cyanobacterial and heterotrophic bacterial abundance were strong despite a minimal contribution of cyanobacteria to total autotrophic biomass

(mean of 6% using $115 \text{ fg C}\cdot\text{cell}^{-1}$, Ray et al. 1988). Overall correlations between heterotrophic bacterial parameters and cyanobacterial abundance were stronger than heterotrophic bacterial correlations with other autotrophic parameters. Additionally, the incidence of highly significant relationships based upon spatial and temporal groupings were more frequent between heterotrophic bacteria and cyanobacteria than with other autotrophic parameters. Recent work by Hagstrom et al. (1988) indicates that grazing removal of cyanobacterial production and subsequent addition to the DOM pool via sloppy feeding may provide an indirect yet strong coupling between cyanobacteria and heterotrophic bacteria. Such an energy flow may have been important in the present study given the high abundances of cyanobacteria observed (mean = $3.02 \times 10^5 \text{ cells}\cdot\text{ml}^{-1}$), and information suggesting high grazing rates upon cyanobacteria in the nearby York River (Ray et al. 1988). It was interesting that although relationships between bacterial abundance and chlorophyll diminished during destratification, new highly significant relationships appeared with cyanobacteria and diatoms. These results suggest that heterotrophic bacteria may be responsive to activities of specific constituents within the autotrophic community, perhaps reflecting changes in the dominant sources or pathways of DOM substrates.

Multiple discriminant and correlative analysis also indicated an importance of the phaeopigment ratio. Phaeopigment ratios indicate the relative proportions of chlorophyll degradation products (such as phaeophytin) which have been suggested to result from herbivory (Welschmeyer et al. 1985; Litaker et al. 1988). Total phaeopigment

ratio and $<15 \mu\text{m}$ phaeopigment ratio were found to be highly loaded on dfl, which discriminated between low and high abundance groupings. Highly significant positive correlations between bacterial parameters and phaeopigment ratios were observed on August 6th, prior to destratification. The role of herbivory by heterotrophic nano and microplankton, as a vector for DOM substrates utilized by bacteria has only recently been addressed (see Hagstrom et al. 1988; and Jumars et al. 1989), and there is evidence that this pathway may be more important during stratified conditions (McManus and Peterson 1988). Although oxygen metabolism experiments conducted during this study suggested that the major bacterial substrates was photosynthetically released DOM, DOM produced via herbivory may be of secondary importance.

Heterotrophic flagellates were considered important in the control of bacterioplankton during the course of the study. Highest flagellate abundances were generally associated with regions of high bacterial abundance and production (surface water, down-estuary stations), and flagellate abundance displayed a highly significant positive correlation with bacterial abundance based upon the entire data set. Correlations between bacterial and flagellate abundances based upon temporal and spatial groupings revealed one highly significant correlation in surface waters. The existence of such a strong correlation was somewhat unexpected, given the generally observed time-lagged relationships between these groups (Fenchel, 1982; Azam et al., 1983; Anderson and Sorenson, 1986; Bjornsen et al., 1988). Sibbald et al. (1987) have shown that flagellates display positive chemotaxis towards amino acids and ammonium. Flagellate-bacterial interactions may be more pronounced

in surface waters for several reasons. First, release of photosynthetically produced dissolved organic matter and the presence of phycospheres with locally high abundances of bacteria around individual phytoplankton cells (sensu; Azam and Ammerman, 1984) would be more likely in surface waters; secondly, release of dissolved organics and amino acids during flagellate grazing activities upon such consortia (Andersson et al., 1985) may promote greater bacterial production and a positive feedback response in flagellate populations; and finally, the probability of encounter of bacteria by flagellates based upon abundances was significantly ($P \leq 0.001$) greater in surface waters (log ratio BA/HNAN=3.38) than in bottom waters (log ratio BA/HNAN=3.31).

Direct measurement of flagellate grazing rates upon bacteria were not obtained in this study, however maximum theoretical rates were estimated from bacterial abundance and cellular production data (Table 11). Calculations assumed the absence of bacterial cell emigration, and non-predatory mortality. Grazing rate values for surface waters ranged from $0.71 - 1.76 \times 10^7 \text{ cells} \cdot \text{ml}^{-1} \cdot \text{d}^{-1}$ (mean = $1.17 \times 10^7 \cdot \text{ml}^{-1} \cdot \text{d}^{-1}$), which are within the range of values reported for estuarine and coastal environments (Fenchel, 1982; Coffin and Sharp, 1987). Conversion of grazing rates to % loss of daily bacterial production revealed that from 85 to 132 % of daily bacterial production was consumed through grazing. A SAS general linear model analysis indicated that total grazing rate (TGR) was significantly different among stations ($P=0.028$), with the difference confined to end-member stations 1 and 4. Although this study is limited in terms of grazing estimates ($n=20$), results appear similar to those obtained by Coffin and Sharp (1987) for the Delaware estuary,

Table 11. Grazing rate calculations in surface waters for stations over sampling intervals. Variables include; TGR (total grazing rate; $\text{cells}\cdot\text{ml}^{-1}\cdot\text{d}^{-1} \times 10^7$) = the difference in bacterial abundance plus mean cellular production divided by time, SGR (flagellate specific grazing rate; $\text{cells}\cdot\text{flagellate}^{-1}\cdot\text{d}^{-1} \times 10^3$) = TGR divided by mean flagellate abundance, and PPG (percent of daily bacterial production grazed; %) = TGR divided by mean cellular production.

INTERVAL	STATION	TGR	SGR	PPG
AUG06-08	1	1.68	2.92	104
	2	1.59	3.19	99
	3	1.44	3.20	117
	4	1.05	3.87	108
AUG08-09	1	1.00	1.79	90
	2	0.98	1.80	98
	3	0.84	2.08	87
	4	1.15	3.00	117
AUG09-10	1	1.19	2.90	107
	2	1.52	5.26	105
	3	0.78	2.26	97
	4	0.93	2.61	89
AUG10-12	1	1.54	2.83	93
	2	1.21	5.96	98
	3	1.01	3.16	97
	4	0.88	3.03	103
AUG12-13	1	1.76	3.49	124
	2	1.01	2.36	132
	3	1.07	3.45	108
	4	0.71	3.67	85

in that both bacterial production and grazing rates were highest in the lower estuary. Temporal patterns indicated depression of total (TGR) and specific grazing rates (SGR) during the onset of destratification. TGR for the interval of August 8-9 was significantly lower ($P \leq 0.05$) compared to the first interval. SGR for the interval August 8-9 was significantly lower than all remaining interval values. This finding agrees with the recent observation of McManus and Peterson (1988) that similar disruptions of flagellate grazing activity occur in the coastal zone off Chile during destratification events.

Bacterial-Nutrient Dynamics

Associations between bacterial abundance and dissolved nitrogen and phosphorus nutrients based upon the entire data set were all negative and highly significant. Rublee et al. (1984) found similar relationships with ammonium and nitrate in the Rhode River estuary, but the relationship with phosphorus was positive. Correlations of these nutrients with bacterial abundance were all more highly significant than analogous correlations to total chlorophyll a, suggesting that the relationship between bacteria and nutrients was not due to coupling with autotrophic biomass.

Multiple discriminant analysis (Figure 10, Table 11) indicated that nitrite concentration was the most important discriminatory variable with regards to bacterial abundance, while ammonium concentration appeared important in discrimination of production groups. Phosphate displayed moderately high loadings on both dfI and dfII and therefore contributed somewhat to separation of all groups. A basic

question with regard to these data is why relationships between nutrients and bacterial abundance should differ from relationships between nutrients and bacterial production. It is possible that various nutrients differentially affect other members of the microbial loop (i.e. grazers) whose interactions with bacteria are important in determining bacterial abundance. For example flagellates displayed highly significant correlations ($P < 0.001$, data not shown) to nitrite concentrations based upon the entire data set and many groupings, and therefore discrimination of bacterial abundance groups by nitrite may be indirectly related to flagellate activity.

Temporal changes in nutrient concentrations revealed patterns similar to those observed by Webb and D'Elia (1980) during a spring-neap tidal cycle in the York River, Virginia. Mean water column concentrations of ammonium, total dissolved inorganic nitrogen and phosphate grouped over stations displayed concentration minimums coinciding with destratification on August 9th (Figures 9a-e). After restratification phosphate concentrations exhibited maximum values greater than observed prior to destratification. The mechanism of this phosphate remineralization is unknown. The most marked temporal change in DIN nutrients occurred at station 3, where integrated concentrations of total DIN, ammonium, and nitrate all decreased 60-70% between August 6th and 9th. This phenomenon coincided with a rapid increase in surface chlorophyll a concentration and therefore may have been due to nutrient uptake associated with autotrophic growth. However, some fraction of ammonium may have served as a substrate for nitrification since nitrate concentrations at upper estuary stations continued to increase over the

duration of the study.

Temporal changes in correlations between heterotrophic bacteria and dissolved nutrients were apparent with regard to various nutrient species. On August 6th bacteria were highly correlated with ammonium, yet by August 9th this relationship had disappeared and was replaced instead by a strong correlation to phosphate and weaker correlations to oxidized nitrogen nutrients. A similar pattern was observed for both total and $<15 \mu\text{m}$ chlorophyll *a*. Autotrophic ammonium uptake has been shown to suppress enzymes responsible for nitrate assimilation (Carpenter and Dunham 1985), therefore, the importance of oxidized nitrogen forms for autotrophic assimilation probably increase as ammonium concentrations decline. The change in correlation pattern coincident with destratification may be the result of increased preference for nitrate and nitrite during the period of diminished ammonium pools. Price et al. (1985) and more recently Harrison and Wood (1988) have demonstrated that uptake rates and preference for particulate nitrogen nutrient species by autotrophs may be dramatically affected by the hydrography (stratified versus frontal) of the system, however similar information on heterotrophic bacteria is lacking. In fact, very little is known about modes and patterns of nitrogen uptake by bacteria in natural systems. Billen (1984) concluded that bacteria primarily utilize amino acids as their nitrogen source, and phytoplankton utilize ammonium, nitrate and urea. However, recent work by Wheeler and Kirchman (1986), and by the author working in the York River (unpublished data), have shown that in the euphotic zone significant amounts of ammonium uptake into particulate material were

due to assimilation by heterotrophic bacteria.

Patterns of nutrient correlations in the vertical plane indicated a weakening in the relationship between bacterial abundance and nutrients with depth in the water column. Strong negative correlations with oxidized forms of nitrogen and weaker relationships with ammonia and phosphate were found in the surface waters. Pycnocline waters exhibited only weak correlations between oxidized nitrogen forms and bacterial abundance. Bottom waters yielded no significant correlations between nutrients and bacterial abundance. It is highly probable that nutrient concentrations under the pycnocline are greatly influenced by benthic regeneration and consumption processes, and these may obscure relationships which may exist with planktonic components in bottom waters. Correlations of nutrients to bacterial abundances based upon stations displayed a pattern similar to that seen for cyanobacteria and chlorophyll *a*, with strong correlations to all nitrogen nutrient species at station 1 with generally decreasing nutrient association progressing upriver.

Hydrographic and Physical Factor Influences

Investigations conducted in oceanic and coastal environments have generally shown heterotrophic bacterial production in the euphotic zone of stratified waters to exceed that observed under mixed conditions (Liney et al., 1983; Turley and Lochte, 1985; Hanson et al., 1988; McManus and Peterson, 1988; Kuosa and Kivi, 1989). Malone et al. (1986), working in the upper Chesapeake Bay, observed a similar trend with bacterial production ranging from 52-680 mg C·m⁻²·d⁻¹ during mixed

conditions, versus $250-1000 \text{ mg C}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ under stratified hydrography. Results of the present study indicate that the same pattern occurred in the James River, a subestuary of the Chesapeake Bay. Integrated bacterial production grouped over stations decreased significantly ($P=0.015$, SAS-General linear model) from $324 \mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ on August 6th to $187 \mu\text{g C}\cdot\text{l}^{-1}\cdot\text{d}^{-1}$ on August 9th, the date of greatest mixing.

Mechanisms proposed to account for reduction of bacterial production with mixing suggest an uncoupling between bacterioplankton and substrate supplied from euphotic phytoplankton components (Malone et al., 1986; McManus and Peterson, 1988). Such a model would be consistent with observations of depressed primary productivity under light limited conditions of destratification (see review by Legendre (1980)). Malone et al. (1986) found that rates of primary production in the upper Bay were lower under mixed conditions. In the present study, although only a limited number of ^{14}C primary production estimates were obtained (data not shown), the lowest values for rates (observed on August 9th) coincided with maximum destratification. Further indications of trophic uncoupling were evident from correlation analysis (Tables 9a-b). Correlation coefficients between bacterial abundance and both measures of chlorophyll were more highly significant on August 6th as compared to August 9th. Relationships between bacterial production and chlorophyll displayed a similar pattern. By August 12th when the stations had begun to restratify more highly significant correlations reappeared between chlorophyll a and bacterial parameters. Malone et al. (1986) also found that correlation coefficients between direct counts of bacteria and chlorophyll a were higher during stratified hydrography as

compared to the mixed period.

The tendency for increases in chlorophyll a in pycnocline and bottom waters combined with a depression in surface water chlorophyll a values at stations 1-3 on August 10th suggested mixing of surface water chlorophyll throughout the water column. Although this would reduce bacterial substrate in the surface waters, increased chlorophyll in euphotic waters would likely increase substrate to those bacterial populations. Such substrate relocation may explain patterns of change in bacterial production. Interpretations of the influence of destratification upon dynamic relationships between heterotrophic bacteria and cyanobacteria are mostly speculative. It has been well documented that Synechococcus sp. (the numerically dominant autotrophs in this study) contribute proportionately more towards total primary production under low light conditions (Morris and Glover 1981). It is possible that cyanobacterial production assumed a greater proportion of total primary production as destratification progressed in the James River. Increased turbidity associated with destratification would be likely to enhance this tendency. Alternatively the appearance of increasing abundances of cyanobacteria in the pycnocline and bottom waters may simply be due to the advection from the surface waters. Regardless of the mechanism bacterial production in pycnocline waters was markedly stimulated with increased cyanobacterial abundances.

CONCLUSIONS

Destratification in the lower James River estuary resulted in decreased bacterial production but relatively little change in bacterial abundance. Coefficients of variation of heterotrophic bacterial abundance and production indicated that spatial variability remained greater than temporal variability. Despite this information, correlative analysis indicated that relationships between bacterial abundance and ancillary parameters changed over destratification. Results of oxygen metabolism experiments suggested that bacterioplankton activity was closely coupled to photosynthetically released dissolved organic matter. Changes in relationships between bacterial and autotrophic parameters indicated that bacteria were responsive to alterations in autotrophic community structure, and were more closely associated with smaller (<15 μm) autotrophic forms, particularly cyanobacteria. Mixing of chlorophyll throughout the water column during destratification appeared to translocate substrate, resulting in the enhancement of bacterial production rates in pycnocline and bottom waters, but depression of rates in surface water. Heterotrophic flagellates were shown to be important in controlling bacterial abundance by consuming nearly all of daily bacterial cell production. Grazing activities of flagellates which were highly correlated with bacterial abundance in surface waters, may have been altered during destratification, as evidenced by depression of total and specific grazing rates during the period of rapid destratification of August 8th-9th.

Bacterial-nutrient relationships suggested that competition for available nutrients with autotrophs was likely. Correlations between bacterial abundance and DIN nutrients were all stronger than correlations between autotrophic parameters and DIN nutrients. Multiple discriminant analysis indicated that bacterial production rates were inversely related to ammonium concentrations, however correlative analysis indicated that this relationship disappeared during the destratified period. Temporal patterns of change in DIN nutrients and phosphate agreed with the observations made by previous investigators that minimal concentrations of these nutrients generally occur during the destratified period. These nutrients were incorporated into planktonic biomass, or converted to other nitrogen forms (i.e. via nitrification) as indicated by increasing nitrite concentrations in the upper estuary. The relative importance of new nitrogen sources (NO_3 and NO_2) were also greatest during the destratified period when ammonium concentrations became low ($<1\mu\text{m}$).

Decreases in bacterial production rates as a consequence of destratification have been observed in many but not all investigations. The consequences of destratification may depend upon the type of controls exerted upon bacterial populations when destratification occurs. Depression of bacterial production rates would be likely if surface water populations were close to substrate limited conditions with regards to carbon and destratification further diluted available substrate. Enhancement of rates could occur if surface populations were nutrient limited and mixing of nutrient rich bottom waters provided increased concentrations throughout a greater portion of the water

column. If the grazing activities of microheterotrophs and bacterial production were depressed during destratification then bacterial abundance would appear constant.

This study has illustrated that bacterioplankton activities and trophic relationships in the microbial loop are markedly altered during destratification. Since hydrographic phenomena such as destratification occur on a regular basis in many temperate estuaries it is of great importance and interest to determine how these changes affect the microbial loop. In particular, more information is needed regarding qualitative and quantitative changes in microbial substrates and alterations in activities of grazers as the result of hydrographic alterations of estuarine milieu.

SECTION 3

THYMIDINE INCORPORATION AND AMMONIUM CYCLING
BY BACTERIOPLANKTON IN THE YORK RIVER ESTUARY:
ESTIMATES BASED UPON A SPRING-SUMMER STUDY

INTRODUCTION

The significance of bacteria in carbon production and nutrient cycling within marine and estuarine ecosystems remains controversial. Bacteria have been reported to be an important carbon source for grazing microheterotrophs and possibly higher trophic levels (Fenchel, 1982; Fuhrman and McManus, 1984; Wright and Coffin, 1984a-b; Roman et al., 1988); sinks for carbon in microbial food webs (Ducklow et al., 1986; Coffin and Sharp, 1987); major mediators of nutrient regeneration (Glibert, 1982; Coffin and Sharp, 1987), and responsible for assimilation of a large proportion of available nitrogen nutrients into particulate matter (Laws, 1985; Wheeler and Kirchman, 1986; Horrigan et al., 1988).

It is likely that bacterial communities contribute to all of these processes with their exact role dictated by environmental conditions. For example, Goldman et al. (1987) has shown that the efficiency of bacteria as nutrient remineralizers is highly dependent upon their physiological state, (exponential growth or senescence) and the C:N ratio of the substrate used by rapidly growing cells. Hopkinson et al. (1989) have developed a model which suggests that the C:N ratio of heterotrophic bacterial substrates affect microbial carbon conversion efficiency, nitrogen remineralization and organic carbon utilization. Physical factors governing hydrographic conditions also appear important. In a study concerning the vertical distribution and partitioning of organic carbon in mixed, frontal, and stratified waters of the English Channel, Holligan et al. (1984) suggested that there was

a greater transfer of carbon from bacteria to flagellates and higher trophic levels at the stratified stations, as compared to the other hydrographic conditions. McManus and Peterson (1988) found that trophic relationships between bacteria, phytoplankton and heterotrophic microflagellates off coastal Chile were highly influenced by hydrographic condition, with bacterioplankton assuming a greater proportion of total planktonic production during upwelling periods.

Bacterioplankton activity within estuaries appears to be of critical importance to nutrient cycling processes. Ducklow and Peele (1987) found that in the upper Chesapeake Bay bacterial biomass often surpassed phytoplankton biomass by a factor of two, while bacterial production exceeded autotrophic production by 50% for extended time periods. Such scenarios can be maintained only through considerable inputs of allochthonous material or as the consequence of a closely coupled microbial loop in which rates of nutrient incorporation are matched by rates of nutrient remineralization. Bacterioplankton and phytoplankton have been shown to be important components with regard to ammonium assimilation (Wheeler and Kirchman, 1986; Pennock 1986). Remineralization processes are less well understood and are generally thought to be mediated by grazing of micro and nanoheterotrophs (Goldman et al., 1985; Roman et al., 1988; Bloem et al., 1989) or by bacterial activities (Goldman et al., 1987; Hopkinson et al., 1987).

Although several investigators have undertaken studies to elucidate the role of bacterioplankton in nitrogen cycling dynamics, only one has compared assimilation and remineralization rates with production of the bacterial component. Hopkinson et al. (1987) found

that suspended bacteria were an order of magnitude more important than microheterotrophs both in terms of carbon production and ammonium remineralization in the water column of an Australian coral reef. Glibert (1982) using a series of size fractionations found that the fractions $>1 \mu\text{m}$ generally accounted for the majority of ammonium uptake and remineralization in the Chesapeake Bay. Although Glibert's (1982) estimates appear legitimate they represent only single samples at three stations in the Chesapeake Bay, and no information concerning bacterial abundance or production were available.

Given the evidence that bacterioplankton may be very important contributors to carbon production and nutrient cycling in microbial food webs, the goal of this study was to measure secondary production of bacterioplankton (utilizing a thymidine incorporation methodology), and to determine the role of bacterioplankton in ammonium cycling at several stations in the York River estuary. The following hypotheses were tested: (1) that values of bacterial abundance and thymidine incorporation, ammonium assimilation and remineralization in whole water would vary significantly on spatial and temporal scales, (2) that bacterioplankton would contribute significantly (i.e. much greater than indicated by their biomass) to ammonium assimilation, and relationships between thymidine incorporation and ammonium assimilation would be highly significant, (3) that ammonium remineralization would be mediated primarily by grazing organisms contained in the $> 1.6 \mu\text{m}$ and $<15 \mu\text{m}$ fractionation and, (4) that rates of ammonium assimilation and remineralization would be coupled and consistent over temporal and spatial axes.

METHODS AND MATERIALS

Study area and sample acquisition

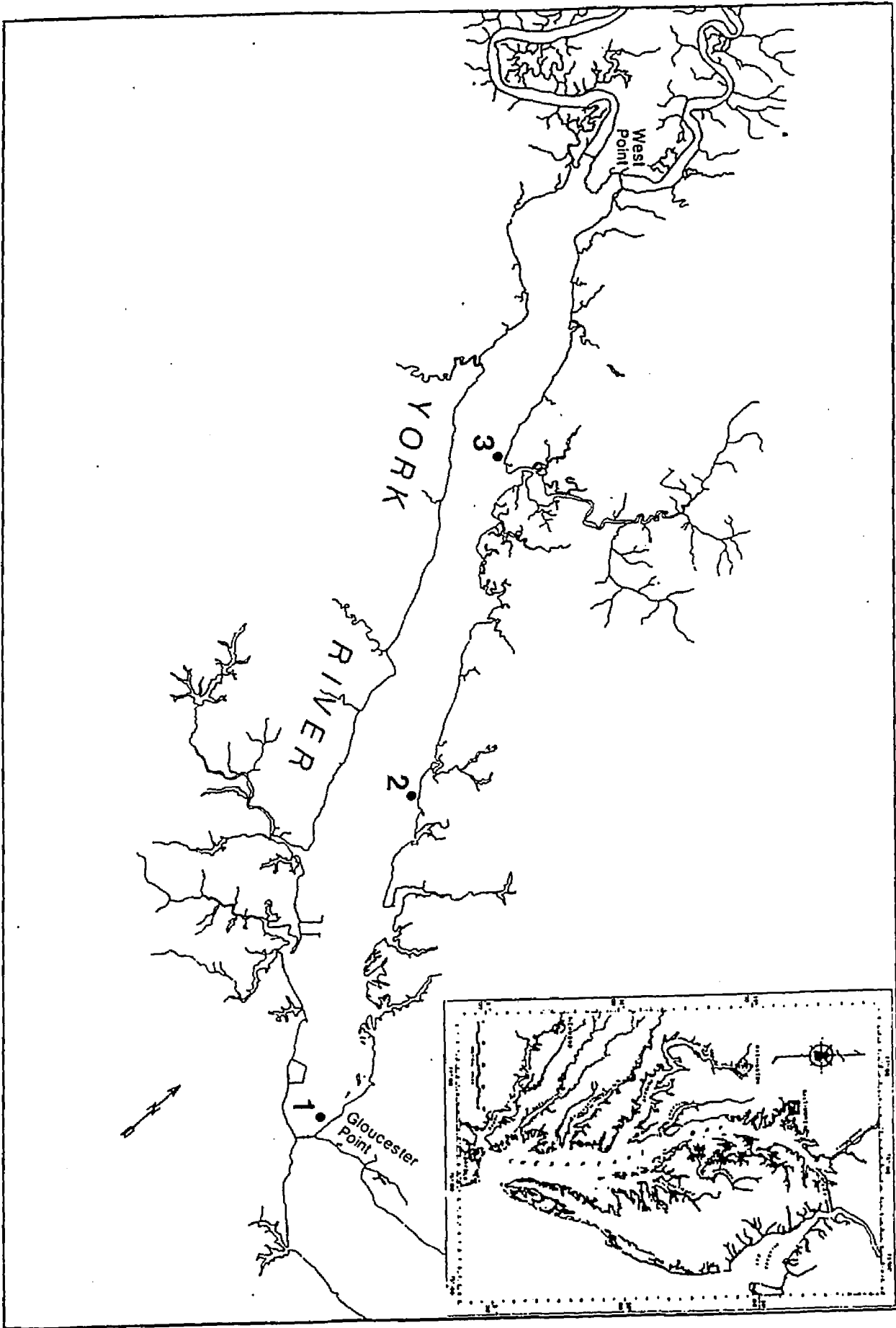
The York River estuary (Figure 12) is formed by the confluence of the Pamunkey and Mattaponi Rivers approximately 50 Km from the mouth, which drains into the lower Chesapeake Bay. Tidal influence extends throughout the length of the York River. The river is considered to be relatively unimpacted compared to other subestuaries of the Chesapeake Bay. Sampling sites (Figure 12) were located in shoal areas (mean depth <1.5 m) on the eastern shore of the river 11.25 Km, 25.5 Km, and 40.25 Km from the river mouth.

Samples were collected monthly from each site during from April through August of 1988. Stations were sampled at slack before flood tide during the morning (0500-0930 hours). Salinity, water temperature, and light attenuation were determined prior to water collection. Triplicate 10 l water samples were retrieved at each station using acid washed, deionized water rinsed black Nalgene® carboys, and were returned to the laboratory for further processing within 1 hour after final station sampling.

Analytical methods

At the laboratory each sample replicate was size fractionated to provide whole water, <15 μm , and <1.6 μm size fractions. The <15 μm fraction was obtained by gravity filtration through a 35 μm nitex mesh followed by a 15 μm nitex mesh. The <1.6 μm fraction was produced from <15 μm fractionated water which was subsequently filtered under low

Figure 12 Location of stations in the York River and
inset showing York River location in the
Chesapeake Bay



pressure (ca. 100mm Hg) through pre-combusted (550° C, 4 hours) Gelman AE glass fiber filters.

Ammonium assimilation and remineralization were determined using ^{15}N tracer techniques. Estimates of both parameters were determined on each sample from a single incubation of 1 litre subsamples spiked with $1\mu\text{g atom}\cdot\text{l}^{-1}$ of 99% $(^{15}\text{NH}_4)_2\text{SO}_4$ (Cambridge Laboratories). Spiked samples were incubated in the dark for 3-4 hours at a temperature equivalent to the mean of in-situ temperatures recorded at the three stations. Immediately after isotope addition (t_0), and at the end of the incubation period (t_f), 250ml (whole water and $<15\mu\text{m}$ treatments) and 500ml ($<1.6\mu\text{m}$ treatments) of samples were filtered through precombusted Whatman GF/F filters. Ammonium in sample filtrates was immediately converted to indophenol-N by the methods of Dudek et al. (1986) and was later recovered (<24 hrs) using solid-phase extraction columns (Selmer and Sorensen 1986). Filters were dried at 80°C for >24 hrs. and then stored under vacuum in a desiccator until further processing. A detailed description of the entire procedure can be found in Kator et al. (1987). Samples were processed for analysis by emission spectroscopy by micro-Dumas combustion according to the protocols of Fiedler and Proksch (1975). ^{15}N enrichment in samples was determined by comparison to a standard curve of ^{15}N ammonium enrichment standards similarly prepared, using a Jasco emission spectrophotometer (Jasco Inc., Easton, Maryland). Ammonium remineralization rates were calculated using the model equation of Blackburn (1979);

$$\ln(R_t) = \ln(R_0) - r/((S_t - S_0) \times \ln(S_t/S_0))$$

Where R_0 and R_t are atom % excess ^{15}N in the NH_4 pool at time equal 0 and t respectively, S_0 and S_t are ammonium concentration at time equals 0 and t respectively, and r equals the remineralization rate per unit time (t). This model assumes that uptake and remineralization rates are constant with time, and that only $^{14}\text{NH}_4$ is produced during remineralization.

Filtered particulate samples were processed to determine ammonium assimilation as follows; CHN analysis performed using a Perkin-Elmer model 240B elemental analyzer (Perkin-Elmer Inc., Norwalk CT, (Patterson 1973)) on filter subsamples established the PON (μg) content on each filter. Filters areas required for a total of $5\mu\text{g}$ sample nitrogen were then subsampled using brass cork borers. This nitrogen content had been previously shown to maintain a stable emission. ^{15}N enrichment in filter subsamples was then determined by emission spectroscopy using a micro-Dumas combustion method as described by Kristiansen and Paasche (1982). Ammonium assimilation rates were calculated using the following equation;

$$a = ((\%^{15}\text{N}_t - \%^{15}\text{N}_0) / \text{SA}) \times \text{PON}_0 \times (1/t)$$

where $\%^{15}\text{N}_t - \%^{15}\text{N}_0$ are atom % excess enrichment in particulate matter at time t and $t=0$ respectively, SA is the mean specific activity of the dissolved ammonium pool for time t and $t=0$, PON_0 is the concentration of particulate organic nitrogen determined at $t=0$, t is the incubation time, and a is the ammonium assimilation rate. This model assumes

insignificant changes in PON and linear depletion of ammonium substrate during the incubation period. This equation is similar to that used in Glibert (1982) and takes into account changing specific activity in the dissolved pool due to remineralization, however it uses the arithmetic mean of initial and final specific activities instead of a geometric mean. Use of the arithmetic mean was felt to be more appropriate since nothing was known concerning the nature of time course changes in dissolved ammonium pool specific activities.

Abundances of heterotrophic bacteria, flagellates and ciliates were estimated by epifluorescence direct counting using a dual staining technique with the dyes proflavin (Haas, 1982), and DAPI (Porter and Feigg, 1978). Counts were made using a Zeiss standard microscope equipped with a 50 W high pressure mercury lamp, 10x calibrated ocular, 100 or 63x Plan apo® (Zeiss) objective and two epifluorescence filter sets. Blue excitation (450-490nm; Zeiss #487709) was used to observe and differentiate heterotrophic from autotrophic cells. Heterotrophic bacterial biomass was estimated using a 20×10^{-15} g C·cell⁻¹ conversion value (Lee and Fuhrman, 1987).

Bacterial production was estimated using the tritiated thymidine (³H-Tdr) incorporation method (Fuhrman and Azam, 1980; 1982). Triplicate, 10 ml samples were incubated with 5 nM of 62 Ci·mmole⁻¹ of ³H-Tdr for 30 minutes in the dark and at in situ water temperatures. Single, 10 ml abiotic control samples (pre-incubation killed with 300 ul of buffered formalin) were treated in a similar manner. Sample incubations were terminated with 300 ul of buffered formalin and samples were stored at 4°C prior to further processing. Samples were prepared

for counting by extraction with ice cold 5% TCA, filtering onto 0.45 μm Gelman GN-6 membrane filters and washing with 5-6ml of ice cold 5% TCA. Filters were then transferred to scintillation vials and acid hydrolyzed in a boiling water bath with 0.5ml of 0.5N HCL for 30 minutes. After cooling 1 ml of ethyl acetate was added to dissolve the filters followed by 10 ml of Aquasol® scintillation cocktail. Disintegrations per minute were determined using a Beckman® scintillation counter, correcting for counting efficiency using an external standard ratio method. When bacterial carbon production was of interest it was calculated using the conversion values of 1.7×10^{18} cells $\cdot\text{mole}^{-1}$ of $^3\text{H-Tdr}$ (Fuhrman and Azam 1982), and 20×10^{-15} .g C $\cdot\text{cell}^{-1}$ (Lee and Fuhrman, 1987).

Chlorophyll a concentrations were determined as described in Ray et al., (1988). Ammonium concentrations were determined by the phenolhypochlorite method (Solorzano 1969).

Statistical Analysis

Two way analysis of variance (ANOVA) was utilized to separately test bacterial production, bacteria abundance, ammonium assimilation, and ammonium remineralization within each size fraction by date and station. Similar tests were performed on the ratio values determined for the $<15 \mu\text{m}:\text{whole-water}$, the $<1.6 \mu\text{m}:\text{whole-water}$, and the $<1.6 \mu\text{m}:\text{<15 } \mu\text{m}$ fractionated samples to examine patterns associated with fractionation. Linear regression and correlation were utilized to examine relationships between parameters. All statistics were derived using the Statistical Package for Social Science (SPSS).

RESULTS

Physical and chemical data obtained at the study sites (Table 12) indicated increases in temperature and salinity over the study period. Total suspended solids displayed no obvious seasonal trends, but were markedly different between stations with concentrations much higher at station 3. Ammonium concentrations varied both temporally and spatially. Temporal patterns of ammonium concentration were similar for stations 2 and 3, with maximum concentrations in May followed by minima in June. Spatial patterns indicated generally higher concentrations of ammonium at station 3. Distributions of chlorophyll *a* tended to increase upriver, however seasonal patterns differed at each station. Particulate organic nitrogen (PON) exhibited a pattern similar to chlorophyll *a*, with values increasing upriver. PON tended to increase over time at stations 1 and 3, whereas station 2 displayed less obvious temporal trends.

Bacterial thymidine incorporation (expressed as disintegrations per minute (DPM)) and bacterial abundance are shown in Figure 13. Both parameters increased significantly after the first sampling date (Tukey multiple range tests, Table 13). Bacterial thymidine incorporation increased several fold between May and June at stations 2 and 3 whereas increases in abundance were more gradual. Values of bacterial thymidine incorporation were significantly lower at station 1 compared to other stations on 3 of 5 sampling dates, whereas bacterial abundance was significantly lower at station 1 on 4 of 5 sampling dates. Temporal and spatial trends of bacterial parameters was similar in all size fractions

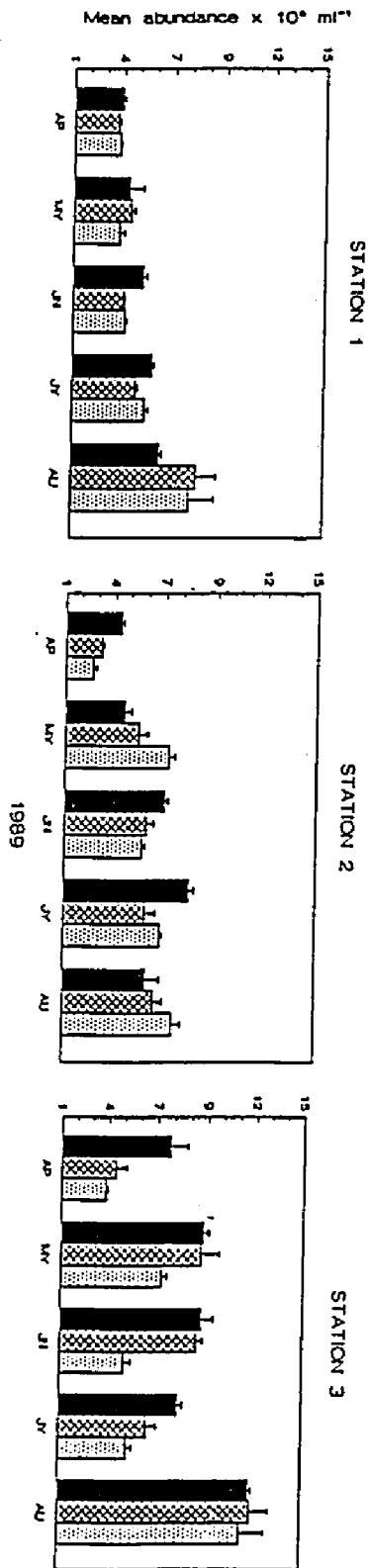
Table 12. Physical and chemical data characterizing the stations on sampling dates. Data include; temperature (TMP, °C), salinity (SAL, ppt), total suspended solids (TSS, mg.l⁻¹), ammonium concentration (NH₄, μM), chlorophyll a concentration (CHL, μg.l⁻¹), and particulate organic nitrogen (PON, μg.l⁻¹).

DATE	STATION																	
	1			2			3											
	TMP	AL	TSS ^a	NH ₄ ^a	CHL ^a	PON ^a	TMP	SAL	TSS ^a	NH ₄ ^a	CHL ^a	PON ^a	TMP	SAL	TSS ^a	NH ₄ ^a	CHL ^a	PON ^a
4/20/88	11.5	16.0	50.7	0.58	14.5	15.7	10.0	12.0	70.3	0.78	19.1	25.9	10.5	10.0	72.0	1.53	20.1	24.4
5/16/88	19.2	18.0	58.7	1.39	12.4	15.1	19.5	16.0	57.6	1.27	14.9	16.4	20.5	10.0	314	6.11	12.6	27.1
6/20/88	23.2	18.0	61.4	1.40	8.52	18.3	24.8	16.0	58.1	0.38	18.9	24.5	25.0	13.0	103	0.50	34.0	45.8
7/18/88	28.0	24.0	74.7	1.81	8.85	21.3	28.8	22.0	79.6	0.53	24.2	23.5	29.0	19.0	105	1.33	27.8	41.3
8/16/88	28.0	28.0	101	0.53	16.4	25.0	28.0	21.0	72.3	0.64	14.9	21.0	28.5	18.0	105	3.31	22.1	40.7

^a mean of n=3

Figure 13 Distribution of bacterial abundance and thymidine incorporation in whole-water and size fractionated treatments

BACTERIAL ABUNDANCE



BACTERIAL THYMIDINE INCORPORATION

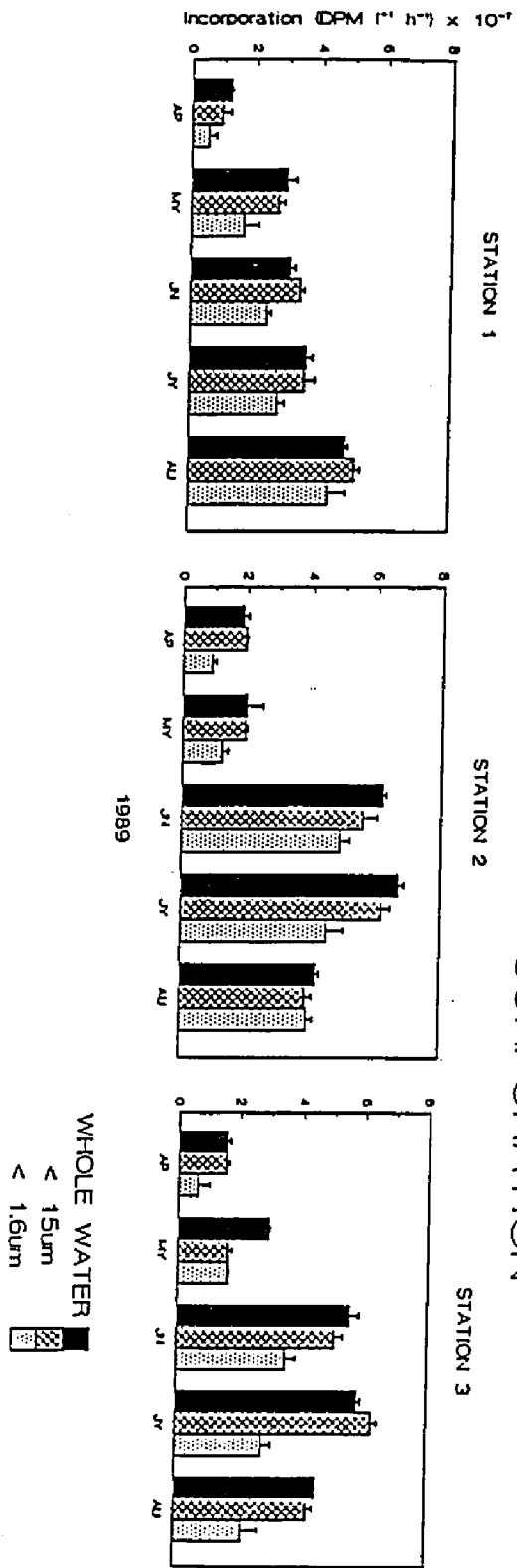


Table 13. Analysis of variance and Tukey multiple range tests of bacterial production (BP), bacterial abundance (BA), ammonium assimilation (AS), and ammonium remineralization (AR) values for whole water treatments. Stations (ST=1-3) and dates (DA=1-5) which are overlined are not significantly different at $\alpha=0.05$. Significance levels for one-way analysis of variance are indicated in parentheses except when not significant (NS) at $\alpha=0.05$.

PARAMETER	<u>BP</u>	<u>BA</u>
DA1 X ST	1 3 2 (.0004)	<u>1 2 3</u> (.0005)
DA2 X ST	2 <u>3 1</u> (.0019)	<u>1 2 3</u> (.0013)
DA3 X ST	1 3 2 (.0000)	1 2 3 (.0001)
DA4 X ST	1 3 2 (.0000)	1 <u>3 2</u> (.0001)
DA5 X ST	2 3 1 (.0005)	<u>2 1 3</u> (.0001)
ST1 X DA	1 <u>2 3 4 5</u> (.0000)	<u>1 2 3 4 5</u> (.0031)
ST2 X DA	<u>1 2 5 3 4</u> (.0000)	<u>1 2 5 3 4</u> (.0000)
ST3 X DA	1 2 5 <u>3 4</u> (.0000)	<u>1 4 3 2 5</u> (.0005)
PARAMETER	<u>AS</u>	<u>AR</u>
DA1 X ST	NS	<u>2 3 1</u> (.0175)
DA2 X ST	NS	<u>3 2 1</u> (.0491)
DA3 X ST	1 <u>3 2</u> (.0004)	<u>1 2 3</u> (.0245)
DA4 X ST	NS	1 <u>2 3</u> (.0016)
DA5 X ST	<u>1 2 3</u> (.0020)	NS
ST1 X DA	<u>5 2 1 3 4</u> (.0011)	<u>4 2 3 5 1</u> (.0012)
ST2 X DA	<u>2 5 1 4 3</u> (.0000)	<u>1 2 4 3 5</u> (.0009)
ST3 X DA	<u>2 1 5 4 3</u> (.0002)	<u>2 1 5 4 3</u> (.0003)

(Figure 13), however abundances and production rates observed in $<15 \mu\text{m}$ and $<1.6 \mu\text{m}$ fractions occasionally exceeded those of the whole water treatment. Tukeys multiple range tests (Table 14) indicated that ratios of $<1.6 \mu\text{m}$ and $<15 \mu\text{m}$ fractions to whole water treatments were significantly different by station on various sampling dates, with station 3 tending to have greater abundance and production associated with the $>15 \mu\text{m}$ fraction. By stations, the ratios of bacterial abundance in $<15 \mu\text{m}$ and $<1.6 \mu\text{m}$ to whole water treatments were significantly greater during May and August samplings. Ratios of bacterial thymidine incorporation between the various size fractions (Table 14) displayed significant temporal differences only at station 2.

Ammonium assimilation rates (Figure 14) differed significantly by station and date (Table 13). Spatial differences were manifested primarily in the last three months of the study, in which stations 2 and 3 displayed higher rates compared to station 1. Differences in assimilation by date were highly significantly ($P < 0.005$) for all stations. Rates of assimilation at stations 2 and 3 peaked in June, increasing 9.5 and 6.3 fold over mean rates of the previous month. Maximum assimilation rates at station 1 occurred in July increasing only 30% over the rates observed during June. The percent of total assimilation attributed to the $<1.6 \mu\text{m}$ fraction tended to increase over the course of the study at stations 1 and 2.

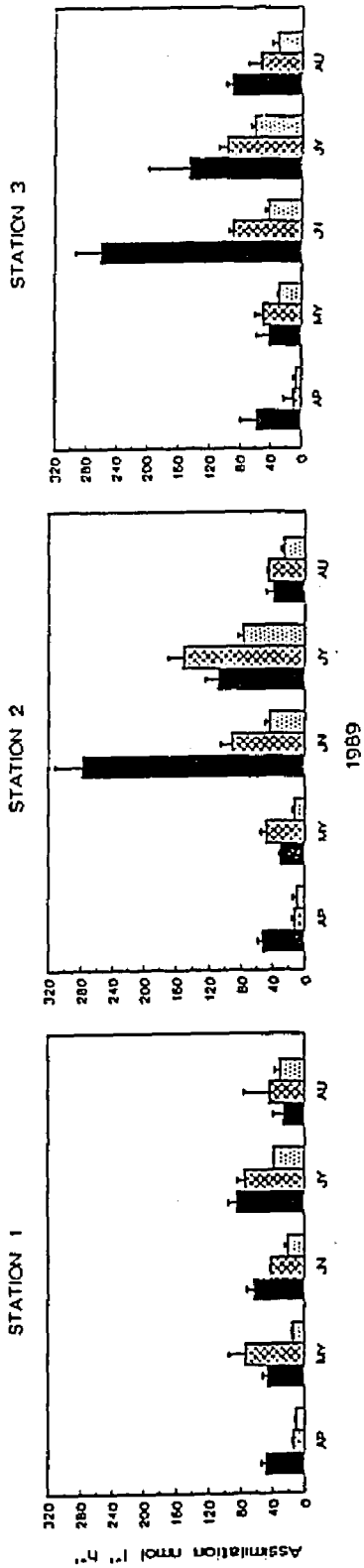
Ammonium remineralization rates (Figure 14) were generally greater than assimilation rates, and patterns of temporal and spatial changes were complex. Remineralization rates at station 1 were significantly greater than those at stations 2 and 3 during April and were also higher

Table 14. Analysis of variance and Tukey multiple range tests of fraction ratios; $15\mu\text{m}$:whole-water treatments (1), $1.6\mu\text{m}$:whole-water treatments (2), and $1.6\mu\text{m}$:$15\mu\text{m}$ treatments (3) for variables BP, BA, AS, and AR. Stations (ST=1-3) and dates (DA=1-5) which are overlined are not significantly different at $\alpha=0.05$. Significance levels for one-way analysis of variance are indicated in parentheses except when not significant (NS) at $\alpha=0.05$.

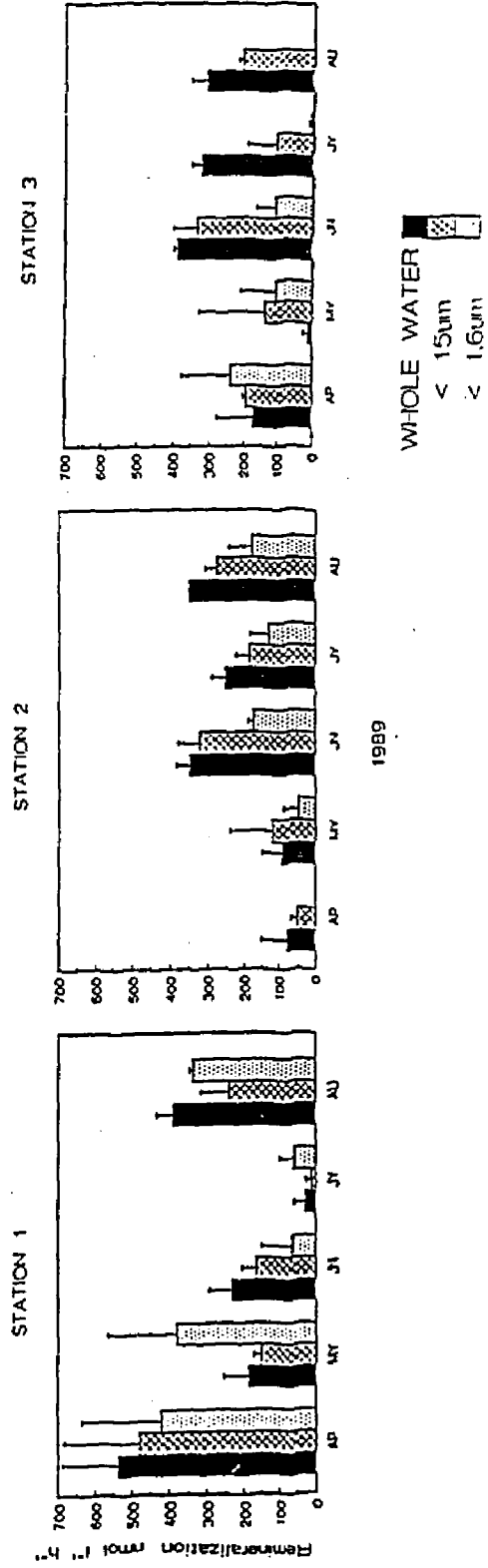
FRACTION RATIO	(1)	(2)	(3)
<u>BP</u>			
DA1 X ST	NS	NS	NS
DA2 X ST	NS	NS	NS
DA3 X ST	<u>2 3 1</u> (.0076)	3 <u>1 2</u> (.0066)	<u>3 1 2</u> (.0174)
DA4 X ST	NS	3 <u>2 1</u> (.0111)	3 <u>2 1</u> (.0024)
DA5 X ST	NS	3 <u>1 2</u> (.0043)	3 <u>1 2</u> (.0004)
ST1 X DA	NS	NS	NS
ST2 X DA	<u>3 5 4 2 1</u> (.0350)	<u>1 2 4 3 5</u> (.0006)	<u>1 2 4 3 5</u> (.0000)
ST3 X DA	NS	NS	NS
<u>BA</u>			
DA1 X ST	<u>3 2 1</u> (.0086)	<u>3 2 1</u> (.0030)	NS
DA2 X ST	NS	NS	NS
DA3 X ST	NS	3 <u>2 1</u> (.0009)	3 <u>2 1</u> (.0002)
DA4 X ST	NS	3 <u>2 1</u> (.0020)	NS
DA5 X ST	NS	NS	NS
ST1 X DA	<u>3 4 1 2 5</u> (.0310)	NS	NS
ST2 X DA	<u>4 1 3 5 2</u> (.0118)	<u>1 4 3 2 5</u> (.0032)	<u>2 1 3 4 5</u> (.0068)
ST3 X DA	<u>1 4 3 5 2</u> (.0055)	<u>1 3 4 2 5</u> (.0025)	<u>3 2 4 1 5</u> (.0472)
<u>AS</u>			
DA1 X ST	NS	NS	NS
DA2 X ST	NS	NS	<u>1 2 3</u> (.0351)
DA3 X ST	2 3 1 (.0195)	<u>2 3 1</u> (.0189)	NS
DA4 X ST	NS	NS	NS
DA5 X ST	NS	NS	NS
ST1 X DA	<u>1 3 4 5 2</u> (.0068)	<u>1 3 2 4 5</u> (.0119)	NS
ST2 X DA	<u>1 3 5 4 2</u> (.0004)	<u>3 1 2 5 4</u> (.0081)	NS
ST3 X DA	<u>1 3 5 4 2</u> (.0363)	<u>1 3 5 4 2</u> (.0130)	NS
<u>AR</u>			
DA1 X ST	NS	NS	NS
DA2 X ST	NS	NS	NS
DA3 X ST	NS	NS	NS
DA4 X ST	NS	NS	NS
DA5 X ST	NS	3 <u>2 1</u> (.0012)	<u>3 2 1</u> (.0112)
ST1 X DA	NS	NS	NS
ST2 X DA	NS	NS	NS
ST3 X DA	NS	<u>5 4 3 1 2</u> (.0003)	<u>5 4 3 1 2</u> (.0103)

Figure 14 Distribution of rates of ammonium assimilation and remineralization in whole-water and size fractionated treatments

AMMONIUM ASSIMILATION



AMMONIUM REMINERALIZATION



WHOLE WATER
 < 15μm
 < 1.6μm

than station 3 during May (Table 13). By June and July the highest rates were observed at stations 2 and 3. Contributions of the $<1.6 \mu\text{m}$ fraction to total remineralization decreased over the course of the study at station 3, whereas it increased at station 2, and was highly variable at station 1.

Abundances of heterotrophic flagellates and ciliates (Figure 15) were much lower the first 3 months of the study compared to July and August. Flagellates increased from $<100 \text{ cells}\cdot\text{ml}^{-1}$ at all stations during May to 1500, 4800 and 6000 $\text{cells}\cdot\text{ml}^{-1}$ at stations 1, 2 and 3 respectively in July. Ciliates peaked one month later increasing at stations 2 and 3 from $< 50 \text{ cells}\cdot\text{ml}^{-1}$ in July to 350 and 150 $\text{cells}\cdot\text{ml}^{-1}$ in August. Spatial patterns of flagellates and ciliates were similar during July and August, with significantly greater abundances at station 3 grading to lower abundances downriver at station 1.

Regressions of bacterial abundance, bacterial thymidine incorporation, particulate organic nitrogen and chlorophyll a versus ammonium assimilation and remineralization are shown in Table 15. Bacterial thymidine incorporation displayed highly significant ($P<.0001$) regressions to assimilation based upon the entire data set and all size fractions. Both log bacterial abundance and chlorophyll a displayed significant regressions against ammonium assimilation in whole water. Regressions of assimilation versus chlorophyll a decreased in significance with smaller fractionations whereas the relationship to log bacterial abundance remained more highly significant. Log bacterial abundance and particulate organic nitrogen in the $<15 \mu\text{m}$ fractionated treatments exhibited less significant regression relationships to

Figure 15 Abundance of heterotrophic flagellates and ciliates by station over dates

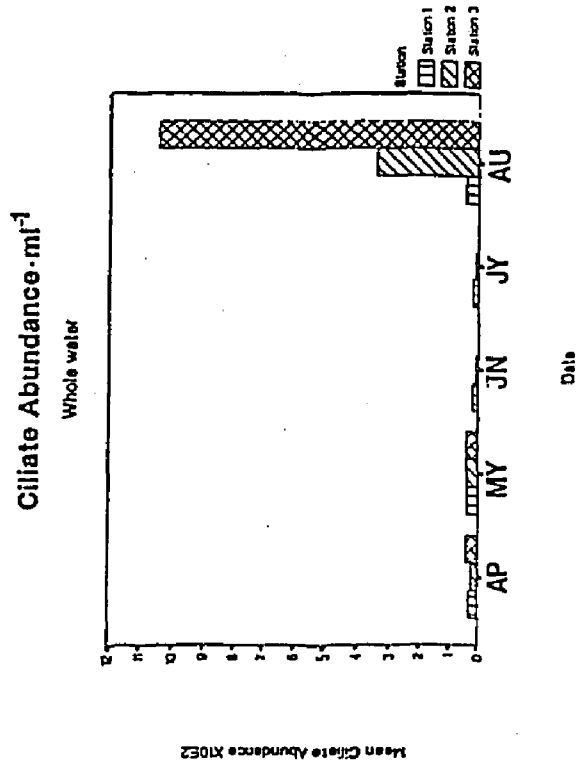
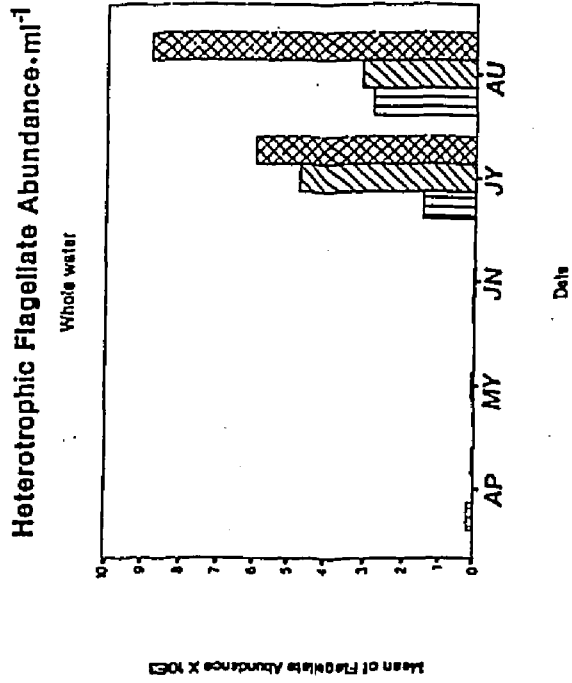


Table 15. Linear regressions of ammonium assimilation (AS) and remineralization (AR) versus bacterial thymidine incorporation (BP), log bacterial abundance (LBA), chlorophyll a (CH), and particulate organic nitrogen (PN). Regressions by size fraction treatments (a.), by stations for whole-water treatments (b.), and by date categories for whole-water treatments.

(a.)

SIZE FRACTION TREATMENTS

REGRESSION	Whole-water			<15 μ m fraction			<1.6 μ m fraction						
	sig.	r ²	m	int.	sig.	r ²	m	int.	sig.	r ²	m	int.	it
AS vs BP	.0000	.404	.740	-19.5	.0000	.531	.420	-2.48	.0000 ^a	.576	.253	65	
AS vs LBA	.0021 ^a	.176	.925	-4.45	.0101 ^a	.127	.979	-4.96	.0000 ^a	.675	.169	-2.77	
AS vs CH	.0000	.328	6.45	-24.5	.0314	.084	1.99	36.9	.0122 ^a	.123	.169	15	
AS vs PN	.0000	.305	4.46	-23.3	ns	--	--	--	.0000	.737	17.2	-21.4	
AR vs BP	.0300 ^a	.105	.008	3.69	ns	--	--	--	ns	--	--	--	
AR vs LBA	ns	--	--	--	ns	--	--	--	ns	--	--	--	
AR vs CH	.0126	.111	7.58	109	ns	--	--	--	ns	--	--	--	
AR vs PN	ns	--	--	--	ns	--	--	--	ns	--	--	--	

(b.)

STATIONS

REGRESSION	Station 1			Station 2			Station 3						
	sig.	r ²	m	int.	sig.	r ²	m	int.	sig.	r ²	m	int.	it
AS vs BP	ns	--	--	--	.0009 ^a	.587	.008	2.95	.0011 ^a	.571	.009	30	
AS vs LBA	ns	--	--	--	.0142 ^a	.381	1.97	-26.3	ns	.735	.085	24	
AS vs CH	.0050	.466	-4.46	107	ns	.386	.148	.955	.0001 ^a	.606	.057	27	
AS vs PN	ns	--	--	--	.0134 ^a	.611	188	-714	.0006 ^a	.503	1.60	-17.8	
AR vs BP	.0455 ^b	.276	-215	1290	.0006 ^b	.380	314	0.00	.0031	.645	15.3	-119	
AR vs LBA	ns	--	--	--	.0143	--	--	--	ns	.524	10.3	-130	
AR vs CH	.0151	.377	35.4	-155	ns	--	--	--	.0003	--	--	--	
AR vs PN	ns	--	--	--	ns	--	--	--	.0023	--	--	--	

(c.)

REGRESSION	DATE				DATE				DATE							
	April-June		June-August		April-June		June-August		April-June		June-August		April-June			
	sig.	r ²	m	int.	sig.	r ²	m	int.	sig.	r ²	m	int.	sig.	r ²	m	int.
AS vs BP	.0001	.810	12.9	-57.5	.0023	.316	10.8	-90.4	.0346 ^a	.167	1.24	-15.0	.0038	.289	5.73	9.68
AS vs LBA	.0088	.244	133	.002	.0132	.222	3.95	6.92	.0363	.164	0.99	97.2	ns			
AS vs CH	.0001	.446	9.08	-59.1	ns				.0063	.262	6.90	155	.0350	.166	4.31	164
AS vs PN	.0002	.425	6.51	-56.9												
AR vs BP	ns															
AR vs LBA	ns															
AR vs CH	ns															
AR vs PN	ns															

^a log transformed first variable

^b log transformed second variable

assimilation versus whole water or $<1.6 \mu\text{m}$ fractionated treatments. Significance levels of regressions of bacterial thymidine incorporation, particulate nitrogen and chlorophyll a versus ammonium assimilation generally increased upriver. Regression relationships between ammonium assimilation and other variables were all stronger during the first half of the study as compared to the last half.

Regressions of ammonium remineralization with bacterial thymidine incorporation, bacterial abundance, particulate organic nitrogen and chlorophyll a were all less significant than regressions using ammonium assimilation. No significant relationships were observed between ammonium remineralization and other parameters in $<15 \mu\text{m}$ and $<1.6 \mu\text{m}$ fractionated treatments. Regressions for whole water treatments indicated a weak ($0.05 > p > 0.01$) relationship to chlorophyll a concentration. Spatial patterns of regressions between remineralization and other parameters were similar to the trend observed with assimilation, with greater significance values at station 3 versus station 1. In contrast to the temporal patterns indicated for regressions using ammonium assimilation, regressions between ammonium remineralization and other variables displayed stronger significance values during the last half of the study.

DISCUSSION

Bacterial Abundance and Production

Results of this study indicated a strong relationship between temperature and bacterial thymidine incorporation. An exponential fit regression of temperature on bacterial thymidine incorporation was highly significant ($P < 0.001$, $r^2 = 0.89$) and indicated a Q_{10} for bacterial thymidine incorporation of 2.18. The influence of temperature upon bacterial abundances and production have been discussed in the literature (Wright and Coffin, 1983; Coffin and Sharp, 1987), yet it is difficult to determine the contribution of this variable due to covariance with other environmental factors. Deviations from a closer temperature dependence may be due to trophic interactions with phytoplankton and bacterial grazers. Mean bacterial thymidine incorporation rates at all stations tripled between April and July samplings whereas bacterial abundance doubled at station 2 and increased only marginally at stations 1 and 3 (Figure 13). Differences in the rates of increase of these parameters suggest that rates of removal of bacterial biomass (grazing) may have increased significantly over the course of the study.

Bacterial thymidine incorporation displayed a strong date-station interaction in which station 1 or 2 displayed either lowest or highest production rates with station 3 always intermediate. Bacterial abundances in contrast were predictable by date, with values at station 3 always significantly exceeding those of station 1 (Table 13). Spatial patterns of bacterial abundance appeared to be strongly related to

chlorophyll a concentration which was also higher at station 3 as compared to station 1 on all sampling dates. This interpretation is supported by regression statistics which indicated a highly significant positive relationship ($P < 0.0001$, data not shown) between bacterial abundance and chlorophyll a. The contribution of the $< 1.6 \mu\text{m}$ fraction to total bacterial abundance and production was always lowest at station 3. This may have been due to a stronger association between bacteria and suspended particles at station 3 which was consistently more turbid and characterized by greater total suspended solids than stations 1 and 2. Although no attempt was made to quantitatively discriminate between densities of free living and particle associated bacteria, particle associated bacteria appeared to predominate at station 3. However, it is also possible that fractionation bias, due to filter retention of and clogging by particles in establishing treatments from station 3 water, may have contributed to the trend for low percent contribution of $< 1.6 \mu\text{m}$ bacterial abundances and production towards whole water totals at station 3.

Ammonium Assimilation and Remineralization

Ammonium assimilation in whole water samples displayed significant variability by date (Figure 14), especially at stations 2 and 3, where assimilation rates in June were higher than all other dates. Seasonal maxima of ammonium assimilation have been observed in the summer in a number of studies. Carpenter and Dunham (1985) found that ammonium assimilation increased markedly in the summer months at a series of stations in the Carmans River Estuary, New York. Fisher et al. (1982),

Glibert et al. (1982), Paasche and Kristiansen (1982), and Pennock (1987) have observed exponential relationships between ammonium assimilation and temperature, but have also stressed the importance of biological covariates. In the present study dark ammonium assimilation in whole water samples did not follow an exponential temperature relationship, but was significantly related to chlorophyll *a*, bacterial thymidine incorporation, bacterial abundance, and particulate organic nitrogen (Table 15). Although significant spatial differences in assimilation rates occurred only in June and August, a general trend for greater assimilation rates at stations 2 and 3 was apparent. Mean assimilation rates grouped over all dates were 2 fold greater at stations 2 and 3 compared with station 1. This trend was also evident in other biological variables as mean values of bacterial thymidine incorporation, bacterial abundances, chlorophyll *a* and PON were also least at station 1. Other studies regarding spatial patterns of ammonium assimilation in estuaries have in contrast indicated peak assimilation rates in middle to lower estuarine locations (Carpenter and Dunham, 1985; Pennock, 1987).

Ammonium remineralization displayed greater spatial variability than assimilation, with significant differences between stations occurring on 4 of 5 sampling dates. As for assimilation, temporal patterns of ammonium remineralization at stations 2 and 3 were similar and tended to increase over the study period. However, the highest remineralization rate occurred at station 1 on April 20th coinciding with the lowest water temperature. Few studies have examined ammonium remineralization in estuarine environments. Glibert (1982) sampled three

stations in the Chesapeake bay on a single occasion and found marked differences in overall rates of ammonium remineralization and the contribution of various size fractions toward total rates. Other studies in coastal environments have either failed to demonstrate spatial patterns (Hanson and Robertson, 1988) or have observed decreasing remineralization progressing offshore (Harrison, 1978). Regression relationships between ammonium remineralization and environmental and biological variables were much weaker than similar relationships for assimilation. Similar findings have been reported by Hanson and Robertson (1988) and suggest that ammonium remineralization is a more complicated process (i.e. governed by more factors) compared to ammonium assimilation.

Bacterial contribution to ammonium assimilation

The contribution of bacterial assimilation to total dark ammonium assimilation was estimated by multiplying the observed rate in the $<1.6 \mu\text{m}$ fraction by the ratio of bacterial abundance in the whole water and $<1.6 \mu\text{m}$ treatments. Assumptions for such estimates were: 1) assimilation by phytoplankton in the $<1.6 \mu\text{m}$ fraction was negligible, and 2) cell specific assimilation rates of bacteria in the $<1.6 \mu\text{m}$ fraction were the same as in the whole water sample. Several observations indicate that assumption 1 has been met. First, if we assume a carbon to chlorophyll a ratio of 20 for picoplankton (Ray et al. 1988; Furnas, 1983) then autotrophic biomass in the $<1.6 \mu\text{m}$ fraction was only 15% of bacterial biomass. Second, regressions of log bacterial abundance and chlorophyll a versus ammonium assimilation in the $<1.6 \mu\text{m}$

fractions revealed a significant relationship with abundance ($P=0.002$) but not with chlorophyll *a* ($P=0.068$). The second assumption may not be totally satisfied. Cell specific assimilation of ammonium by bacteria may be depressed in the $<1.6 \mu\text{m}$ fraction because mean cell specific thymidine incorporation rates in the $<1.6 \mu\text{m}$ fraction were 70% of rates for whole water samples (data not shown). Indications that the second assumption was violated suggests that our estimates of bacterial ammonium assimilation are conservative. Percent bacterial assimilation of total assimilation (Table 16) ranged from a minimum of 19% at station 2 in June to 95% at station 3 in May. Although mean contribution of bacterial assimilation averaged over dates were similar at each station ranging only from 48-50%, temporal variability at each station was apparent. Carbon specific ammonium assimilation rates of bacteria displayed similar temporal patterns at all stations peaking in July, and were closely related to patterns of bacterial thymidine incorporation (Figure 13).

Though the experimental design of the present study may have been biased towards heterotrophic uptake as all incubations were conducted in the dark, other studies (Hansen and Robertson, 1988) have indicated that dark uptake of ammonium typically exceeds 70% of light uptake especially in shallow photic zone waters. Accordingly, a reasonable mean value for bacterial contribution towards light-mediated assimilation of ammonium for this would lie in the range of 35-40%. This range would be in close agreement with rates observed by Glibert (1982) for station 724R in the lower Chesapeake Bay (40%), and supports recent findings of Fuhrman et al. (1988) that bacteria can be directly responsible for 33% of total

Table 16. Ammonium assimilation ($\text{nmol}\cdot\text{l}^{-1}\cdot\text{h}^{-1}$) partitioned into % assimilation by bacterial and autotrophic components. % bacterial assimilation was determined by assuming solely bacterial assimilation in the $<1.6\mu\text{m}$ fraction, the value of which was multiplied by the ratio of bacterial abundance in the whole-water: $<1.6\mu\text{m}$ treatments. % autotrophic assimilation was determined from the difference in total particulate and bacterial assimilations. Carbon specific assimilation rates ($\text{nmol}\cdot\text{l}^{-1}\cdot\text{h}^{-1}\cdot\mu\text{g C}^{-1}$) were determined by dividing component assimilation rates by respective biomasses.

	1988				
STATION 1	4/20	5/16	6/20	7/18	8/16
Total assimilation	48.20	45.00	62.97	84.93	25.73
% Bacterial assim.	22.38	46.76	40.42	48.99	93.39
% Autotrophic assim.	77.62	53.24	59.57	51.01	6.61
Carbon specific bact.	0.145	0.256	0.258	0.383	0.204
Carbon specific auto.	0.052	0.039	0.088	0.098	0.002
STATION 2					
Total assimilation	52.77	29.60	278.0	108.7	38.70
% Bacterial assim.	30.31	49.80	19.14	88.13	53.33
% Autotrophic assim.	69.69	50.20	80.86	11.87	46.67
Carbon specific bact.	0.194	0.169	0.404	0.604	0.182
Carbon specific auto.	0.038	0.020	0.238	0.011	0.024
STATION 3					
Total assimilation	57.20	41.33	259.7	145.3	89.87
% Bacterial assim.	24.30	94.72	31.28	65.40	34.75
% Autotrophic assim.	75.70	5.28	68.72	34.60	65.20
Carbon specific bact.	0.096	0.214	0.448	0.609	0.131
Carbon specific auto.	0.043	0.003	0.105	0.036	0.053

ammonium uptake in spring and summer in Long Island Sound, New York.

The regression between bacterial carbon production (determined from thymidine incorporation and conversion values) and ammonium assimilation in the $<1.6 \mu\text{m}$ fraction revealed that a large proportion of the nitrogen requirements for bacterial growth were satisfied by assimilated ammonium. The slope of this regression yielded a C:N assimilation ratio of 7.1 whereas the most accurate C:N ratios for bacterial biomass are probably those determined by Goldman et al. (1987) of 5.1. This differential indicates that ca. 70% of bacterial nitrogen requirements for growth were being satisfied by observed rates of ammonium assimilation. These findings agree with recent work by Wheeler and Kirchman (1986) who found that a significant portion of total nitrogen uptake by bacteria is of ammonium, and Horrigan et al. (1988) who found that bacterioplankton could utilize significant amounts of DIN in seawater chemostat systems.

In the present study, although bacterial biomass averaged only 8% of chlorophyll *a* indicated autotrophic biomass, bacteria were responsible for 40-50% of total assimilation. Mean carbon specific uptake rates for bacteria were 5 times that for autotrophic biomass. The implications of such bacterial assimilation with regards to nitrogen nutrient uptake in regions of the upper Bay, where it is not uncommon to find bacterial biomass exceeding autotrophic biomass for extended periods of time (Ducklow and Peele, 1987), are significant. It seems likely that bacterial assimilation of ammonium could limit phytoplankton growth if nitrogen regeneration is not as rapid as uptake. The roles of flagellates, ciliates and other grazers are thought to be important with

respect to nutrient generation and will be discussed in the following section.

Factors influencing ammonium remineralization

Grazing of phytoplankton and bacterioplankton by microheterotrophs has been invoked as the "main-spring" of the microbial loop. In the present study the significant seasonality of both heterotrophic flagellates and ciliates (Figure 15), did not appear to account for temporal variability in chlorophyll *a* or bacterioplankton abundance. Bacterial abundance at station 3 peaked in August, coinciding with maximum abundance of both flagellates and ciliates, yet significantly decreased rates of bacterial thymidine incorporation versus July. Bacterial abundance at station 2 decreased between July and August, but this occurred coincident with a decrease in thymidine incorporation, thereby suggesting that the decrease in abundance may have been independent of grazing rate. Other data, from later months of sampling, did indicate grazing effects. Mean bacterial thymidine incorporation rates in the <15 μm fraction at stations 1 and 3 in August, exceeded those of the whole water. At stations 1 and 2 bacterial abundance in the <1.6 μm fraction exceeded whole water abundance in August. Since all samples were fixed within 1 hour of fractionation this increased abundance suggests an rapid response to release from predation pressure. Decreases in chlorophyll *a* standing stocks between June and July at stations 2 and 3 may have also been in part due to increased grazing.

The most surprising aspect of the observed flagellate and ciliate seasonality was the relatively minor effect these groups appeared to

have upon ammonium remineralization. We had expected more dramatic changes in remineralization rates, given the several order of magnitude increase in the observed abundance of heterotrophic grazers between June and August of the study. Despite a strong relationship between temporal patterns of microheterotroph abundance and remineralization rates, regression data did indicate stronger associations between chlorophyll *a* and remineralization for the last three sampling dates (Table 15), suggesting that the importance of autotrophic biomass as a substrate was greater at a time when microheterotroph densities were high. Further, albeit indirect evidence of the importance of microheterotrophs comes from date-averaged means of remineralization for the various size fractions. Values of 8, 52, and 62% of total remineralization at stations 1, 2, and 3 were determined for the fraction $>1.6 \mu\text{m}$ (Table 6). These values would rank the same as the date-averaged mean of flagellate and ciliate abundances at these stations. Although, the influence of heterotrophic flagellates on remineralization of ammonium has been well documented (Bloem et al., 1989; Goldman et al, 1985; Gude, 1985), in certain cases larger organisms appear responsible. For example, Paasche and Kristiansen (1982) found that ammonium remineralization rates were related to abundances of juvenile copepods, rotifers, tintinnids and heterotrophic dinoflagellates. Glibert (1982), found that stations in the Chesapeake Bay displayed differences regarding the size fraction responsible for ammonium remineralization, with remineralization at the lower Bay station dominated by a size fraction $> 35 \mu\text{m}$. Thus it is possible in the present study that larger microzooplankton, which were not enumerated, were responsible for a significant amount of grazing and

ammonium remineralization.

Relationships between bacterial parameters and remineralization were weaker than those observed with assimilation. Similarly, regressions of particulate organic nitrogen and chlorophyll a on ammonium remineralization for various size fractions, were all less significant than analogous regressions with ammonium assimilation (Table 15). Regression analysis generally indicated closer associations between total ammonium remineralization and chlorophyll a than to either particulate organic nitrogen or bacterial abundance. Cochlan (1986) also found that ammonium remineralization was significantly correlated with chlorophyll a in a study on the Scotian Shelf. The potential contribution of grazers towards the removal of phytoplankton and concomitant nutrient regeneration have been strongly suggested in recent work by Welschmeyer and Lorenzen (1985), who found that macrozooplankton and microzooplankton grazing were responsible for the removal of ca. 94% of annual chlorophyll production in Dabob Bay, Washington, and more recently by Litaker et al. (1988) who observed strong diel patterns linking grazer removal of chlorophyll with increasing ammonium concentrations in the Newport River Estuary, North Carolina.

Despite indications concerning the importance of autotrophic carbon in the remineralization process, rates of ammonium remineralization in the $<1.6 \mu\text{m}$ fraction still represented 92, 48, and 38% of seasonal means of total ammonium remineralization at stations 1, 2, and 3 respectively (Table 17). Since autotrophic biomass averaged only 15% of bacterial biomass in this fraction it is apparent that bacteria were still a major factor in the remineralization process. It

Table 17. Mean and standard deviation (in parentheses) of ammonium remineralization ($\text{nmol}\cdot\text{l}^{-1}\cdot\text{h}^{-1}$). Values for whole-water and $<1.6\mu\text{m}$ treatments by station and date, and ratios of means grouped over dates of remineralization in $<1.6\mu\text{m}$ versus whole-water treatments.

STATION/FRACTION	1988				
	4/20	5/16	6/20	7/18	8/16
1 whole-water	533(155)	186(70)	233(61)	29(28)	390(41)
< 1.6 μm	418(217)	379(184)	62(87)	57(41)	338(10)
2 whole-water	75(75)	88(61)	347(37)	253(37)	348(2)
< 1.6 μm	0(0)	50(36)	174(16)	130(53)	179(62)
3 whole-water	171(106)	11(16)	386(10)	319(28)	304(42)
< 1.6 μm	237(138)	102(106)	104(57)	4(6)	0(0)

Ratio of means grouped over date of
 $<1.6\mu\text{m}$: whole-water treatments

Station 1 = 0.92

Station 2 = 0.48

Station 3 = 0.37

is likely that autotrophs provided the major substrate in a grazer mediated remineralization scenario, while bacteria were involved secondarily after the grazing process, by remineralizing nitrogen from small particulate and dissolved detrital material which was in excess of their cellular nitrogen needs (Goldman et al. 1986). Significant regressions between bacterial thymidine incorporation and remineralization provide some support for this hypothesis.

Rates of remineralization compared to assimilation

The degree to which ammonium remineralization and assimilation are coupled appear to depend upon the system and scales of observation. Glibert (1982) found that rates of uptake and remineralization at a number of locations were roughly in balance. Other studies have shown this ratio to favor assimilation (Paasche and Kristiansen, 1982; Roche, 1983) or remineralization (Hansen and Robertson, 1988; Cochlan, 1986; Harrison, 1978). The daily net flux will be an integration of dark activity which will favor remineralization, and light activity which should favor uptake. Such processes can contribute to strong diel patterns of ammonium concentrations, as has been shown for the Newport River estuary, North Carolina (Litaker et al. 1988).

In the present study the ratio of grand means of assimilation to remineralization for whole water is 0.38 and assimilation exceeded remineralization on only 2 of 15 station/date combinations (Table 18). If it was assumed that light incubated assimilation rates were 50 % greater than rates determined for dark assimilation (a reasonable estimate based on Hansen and Robertson, 1988; Pennock, 1987; and J.N.

Table 18. Ammonium assimilation (AS) and remineralization (AR) rates ($\text{nmoles}\cdot\text{l}^{-1}\cdot\text{hr}^{-1}$) in unfractionated treatments, ratios of assimilation to remineralization rates (AS:AR), and ammonium pool turnover times (hr^{-1}) based upon rates of assimilation (T-AS) and remineralization (T-AR).

STATION		1988				
		4/20	5/16	6/20	7/18	8/16
1	AS	48	45	63	85	26
	AR	533	186	233	27	290
	AS:AR	0.09	0.24	0.27	3.51	0.66
	T-AS	12.2	30.8	22.2	21.3	20.3
	T-AR	1.09	7.45	6.01	67.2	1.35
2	AS	52	30	278	109	39
	AR	75	88	347	253	348
	AS:AR	0.69	0.36	0.80	0.43	0.11
	T-AS	15.0	75.5	1.35	4.83	16.5
	T-AR	10.4	25.8	1.08	2.08	1.85
3	AS	57	42	260	145	90
	AR	171	11	386	319	304
	AS:AR	0.33	3.82	0.67	0.45	0.30
	T-AS	26.9	145	1.93	9.19	36.8
	T-AR	8.97	556	1.30	4.18	10.89

Boyer (personal communication)), then the assimilation to remineralization ratio would increase to only 0.56, and assimilation would have exceeded remineralization on 5 of 15 station/date combinations.

Although measurements of primary production were not obtained in this study, information on ammonium remineralization (from station 1) were compared to findings of Ray et al. (1988) concerning primary production (P_{max}) in the lower York River, to determine whether observed remineralization rates would be adequate to support primary production. Ray et al. (1988) found the mean autotrophic carbon uptake rate for the months of July and August to be ca. $4.2 \mu\text{moles C}\cdot\text{l}^{-1}\cdot\text{hr}^{-1}$. Assuming a 12 hour photoperiod and the Redfield ratio (of 106:16) for molar uptake of C to N (Redfield 1958), rates of remineralization could account for 66% of maximum photosynthetic nitrogen uptake. It would appear that rates of remineralization would be inadequate to support primary production. However, modeling the Ray et al. (1988) photosynthetic carbon uptake rate upon a typical daily photosynthetically active radiation (PAR) light curve would lower the autotrophic nitrogen requirement such that ammonium remineralization rates may provide close to 100% of autotrophic nitrogen requirements for growth during this study period.

CONCLUSIONS

Bacterial thymidine incorporation and abundance displayed significant differences between stations and over the course of the spring-summer study period. Temporal patterns appeared to be highly influenced by temperature, but spatial differences were correlated primarily with chlorophyll *a* concentration. Contributions of heterotrophic bacteria towards planktonic carbon production and ammonium cycling were much greater than their proportional biomass would indicate. Though bacterial biomass averaged only 8% of the autotrophic biomass, bacterioplankton contributed significantly to dark uptake of ammonium (date averaged means of 50%). Estimates of bacterial contributions, assuming higher light-mediated total uptake rates, were 35-40%, and were still much greater than autotrophs on a carbon specific basis.

Differences in the rate of increase of bacterial thymidine incorporation compared to bacterial abundance over the course of the study, suggested that bacteria were closely controlled through grazing processes. The activity of grazer organisms was also important in the ammonium remineralization process. However, regression analysis indicated that autotrophic as compared to heterotrophic bacterial biomass was more important as a substrate for the remineralization process. Despite indirect evidence suggesting the importance of heterotrophic flagellates and ciliates in the remineralization process, temporal patterns of their abundance did not suggest a direct relationship to remineralization and larger grazers may have been of

greater importance. Remineralization rates in the $<1.6 \mu\text{m}$ fraction indicated that bacteria were very important in the remineralization process accounting for seasonal means of between 92 and 38% of total remineralization at stations 1 and 3 respectively. It is postulated that bacteria were important secondarily in the remineralization process by interception of DON excretion or very small detrital particles from grazing activities of microheterotrophs.

Remineralization rates exceeded assimilation rates for 13 of 15 date/station groupings indicating that particulate nitrogen was rapidly recycled and the resultant ammonium may represent a significant source for autotrophic growth. Using estimates of primary production in the lower York River, calculations showed that ammonium remineralization supplied 66 - 100% of autotrophic production needs for nitrogen.

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