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FLUX OF ENERGY AND ESSENTIAL ELEMENTS THROUGH THE CONTINENTAL SHELF ECOSYSTEM

PROGRESS REPORT

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I. Scope of the Research of the Past Three Years

Abstract

There are three distinct but not mutually exclusive areas of research in this contract, studies of intrusions of the west wall of the Gulf Stream onto the outer continental shelf, studies of the flux of materials across nearshore density fronts, and advances in our understanding of the planktonic food web of the continental shelf. Our studies of frontal events on the outer and inner continental shelf involve distinctive physical and chemical regimes and have proven to require distinctive biological approaches. The studies of the food web run through our work on both of the frontal regimes, but certain aspects have become subjects in their own right. We have developed a simulation model of the flux of energy through the continental shelf food web which we believe to be more realistic than previous ones of its type. It represents realistically both details of the energy transfers within the plankton community and the termanal production of fishes. We have examined several of the many roles of dissolved organic compounds in sea water which originate either from release by phytoplankton, digestive processes or metabolites of zooplankton, or extracellular digestion of microorganisms. We have found that the uptake of dissolved materials, in this instance represented by dissolved adenosine triphosphate, is nearly proportional to bacterial biomass, and that free-living minibacteria and large bacteria attached to particles are equally active in scavenging that material from the water. Many naturally occurring dissolved organic compounds are chelators of heavy metals, and it has been realized for some time that much of the total metal content of sea water is in a chelated form. This would have a significant effect on the toxicity of metals to plankton. Methods have been developed under this contract to measure both the chelating capacity of naturally occurring organic materials and the copper concentration in the water. Using these methods in conjunction with the Schindler modification of the ^{14}C method of measuring photosynthesis, it has been possible to characterize the effects, both toxic and stimulatory, of copper on photosynthesis of naturally occurring phytoplankton populations.

Our understanding of biological, as well as physical and chemical, events on the outer continental shelf is now on a firm basis. Thanks to our knowledge of the physical forcing of the regime by the west wall of the Gulf

Stream, it is possible to characterize in considerable detail the course of biological events associated with meanders of the Gulf Stream. Although there are a number of variations on the theme, the general course of events is a predictable one. We are now in a position to explain the limits to biological productivity of the outer continental shelf of the southeastern U.S. and the reasons why that biological production moves through the food web in the characteristic way that it does.

1. Cooperative Studies of Gulf Stream Intrusions

1.1 The Research Program

During the past three years we have participated in six cooperative studies of intrusions of nutrient-rich water from the cold wall of the Gulf Stream over the outer continental shelf off Georgia and northern Florida. On four of these occasions the scientific party included physical oceanographic contractors as well as other biological DOE contractors and ourselves. This cooperative work between physical and biological oceanographers has contributed significantly to the success of the biological research program. We have been able to find the physical events we are seeking with minimal loss of valuable ship time. The use of infrared imagery from weather satellites, processed and relayed from Miami (RSMAS), was particularly helpful not only in determining the position of the Gulf Stream but in finding intrusions from the west wall of it, so the ship could be positioned in advance of a northward-moving eddy. This permitted us on several occasions to obtain daily observations of the succession of planktonic populations as an intrusion event arrived and was subsequently drawn back over the shelf break. We have, of course, learned the basic pattern of events from the physical oceanographers, and on EASTWARD cruise E1B-79 we were readily able to locate and follow an intrusion off Jacksonville, Florida without the participation of physical oceanographers.

1.2 The Physical Regime

While the detailed information on physical processes on the outer continental shelf of the southeast will be found in the reports to DOE by Atkinson, Blanton, Lee, and Peitrafesa, we present here an outline of the general features of the system which are important to an understanding of biological processes on the outer shelf.

Meanders of the Gulf Stream appear to be perturbations of the Rossby wave type generated downstream of topographic features, such as Cape Canaveral or the so-called Charleston bump (Atkinson 1977; Blanton et al. 1981; Lee and Brooks 1979). Because of this relationship to fixed physical features, the intensity and frequency of intrusions varies along the coast, with a region of high frequency of intrusion events off St. Augustine and Jacksonville, Florida, and in Onslow Bay in North Carolina. The Georgia shelf is a region of comparatively low activity, although many events take the form of moving eddies which sweep up the shelf break through both Florida and Georgia. The period of Gulf Stream meanders is 5-10 days (Blanton et al. 1981), and complete cycles of intrusion and relaxation occur in the active areas at about that rate. South winds during an intrusion may have an Ekman effect, causing the intruded water to remain on the shelf. It is not unusual in summer, when consistent, brisk southerly winds occur, to observe one intrusion move in under a former one, and even a third move in under the others. Cold water stranded on the continental shelf may remain for a week or more as a distinct water body, isolated by density differences from the water above it and around it. Such bodies of nutrient-rich water are the sites of phytoplankton and zooplankton blooms, because they are a persistent source of nutrients (Atkinson et al. 1978). Intrusions which do not strand on the shelf will be drawn back over the shelf break into deep slope water after two or three days, just as a phytoplankton and bacterial bloom is well established. Intrusions are major source of new nitrogen for phytoplankton production on the southeastern continental shelf (Dunstan and Atkinson 1977). Their effect is by far the greatest off northern Florida and in Onslow Bay, particularly during summer (Blanton et al. 1981). Their impact on shelf water off Georgia is substantially less. While frontal intrusions and related frontal processes are widespread (Savidge and Foster 1978; Pingree et al. 1979; Floodgate et al. 1981), these take a unique form on the southeastern continental shelf because of the proximity of the west wall of the Gulf Stream to the shelf break. It is curious that the positive effect on productivity of intrusions of the west wall is nearly nullified by the relaxations which follow closely, before secondary production of plankton larger than bacteria can occur. The northerly and westerly regional wind fields, which predominate except in summer, tend to reenforce the loss of intruded water rather than its retention. This is in contrast to the typical regime of coastal upwellings in which edge waves

interact positively with the regional wind field to induce upwelling of nutrient-rich water to the surface, where it remains, allowing primary and secondary biological production processes to go to completion.

1.3. The Planktonic Community

Because of their short generation times, microbial populations respond to even the most transitory intrusions of nutrient-rich water over the contiental shelf. As this water enters the zone of >1% of surface solar illumination, a bloom of phytoplankton begins to develop. The initial bloom organisms are small, one of those repeatedly found being Thalassiosira subtilis (J. Yoder, pers. comm.), a 5 μm diatom. This is accompanied by blooms of bacteria, although these are not usually congruent in space. The greatest bacterial densities are typically above or toward the land with respect to the center of the bloom of phytoplankton. It is not clear what causes this physical separation of the bacterial and phytoplankton blooms but it has also been observed in the Irish Sea by Floodgate et al. (1981). In the case of Gulf Stream intrusions, the blooms of bacteria are not in the intrusion itself but in the old shelf water adjacent to it. There is, of course, some diffusion of water between the intrusion and the old shelf water as a result of the combined action of internal waves running on the top of the intruded water, tidal currents on the continental shelf, and eddy diffusion and, perhaps more significant, there is migration of zooplankton. Bacterial numbers, or bacterial adenylates as shown by A/C>10 (Campbell et al. 1979) are a very good indicator of short-term secondary production.

Bacteria of the shelf waters are composed of two morphologically distinct groups, the large (>1 μm) bacteria usually associated with particulate organic material, and the minibacteria (<0.5 μm), originally described by Watson et al. (1977), which are free in the water. The latter may be seen doubling but never form colonies in the water. The former often form colonies on seston particles. There are 10^5 minibacteria/ml in continental shelf water. The number may reach 10^6/ml in blooms associated with intrusions. Minibacteria are more abundant than large, attached bacteria by about two orders of magitude, but because of their small size they constitute only about half of the bacterial biomass, and probably they are responsible for half of the production of new bacterial biomass (Hodson et al. 1981).

What is surprising is that free and attached bacteria vary in numbers together, and in fact both vary synchronously with populations of flocculent organic seston. The greatest population densities of chroococcoid cyanobacteria (Johnson and Sieburth 1979; Waterbury et al. 1979) also are associated with dense concentrations of flocculent organic matter. The flocculent organic matter is frequently associated with intrusions but is in the water above them as well as in the intrusions proper. We have associated some of this seston with feces of pelagic tunicates (Pomeroy and Deibel 1980), which are also abundant at the interfaces of intrusions with shelf water and above them. It is reasonable to suppose that the pelagic tunicates are responding to the bloom of bacteria, as well as phytoplankton, by feeding actively and producing feces. According to King et al. (1980), pelagic tunicates can be expected to derive significant nutrition from bacteria but not to greatly influence the total numbers of bacteria in the water. However, the most dense populations of tunicates encountered on the outer shelf may even depress bacterial numbers (Deibel 1980). While the activity of pelagic tunicates may explain the increase in both the numbers of flocs and of bacteria, other mechanisms of production of flocculent aggregates are known (Johnson and Cooke 1980; Kranck and Milligan 1980), it is not clear how they operate in the regime of the outer continental shelf to produce distinctive, localized populations of flocculent seston. Whatever the mechanism, there is an increase in populations of bacteria and flocculent aggregates associated with intrusions of nutrient-rich Gulf Stream water. Secondary production in these water bodies involves the production of bacterial biomass and probably the production of organisms which consume bacteria, such as ciliates and pelagic tunicates. The role of the ciliates in consuming bacteria in fecal flocs has been described by us elsewhere (Pomeroy and Deibel 1980)

The picture which emerges from our experience with about ten intrusions over the past six years is one of a rapid increase in primary production during the first 24 hours with a phytoplankton bloom localized in the intrusion or at the interface between the intrusion and the shelf water above it. Concurrent with this is a bloom of bacteria in the water above or inshore of the intrusion. Where these events persist for several days, dense populations of pelagic tunicates develop in the water in the vicinity of the thermocline between intrusion and shelf water. These are often associated with an increase in flocculent seston in the water. They are extremely dynamic events

Table 1. Combined data from cruises E3B-79, FRNFLX I, and BLUE FIN 10-80.

FREEBAC = free bacteria
ATTBAC = attached bacteria
TBAC = total bacteria
PC-ATT = per cent of bacteria attached
FLOCFEC = fecal-type floc. aggregates
PHYTO = phytoplankton (counted)
NVPHYTO = non-viable phytoplankton
CYANO = chroococcoid cyanobacteria
NO3 = nitrate
SAL = salinity
CHL-A = chlorophyll a
PHEO-A = pheophytin a
ATP = extracted ATP
AXP = extracted total adenylates
EC = adenylate energy charge
HPINDEX = heterotrophic-photoautotrophic index (AXP/CHL-A)

VARIABLE	N	MEAN	STD DEV
FREEBAC	219	5041598.1735160	7040572.5699707
ATTBAC	219	17205.4794521	21453.4714610
TBAC	219	5058803.6529680	7045409.2209031
PC_ATT	219	2.4889947	4.4174227
FLOCFEC	219	8017.8082192	11349.8778533
PHYTO	219	5431.9634703	12516.9676135
NVPHYTO	112	918.7500000	1116.5851456
CYANO	219	1310.9589041	2641.9982174
DEPTH	217	15.8027650	16.9150490
TEMP	147	21.1948980	3.9925122
NO3	83	4.1659036	5.2491934
SAL	143	34.1124182	2.1497517
CHL_A	187	0.5701632	0.6528446
PHEO_A	112	1.4439040	2.4625830
ATP	144	0.5842549	0.3754986
AXP	144	1.6820093	1.1388165
EC	144	0.5741583	0.1179670
HPINDEX	140	32.3308069	75.9859062

Table 2. Combined data from cruises E3B-79, FRNFLX I, and BLUE FIN 10-80. Pearson correlation coefficients, probability, and number of observations. Variables are the same as in Table 1.

EC

PHYTO	TBAC	FREEBAC	CYANO	NVPHYTO	PC_ATT	FLOCFEC	ATTBAC
-0.13931	-0.12654	-0.12652	0.08715	-0.06557	0.06438	0.05319	-0.03309
0.0958	0.1307	0.1308	0.2989	0.5897	0.4433	0.5266	0.6938
144	144	144	144	70	144	144	144

HP INDEX

TBAC	FREEBAC	FLOCFEC	PHYTO	NVPHYTO	PC_ATT	ATTBAC	CYANO
-0.36020	-0.36025	0.25969	-0.19824	0.16404	0.11562	-0.08325	-0.02044
0.0001	0.0001	0.0043	0.0189	0.1748	0.1737	0.3281	0.8106
140	140	140	140	70	140	140	140

FREEBAC

FREEBAC	TBAC	PC_ATT	NVPHYTO	ATTBAC	PHYTO	FLOCFEC	CYANO
1.00000	1.00000	-0.35296	-0.31544	0.22400	-0.17552	-0.10428	-0.10385
0.0000	0.0001	0.0001	0.0007	0.0008	0.0092	0.1239	0.1255
219	219	219	112	219	219	219	219

ATTBAC

ATTBAC	PC_ATT	TBAC	FREEBAC	PHYTO	CYANO	FLOCFEC	NVPHYTO
1.00000	0.60635	0.22689	0.22400	0.16339	0.05177	0.05015	-0.03091
0.0000	0.0001	0.0007	0.0008	0.0155	0.4459	0.4603	0.7463
219	219	219	219	219	219	219	112

TBAC

TBAC	FREEBAC	PC_ATT	NVPHYTO	ATTBAC	PHYTO	FLOCFEC	CYANO
1.00000	1.00000	-0.35089	-0.31533	0.22689	-0.17491	-0.10406	-0.10362
0.0000	0.0001	0.0001	0.0007	0.0007	0.0095	0.1247	0.1263
219	219	219	112	219	219	219	219

PC_ATT

PC_ATT	ATTBAC	FREEBAC	TBAC	NVPHYTO	FLOCFEC	CYANO	PHYTO
1.00000	0.60635	-0.35296	-0.35089	0.26315	0.09006	0.06231	0.05047
0.0000	0.0001	0.0001	0.0001	0.0051	0.1842	0.3549	0.4574
219	219	219	219	112	219	219	219

FLOCFEC

FLOCFEC	CYANO	NVPHYTO	FREEBAC	TBAC	PHYTO	PC_ATT	ATTBAC
1.00000	0.21200	0.17622	-0.10428	-0.10406	0.10029	0.09006	0.05015
0.0000	0.0016	0.0631	0.1239	0.1247	0.1390	0.1842	0.4603
219	219	112	219	219	219	219	219

PHYTO

PHYTO	CYANO	FREEBAC	TBAC	ATTBAC	NVPHYTO	FLOCFEC	PC_ATT
1.00000	0.22673	-0.17552	-0.17491	0.16339	0.10183	0.10029	0.05047
0.0000	0.0007	0.0092	0.0095	0.0155	0.2854	0.1390	0.4574
219	219	219	219	219	112	219	219

NVPHYTO

NVPHYTO	FREEBAC	TBAC	PC_ATT	CYANO	FLOCFEC	PHYTO	ATTBAC
1.00000	-0.31544	-0.31533	0.26315	0.24430	0.17622	0.10183	-0.03091
0.0000	0.0007	0.0007	0.0051	0.0094	0.0631	0.2854	0.7463
112	112	112	112	112	112	112	112

CYANO

CYANO	NVPHYTO	PHYTO	FLOCFEC	FREEBAC	TBAC	PC_ATT	ATTBAC
1.00000	0.24430	0.22673	0.21200	-0.10385	-0.10362	0.06231	0.05177
0.0000	0.0094	0.0007	0.0016	0.1255	0.1263	0.3549	0.4459
219	112	219	219	219	219	219	219

Table 3. Combined data from cruises E38-79, FRNFLX I, and BLUE FIN 10-80. Spearman correlation coefficients, probability, and number of observations. Variables are the same as in Table 1.

CHL_A

TBAC	FREEBAC	ATTBAC	PC_ATT	PHYTO	NVPHYTO	FLOCFEC	CYANO
0.82561	0.82423	0.41450	-0.38130	0.31780	-0.26495	-0.11626	-0.05448
0.0001	0.0001	0.0001	0.0001	0.0001	0.0048	0.1070	0.4589
187	187	187	187	187	112	187	187

PHEO_A

FREEBAC	TBAC	PHYTO	PC_ATT	FLOCFEC	CYANO	ATTBAC	NVPHYTO
0.45770	0.45770	-0.34505	-0.27458	-0.20200	0.15823	0.12085	-0.11217
0.0001	0.0001	0.0002	0.0034	0.0327	0.0956	0.2043	0.2390
112	112	112	112	112	112	112	112

ATP

FREEBAC	TBAC	PC_ATT	ATTBAC	PHYTO	FLOCFEC	CYANO	NVPHYTO
0.72265	0.72020	-0.40858	0.31902	0.26057	-0.21181	-0.09541	0.00977
0.0001	0.0001	0.0001	0.0001	0.0016	0.0108	0.2553	0.9360
144	144	144	144	144	144	144	70

AXP

TBAC	FREEBAC	ATTBAC	PC_ATT	PHYTO	FLOCFEC	NVPHYTO	CYANO
0.81254	0.81156	0.44682	-0.39738	0.33085	-0.12792	0.11949	-0.09321
0.0001	0.0001	0.0001	0.0001	0.0001	0.1265	0.3245	0.2665
144	144	144	144	144	144	70	144

EC

TBAC	FREEBAC	PC_ATT	ATTBAC	NVPHYTO	FLOCFEC	CYANO	PHYTO
-0.28933	-0.28223	0.13404	-0.13295	0.06050	0.03182	0.00556	0.00205
0.0004	0.0006	0.1076	0.1122	0.6188	0.7050	0.9473	0.9806
144	144	144	144	70	144	144	144

HPINDEX

TBAC	FREEBAC	PC_ATT	PHYTO	ATTBAC	FLOCFEC	CYANO	NVPHYTO
-0.55605	-0.54721	0.37529	-0.35000	-0.25931	0.18737	0.14838	0.05602
0.0001	0.0001	0.0001	0.0001	0.0020	0.0266	0.0802	0.6451
140	140	140	140	140	140	140	70

FREEBAC

FREEBAC	TBAC	PC_ATT	ATTBAC	NVPHYTO	PHYTO	FLOCFEC	CYANO
1.00000	0.99834	-0.44722	0.44333	-0.34440	0.25927	-0.11135	0.01432
0.0000	0.0001	0.0001	0.0001	0.0002	0.0001	0.1003	0.8331
219	219	219	219	112	219	219	219

ATTBAC

ATTBAC	TBAC	FREEBAC	PC_ATT	PHYTO	FLOCFEC	CYANO	NVPHYTO
1.00000	0.47215	0.44333	0.43655	0.41727	0.34412	0.18578	0.05200
0.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0058	0.5861
219	219	219	219	219	219	219	112

TBAC

TBAC	FREEBAC	ATTBAC	PC_ATT	NVPHYTO	PHYTO	FLOCFEC	CYANO
1.00000	0.99834	0.47215	-0.42501	-0.34277	0.27032	-0.10565	0.01808
0.0000	0.0001	0.0001	0.0001	0.0002	0.0001	0.1191	0.7905
219	219	219	219	112	219	219	219

Table 3, continued.

PC_ATT

PC_ATT	FLOCFEC	FREEBAC	ATTBAC	TBAC	NVPHYTO	CYANO	PHYTO
1.00000	0.51020	-0.44722	0.43655	-0.42501	0.40740	0.24680	0.19642
0.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0035
219	219	219	219	219	112	219	219

FLOCFEC

FLOCFEC	PC_ATT	NVPHYTO	ATTBAC	PHYTO	CYANO	FREEBAC	TBAC
1.00000	0.51020	0.44522	0.34412	0.33964	0.29946	-0.11135	-0.10563
0.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.1003	0.1191
219	219	112	219	219	219	219	219

PHYTO

PHYTO	ATTBAC	NVPHYTO	FLOCFEC	TBAC	FREEBAC	CYANO	PC_ATT
1.00000	0.41727	0.37989	0.33964	0.27032	0.25927	0.24953	0.19642
0.0000	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002	0.0035
219	219	112	219	219	219	219	219

NVPHYTO

NVPHYTO	FLOCFEC	PC_ATT	PHYTO	CYANO	FREEBAC	TBAC	ATTBAC
1.00000	0.44522	0.40740	0.37989	0.35967	-0.34440	-0.34277	0.05200
0.0000	0.0001	0.0001	0.0001	0.0001	0.0002	0.0002	0.5861
112	112	112	112	112	112	112	112

CYANO

CYANO	NVPHYTO	FLOCFEC	PHYTO	PC_ATT	ATTBAC	TBAC	FREEBAC
1.00000	0.35967	0.29946	0.24953	0.24680	0.18578	0.01806	0.01432
0.0000	0.0001	0.0001	0.0002	0.0002	0.0058	0.7905	0.8331
219	112	219	219	219	219	219	219

DEPTH

FREEBAC	TBAC	PC_ATT	NVPHYTO	CYANO	PHYTO	FLOCFEC	ATTBAC
-0.35347	-0.34457	0.27365	-0.11506	0.02951	-0.01347	0.01137	0.00101
0.0001	0.0001	0.0001	0.2270	0.6655	0.8436	0.8678	0.9882
217	217	217	112	217	217	217	217

TEMP

PHYTO	FLOCFEC	TBAC	NVPHYTO	ATTBAC	FREEBAC	PC_ATT	CYANO
-0.31481	0.20409	-0.15507	-0.15403	-0.15314	-0.14637	0.10550	0.03617
0.0001	0.0132	0.0607	0.2064	0.0640	0.0769	0.2035	0.6636
147	147	147	69	147	147	147	147

NO3

PHYTO	FREEBAC	TBAC	PC_ATT	ATTBAC	FLOCFEC	CYANO	NVPHYTO
0.31536	-0.31351	-0.26754	0.21852	0.12323	0.03816	-0.03788	0.00000
0.0037	0.0039	0.0145	0.0472	0.2670	0.7320	0.7334	
83	83	83	83	83	83	83	0

SAL

TBAC	FREEBAC	ATTBAC	PC_ATT	PHYTO	NVPHYTO	FLOCFEC	CYANO
-0.78632	-0.78009	-0.38364	0.37792	-0.23269	-0.15468	0.11901	0.06362
0.0001	0.0001	0.0001	0.0001	0.0052	0.2044	0.1569	0.4503
143	143	143	143	143	69	143	143

in both space and time. Because water is usually moving laterally along the shelf as well as in and out over the shelf break, it is not possible to follow events in exactly the same body of water through time. Doing so would require three-dimensional mapping on a local scale, and would require more personnel and ship time than we have at our disposal.

1.4. Analysis of Microbiological Methods

On several cruises over the past three years we have done microscopic counts of bacteria as well as the more rapid biochemical analyses which are much less costly in processing time. Because there appears to be no perfect biochemical indicator of bacterial numbers or biomass at this time, we have done a partial correlation analysis of data from three cruises, EASTWARD E3B-79, FRNFLX I (GILLISS and BLUE FIN), and BLUE FIN 10-80. These data cover the entire width of the continental shelf, from the nearshore front to the Gulf Stream (Table 1). While this approach is a relative one, and one which says nothing about causation, it gives some indication of the interpretation which can be placed on the biochemical parameters of microbial abundance that we are using.

Correlation of total bacteria and free bacteria with ATP concentration was 0.72, while correlation of total and free bacteria with total adenylates was 0.82 (Table 3). Thus, the analysis of total adenylates does offer some improvement over ATP alone, but perhaps not one that is commensurate with the additional costs and processing time involved.

Chlorophyll has a rather high correlation (0.60) with both free and total bacteria, suggesting that although the peak densities of the two populations, phytoplankton and bacteria, appear to be offset in plots of oceanographic sections, bacteria do occur where phytoplankton (as indicated by chlorophyll concentration) are most abundant. This is what one would expect. However, it unfortunately tends to nullify any attemp to seek correlations of chlorophyll with ATP or adenylates, since bacteria and phytoplankton converge in our data set. Therefore, we must continue to fall back on the use of the HP index (Campbell et al. 1979) to separate phytoplankton dominance from microbial heterotroph dominance in individual samples. It is also worth noting that the distribution of bacteria counts is highly non-random (variance >> mean) while that of ATP and adenylates approaches a Poisson distribution. Therefore, some of the apparent increased precision offered by microscopic counting cannot be

realized because of the statistical problems of dealing with a negative binomial distribution.

1.5 GABEX II

In August, 1981, two vessesl, R/V CAPE FLORIDA and R/V CAPE HENLOPEN, were used simultaneously to follow intrusion processes on the outer continental shelf off northern Florida and Georgia. This was a summer of intense intrusion activity, with extensive stranded intrusions on the shelf throughout the summer (G.-A Paffenhöfer, pers. comm.). Although the data are not fully processed, we have found that bacterial populations are an order of magnitude larger than usual. This appears to be a response to the sustained high primary and secondary production associated with the large stranded intrusions. This tends to support the hypothesis that bacteria are substrate-limited and relatively less influenced by grazing. If we can indeed show that this is the case, then bacteria are useful indicator organisms on the continental shelf, responding to changing physical and biological regimes with little influence by grazers. We are still processing samples from GABEX II, and the full results will be available at a later time.

1.6 GABEX I

In April, 1980 R/V ISELIN and R/V EASTWARD were used concurrently to study mesoscale events on the outer continental shelf between Cape Canaveral and Savannah, Georgia. This was supported by overflights of remote-sensing aircraft (a NASA U-2 high altitude flight and Coast Guard low-altitude flights) and by deployment of an extensive array of current meters by T.N. Lee. The two-ship plan gave continuous coverage of the Georgia and Florida continental shelf while permitting flexibility to follow intrusion events as they occurred. R/V EASTWARD maintained a regular cruise track, repeatedly sampling along a series of transects from Savannah to St. Augustine. R/V ISELIN began by covering the outer portion of the grid out of phase with the EASTWARD, but as the three-week study progressed ISELIN turned to more specialized mapping and intrusion hunting. Our program had three persons on EASTWARD and one on ISELIN. This was the maximum space available to us. One of the inevitable consequences of cooperative studies is the limitation on the size of each of the research teams. As a result, we were unable to sample all transects continuously as we would have liked. We concentrated on the St.

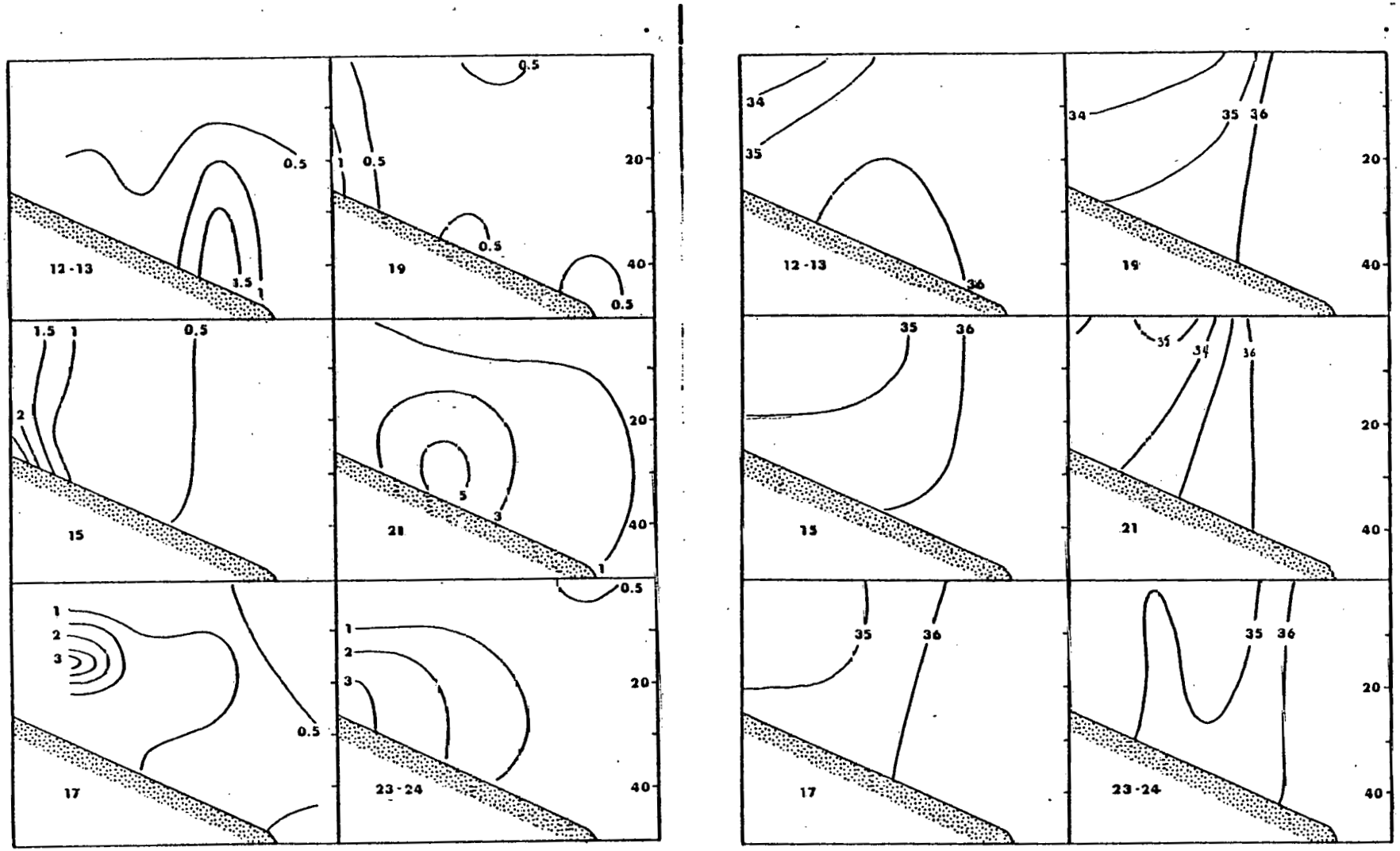

Figure 1. GABEX I. April 12-24, 1979. St. Augustine section. Distribution of chlorophyll a (left) and salinity (right).

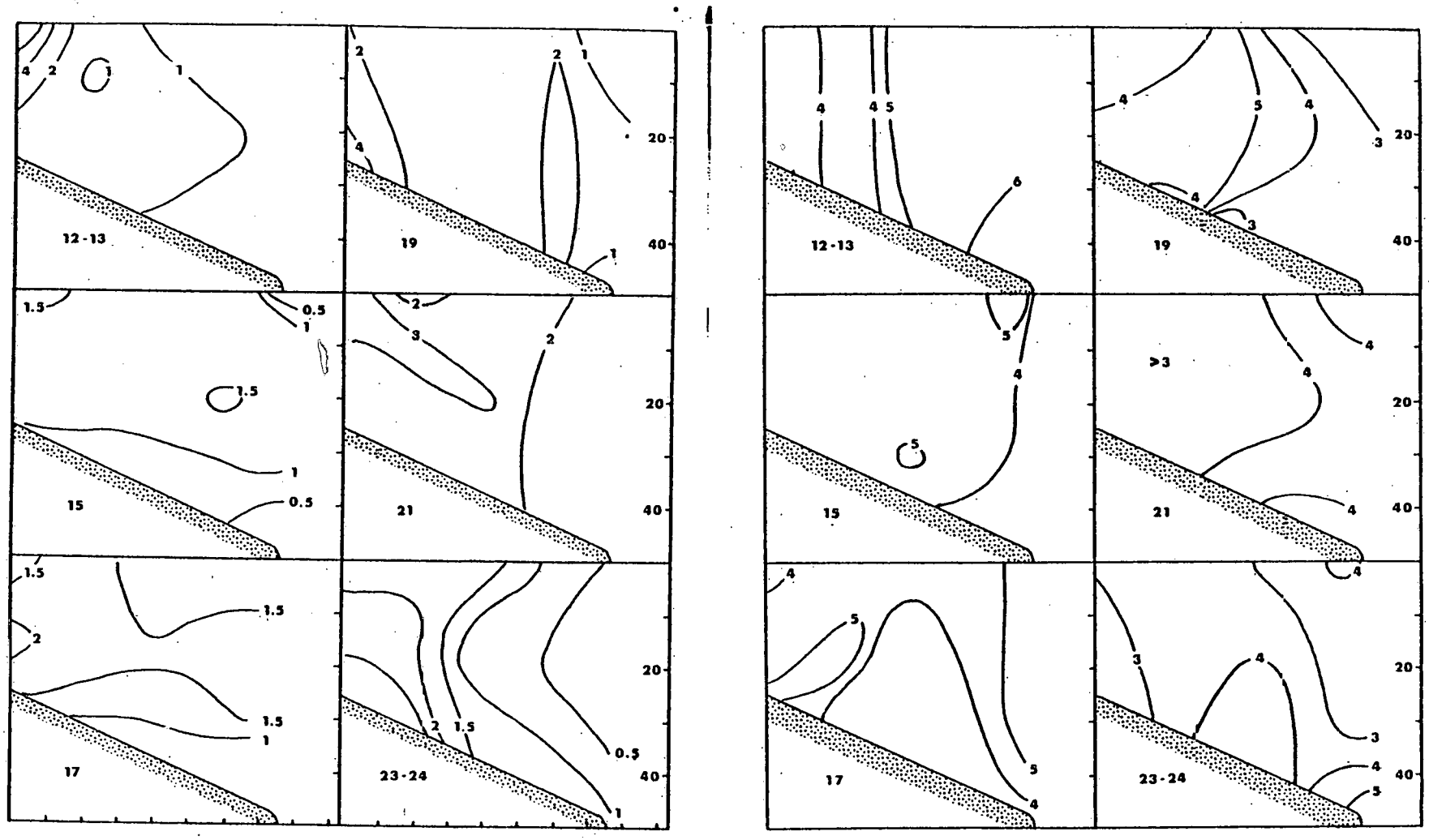

Figure 2. GABEX I. April 12-24, 1979. St. Augustine section. Distribution of total adenylates, micrograms/liter (left) and adenylate energy charge ratio (right).

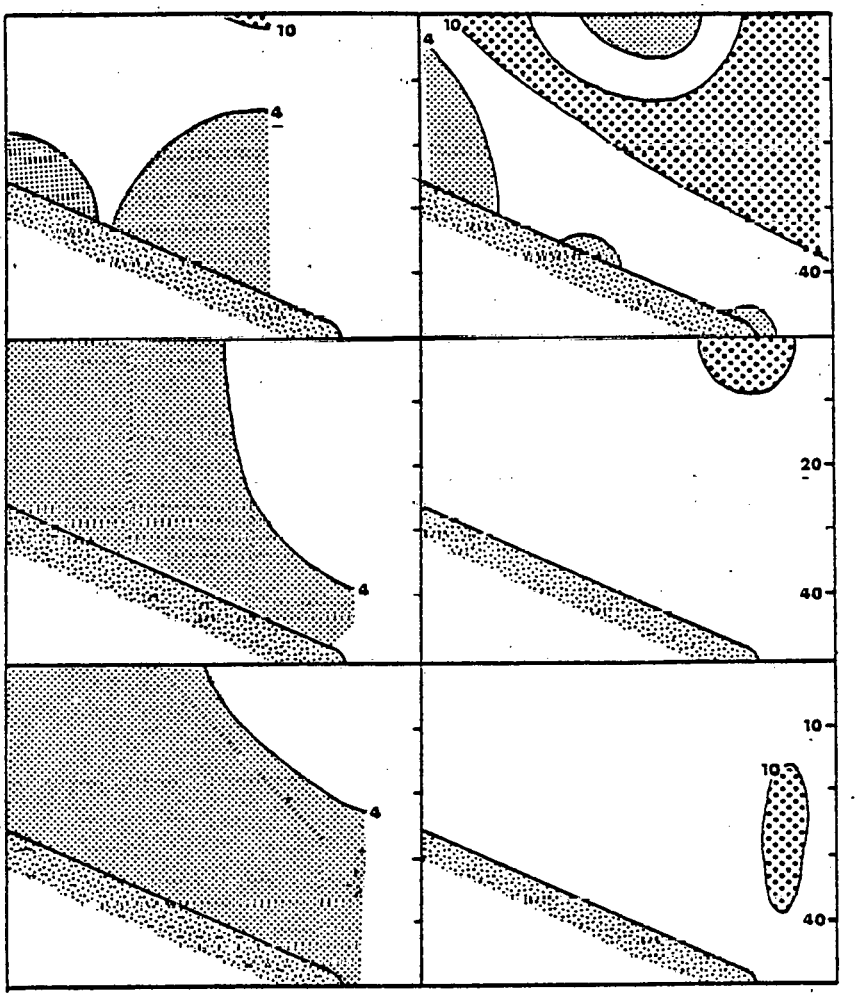

Figure 3. GABEX I. April 12-24, 1979. St. Augustine section. Total adenylate/chlorophyll a ratio.

Augustine transect, because experience had shown this to be a region of frequent intrusions from the west wall of the Gulf Stream.

April, 1980, proved to be an unusual period of runoff of freshwater from the coast, with 34 $^{o}/_{oo}$ salinity extending far out across the shelf at times. Intrusion activity was weak, without the appearance of prominent eddies, but it was essentially continuous through the period, with some nutrient-rich water on the outer continental shelf much of the time. This is indicated by the presence of lenses of 18° water at the bottom on the outer continental shelf during most of the period. The development of the phytoplankton bloom on the outer continental shelf in April, 1980 was unusually extensive and was not limited to the immediate vicinity of the intrusions (Figure 1). On several occasions the bloom extended toward shore beyond the region of the outer shelf that we sampled, and it extended to the surface, or very nearly so in some cases. Day-to-day variation along the section was great, as it usually is on the outer shelf, because water is moving through the section as well as back and forth along it. For example, the absence of bloom conditions in the 19 April section should not be taken to indicate that the bloom seen on the previous section two days earlier had been dispersed or eaten by zooplankton.

The A/C index (Campbell et al. 1979) shows autotrophic dominance of the microbial community over most of the shelf waters, including all bloom areas, with small patches of heterotrophic dominance outside the bloom areas (Figure 3). The exception is April 19, when heterotrophic dominance is ubiquitous. The extent of autotrophic dominance is in sharp contrast to typical intrusion situations uncomplicated by fresh-water runoff. Compare, for example the HP index distribution of April 1980 with the of April 20-21.

1.7 BLUE FIN Cruises, 1980

Additional sections of the continental shelf off northern Florida and Georgia were run on cruises of R/V BLUE FIN. With the possible exception of a region of high chlorophyll off Fernanadina, October and December, 1980, no Gulf Stream events were present at those times, but the presence of somewhat brackish (34 °/oo) coastal water was evident on both occasions. According to Blanton's (JPO ms.) model, this coastal water is held in place by bouyancy in the absence of sufficient tidal and wind stress to mix it with shelf water. Further, Blanton postulates that this water tends to move southward along the

coast, turning offshore across the shelf off Florida. This may be what we see in these, as well as some earlier sections of the shelf (cf. Pomeroy et al., ms. in this report) off St. Augustine, where there is a consistent bulge in chlorophyll, somethimes associated with enhanced ATP or reduced salinity. Probably some other process is at work in the St. Augustine region, in addition to the southward movement of coastal water which we do not yet understand. This is a region where the turbid, low-salinity coastal water generated off Georgia mixes with clear, high-salinity Florida shelf water. This mixing may produce conditions favorable for phytoplankton production and associated secondary production. In any case this is one of the most consistent local features we have found.

1.8 EASTWARD'Cruise E3B-79

The EASTWARD cruise of August 7-14, 1979 (Brunswick, Ga. to Beaufort, N.C.) was focused on microbial processes in an intrusion off St. Augustine. This was a situation in which weak intrusions were coming with a high frequency. An intrusion which was located immediately upon arrival at the shelf break off St. Augustine, was already near its maximal advance over the shelf, with 15° water at least 20 km west of the shelf break. Nitrate concentrations of 5 µg atoms N/l extended along the bottom to the west of our section A midwater chlorophyll maximum above the thermocline or in it remained throughout the three days of observation. Counts of bacteria were done on every sample taken on the section for three days. Included in the enumerations were free minibacteria, large attached bacteria, flocculent aggregates, chroococcoid cyanobacteria, and phytoplankters. Three manuscripts based on the results of this cruise are included as parts of this report (Hodson et al., Pomeroy et al., and Jacobsen et al.), and the results are discussed further in section 3.2. If one can speak of a typical intrusion, this example approaches it, based on our experience to date. Although the bloom of both phytoplankton and bacteria was still active when ship scheduling forced us to depart at the end of the third day, there was evidence that the intrusion was relaxing, and probably the whole thing went over the shelf break on the fourth day.

1.9 R/V GILLISS cruise, April 19-May 2, 1979

An intrusion at the shelf break due east of Jacksonville, Florida was followed for four days by running daily sections at 30° 20' N as the intrusion

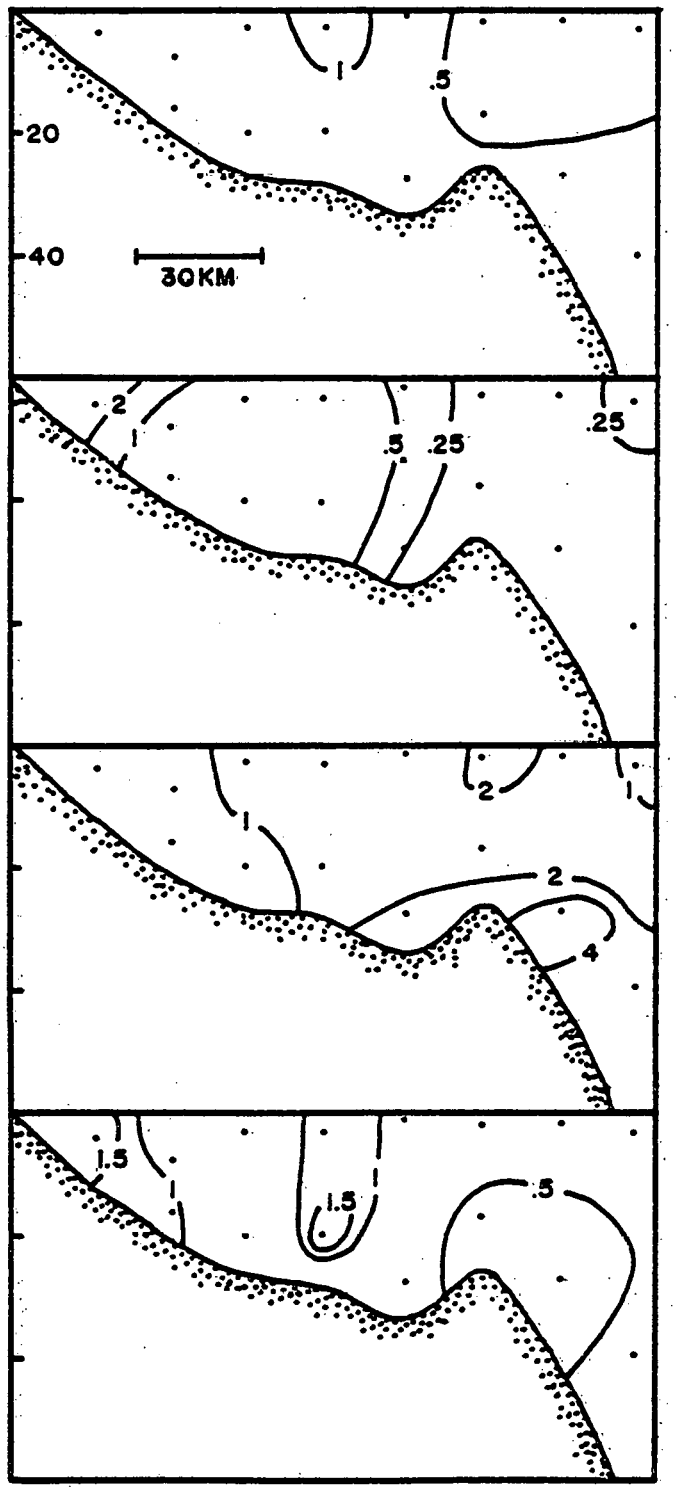

Figure 4. BLUE FIN Cruise, October, 1980. Fernandina section. From top to bottom: ATP, micrograms/liter; chlorophyll a, micrograms/liter; ATP/chlorophyll; free bacteria x 10^6/ ml.

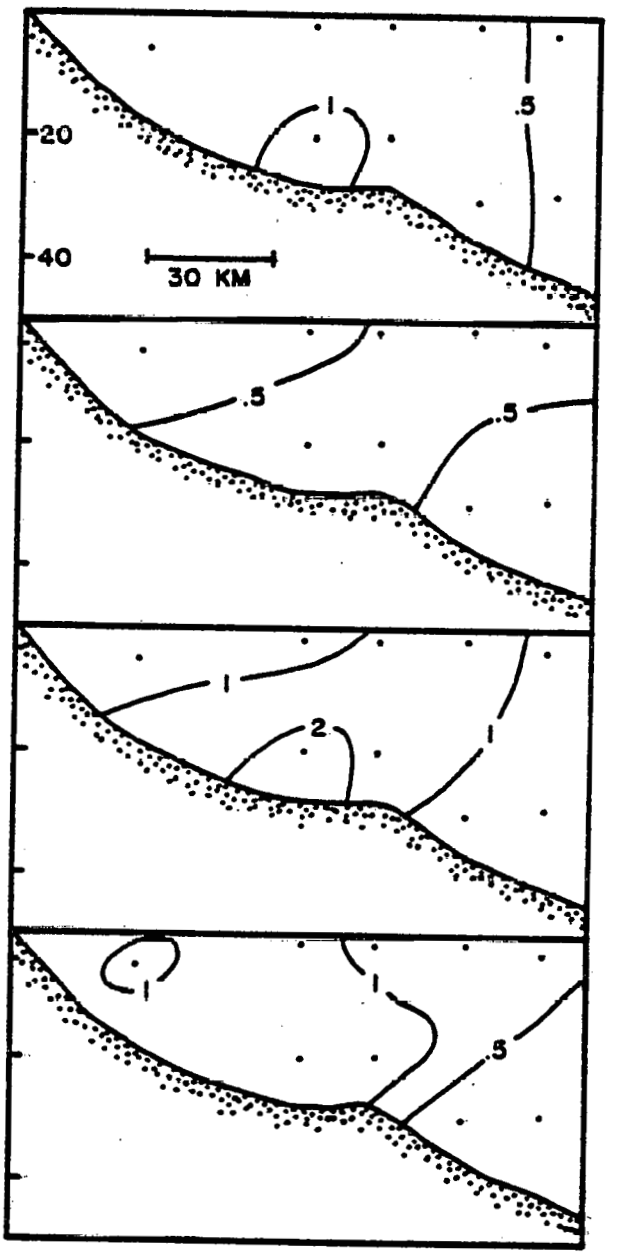

Figure 5 BLUE FIN Cruise, October 1980. St. Andrews section. From top to bottom: ATP micrograms/liter); chlorophyll a (micrograms/liter); ATP/chlorophyll; free bacteria x 10^6/ ml.

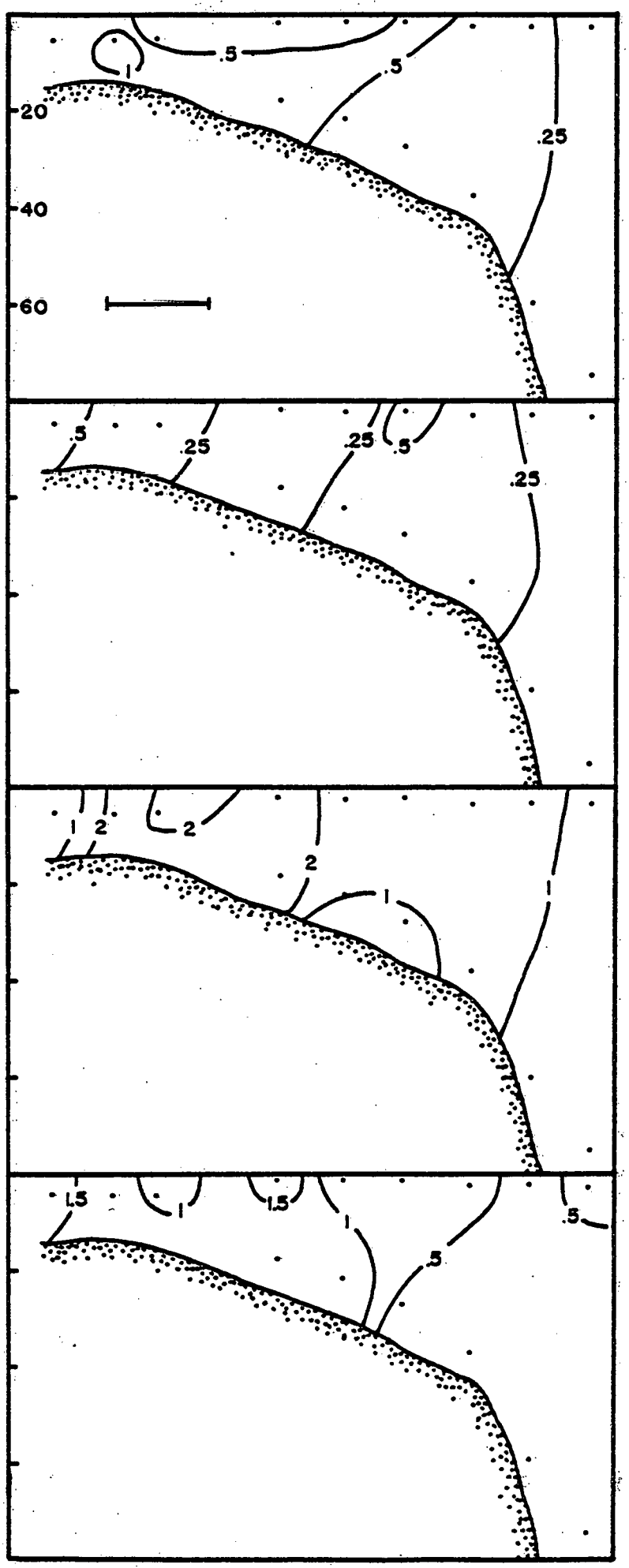

Figure 6. BLUE FIN Cruise, October, 1980. Wassaw section. From top to bottom: salinity; ATP (micrograms/liter); chlorophyll a (micrograms/liter); ATP/chlorophyll; free bacteria x 10^6 per liter.

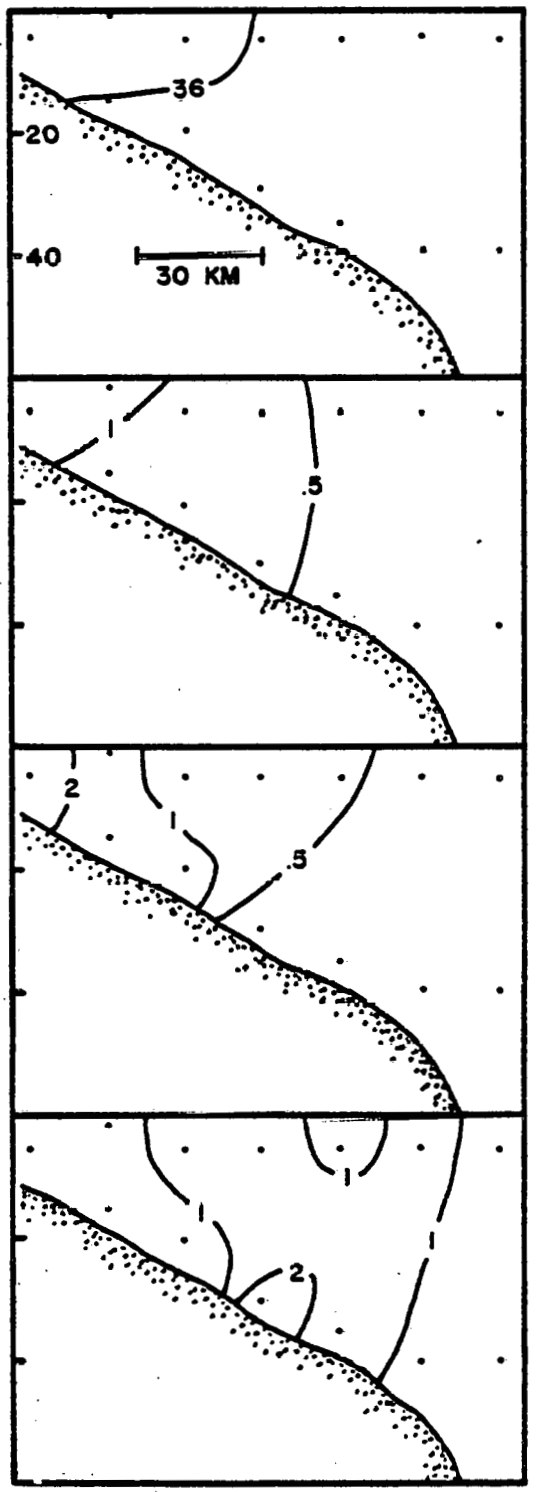

Figure 7. BLUE FIN Cruise, December, 1980. St. Augustine section. From top to bottom: salinity; ATP (micrograms/liter; chlorophyll a (micrograms/liter); ATP/chlorophyll.

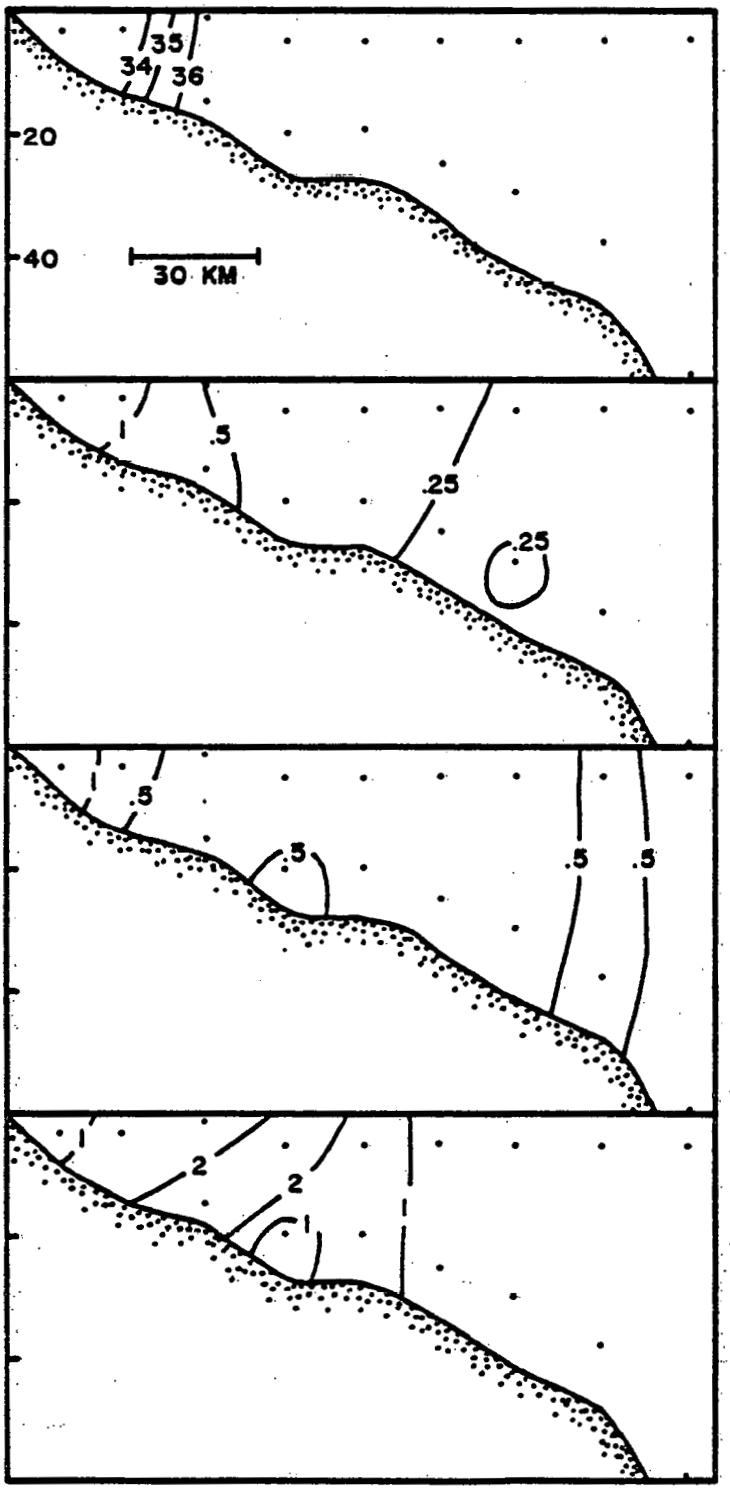

Figure 8. BLUE FIN Cruise, December 1980. St. Andrews section. From top to bottom: salinity; ATP (micrograms/liter); chlorophyll a (micrograms/liter); ATP/chlorophyll.

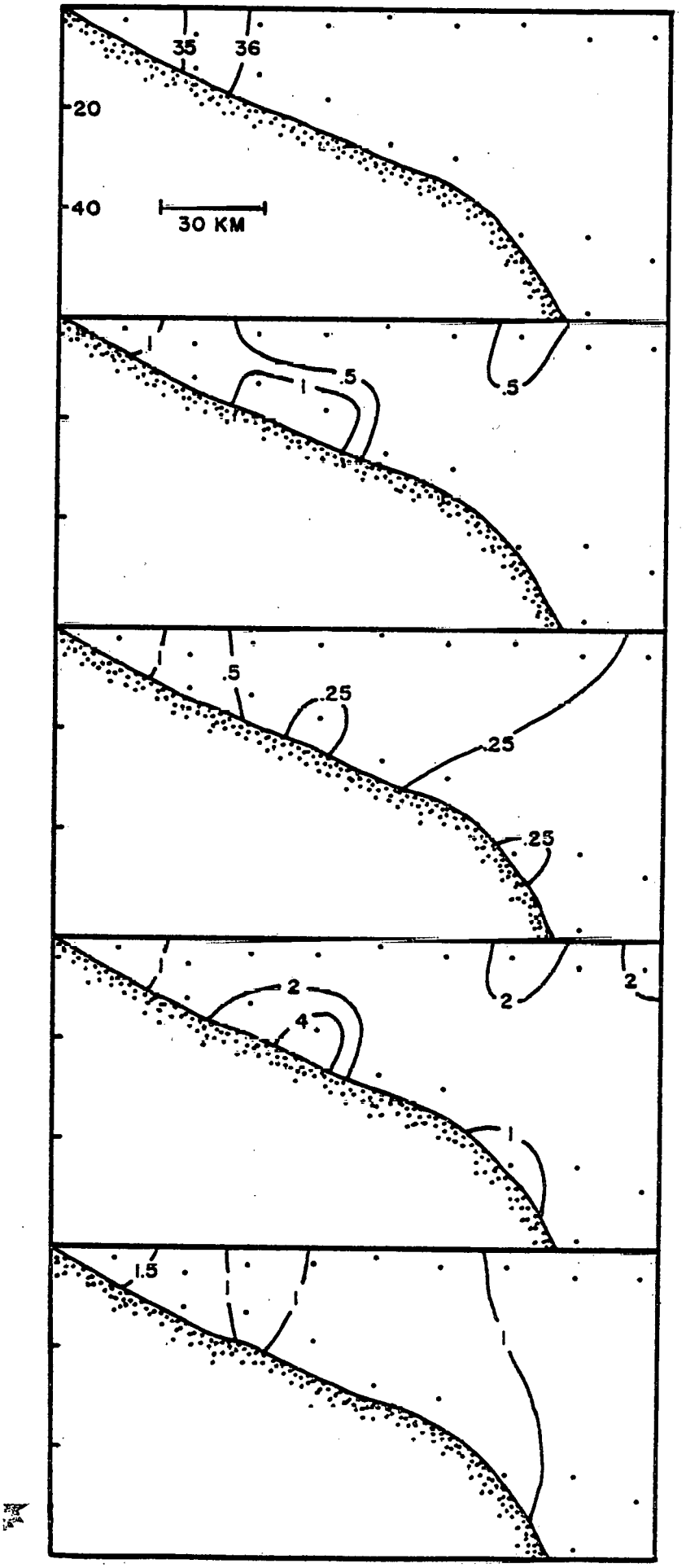

Figure 9. BLUE FIN Cruise, December, 1980. Wassaw section. From top to bottom: ATP (micrograms/liter); chlorophyll a (micrograms/liter); ATP/chlorophyll; free bacteria x 10^6/ml.

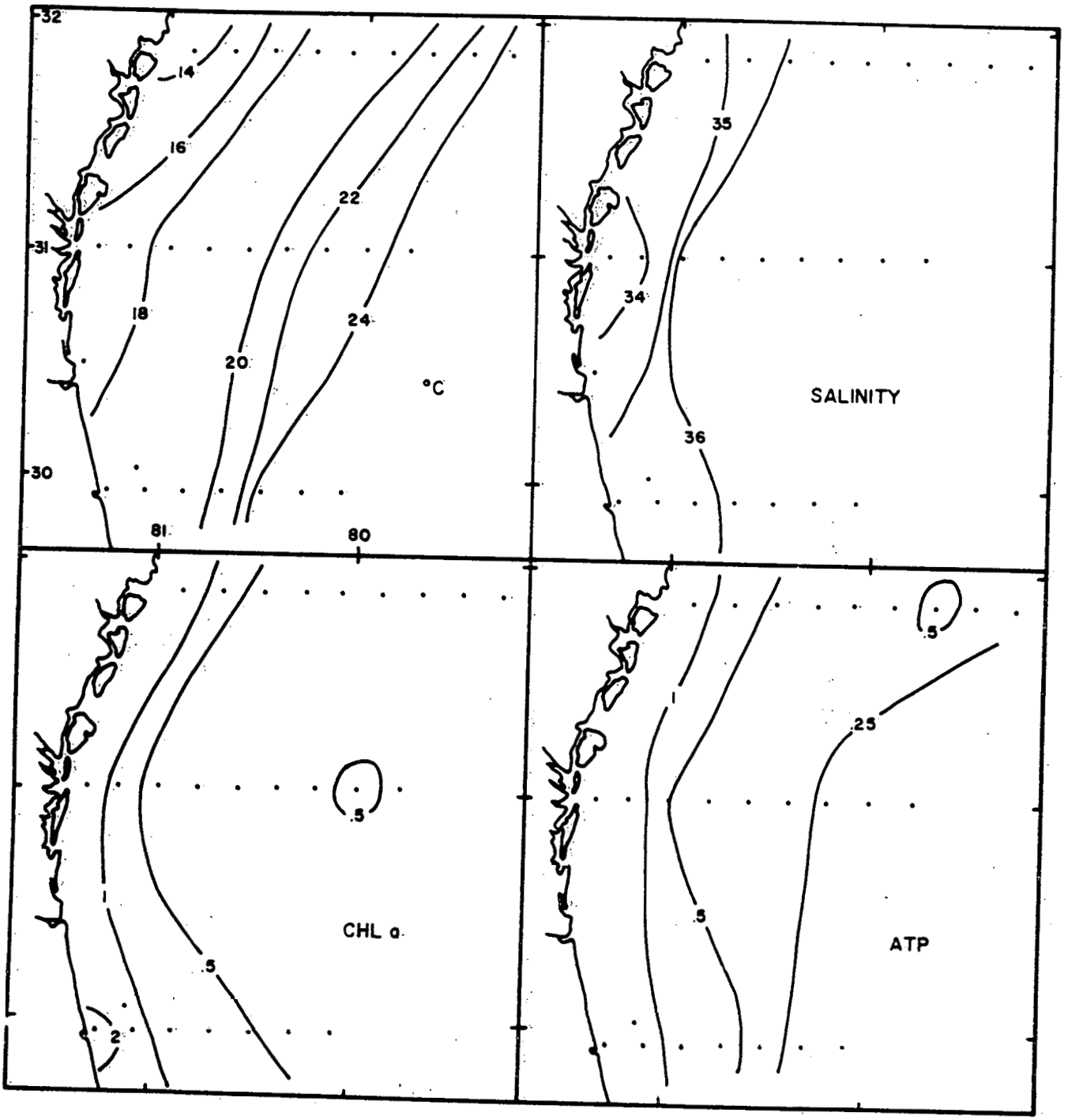

Figure 10. Results of R/V BLUE FIN cruise, October, 1980. Free bacteria are 10^5/ ml. Chlorophyll a and ATP are micrograms/liter.

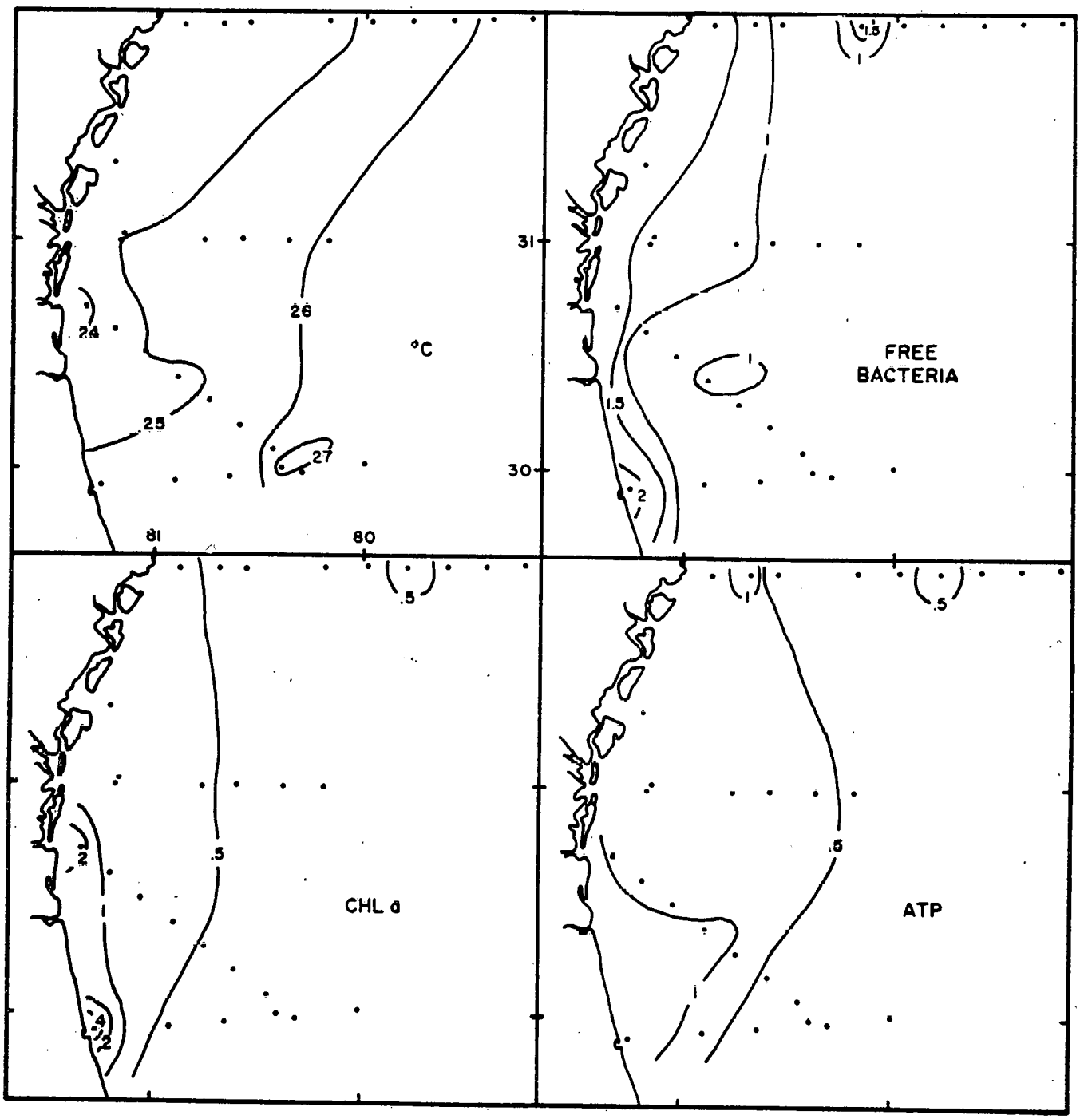

Figure 11. Results of R/V. BLUE FIN cruise, December, 1980. Chlorophyll a and ATP are micrograms/liter.

event moved north ward through the position. This was a cooperative study involving Atkinson, Paffenhöfer, and Yoder. Physical and chemical data reported here were collected by Atkinson's group. The results are included in the manuscript, Pomeroy et al., which is a part of this report.

1.10 R/V ISELIN Cruise April 25-24, 1978

This was the first combined cooperative cruise by the intrusion research group, in which physical, chemical, and biological oceanographers combined efforts on the study of a single intrusion event. Using satellite imagery, an intrusion was located east of Brunswick, Georgia, and a section was run on two successive days. The first day was that of maximal intrusion, and the water was moving over the shelf break on the second day. This was the first use of our concept of the heterotrophic-photoautotrophic index (HP index) as a qualitative indicator of the relative dominance of autotrophs and heterotrophs in water samples. Data from this cruise, together with a description of the HP index and the rationale behind it are published in the paper by Campbell et al. (1979) which is a part of this report.

While this cruise was short, and only two consecutive sections were run through an intrusion, it was important in establishing a research strategy which has proven very successful for us. This involves the cooperative use of a ship by physical, chemical, and biological contractors, satellite data to locate the Gulf Stream and eddies on the west wall (at least in seasons when temperature differences make this possible), and running daily hydrographic sections at an establsihed latitude. Each station is sampled only once, using an STD and rosette sampler, with all scientists using water from the single cast.

1.11 R/V ISELIN Cruise August 3-10, 1978.

This was a cooperative cruise whose purpose was to follow a stranded intrusion. August is the most favorable time for stranding of intrusions, and indeed one was found off Fernandina which we were able to follow over several days. Unfortunately, it is difficult to set up a two-dimensional sampling regime, such as that used for intrusions at the shelf break, because the movement of stranded intrusions is slow and unpredictable. Moreover, they lie on the bottom, so there are no surface clues. Satellite imagery does not help. We have since developed a towed subsurface thermistor, but at that time

the only approach was many STD or XBT casts. Attempts to follow a specific water body with a drogue were of limited success, apparently because of the shear between the intrusion and the overlying shelf water. Therefore, continuity of sampling was not satisfactory.

From the point of view of the DOE mission, this was a useful experience in that it gave us good insight into water movement on the continental shelf. Movement is comparatively slow in the absence of forcing from the Gulf Stream, but when stratification is present surface and bottom waters may not be moving together.

2. Coastal Fronts

Density fronts are a feature of the ocean which has long been recognized. The importance and ubiquity of mesoscale density fronts has been appreciated relatively recently (Savidge and Foster 1978; Floodgate et al. 1981). The water of the inner continental shelf of the southeastern U.S. is well mixed vertically, but it is segmented horizontally by a number of density fronts. The fronts are often prominent on satellite imagery. They can usually be seen from the bridge of a ship as well. This is because the water on the coastal side of a front is typically more turbid. The fact that there are significant density differences across the fronts is demonstrated by the presence of a convergence at the front, marked by a foam line. Forming in the first 5-10 km of the ocean, fronts influence the flux of materials, as well as water itself, across the inner continental shelf (Blanton, JPO ms.; Blanton 1980; Blanton and Atkinson 1978). To evaluate that flux and its chemical and biological impact, a series of cooperative studies were undertaken which were planned and coordinated by J.O. Blanton. The first, in 1977, was an anchor station in Wassaw Inlet to evaluate transport through the frontal zone. Two persons from this research group participated in the collection of data. In 1978 two vessels, R/V KIT JONES and R/V BLUE FIN, were anchored in the Wassaw Inlet area. Four persons from our research group participated. In 1979 two vessels, R/V BLUE FIN and R/V ISELIN, were anchored in the Wassaw Inlet area. Five persons from our research group participated. In April, 1981 R/V BLUE FIN and R/V CAPE HENLOPEN were anchored off Wassaw inlet. Seven persons from our research group participated. The latter three studies are described in the following sections. It must be emphasized that these studies, at least

from our point of view, are preliminary in nature. The objective is as much to define the system as to actually measure it. For this reason we have tried many things, sometimes collecting only partial data sets, to ascertain what measurements will be useful.

2.1. Cooperative Front Study, May 9-12, 1978

Participants in the study included J.O. Blanton, H. Windom, and D. Hayes. The overall objective of the project was to measure water flux through the region of the coastal front off Wassaw Inlet. The primary objective of all participants was to evaluate various tracers of water movement, including tritium (Hayes), trace metals (Windom), and such biological parameters as chlorophyll, pheophytin, and adenylates (Pomeroy). BLUE FIN and KIT JONES were anchored for four tidal cycles in positions which we hoped would provide sequential sampling of the Savannah River plume. The positions and a summary of the physical data are given by Blanton (1979). Measurements of active chlorophyll a, ATP, and total adenylates were made at several depths every two hours. Sample depths were based on temperature and salinity profiles from a CTD cast to place the water samplers in distinct upper and lower water layers associated with the frontal convergence. In addition, in vivo fluorescence was measured with a Turner Designs fluorometer equipped with a submersible pumping system.

Plots of in vivo fluorescence against time (Figure 13) suggest that the tow vessels were seeing different water bodies, and the physical data support this. Location A was in the Savannah River plume, while location B was in the Ogeechee River plume as well as frontal and nearshore circulation. The dif ference between fluorescence of in vivo "chlorophyll" and active chlorophyll a measured by the method of Jacobsen (1978) in the time series (Figures 14 and 15) is striking. In vivo fluorescence at location A remained relatively constant at about 2.5 µg/l, while active chlorophyll a was only about 0.5 µg/l but with considerably more variation. The higher concentrations of active chlorophyll a in the bottom samples probably represent highly active phytoplankton which have recently been carried down in the frontal convergence.

While active chlorophyll a is demonstrably a superior parameter of phytoplankton biomass and is useful in understanding the movement of phytoplankton in the frontal zone, in vivo chlorophyll fluorescence was the more useful indicator of different water bodies in this instance. The difference between

in vivo chlorophyll fluorescence at location A and location B is one of the best indications that the two vessels were seeing different bodies of water.

2.2 FRNFLX I, November 6-9, 1979.

Two vessels were placed in Wassaw Inlet, one seaward of the other so water would move directly from one station to the other. Their distance was such that some of the same water would be sampled by both vessels. Replacement of the very small and cramped KIT JONES with R/V ISELIN made it possible to have more technical assistance, to undertake more elaborate kinds of measurements, and to set up the Zeiss epifluorescence microscope to look at living microbial samples during the anchor station. Rather than focusing on the identity of water bodies, our goal during this study was to evaluate a number of microbiological parameters which might be indicative of biomass or activity in the nearshore zone. Some of the parameters which had been most useful in our work on Gulf Stream intrusions proved to be of less interest in the nearshore zone, while others showed considerable promise for future studies. In several cases we took only partial data sets during the five tidal cycles of the study. This permitted us to examine more parameters. As in the offshore work, we were limited by the number of personnel the vessels could accomodate.

Absolute numbers of bacteria are nearly an order of magnitude higher in the nearshore zone than in the water of the outer continental shelf. The numbers vary relatively little. In this study the range was from 0.9 to 3 x 10^6/ml (Figure 17). Most of the bacteria are free-living. Those on particles are much fewer but they are more variable in number. Based on our experience on this and several other coastal cruises on R/V BLUE FIN, it is necessary to prepare a separate sample, in which more water is filtered, to get a statistically significant count of attached bacteria. There may in fact be some difficulty in getting enough material on the slide for a significant count without incurring a coincidence loss effect from clumping of particulate material.

The alternative approach to measuring microbial biomass, which we found to be quicker and equally precise on the outer continental shelf, is the measurement of ATP or total adenylates. In the nearshore zone we found that total adenylates varied little (as did counts). However, the H-P index shows that most of that biomass, most of the time, is phytoplankton (Figure 16). Therefore, adenylate measurements are telling us essentially the same thing as

chlorophyll a measurements in this water. Moreover, energy charge calculated from the adenylate measurements is in most cases that of the phytoplankton. We cannot ascertain the energy charge of the bacterial population in the presence of so many phytoplankton. For these reasons probably we will not measure adenylates in the future in studies of the nearshore zone in Georgia.

Chlorophyll a measurements showed more phytoplankton in the bottom layer than in the surface (Figure 17). Measurements of photosynthetic potential showed these phytoplankters near the bottom to have a higher potential than those near the surface, all being exposed to the same, moderate illumination from a fluorescent light source (Figure 17). Evidently the phytoplankton in the bottom water have recently been drawn down in the frontal convergence. However, something more than that has occurred to increase their photosynthetic potential. Enhanced nutrients seem unlikely in this nutrient-rich coastal water. Possibly there is an infusion of a different phytoplankton population from the other side of the front. While the measurement of photosynthetic potential produced some interesting and unexpected results, probably it is not an item of high priority for future work in the frontal zone.

Heterotrophic uptake of ^{14}C-labeled glucose showed as much variation as any parameter of microbial activity we investigated (Figure 17). It varied a full order of magnitude over the course of the three tidal cycles in which the measurements were done. This made heterotrophy a parameter of interest for future studies. Uptake of labeled glucose, however, may not be the best possible approach. It is not indicative of total uptake of organic matter, and probably it underestimates it by more than an order of magnitude (Wiebe and Smith 1979). While routine use of even an abbreviated measurement of uptake of a defined labeled compound is almost prohibitively time consuming for a fast-paced series of samples at an anchor station, the possibly more realistic approach of Smith and Wiebe (1978) of using labeled naturally occurring dissolved organic compounds (but chemically undefined) is even more time consuming. Perhaps the ideal parameter would be the measurement of respiration, although this would have a substantial phytoplankton bias. We shall consider doing that in the future, using Coolometric measurement of changes in total CO_2.

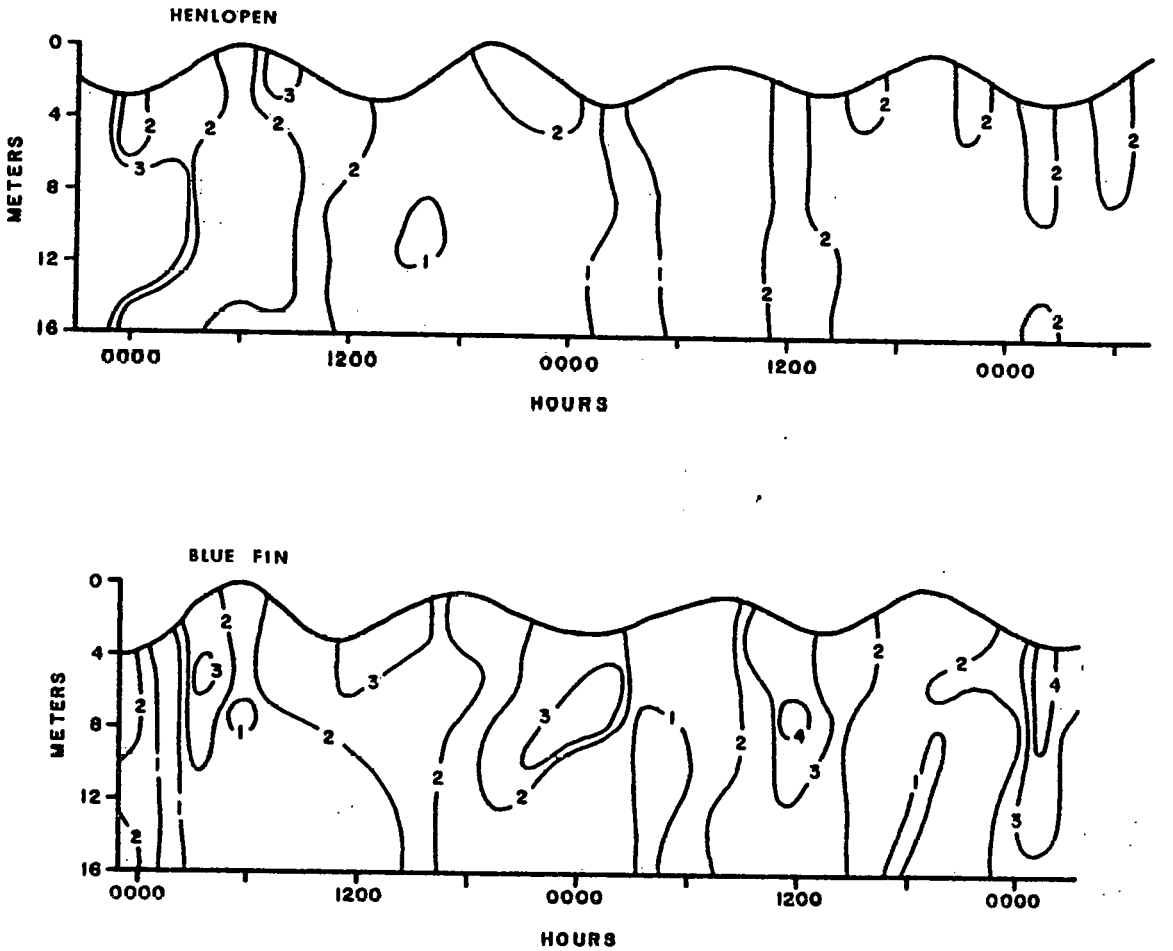

Figure 12. FRNFLX II. Heterotrophic uptake of glucose by microorganisms, 10^{-3} micrograms glucose·liter^{-1}·hour^{-1}. Activity collected on 0.2 micron Nuclepore filter corrected for sorption. R/V HENLOPEN was stationed offshore and R/V BLUE FIN was stationed inshore.

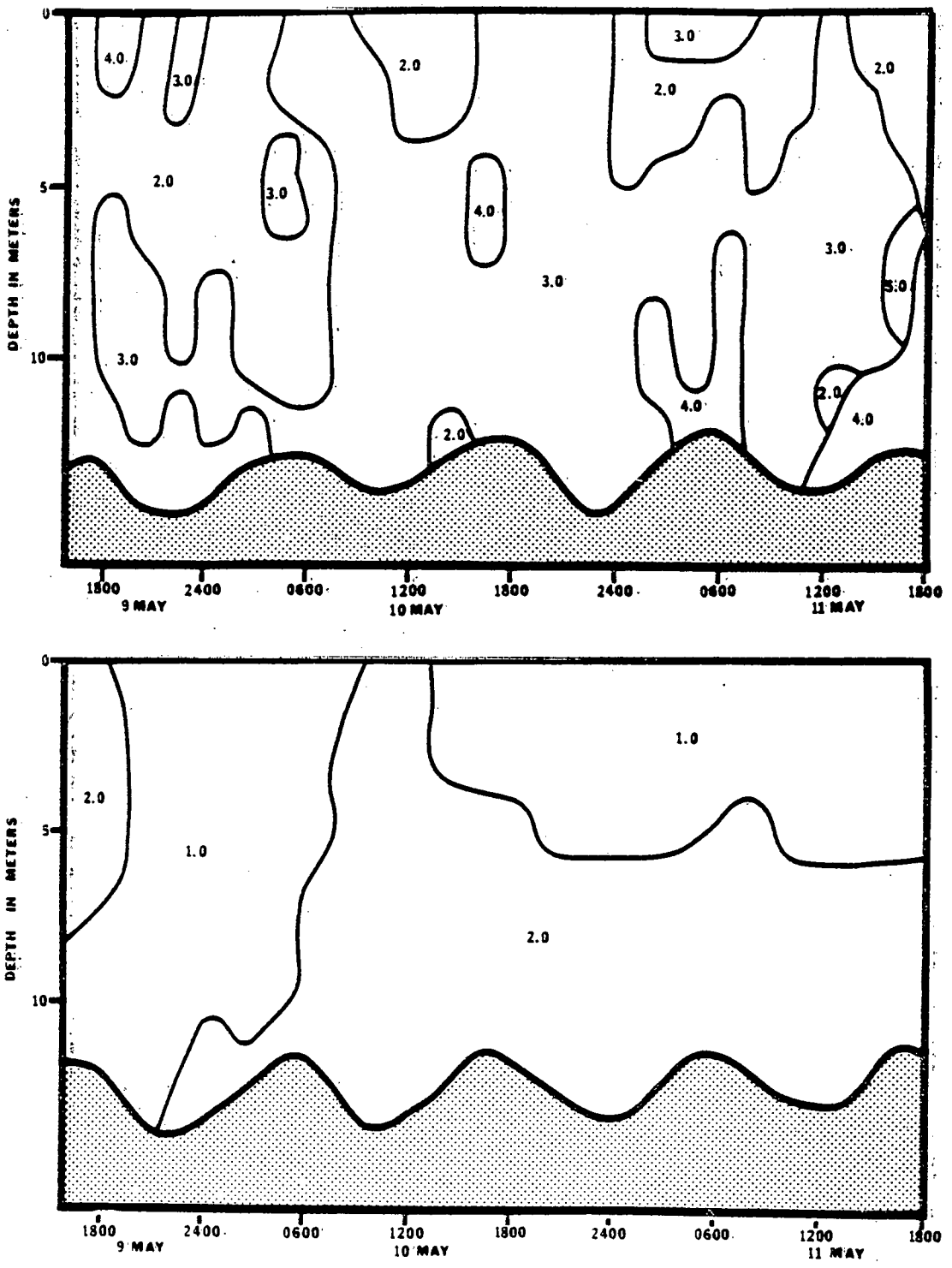

Figure 13. In vivo fluorescence time series at anchor stations off Wassaw Inlet, May 9-11, 1978. Top: R/V BLUE FIN. Bottom: R/V KIT JONES. Calibrated against purified chlorophyll a.

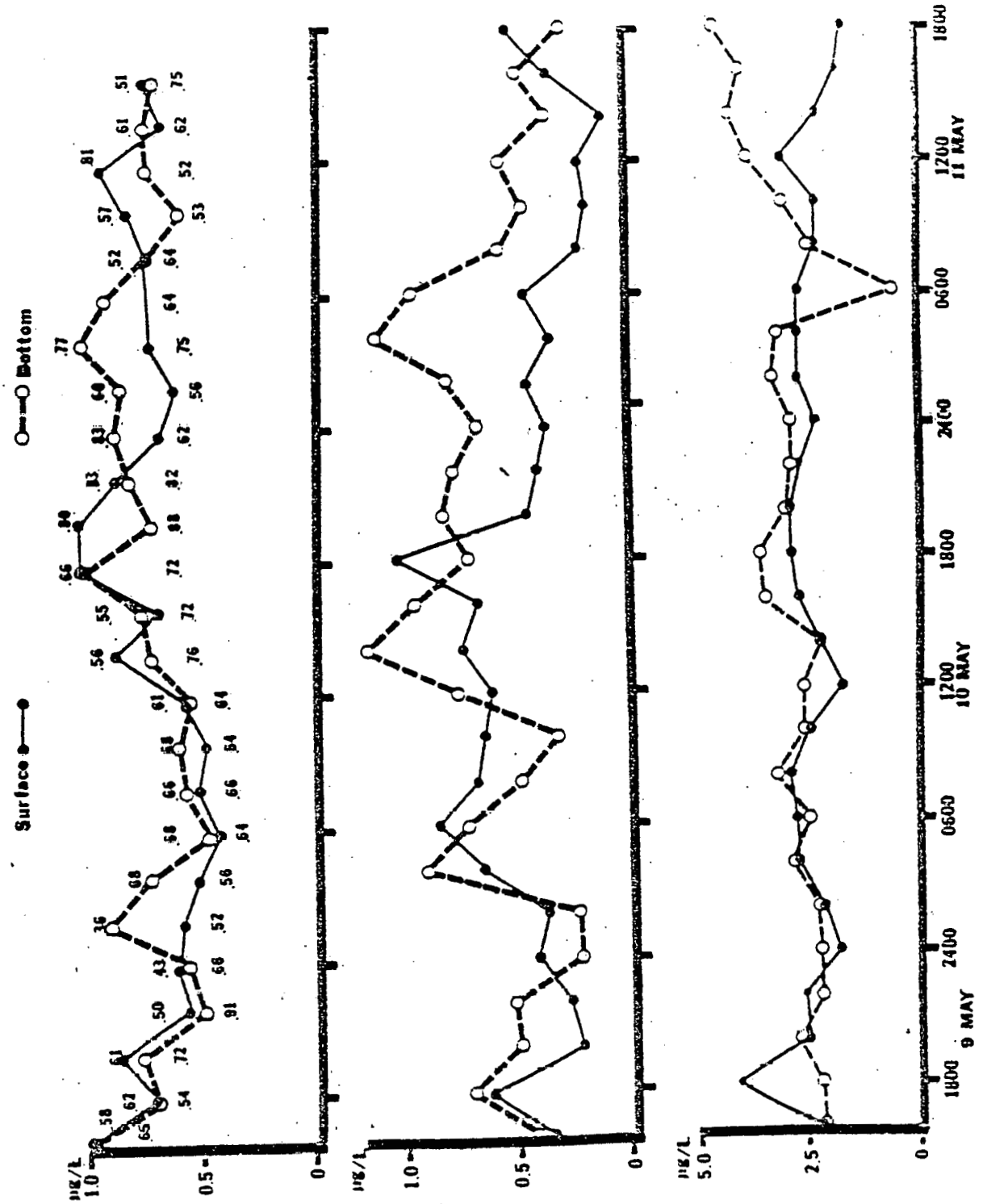

Figure 14. Time series at BLUE FIN anchor station off Wassaw Inlet, May 9-11, 1978. Top: total adenylates and adenylate energy charge. Center: chlorophyll a (HPLC), mg/m^3. Bottom: In vivo fluorescence, mg/m^3.

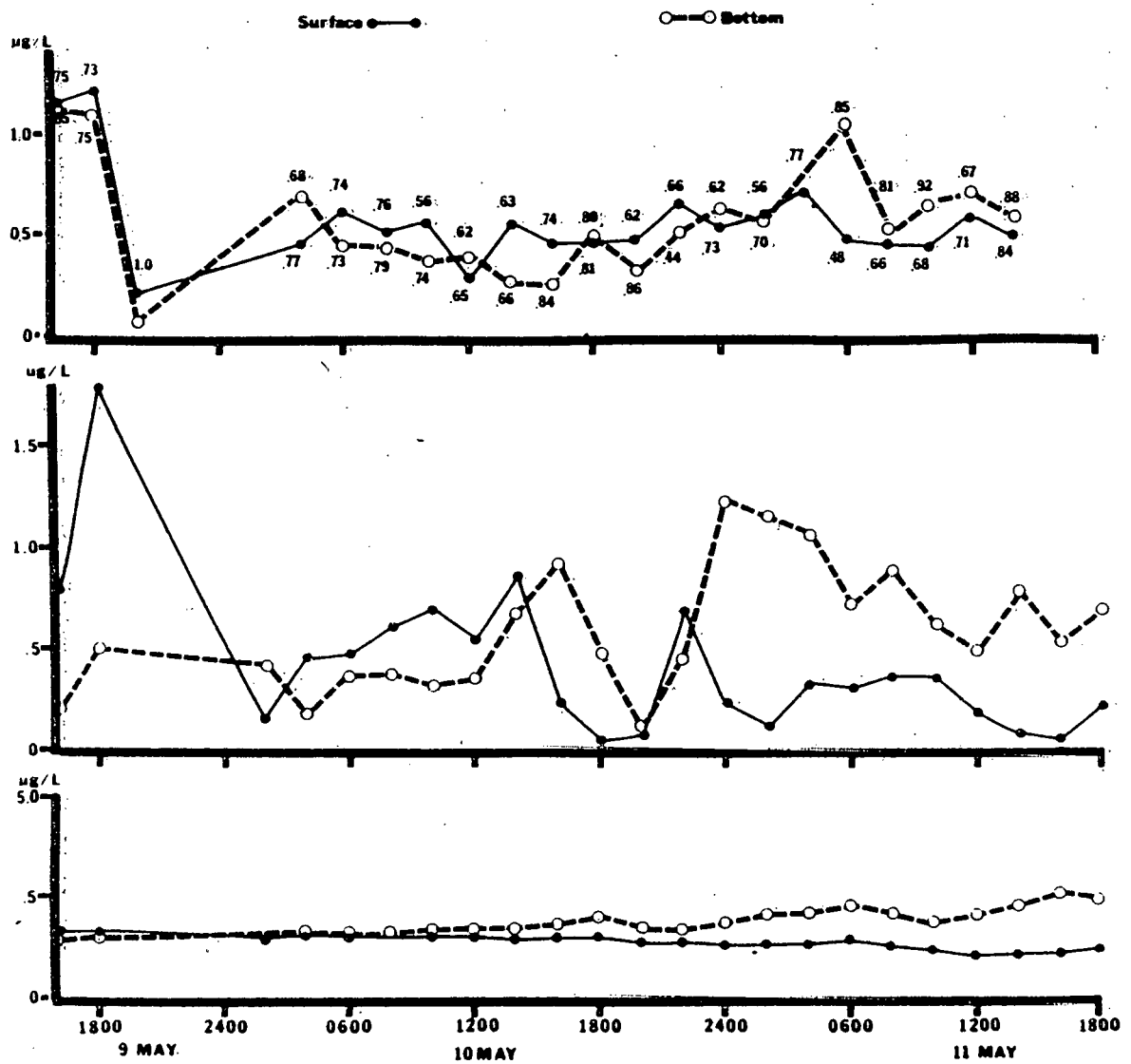

Figure 15. Time series at KIT JONES anchor station off Wassaw Inlet, May 9-11, 1978. Top: Total adenylates and adenylate energy charge. Center: chlorophyll a (HPLC), mg/m^3. Bottom: In vivo fluorescence, mg/m^3.

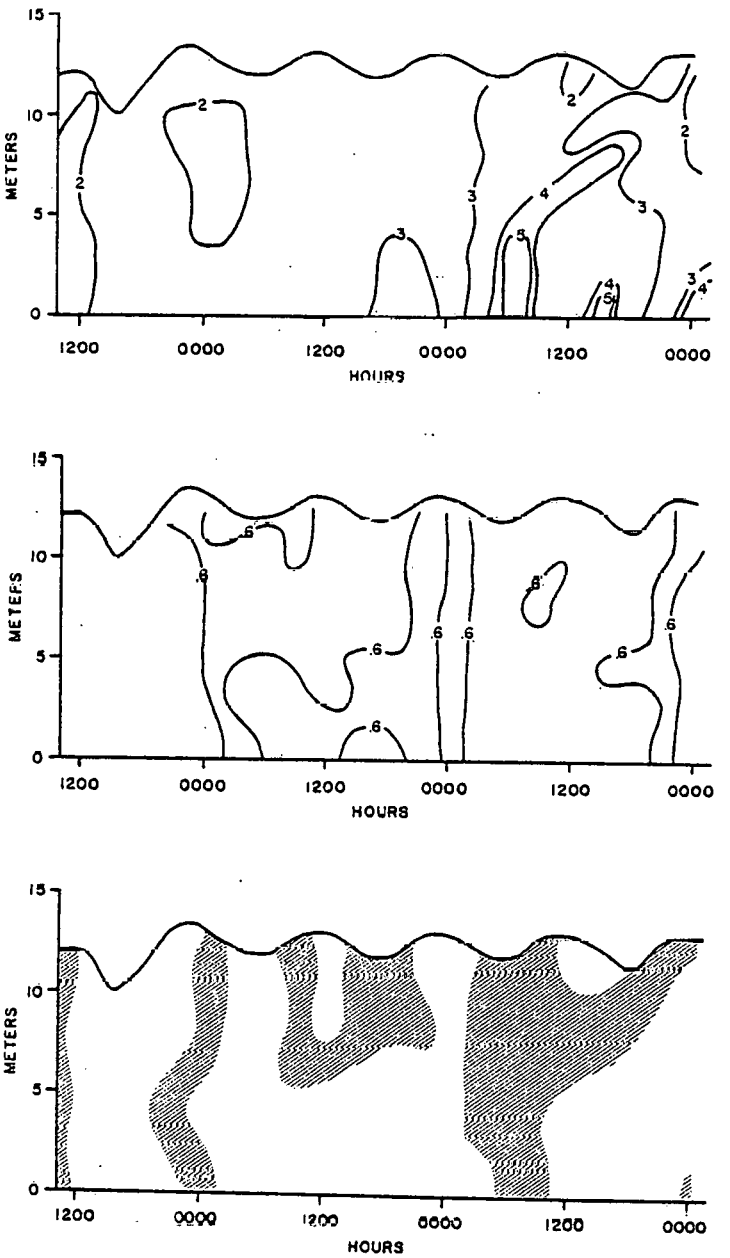

Figure 16. FRNFLX I anchor station A, 7-9 November, 1979. Top, total adenylates. Center, adenylate energy charge ratio. Bottom, heterotrophic-photoautotrophic index. Shaded areas are mixed autotrophic-heterotrophic biomass (HP >3<10). Unshaded areas are autotroph-dominated (HP <3).

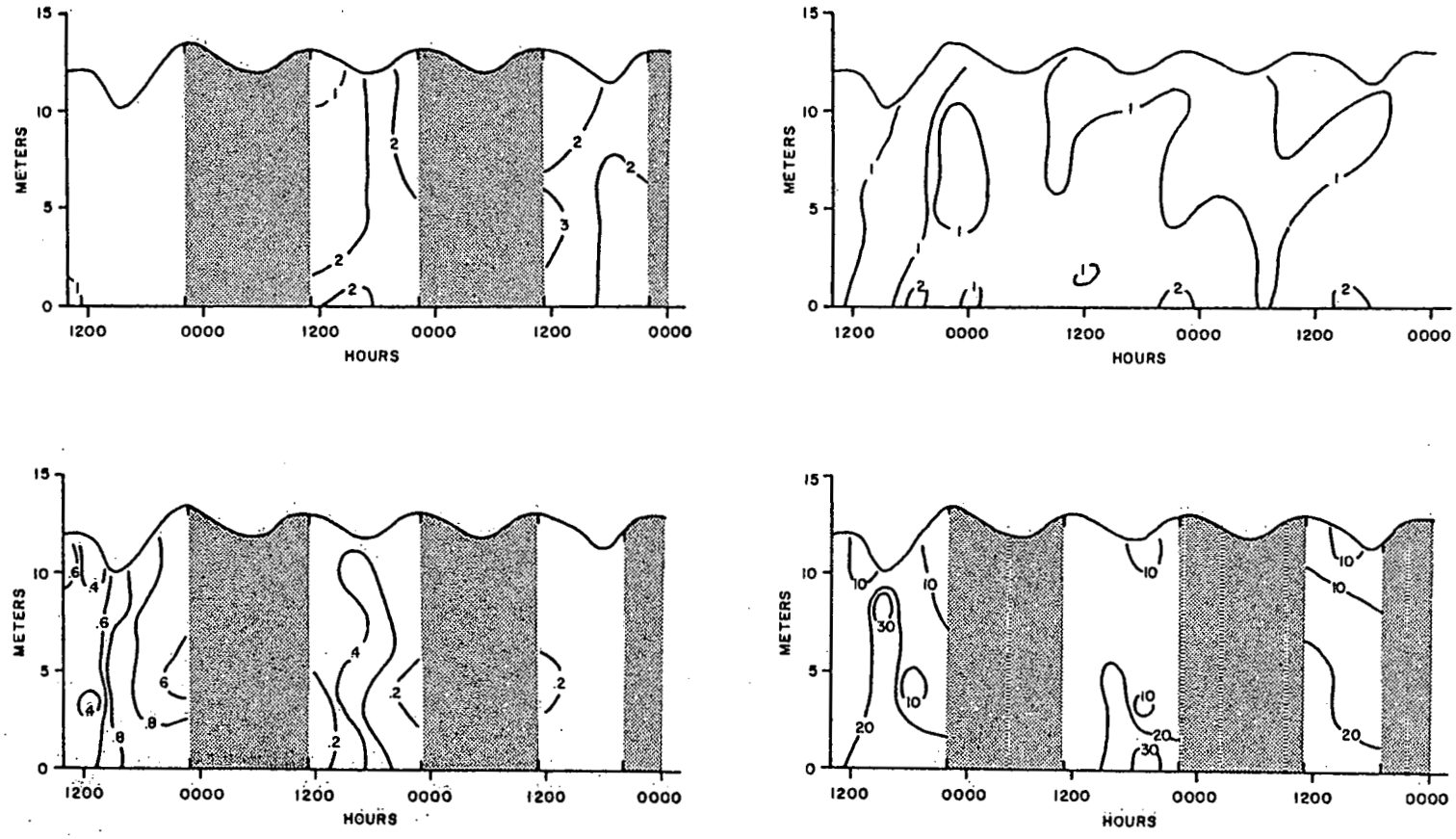

Figure 17. FRNFLX I anchor station A, 7-9 November, 1979. Top left, AO counts of total bacteria, 10^6 cells/ml. Bottom left, heterotrophic uptake of ^{14}C-labeled glucose, µg C l^{-1} hr^{-1}. Top right, chlorophyll a, mg m^{-3}. Bottom right, potential photosynthesis, mg C m^{-3} hr^{-1}. Shaded portions are times when samples were not taken.

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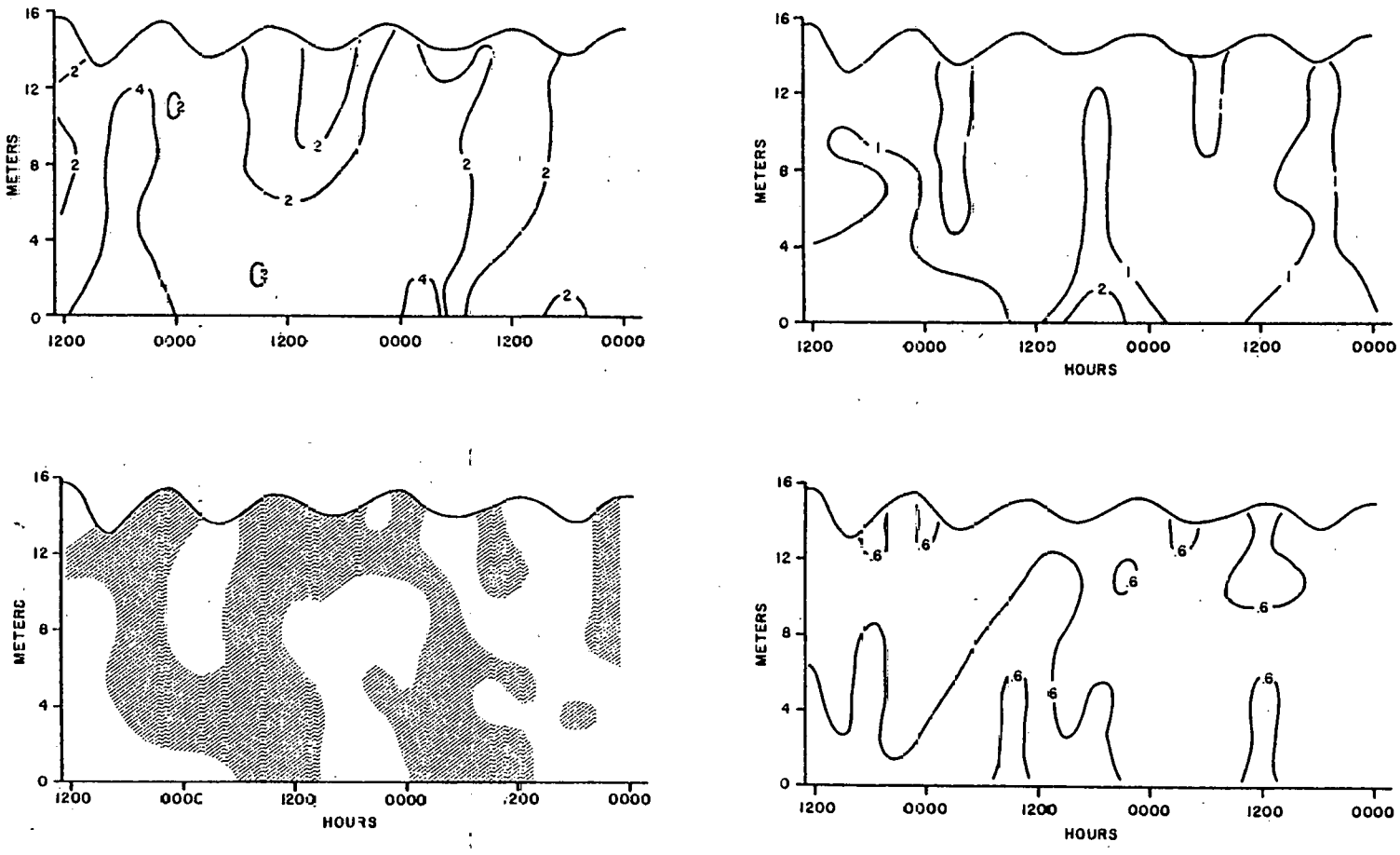

Figure 18. FRNFLX I anchor station B, 7-9 November, 1979. Top left, total adenylates. Bottom left, heterotrophic-photoautotrophic index. Shaded portion is mixed autotrophic-heterotrophic biomass (HP >3<10). Remainder is autotrophic-dominated biomass (HP <3). Top right, chlorophyll a, mg m^{-3}. Bottom right, adenylate energy charge.

2.3 FRNFLX II

On April 1-4, 1981 R/V CAPE HENLOPEN and F/V BLUE FIN were engaged in a two-ship anchor-station study of the region off Wassaw inlet. Seven personnel from our research group were aboard the two vessels. Samples were taken for counting bacteria and other microorganisms, and a study of the rate of heterotrophic uptake of glucose by free and attached bacteria was performed on both vessels. On the basis of a preliminary study conducted during a part of FRNFLX I, we expected to see an order of magnitude variation in the rate of glucose assimilation. However, we actually saw variation only by a factor of 4, with maximum uptake occurring during ebb tide and low water periods (Figure 12).

Counts of bacteria are not yet completed. However, on the basis of the results of the heterotrophic uptake experiment we do not expect to see large variations, at least in the counts of total or free vs. attached bacteria. Evidently this scale of observation, which was established by the physical oceanographers and is useful to them, is not the right one for observing biological variability in the coastal water.

Up to this point we have been interpreting the results of our own data in the absence of the physical and chemical data. As the latter become available in the near future, further interpretations of our data may be possible. These may tell us how future observations should be designed from the point of view of understanding microbiological processes in the coastal water. While the present observations, on both FRNFLX I and FRNFLX II, suggest that microbial activity is high but not variable in the coastal water, other observations lead us to believe that there is in fact considerable patchiness on a scale not much larger than that on which we were sampling during these investigations. However, we need to continue to coordinate our efforts with those of the physical oceanographers in order to have ocrrect, detailed interpretations of water movement and exchange.

3. Microbial Populations and the Detritus Food Web

Because this contract is concerned with the flux of energy and materials, particularly as mediated by microorganisms, in the water over the continental shelf, it is concerned with the production and fate of particulate and dissolved organic matter that is non-living as well as living. While this work emphasizes the non-living components to some degree, any complete separation

of living and non-living would be artificial and unworkable. Therefore, we have to begin with primary production, as do Paffenhöfer and Yoder, who are concerned with the flux of energy through the zooplankton. We also have to consider some aspects of zooplankton ecology, particularly the production of fecal material which is a major input of non-living particulate organic matter to the system. Since we are working cooperatively in a coordinated research program, unnecessary duplication can be avoided.

The flux of energy through non-living components of the pelagic ecosystem involves both very rapid responses and stabilizing influences. The dissolved organic matter, produced both as a primary source by phytoplankton and as a secondary energy source by zooplankton and bacteria, is utilized by bacteria very rapidly. In fact, utilization is so rapid that evaluation of the rate of production of DOM is difficult. This is one of several reasons why its significance as an energy flux in planktonic ecosystems has been underestimated. Most of the particulate matter in the sea is non-living, indicating that much of it has a comparatively long residence time in the water. Since it is both a substrate and a structural component of the environment for microorganisms, its production and fate are important considerations. However, we know surprisingly little about the non-living seston. Both biological and abiotic origins have been postulated (Riley 1963; 1970; Wiebe and Pomeroy 1972; Zimmermann 1977; Pomeroy and Deibel 1980). Probably both occur in the real world, but the actual rates are not known. Chemical analysis of seston has been of little help, because all of it probably originates from phytoplankton, and therefore its chemistry is all much alike and like the phytoplankton themselves. While it is not the major thrust of this contract to develop new ideas or data on the detritus food web for its own sake, these are questions which have to be taken into consideration in our interpretations of processes on the continental shelf. In the course of understanding processes on the southeastern continental shelf some new ideas and information on the detritus food web have emerged.

The turnover of dissolved organic matter and its relation to bacterial populations in the water has been examined. The nature, source, and fate of particulate seston has been re-examined, and some of it identified as the fecal material of zooplankton. A simulation model of the food web as been constructed which includes the major features of the flux of dissolved organic matter and detritus.

3.1 Methods of Microscopy.

Although we are routinely using the acridine orange epifluorescence method for counting marine bacteria, it does have some limitations, and we are continually exploring new methods. We have modified the acridine orange method somewhat to increase the time samples can be stored before they are counted. This is a significant problem. It is not feasible to count samples in large numbers at sea. They must be brought back to the laboratory and counted later. Samples preserved in formalin last only about two weeks after collection, not long enough to count a large set. We have extended the storage time to about six weeks by preserving the samples in buffered glutaraldehyde, just as one would for scanning electron microscopy. The time can be extended further by preparing the stained filters, mounting them on slides in immersion oil, and storing them in the refrigerator. The technique of storing dried samples on filters, proposed by Meyer-Reil, yields low counts in our laboratory.

We have also worked with Karen Porter on the development of DAPI staining for epifluorescence counting (Porter and Feig 1980). Although DAPI staining has some advantages, we will not switch to it as a routine counting procedure unless data we are now collecting indicate that there is a significant difference in total counts. One of the minor disadvantages of DAPI staining is the necessity to prepare stained filters immediately at sea and to do so in near darkness. The filters must then be kept cold until they are returned to the laboratory and counted.

3.2 Utilization of Dissolved Substrates by Bacteria in Gulf Stream Intrusions

To evaluate microbial responses to intrusions it is necessary to develop methods and criteria of activity appropriate for the populations in question. We have found that specific (per cell) uptake rates (Wright 1978) present a problem when cell volumes vary over two orders of magnitude, as they do in oceanic bacteria. Using dissolved adenosine triphosphate (DATP) labeled with ^{32}P as a tracer, we measured uptake of naturally occurring DATP by natural populations of free and attached bacteria in a Gulf Stream intrusion and in adjacent Gulf Stream and continental shelf water. Free bacteria in the ocean, including the outer continental shelf are, with rare exceptions, less than 0.5 micrometers in size, while attached bacteria are usually greater than one

micrometer in size. The greater size is generally taken to indicate greater metabolic activity and active growth on the part of attached bacteria.

DATP is a convenient naturally occurring organic compound to study, because a good analytical method exists (Azam and Hodson 1977), and ^{32}P-labeled ATP can be used as a sensitive tracer. Azam and Hodson (1977) have shown that DATP is biologically active: its is readily taken up by bacteria. Uptake by free and attached bacteria can be distinguished in ocean water by counting ^{32}P activity retained on 0.2 and 0.6 μm Nuclepore filters. Aliquots from the same water samples were also preserved in cold glutaraldehyde for counting free and attached bacteria by acridine orange fluorescence.

Specific uptake of DATP was highly correlated with ambient DATP concentration. Specific uptake of DATP by large, attached bacteria was 4-430 times that of small, free-living bacteria. Specific uptake by both free and attached bacteria increased up to 200 times within 24 hours of the arrival of an intrusion on the continental shelf. Uptake rates of free and attached bacteria are significantly correlated ($r^2 = 0.8$), suggesting that both are dissolved substrates and both are stimulated by intrusion events.

While on a per-cell basis the rate of uptake of DATP by attached bacteria was 66 times higher than that by free bacteria, the uptake per unit of biomass was essentially the same for both populations, because attached bacteria have approximately 40 times the volume of free bacteria. Therefore, on the basis of equal units of biomass, attached bacteria do not appear to be more active than free minibacteria. Of course, this may be because a significant fraction of the population of free bacteria is inactive. However, when bacterial populations are widely divergent in size, as are the free and attached bacteria of the ocean, the best basis of comparison appears to be activity per unit biomass rather than per cell. A more complete account of this is in the manuscript by Hodson, Maccubbin and Pomeroy (1981) which is a part of this report.

3.3. Interactions of populations of Free Bacteria, Attached Bacteria, and Aggregates

The work of Hodson et al. (1981) showed that both free and attached bacteria respond to Gulf Stream intrusions by increasing their numbers and their uptake of dissolved substrates. In a further examination of the interactions of these populations we examined the relationship of bacteria, phyto-

plankton, and chroococcoid cyanobacteria to the abundance of flocculent aggregates. The abundance of flocs varies greatly, sometimes dominating the population of particulate matter and sometimes being a comparatively rare component. In general, dense populations of flocs are associated with intrusions of Gulf Stream water (but are in surface water as well as in the subsurface intrusion) and with biologically productive nearshore fronts.

A set of 45 samples from all parts of the continental shelf was examined. Each sample was counted with acridine orange epifluorescence for free and attached bacteria, phytoplankton, chroococcoid cyanobacteria, and flocculent aggregates. In each case a sample was categorized as having a dense flocculent background or not having one. Because a dense population of flocs may obscure free bacteria, this in itself might tend to reduce the count of free bacteria in the presence of dense flocs. Of the 45 samples examined, 29 had a dense flocculent background and 16 did not. Using the Wilcoxon two-sample test, the following parameters were tested with respect to the two categories of samples.

1. Number of free bacteria per ml.
2. Variance of the free bacteria counts as a per cent of the mean.
3. Per cent of flocs with attached bacteria.
4. Mean number of attached bacteria per floc.
5. Per cent of time viable phytoplankton occur with flocs.
6. Per cent of time flocs occur with viable phytoplankton.
7. Ratio of viable phytoplankton to flocs.
8. Per cent of time cyanobacteria occur with flocs.
9. Per cent of time flocs occur with cyanobacteria.
10. Ratio of cyanobacteria to flocs.
11. Floc/free bacteria ratio.
12. Viable phytoplankton/free bacteria ratio.

The number of free bacteria was significantly greater at the 99% confidence level for samples with a dense flocculent background, in spite of the likely possibility that some free bacteria were obscured by the flocs. The per cent of flocs with attached bacteria on them was significantly greater at the 95% confidence level in samples with a dense flocculent background. Also, the per cent of flocs that occur with cyanobacteria is significantly greater at the 95% confidence level in samples with a dense flocculent background.

Particularly when viewed in the light of the findings of Hodson et al., these additional findings suggest that dense populations of flocculent aggregates are a phenomenon of productive conditions where bacterial numbers have increased. There is no substantial agreement in the literature on the nature and origin of flocculent aggregates. Over the years they have been postulated to be condensations of dissolved organic matter, extracellular products of bacteria, and fecal matter. Very likely they are all of the above in various proportions. Their presence in situations of high productivity and their relative absence elsewhere suggests that they have a comparatively short residence time in the water, either because they aggregate further and sink or because they are degraded by bacteria, Again, these may not be mutually exclusive fates, and both fates provide flocculent aggregates with a potential role in the food web of the continental shelf. These potential roles are explored in a different manner in the simulation model of the food web (section 3.5).

3.4. Production of Fecal Aggregates by Zooplankton

A novel finding in the course of our work on microbial processes in continental shelf water was that some of the flocculent organic aggregates which are a common feature of the seston of productive surface water are fecal. Some of them are identifiable as the fecal ribbons of pelagic tunicates (salps, doliolids, and appendicularians) which have broken up in the water (Pomeroy and Deibel 1980). Our assertions about finding this fecal material in the water in small (50 μm) fragments has been contested by some workers on the grounds that salp feces are found in sediment traps, and some other studies of salp feces have shown that they sink rapidly. What these investigators do not seem to appreciate is that pelagic tunicates do not produce fecal pellets but a fecal ribbon which breaks away. Within the ribbon are boluses of material which do remain intact and do sink rapidly. This is the material found in sediment traps and, because it is big enough to see with the unaided eye, it is what other workers have studied exclusively. These large boluses are only a part of the fecal matter, however. Studies of the respiratory rate of fecal material and of the uptake of adenine by the material (R. Hanson, pers. com.) show that there is more activity in the small fragments which remain in suspension than in the large boluses which sink out of the water column. Since the fragments are seen only by oil immersion microscopy, they have been missed by other workers.

Probably a number of other zooplankton contribute to the fecal aggregate population in the seston, including the smaller larval stages of copepods (Paffenhöfer and Knowles 1979, Hofmann et al. 1981). We find very dense populations of what appear to be fecal aggregates in the nearshore zone where pelagic tunicates do not live. These may be benthic or may be produced by meroplankton. A project to pursue this line of research has been funded by the National Science Foundation. It is not our intention to continue along this line under this contract.

3.5. A Simulation Model of the Shelf Ecosystem

In 1978 a conceptual model of the detritus food web on a continental shelf subject to intrusions of nutrient-rich water was developed in connection with this contract (Pomeroy 1979). The conceptual model demonstrated that, using recent estimates of gross growth efficiency of oceanic organisms, it was possible to have production of higher trophic levels, including fishes, while much of the energy of primary production was channelled through a detrital food web. This was in sharp contrast to the North Sea model of Steele (1974) in which all production was directed through a short, linear chain of grazing and predation, with fecal pellets going quantitatively to the benthos. The latter was not based on observed fate of fecal pellets but rather on the necessity to provide a sufficient flux of energy to the benthos in the model to support a reasonable production of demersal fishes. Pomeroy's continental shelf model demonstrated that it is possible to support fishes, both demersal and pelagic, without drastic and unrealistic assumptions about transfer of fecal pellets to the benthos, and indeed without such unrealistic assumptions about the flux of energy through a short, linear food chain, in which copepods eat 100% of the phytoplankton production. Not all of phytoplankton production is available to copepods. Much of it is rather small for them. On the other hand, Steele's model clearly demonstrated that the so-called ecological efficiency of 10% per trophic level cannot work in the real world, even if the world were constrained to clear-cut trophic levels, as was Steele's model. In the real marine environment trophic levels are blurred by bacteria, mucus net feeders, protozoans, and other omnivores.

After the crude demonstration that a model could be constructed which would produce fishes through various detrital food webs (Pomeroy 1979), J.E. Hagner was supported through this contract to develp a simulation model of

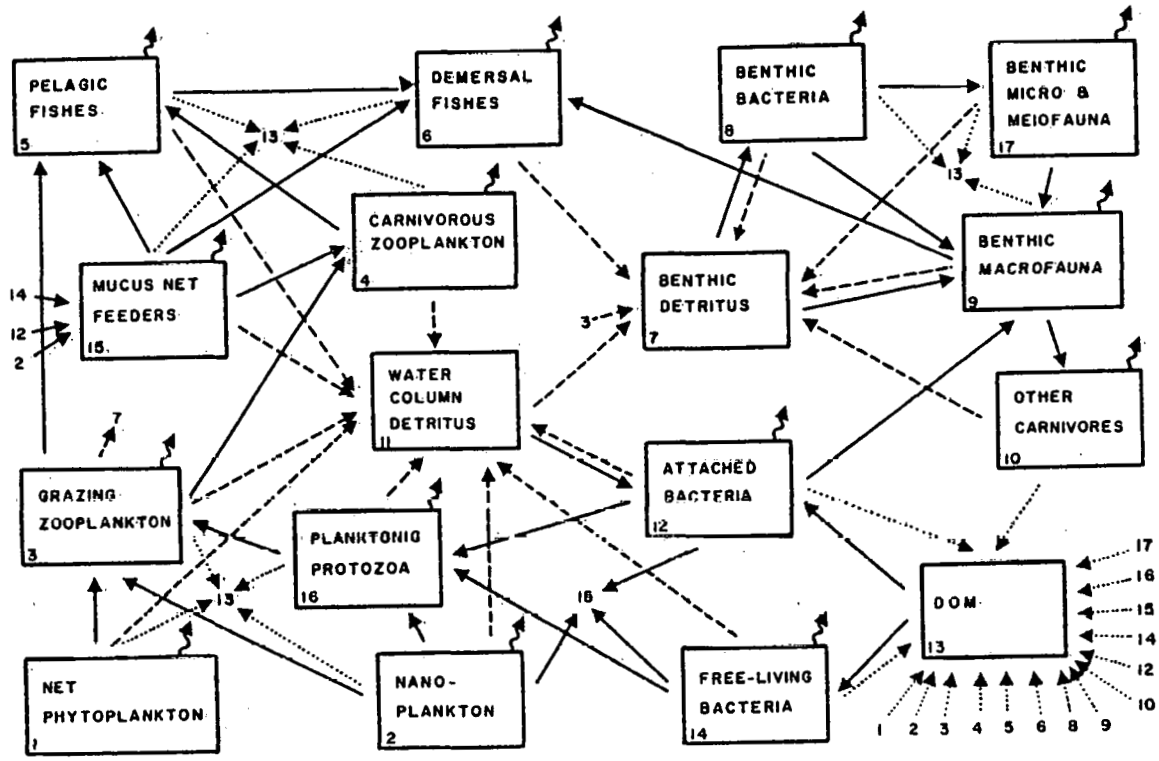

Figure 19. Simulation model of the flux of energy through continental shelf food webs. Continuous lines = trophic fluxes. Dotted lines = dissolved fluxes. Broken lines = detrital fluxes. Wavy lines = respiratory fluxes. Arrows to or from a number are additional fluxes to the compartment numbered.

such a food web. A 17-compartment model was developed (Figure 1) which has a number of unusual features. Phytoplankton are divided into two size groups. Phytoplankton produces dissolved as well as particulate organic matter. Some of the former becomes detritus directly. There are three bacterial populations: free, attached, and benthic. There are four zooplankton populations: grazers, carnivores, mucus-net feeders, and Protozoa. This model provides for the possibility of many alternative pathways for energy and materials between primary producers and terminal consumers (benthic and demersal fishes, other carnivores, and benthic invertebrates). We propose that indeed the pathways probably do vary with the circumstances. For example phytoplankton blooms are situations in which much of the primary production is not being grazed and is going to detritus production.

Hagner developed a model along the lines of those developed for other ecosystems by Wiegert (1975; 1979a; 1979b; Wiegert and Wetzel 1979; Wiegert et al. 1975). Non-linear equations describe growth, assimilation, mortality, refuge levels, upper population limits, and other known parameters of organisms. The groups thus described are trophic groups of similar species, not individual species populations. We are postulating that ecosystem function will be similar in the presence of certain trophic groups, regardless of the species that predominate at any time. The models thus are intended to simulate ecosystem function, not species population dynamics.

There are several subsets within the model which can be varied individually to explore their effect on the entire model. There is a pelagic subset which is very similar to Steele's (1974), except for the division of phytoplankton into two catagories based in size (and the properties which go with size, such as k_s and r). There is benthic subset which includes benthic

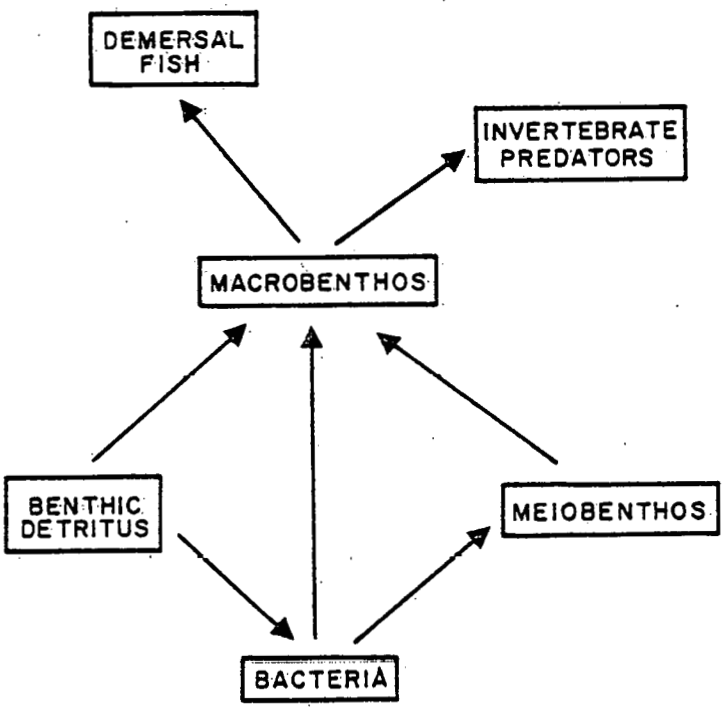

Figure 20. Subset of benthic energy fluxes on the 17-compartment model of energy flux on continental shelves.

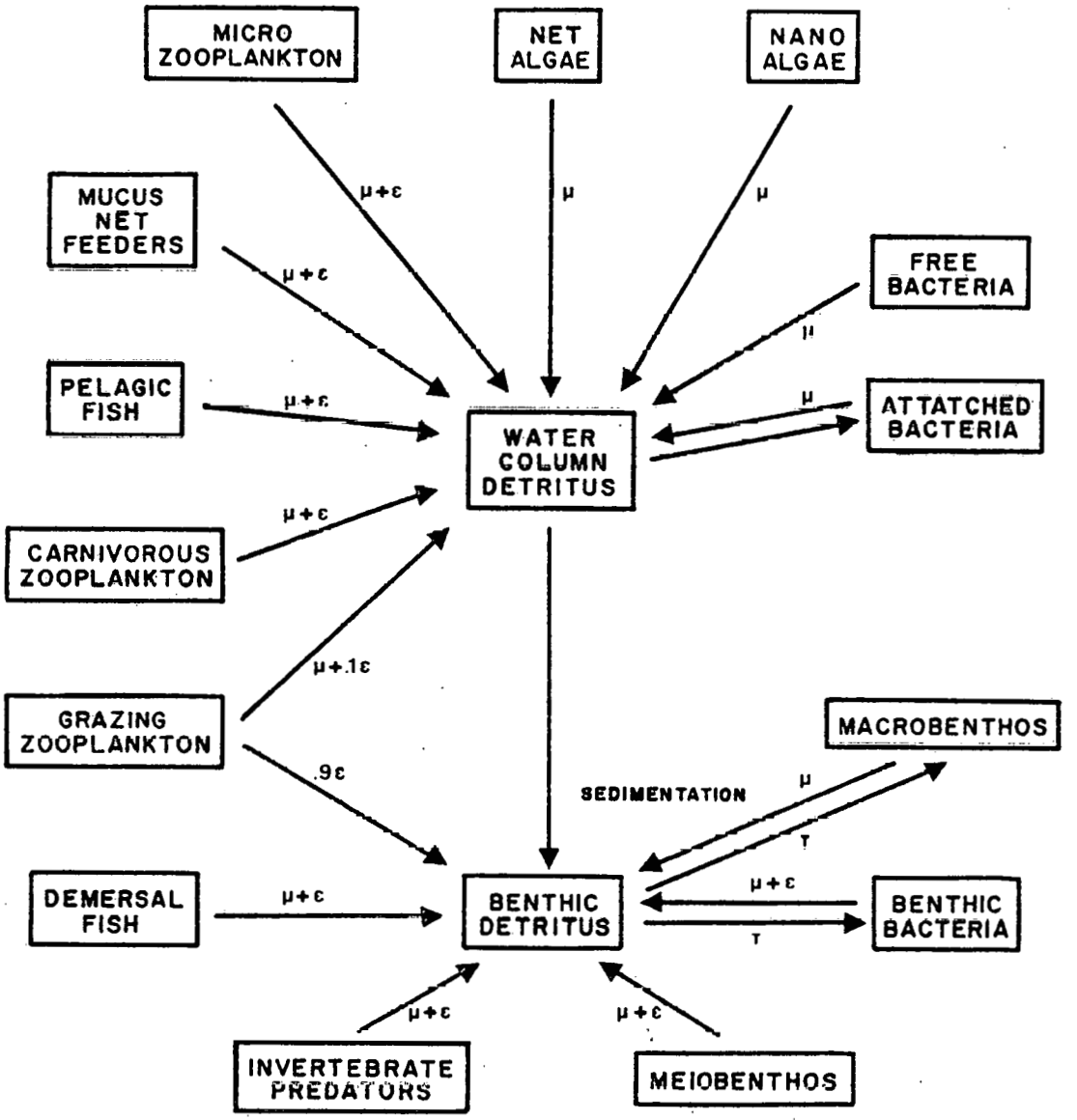

Figure 21. Pathways of energy to and from detritus in the simulation model of the flux of energy in a continental shelf ecosystem.

Table 4. Net primary production in simulations of various intrusion and upwelling events used for modeling the detrital food web.

	g C $m^{-2}yr^{-1}$	K Cal $m^{-2}yr^{-1}$	KJ $m^{-2}yr^{-1}$
4 intrusions, regular	143	1630	6820
4 intrusions, regular, yr 1	143	1629	6816
yr 2	149	1704	7130
yr 3	150	1706	7140
yr 4	149	1697	7100
yr 5	149	1694	7088
4 intrusions, stochastic	141	1607	6742
constant upwelling	761	8671	36279
Peru upwelling	326	3715	15544
spring bloom 1	182	2069	8657
spring bloom 2	140	1599	6690

Table 5. Simulated zooplankton production, K Cal m^{-2} yr^{-1} in a mixed detritus and phytoplankton based food web. A. Four pulses of nitrogen at regular intervals. B. Short pulses of nitrogen at 20-day intervals.

A	grazers	mucus-net feeders	Protozoa	Total
Ingestion	561	404	393	1358
Assimilation	393	263	275	931
Respiration	181	117	117	415
Excretion: loss of DOC	82	61	70	212
Physiological mortality	41	30	7	78
Predatory mortality	96	58	85	239
Egestion	168	142	118	428
Gross growth efficiency	0.24	0.22	0.30	0.25
Production	137	89	118	343
B				
Ingestion	633	422	488	1543
Assimilation	443	274	342	1059
Respiration	178	115	149	442
Excretion	84	60	96	240
Physiological mortality	42	30	10	82
Predatory mortality	137	69	87	294
Egestion	190	147	147	484
Gross growth efficiency	0.28	0.24	0.20	0.24
Production	180	100	97	376

bacteria, benthic detritus, meiofauna, macrofauna, predators, and demersal fishes (Figure 20). There is a detritus subset (Figure 21) and a dissolved organic matter subset (Figure 22). By varying these sets, as well as components of them, it is possible to simulate many situations which have been documented in the literature and others which are postulated. Assimilation efficiency can be set to correspond with values in the recent literature or the model can seek efficiencies which lead to a stable system.

Unfortunately, Hagner had to leave the work before it was completed, and after a lapse of about a year the work was taken up again by Michael Pace, who is now completing work with the first-generation model. It is an energy model with nitrogen forcing on primary production. Productivity is adjusted to reasonable levels by varying the number or intensity of intrusions of nitrate-rich water. The space-averaged model does not simulate the two-layered

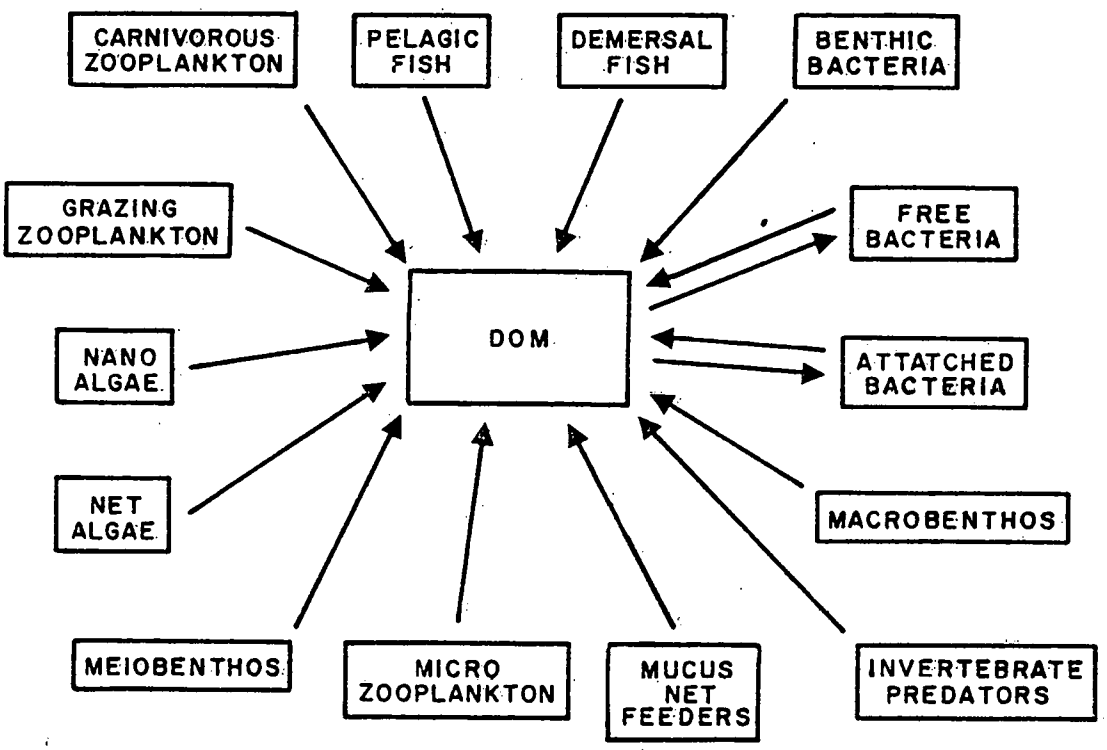

Figure 22. Fluxes of dissolved organic carbon in the 17-compartment simulation model of the flux of energy through continental shelf ecosystems.

Table 6. Simulated bacterial production, K Cal m^{-2} yr^{-1} in a mixed detritus and phytoplankton based food web. A. Four pulses of nitrogen at regular intervals. B. Short pulses of nitrogen at 20-day intervals.

A	Benthic	Planktonic		Total	Plankton Total
		attached	free		
Ingestion	1695	209	684	2589	893
Respiration	257	99	239	595	338
DOM loss	90	36	86	212	122
Physiological mortality	26	10	25	61	35
Predatory mortality	1321	66	335	1722	402
Gross growth efficiency	0.79	0.37	0.53	0.69	0.49
Production	1347	76	361	1784	437
B					
Ingestion	1891	250	753	2894	1004
Respiration	286	109	248	643	356
DOM loss	103	39	86	228	125
Physiological mortality	30	11	26	67	37
Predatory mortality	1470	93	393	1956	486
Gross growth efficiency	0.79	0.42	0.56	0.70	0.52
Production	1500	104	419	2023	523

Table 7. A comparison of metabolic rates and production of zooplankton and bacteria in a simulated food web which includes both phytoplankton and detrital components (K Cal $m^{-2}yr^{-1}$).

	Four regular pulses of nitrogen per year		Short pulses of nitrogen every 20 days	
	Zooplankton	Bacteria	Zooplankton	Bacteria
Ingestion	1358	893	1543	1004
Respiration	415	338	442	356
Production	343	437	376	523

Table 8. A comparison of production data (K Cal m^{-2} yr^{-1}) from studies of several ecosystems (from Mills 1980) with production values from a simulation model of a mixed phytoplankton and detritus food web in a continental shelf subject to periodic intrusions of nitrate-rich water.

System	Net Primary Production	Zooplankton	Macro-benthos	Demersal Fishes	Pelagic Fishes
Scotian Shelf	1160	89	35	4	4
Scotian Slope	1148	79	27	2	16
North Sea	1026	128	20-50	3.3	10
Intrusion model	1630	137	36	1	3
Spring Bloom model	1599	141	39	2.5	4.4
Peru upwelling model	3715	701	141	1.2	55

condition of an intrusion on the outer continental shelf. This is a potential refinement for a second generation model. There are no lags in responses of populations in the present model, so there is some foreshortening of time of the responses to the pulses of nitrogen.

By varying the nitrogen forcing, the model can be made to simulate various coastal upwelling situations, as well as the spring bloom conditions of temperate oceans and epicontinental seas (Table 4) At this level of aggregation there is really nothing unique about a particular marine ecosystem, so long as it is at least 50 meters deep. The model is very stable, as indicated by the means of each of tive years of simulation, and this stability carries on through the food web.

In a preliminary run of the simulation, secondary production is divided between various zooplankton groups in what is, so far as we know, a realistic way. We cannot validate the relatively large production of Protozoa at this time, either from our own work or from the literature, although there are indications that probably it is realistic. Gross growth efficiencies are similar to those in the recent literature (Pomeroy 1979; Mills 1980; Banse and Mosher 1980). Secondary production of bacteria is nearly as high as that of zooplankton (Tables 6 and 7). Although this would be missed by a highly condensed model such as Steele's, probably it is a realistic representation of the partitioning of energy fluxes in a planktonic ecosystem. The recent estimates of bacterial production using tritiated thymidine (Fuhrman and Azam 1980) tend to support it. Various levels of production in the simulation model compare favorably with data from continental shelf ecosystems (Table 8). When run as an intrusion model, the simulation yields of demersal fishes are low. Everything we know about the outer continental shelf of the southeastern U.S. supports this (Tenore et al. 1978). The sporadic nature of intrusions is not conducive to development of demersal fish populations, and except for some small populations associated with ledges and rock outcrops, there is little demersal fish production, so far as we know at present.

The simulation model of the continental shelf ecosystem will serve several functions. It provides a richer simulation of the pelagic food web than most extant models, allowing us to explore the potential fluxes of energy and materials through detritus, bacteria, non-crustacean zooplankton, and even dissolved materials. This in turn serves as a heueristic device to show us the populations and processes not well represented in the present DOE research

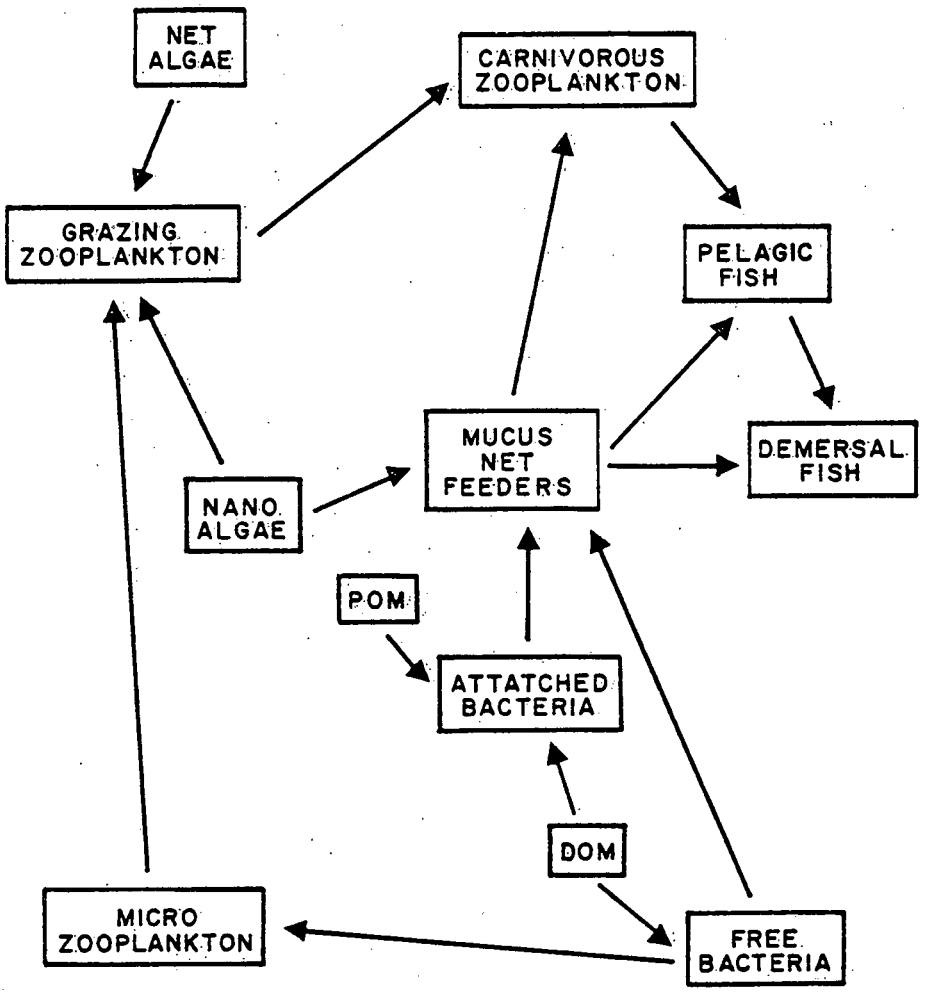

Figure 23. Simplified conceptual model of the food web of the Southeastern continental shelf, showing the major pathways for energy and materials from phytoplankton to terminal consumers.

program in the southeast. Protozoa immediately appear as one such under-investigated group. The reader should, of course, be careful not to ask this model to perform simulations it was not designed to do. Response times of trophic groups may not be realistic, although they will be in relative proportion to each other. Of course, any model such as this suggests what is possible or impossible and does not tell us what indeed happens in the real world. Prediction is not an immediate goal of this modeling effort.

Because the first generation simulation model is space averaged, it would have limited value if coupled with more detailed physical models of intrustion phenomena. However, a two-layered model, which could easily be developed would not only add interesting biological details but could be meaningfully coupled to models or data sets of the physical regime of the southeastern continental shelf. The inherent strength of this kind of model is its basis in biological principles. It is not a curve-fitted model which simply reproduces in any convenient mathematical way the behavior of the ecosystem. Rather, the equations are developed as descriptions of biological function, just as good physical oceanographic models are descriptions of physical functions. The fact that the model produces realistic production values is therefore, a first-stage validation.

4. The Effect of Copper on Photosynthesis of Marine Phytoplankton

The interaction of naturally occurring dissolved organic matter with heavy metals in solution in sea water may significantly modify the uptake of the metals by organisms. Therefore, analysis of total concentration of a metal in sea water does not permit inferences about its toxicity. A.M. Wood, who is now a Research Associate in the Biology Department of the University of Chicago, completed a doctoral dissertation on the effect of naturally occurring chelators on photosynthesis of naturally occurring phytoplankton populations while she was associated with this contract as a research assistant (Wood 1980).

To evaluate the effect of binding capacity of dissolved organic materials on copper toxicity it is necessary to measure binding capacity as well as copper toxicity. In collaboration with David Evans and James Alberts of the Savannah River Ecology Laboratory, Wood developed an ion-exchange method for measuring copper binding capacity in sea water. Toxicity was evaluated with a bioassay which consisted of a ^{14}C measurement of photosyhthetic carbon assimilation in the presence of known copper additions. This is the first time that a copper bioassay has been combined with a binding capacity measurement in a marine environment.

Binding capacity was highly correlated with total dissolved organic carbon concentration. Therefore, coastal waters and estuaries showed the highest binding capacities. At one station in Doboy Sound, Georgia, the binding capacity was so high that no copper toxicity could be demonstrated at

600 μg Cu l^{-1}. Other stations in Doboy Sound showed 50% inhibition at Cu concentrations greater than 100 μg l^{-1}. A series of samples from the Gulf of Mexico in the vicinity of Barataria Bay and the Mississippi River plume showed 50% inhibition of photosynthesis at Cu concentrations of 50-100 μg l^{-1}. In contrast, Sargasso Sea samples showed 50% inhibition at 15-25 μg l^{-1}.

These findings have implications for anthropogenic copper inputs into the ocean and continental shelves, such as those associated with the cooling systems of power plants. The binding capacity on the southeastern continental shelf shows the full range of values, with very high capcity near shore to very low at the edge of the shelf. Release of equal amounts of copper on the inner and outer shelf would have very different biological consequences. The principle will apply to other heavy metals. Therefore this work should prove to be a benchmark in the bioassay of heavy metal toxicity in the ocean. Two manuscripts which have been submitted for publication are included in the appendix to this report.

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Microbial Roles In Aquatic Food Webs

LAWRENCE R. POMEROY

Classical ecological food web theory relegates to bacteria the role of degrading dead and refractory organic materials, an essential but presumably minor role compared to predator-prey and grazer-herbivore processes of macroorganisms (Elton 1927). There is growing evidence, however, that bacteria mediate a substantial part of the energy which moves through aquatic food webs. Much nonliving material, in a wide variety of particulate and dissolved forms, is available to be exploited, even in purely pelagic food webs which begin with the production of microscopic plankton (Andrews and Williams 1971; Derenbach and Williams 1974; Pomeroy 1974; Pomeroy 1979; Saunders 1976; Watson 1978; Williams 1970; and Williams and Gay 1970). Certainly, any coastal, estuarine, or freshwater system in which macrophytes are dominant producers supports a major flow of energy and materials through microbial pathways. Mann (1972) has said,

> When we try to generalize about the functioning of ecosystems, I wonder

> if it would be more correct to say that plants are usually consumed by microorganisms, which in turn are consumed by animals, rather than to give the usual story about plants, herbivores, and carnivores.

Microbiologists have shown little interest in providing the observational and experimental basis for the major revision of food web theory which seems to be needed. Rapid progress toward a better understanding of food webs is now possible because of recent advances in methods of aquatic microbiology.

Populations and Communities of Aquatic Microorganisms

The standard and rigorous culture techniques for identifying populations of microorganisms in many branches of microbiology have not been satisfactory to deal with questions about the abundance and ecological roles of aquatic microorganisms (Jannasch and Jones 1959). Advances in direct visualization of bacteria by light and electron microscopy together with assays for specific cell components, ranging from adenylates to cell wall lipids, have provided for the first time an emerging picture of the structure and function of aquatic microbial populations and of the taxa of bacteria in them (King and White 1977; Menzel and Ryther 1970; Watson et al. 1977; White et al. 1977; White et al. 1979). Each species has a distinct niche, or set of niche dimensions in the sense of Hutchinson (1965). As in phytoplankton, niche dimensions are determined by the biochemical interactions of the environment with the genotype (Pomeroy 1975), but some of the consequences of these modes of niche selection are complex and cryptic in cases of aquatic bacteria. For example, pleomorphism occurs in response to changing

conditions in the microenvironment (Wiebe and Pomeroy 1972). Therefore, some of the morphologically and functionally different populations of bacteria we now recognize in natural waters may be different phenotypic expressions of genetically similar organisms. In other cases, they do indeed represent distinct taxa, but it is not always evident which case we are observing.

Distinctive communities of bacteria occupy specific parts of the pelagic microenvironment (Sieburth 1979; Sieburth et al. 1977; Sieburth et al. 1978). An important recent discovery is the existence of relatively large numbers of free-living bacteria of very small size in virtually all natural waters (Figure 1). Ranging from 0.2 to 0.5 µm in size, they have been called minibacteria by Watson et al. (1977), and they are the numerically dominant bacteria in both fresh and salt waters, sometimes becoming the major component of total microbial biomass, including even the phytoplankton (Watson 1978). Presumably they are non-exacting heterotrophs which utilize dissolved organic compounds. It is a curious observation, recently confirmed again by this writer, that when water is held for 24 hours in a bottle or on a culture slide, the minibacteria become larger (Wiebe and Pomeroy 1972). Whether this transformation is the result of some subtle change in the microenvironment or the result of reduced grazing pressure is not known.

Fecal matter supports a distinctive microbial community in which the bacteria are larger, almost by an order of magnitude, than the free-living minibacteria (Figure 2). Large, motile rods tend to develop within 24 hours, and within 48 to 72 hours they have degraded much of the less refractory particulate matter in the feces, and are themselves being consumed by protozoans. The chitinous casing of crustacean fecal pellets is degraded by chitinoclastic bacteria in about 72 hours, and the

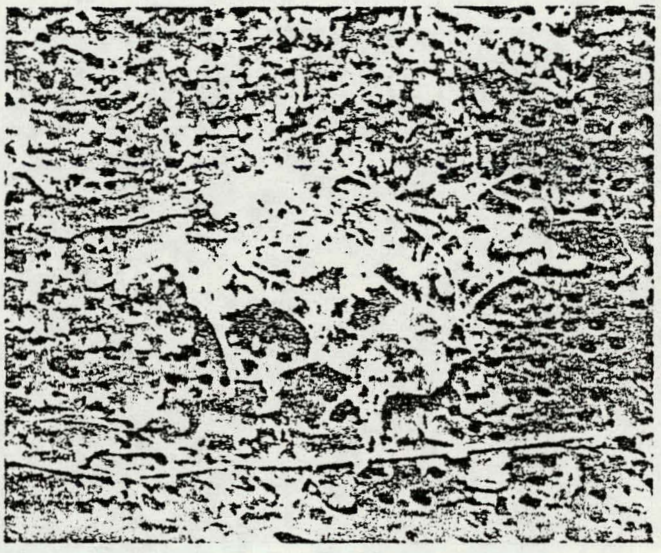

Figure 1. Free-living minibacteria collected on a 0.2 μm filter. Some of them have developed extensive extracellular processes, of which some have adhered to form a small aggregate in the center of the field. Scanning electron micrograph by Kenneth Kerrick.

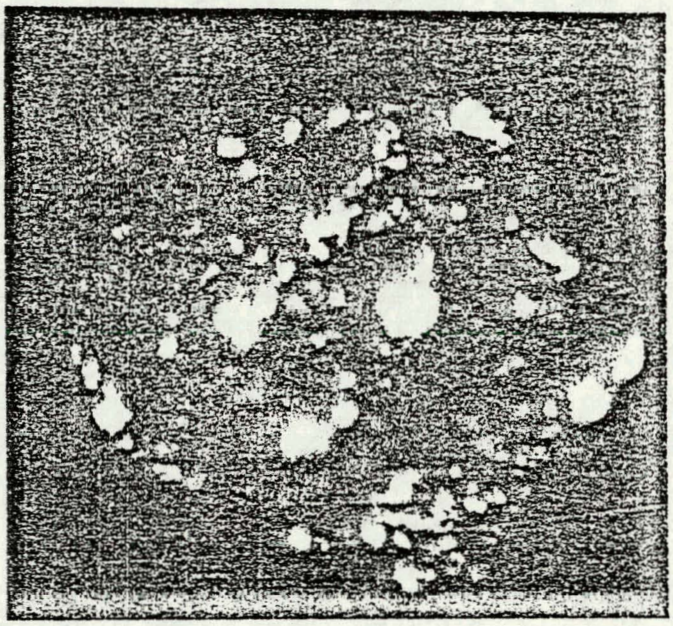

Figure 2. Fecal mass, 48 hours old, produced by the salp, *Thalia democratica*. Epifluorescence photomicrograph of acridine orange stain shows growth of bacteria within the mass. The entire particle is approximately 25 µm in its longest dimension.

pellet is then rapidly dispersed (Johannes and Satomi 1966). The fragmentary remains of old fecal pellets are largely devoid of bacteria (Wiebe and Pomeroy 1972). Whether this is because of the refractory nature of the substrate or because of the grazing pressure on such particles is not known.

Some other populations of bacteria occupy the surfaces of living phytoplankton and macrophytes (Sieburth 1979). They are in a favorable position to utilize low molecular weight dissolved organic compounds that are released from the plants.

The classification of microbial populations into a rational scheme of community structure proves difficult and challenges classical ecological theory. While it is relatively straightforward to organize the classical plant-grazer-carnivore structure into trophic levels, the microbial community, living at the expense of a variety of particulate and dissolved substrates, does not fit that scheme. Although there are several energy transfer steps between substrate, bacteria, and grazers, both protozoan and metazoan, they cannot be separated into neat, distinct trophic levels. This would be a trivial problem had not ecologists given the trophic level concept such a strong sense of reality. It must be remembered that the trophic level is a simplifying abstract concept that is, in this case, too simple to be useful (Pomeroy 1979).

Substrates for Bacteria in Natural Waters

Most of the open waters of lakes, estuaries, and oceans are aerobic and are the domain of the nonexacting heterotrophs. The bottom waters of stratified lakes, the Black Sea, the Cariaco Trench, and the sediments of shallow, eutrophic systems are anaerobic. These anaerobic zones and

the interfaces with the aerobic zones provide habitats for chemolithotrophs and other metabolically specialized bacteria. Although the anaerobic zones represent only a small fraction of the total area of natural waters of the world, bacterial activity in them probably has a significant impact on the planetary cycles of nitrogen. However, the mediation of energy is dominated by the communities in aerobic waters.

The largest single pool of organic matter potentially available to bacteria is that dissolved in natural waters. Total dissolved organic matter varies from less than 1 mg/l in the open ocean to more than 10 mg/l in some estuaries and lakes (Duursma 1965; Menzel and Ryther 1970). The long controversy about the availability of this dissolved organic matter to organisms of all kinds has now been largely laid to rest (Jørgensen 1976). Most of that fraction of it which can be utilized probably is degraded by bacteria. However, it is clear that much of the dissolved organic matter of the ocean is highly refractory. The mean radiocarbon age of marine humates is around 3000 years (Williams, Oeschger and Kinney 1969), and this sort of refractory material makes up most of the standing stock. Therefore, the available standing stock of dissolved material is probably less than 1% of the total. In reality, there must be a continuum from totally refractory compounds to those like glucose, glycolate, and amino acids which require no extracellular degradation, and for which permeases are likely to be mobilized. Therefore, such compounds, which are most readily available and most actively used, will have very short residence times in the water. Estimates of the residence time of amino acids in the surface waters of the ocean are on the order of days or weeks in most cases (Andrews and Williams 1971).

The sources of dissolved organic matter are what is released by living plants (including

phytoplankton), the products of defecation and excretion of animals (quantitatively mostly from zooplankton and protozoans), and the products of extracellular degradation of particulate substrates by bacteria. The rate of production of dissolved organic matter from these various sources is much less well quantified than is the standing stock. The release of dissolved material by phytoplankton and other plants is highly variable. Under the stressful conditions typical of the central gyres of the ocean and oligotrophic lakes, nearly half of the carbon fixed may be released as glycolate and other low molecular weight compounds. In the relatively favorable conditions in coastal waters, coastal upwellings, and eutrophic lakes, less than 5% of the carbon fixed may be released (Fogg 1971). However, the absolute amount of dissolved matter released by phytoplankton is greatest in coastal upwellings and eutrophic lakes. The production of dissolved materials might be on the order of 50 to 100 mg/day, while in an oligotrophic lake or a central ocean gyre it might be on the order of 10 mg/day. Since these compounds do not accumulate beyond concentrations of a few micrograms/liter, they must be utilized by bacteria, principally the free-living minibacteria. Organic excretory and fecal products also will be produced in greatest amount in eutrophic waters and probably will amount to several times the rate of production of products released by phytoplankton; in oligotrophic waters they will not be more than the amount released by phytoplankton. While these rates are not well quantified, apparently the production of dissolved materials available to free-living bacteria is greater in eutrophic waters by an order of magnitude, and might be greater by several hundred mg/day.

The primary and principal source of particulate matter available to bacteria is from the growth of plants. In pelagic systems this means phytoplankton. Although it has often been

suggested that phytoplankton are consumed quantitatively by zooplankton (Steele 1974), both examination of naturally occurring detritus and studies of the feeding of zooplankton indicate that a substantial, if variable, fraction of phytoplankton is not eaten by zooplankton. Some species are noxious, notably cyanobacteria and some spiny or armored species, and these are but rarely eaten (Porter 1973; Porter 1977). The feeding efficiency of zooplankters varies with the size and species of food organisms available. While some species are ingested with very high efficiency, others, such as large, chain-forming diatoms, tend to be broken, with substantial losses of fragments. Those not eaten by zooplankton for one reason or another become substrates for bacteria. Quantification of the rate of production of this primary detritus is lacking.

Those phytoplankton which are ingested by zooplankton pass through the gut, with about 30% ultimately released as feces. Some zooplankton, such as crustaceans, have grinding mechanisms to break up food materials. Even so, it is commonly observed that some phytoplankters come through into the feces alive and well (Porter 1973; Porter 1977), while others are dead but little digested. Other organisms, such as salps, lack any grinding mechanism, and digestion is even less complete. Such fecal matter is a rich substrate for bacteria (Figure 2).

In shallow water environments, large, rooted vegetation, either submerged or emergent, is the major source of plant biomass production. In the coastal zone, sea grasses, kelps, and other macroalgae have very high production rates (Mann 1972); on coral reefs, a thin, heavily grazed mat of red and blue-green algae is responsible for more primary production than are the zooxanthellae of the corals themselves (Johannes et al. 1972). In terrestrial ecosystems generally the direct

consumption of plants by grazers amounts to about 10% of total primary production, the rest becoming litter and humus. Where it has been measured, the proportion of direct grazing of aquatic macrophytes also amounts to about 10% of primary production (Teal 1962). This means that most primary production goes directly into the detritus food web to be mediated by bacteria, and therefore, the flow of energy through detritus and bacteria is in fact the predominant one in most, if not all, ecosystems. It is therefore important to understand and to quantify the roles of bacteria in food webs.

Although a case is made in this review for the importance of bacteria in food webs, it must be emphasized that bacteria alone do not make a community in which varied substrates are utilized quickly and efficiently. It is necessary to have some higher organisms in the community as well to provide some functions of processing substrates. It has been known for many years that the degradation of litter, and even animal carcasses on the forest floor, depends on the activity of small invertebrate animals that chew, grind, and partly digest the material, making it more readily available as a bacterial substrate. Material enclosed in exclosure cages, which eliminate the activity of the small invertebrates, remains whole and relatively undegraded for a much longer time than does material not so protected. We now know that the same is true in aquatic environments. In streams, where the principal input is leaves, there is a guild of insect larvae which processes leaves, rapidly rendering them into fine detritus, and facilitating attack by both bacteria and fungi. Experiments similar to those done in forests have shown that in broad terms the community of the deep sea bottom functions in much the same way. They were inspired by the finding that the lunches which went to the sea bottom in the sinking of the ALVIN were little decomposed after many months (Jannasch et al. 1971; Jannasch and Wirsen 1973). While the unusual

conditions of high pressure and low temperature at the bottom of the ocean certainly contribute to the slow rate of degradation found there, it also appears that the action of benthic invertebrates is important in facilitating degradation (Sieburth and Dietz 1974).

Not only does the rate of degradation of large particulate material depend upon the action of invertebrates as well as bacteria, but so does the regeneration of essential nutrient elements, such as nitrogen and phosphorus. If conditions remain strictly aerobic, as they do in most natural waters, bacteria appear to retain all phosphate they assimilate as polyphosphates. It is released only when the bacteria are eaten and digested by protozoans or higher animals (Buchler and Dallon 1974; Johannes 1964; Johannes 1968). Under anaerobic conditions, where the excess metabolic energy necessary for synthesizing polyphosphates may not be available, phosphate is regenerated by bacteria. The fate of nitrogen may differ from that of phosphorus because of its different structural and functional roles. There appears to be no open-ended storage mechanism for nitrogen, as there is for phosphorus, in bacteria. Therefore, an excess might be released as ammonia, even under aerobic conditions. At the same time, there may be nitrogen fixation by specific bacteria and cyanobacteria. In anaerobic environments, where excess ammonia is being produced, much of it may be denitrified.

Bacteria in Aquatic Food Webs

Because they utilize a variety of dissolved and particulate substrates, bacteria occupy many positions in aquatic food webs. Distinct populations are associated with particles, including feces, while free bacteria utilize dissolved substrates from both primary and secondary sources.

The reality of any food web is much more complex than that shown in Figure 3. However, that is in itself much more complex than, and fundamentally different from, an Eltonian food chain. What would be recognized as the food chain of this generalized aquatic food web is connected by dashed lines in the upper right. All the rest is ignored by most aquatic ecologists, and most of it is the domain of bacteria, together with the specialized metazoans which feed on bacteria.

It has been argued that this part of the food web is not of interest to ecologists who are concerned with the total flux of energy and materials. One school of thought maintains that very little energy flows through these pathways. There is now substantial evidence that a relatively large proportion of the total flux of energy is mediated by microorganisms, however (Pomeroy 1974; Pomeroy 1979; Sieburth et al. 1977; Watson 1978; Table 1). Another school of thought maintains that even if more than half of the total flux of energy is through microbially mediated pathways, these pathways lead to a dead end so far as fishes and other terminal consumers are concerned, and are therefore of limited interest. While we do not have data from the real world to evaluate quantitatively the ultimate fate of energy that passes through microbial biomass, simple heuristic modeling exercises demonstrate that we cannot exclude the possibility that virtually all terminal consumers may be able to utilize energy from microbial pathways (Pomeroy 1979).

The principal basis for the conclusion that microorganisms create dead-end food webs is the assumption that there are too many transfers of energy between so-called trophic levels. Ecological dogma has it that the transfer of energy between trophic levels is about 10% efficient, and therefore can never go more than four or five steps before all of the energy from primary sources has

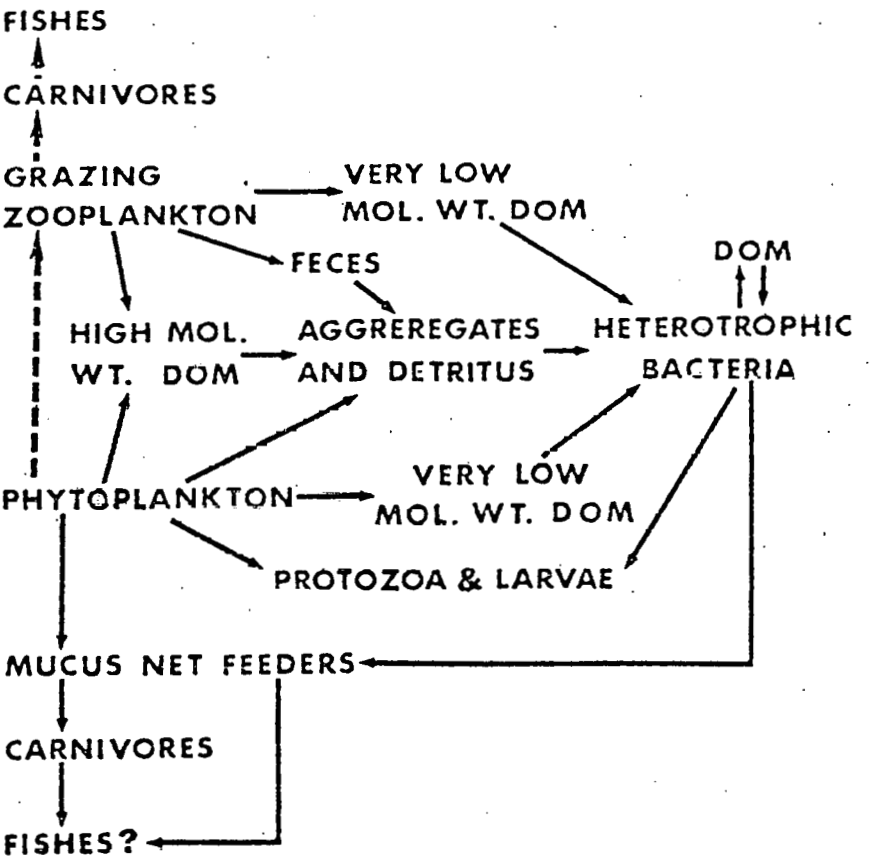

Figure 3. A generalized and simplified diagram of aquatic food webs. The compartments in the upper left joined by the heavy, dashed lines are the classical food chain. The remainder shows other compartments and pathways now recognized to exist, and illustrates the role of heterotrophic bacteria in utilizing a variety of dilute, dispersed, or somewhat refractory materials, while in turn becoming a food source for a variety of higher organisms.

Table 1. Bacterial growth rate in natural waters. In some cases mean values were calculated and units of reference changed for uniformity.

Location	Production Rate mg C m^{-3} day^{-1}	Production Rate mg C m^{-2} day^{-1}	Method	Reference
Arctic pond		5*	direct count	11
Lake Biwa, Japan	11.5		dark HCO_3	27
Lake Biwa, Japan, summer		900	direct count	27
Lake Biwa, Japan, winter		100	direct count	27
Frains Lake, Michigan	300		respiration	34
Dalnee Lake, Kamchatka	1,530		respiration	40
Ryblinsk Reservoir	57	457	dark HCO_3	24
Narragansett Bay	202		direct count	38
Butaritari Atoll Lagoon		410	respiration	40
Continental shelf water behind Great Barrier Reef		810	respiration	40
Black Sea, euphotic zone	2		respiration	40
Black Sea, chemosynthesis zone	6		respiration	40
N. Africa, coastal upwelling	3-30		rate of natural substrate production	43
Peru, coastal upwelling	11	1,600	respiration	40
N. Atlantic E. of Azores	120		direct count	38
Sea of Japan	45		respiration	40
N. & S. Pacific, mid and low latitudes	0.6	126	respiration	40

* Assuming 60 day active season

been dissipated as metabolic heat. In a recent analysis (Pomeroy 1979), the writer has shown that most of the transfers of energy between producer and consumer populations exceed 10% efficiency, and there is evidence that transfers involving microbial consumers operate at an efficiency of around 50%. Longer and more complex food webs appear to be possible, and observations of real communities of organisms tells us that they are the rule. What appeared to Hutchinson (1959) to be a paradox in classical food chain theory is probably a major stabilizing influence for complex communities (Pomeroy 1975; Pomeroy 1979).

If bacteria are metabolically active and responsive to the availability of substrates in natural waters, as they now appear to be, are they indeed links in the food web or merely energy sinks, as some ecologists now believe? If their supply of energy is intermittent, and they continue to respire when external energy sources are not available, bacteria may respire most of the organic matter they assimilate. On the other hand, if bacteria are consumed rapidly and efficiently by grazers and filter feeders, their excess production will be utilized efficiently (Pomeroy 1974). The standing stocks of bacteria in most natural waters usually are not great (10^4-10^6/ml), and do not seem to vary greatly (Ferguson and Rublee 1976; Watson et al. 1977). Yet, there is evidence that their production rate is substantial. This suggests that bacteria are consumed down to some refuge concentration at the same rate that they grow. We know that a wide variety of aquatic organisms, from protozoans to prochordates, are capable of consuming bacteria. While some uncertainty exists about the fate of bacteria in the food web, their assimilation efficiency appears to be high. A number of studies of assimilation efficiency of bacteria show it to be 50% or greater (Ho and Payne 1979; Payne 1970), but most of this is experimental work at substrate concentrations that are several orders of

magnitude greater than those found in natural waters. However, relative assimilation and respiration of defined substrates by natural assemblages of bacteria, at natural concentrations of substrates, also show efficiencies better than 50% in most cases (Hobbie and Crawford 1969). Therefore, food webs with a bacterial link may be as efficient as those without one, because of their relatively high efficiency coupled with their versatility in assimilating dilute and refractory substrates.

Contemporary techniques of microscopy and biochemistry make it possible to enumerate bacteria in natural waters reasonably well (Hobbie, Daley and Jasper 1977; King and White 1977; Watson et al. 1977; White et al. 1977; White et al. 1979), but measuring their rate of production remains a difficult problem. Direct counts on confined water samples over time are likely to be flawed by the induction of a latent period as the result of handling. It is also difficult to exclude all grazers, especially the small protozoans. Indirect measures of production, based on respiration and uptake of organic or inorganic substrates, depend upon conversion factors usually arrived at through laboratory manipulation of bacterial populations quite unlike those found in natural waters. Such factors are not really constants, even if they are carefully determined. In the end, one gets almost as good an estimate of production by inferring it from a known rate of substrate production (Watson 1978). Because of these uncertainties, the production values in Table 1 must be viewed as provisional ones, particularly since they involve the entire range of methods. While they may not be absolutely correct, they produce a credible picture of the production rate of bacteria in natural waters, which varies over six orders of magnitude. The arctic pond is, not surprisingly, lowest by an order of magnitude. The disparity between the Peruvian coastal upwelling and Narragansett Bay

indicates that the latter is not one of the most eutrophic of estuaries. Lakes have a wide range of bacterial production values, with smaller eutrophic lakes actually approaching sewage oxidation ponds in their level of activity. The larger oligotrophic lakes are not well represented in the table.

Bacterial biomass may at times equal or even exceed that of phytoplankton, and bacterial production may sometimes be equivalent to a significant fraction of primary production (Sieburth et al. 1977; Watoon 1978; Watson et al. 1977). Therefore, it is potentially a major food chain link. Of course, not all higher organisms can consume bacteria, especially the free-living minibacteria. They must be utilized either by protozoans or by mucus net feeders, such as salps and appendicularians. We must then ask what organisms consume the protozoans and mucus net feeders, and what is the efficiency of assimilation of energy in such a pathway. It is possible that the bacteria are efficiently used by organisms that are themselves more or less dead ends. If this is so, an environment which promotes production of bacteria might stimulate secondary productivity, but at the expense of other secondary pathways that lead to the production of fishes. There are many gaps in our knowledge of food webs, not only at the level of bacteria and protozoans, but at higher levels as well. A clear overall picture of aquatic food webs has not emerged, in part because there has not been enough interaction between aquatic microbiologists and aquatic ecologists. Neither alone can see the entire system and how it works.

Questions not Answered

Aquatic microbial ecology is a fast growing field in which there have been some notable recent successes. Substantial progress has been made in bringing the best existing techniques in

epifluorescence and scanning electron microscopy to bear on aquatic problems. There has also been rapid development of applications of biochemcial methods to the problems of microbial ecology through the use of adenylates, lipopolysaccharides, and muramic acid as parameters of microbial biomass. However, it is still difficult to make clear distinctions between major taxa. Adenylates do not distinguish taxa at all. Muramic acid does not distinguish cyanobacteria from other bacteria. Because direct counts, while very useful, are terribly time-consuming, there remains a need for rapid, relatively straightforward analytical methods to measure the biomass of distinct taxa of aquatic microorganisms. Although the methods mentioned appear not to be sufficiently specific for major taxa, immunofluorescence appears to be too highly specific for most of the kinds of questions addressed here.

Measures of such seemingly well understood processes as photosynthesis and respiration do not always yield values which can be rationalized (Joiris 1977). The standard method for measuring photosynthesis in aquatic systems appears to underestimate the rate by a highly variable amount. There is no accepted method for measuring respiration in oligotrophic natural waters, and one would be especially useful if it were possible to use it to distinguish the respiratory rates of at least the autotrophs from the heterotrophs.

Surprisingly little is known about the food webs of aquatic systems and least of all is known about the microbial aspects of them. Most models of the flow of energy and materials through aquatic ecosystems either do not include bacteria at all, or include them implicitly in some other category, such as feces. Most aquatic ecologists and fisheries biologists are not convinced that bacteria play a significant role in the flow of energy through aquatic food webs. At the same time, most aquatic

microbiologists are not interested in the interdisciplinary questions concerning the production of substrates for microorganisms or the consumption of microorganisms by grazers and mucus net feeders. Many of these problems will yield only to team research, of which microbiology must be an integral part.

During the decade of the 1970s, the microbial populations of pelagic waters were identified by direct observation; their biomass was determined by both direct observation and biochemical means, and their metabolic activity was estimated using tracer methods. On the basis of these and other studies of pelagic ecosystems, a paradigm of the microbial pelagic food web was developed out of earlier concepts of the detritus food web of shallow waters. In the coming decade the details of this microbial food web will be revealed and quantified. If the rates of microbial consumption and production of energy-containing organic compounds prove to be as great as present indications suggest, several basic ecological concepts will have to be reexamined, not only in natural waters, but in ecosystems generally. The paradigm of a short, direct food chain from plants to grazers to carnivores with relatively low assimilation efficiency will have to be fundamentally modified to encompass the varied activities of microorganisms and their relatively high assimilation efficiency.

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Phosphorus Uptake by Microplankton in Estuarine and Coastal Shelf Water near Sapelo Island, Ga., U.S.A.

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ABSTRACT

The residence times of orthophosphate in estuarine and coastal shelf waters near Sapelo Island, Ga., ranged from 1.6 to 105 hours. Rates of orthophosphate uptake by microplankton varied from 1.4 to 62.2 $\mu g\ P\ l^{-1}\ h^{-1}$. Generally when isotopic equilibrium was reached after the addition of ^{32}P orthophosphate, significant amounts of ^{32}P remained in solution, suggesting that the supply of phosphorus to microplankton was not limiting in these waters. In coastal shelf waters, the majority of phosphorus uptake (>60%) was associated with 'single' bacteria (<1 μm); whereas in estuarine waters or in a Gulf Stream intrusion usually a proportionately greater amount of phosphorus was incorporated into algae, clumped or attached bacteria (>1 μm). The ^{32}P-orthophosphate incorporation into a cold 10% TCA precipitable cellular fraction was more consistently linear with time than into whole cells and this procedure may be useful for future comparative studies of phosphorus utilization by microplankton.

Although considerable work on nutrient cycling within the Spartina salt marshes has been reported (Pomeroy, et al., 1972) there is relatively little information concerning phosphorus flux into the microplankton of the tidal waters in these estuaries. Correll, Faust and their collabrators, in an extensive series of studies (Correll et al., 1975; Faust and Correll, 1976; 1977; Friebele et al., 1978), have elucidated details of the uptake of orthophosphate by phytoplankton and bacteria in the Rhode River, a sub-estuary of Chesapeake Bay, and Taft et al., (1975) reported on phosphorus uptake and release by phytoplankton in the Chesapeake.

In this paper, I present data on phosphorus residence times and uptake rates from tidal creeks at Sapelo Island, Ga., and compare these with results from offshore coastal shelf waters. By using differential filtration (Sheldon, 1972; Berman, 1975; Faust and Correll, 1976) I have attempted to determine the relative activity of the bacterial components of the total microplankton assemblage. I also tested a method of chemical fractionation of natural microplankton populations with cold trichloracetic acid (TCA) in order to estimate the rate of phosphorus incorporation into a 'macromolecular' fraction of the organisms and suggest that this might prove a useful technique for comparing metabolic activities of various microplankton populations.

Methods

The sampling sites in the Duplin River, estuarine creeks, and Doboy Sound off Sapelo Island, Ga., are shown in Fig. 1. In actuality, the Duplin is a tidal slough and not a river in the usual sense, because the only fresh water sources at its headwaters are rainfall on the surrounding marshes and island groundwater runoff. Water movement and flushing are controlled

mainly by tidal currents (tidal amplitude, 2 to 3 m) and salinities range from 15 to 20%. The salinities at the mouth of the Duplin River and in Doboy Sound which were ~28% during this study are strongly affected by seasonal changes in discharge of the Atlantic River. Salinities at the headwaters of the Duplin River can fluctuate widely and after severe rainstorms can fall as low as 3% (Imberger et al., 1980).

Sampels of coastal shelf waters were taken during a cruise on the R.V. Kit Jones, at a single station (approximately 30^{o} 30' N, 80^{o} 30' W).

Surface samples were taken with a bucket from the tidal creek stations and at various depths with a 5 l Nisken sampler at offshore stations. After prefiltration through a 150 µm net to exclude the larger zooplankton, water samples (20 or 50 ml) were placed in sterilized Ehrlenmeyer flasks. Experiments were begun by adding appropriate amounts of carrier-free ^{32}P-orthophosphate which had been previously diluted into a small volume (0.5 to 1 ml) of autoclaved seawater and filtered through a sterile 0.45 µm Millipore filter immediately prior to use. The experimental flasks were incubated in low to medium intensily light (40-200 µEin $m^{-2}sec^{-1}$) at the temperatures of the sampling locations. At appropriate times, duplicate or triplicate 3 to 5 ml portions were filtered onto 0.45 µm Millipore filters, presoaked in 0.05 M K_2HPO_4. The filters were rinsed with 5 ml filtered sea water containing 0.05 M K_2HPO_4, dried, placed in Instagel (Packard) scintillation fluid, and counted in a Packard Tricarb Scintillation Spectrometer.

For each experiment, poisoned controls were prepared by adding Lugol's iodine to subsamples. Alternatively, "zero time" controls were made with chilled water samples. Both these methods gave similar levels of background radioactivity. Incorporation rates for phosphorus were estimated from the initial slopes of the uptake curves.

In order to prepare estuarine or sea water containing only "free bacteria" natural water samples were prefiltered at low vacuum (< 100 mm Hg) through a 1 μm Nuclepore filter (47 mm diam) prior to the addition of the radioactivity. Although this procedure certainly removes almost all of the phytoplankton (Berman, 1975; Azam and Hodson, 1977; Harrison et al., 1977), it is possible that some very small chlorophyll bearing cyanobacteria also pass through such filters (Waterbury et al., 1979).

In some experiments the incorporation of ^{32}P orthophosphate into intact cells and into a "macromolecular fraction" was measured in parallel. At each time one set of duplicate sub-samples (3 to 5 ml) was filtered directly, as described previously, while a second set was placed in test tubes containing equal volumes of chilled 20% trichoroacetic acid (TCA). After 30 mins at 0°C, the contents of the test tube were filtered on 0.45 μm Millipore, rinsed with two 2 ml portions of cold 10% TCA, dried and the radioactivity counted as described above. (Subsequent experiments in a freshwater lake showed no significant differences between microplankton samples prescipitated with 5% or 10% cold TCA).

Results and Discussion

Orthophosphate Residence Times and Uptake Rates

Residence times for orthophosphate (P_i) in these waters ranged from 1.6 to 105 hours (Tables 1 & 2). The shortest residence time occurred in the presence of a dense bloom of Kryptoperidinium sp. in the upper Duplin River (station 175) when chlorophyll concentrations reached 78 μg l^{-1}. The residence times of P_i in the estuarine system generally increased

from the head of the Duplin River towards the bay (Doboy Sound). In coastal shelf water, residence times were much longer but varied considerably. I never measured extremely rapid cycling (e.g. several minutes) that others have reported in oligotrophic, phosphorus limited lakes (Rigler, 1956; Lean, 1973) the Rhode River system (Correll et al., 1975) or Chesapeake Bay (Taft et al., 1975).

At stations where ambient P_i concentrations were also measured the absolute rates of orthophosphate uptake could be determined (Table 1). These rates reflected the same spatial pattern as phosphorus residence times. Within the Kryptoperidinium bloom, a maximum uptake rate of 62.2 $\mu g\ P\ l^{-1}h^{-1}$ was measured while in the open waters these uptake rates decreased to 1.4 $\mu g\ P\ l^{-1}h^{-1}$. When related to concentrations of chlorophyll these rates are considerably faster than noted by Harrison et al., (1977) for Pacific water but similar to those given by Correll et al., (1975) and Faust and Correll (1976) for Chesapeake Bay. The highest phytoplankton biomasses, (measured as chlorophyll) and the most rapid turnover times were found at stations with the greatest ambient P_i concentrations.

Because of the relatively slow uptake of P_i that was observed, the determination of ^{32}P uptake by incorporation into the particulate fraction rather than by the drop of radioactivity in the filtrate was probably justified (Correll and Faust, 1975). Obviously, if there was extensive grazing and subsequent excretion of dissolved phosphorus by microplankton during the incubation period (Sheldon and Sutcliffe, 1978) the actual residence times may be shorter than indicated by my data. The difficulty of determining the true initial phosphorus uptake rates accurately may be similar methods, Pomeroy (1960) reported residence times ranging from 1 to 169 hours and uptake rates from 0.1 to 19 $\mu g\ P\ l^{-1}h^{-1}$ for these waters.

The partitioning of ^{32}P between particulate and dissolved forms was measured after 24 hours incubation (Table 3). By this time isotopic equilibrium had probably been reached (Berman and Skyring, 1979). Further incubation for another 24 hours did not change the ^{32}P partitioning greatly (Station 7, and Station 9, 24 July 1977). In two experiments (12 and 24 July, Stations 1 and 9) I measured the changes in dissolved inorganic phosphorus (P_i) concentrations over 24 hours by standard chemical methods and compared these to the uptake estimated from ^{32}P incorporation into particulate matter. The P_i level fell by 48% and 44% while uptake calculated from ^{32}P uptake was 46% and 49% respectively. Although in a few cases most of the ^{32}P orthophosphate was taken up into organisms after 24 hours, usually a significant amount remained in dissolved forms. Presumably this observation reflected conditions of generally adequate P supply for the microplankton in these waters. The exceptional results (e.g. Station 3, 8 July; Station 7, 27 July) may be experimental artifacts or more likely are due to localised inhomogeneties of phosphorus supply and microplankton uptake.

In these studies I did not investigate any diurnal variations within a defined water mass. The effects of light on phosphorus uptake by natural microplankton is still unclear. Harrison et al., (1977) found that very large fluctuations of phosphorus uptake occurred over a 24 hour period in Southern Californian waters but Perry (1976) observed no diel periodicity of uptake in the North Pacific. Other investigators have also reported little influence of light (Correll et al., 1976; Taft et al., 1975; Berman and Stiller, 1977). Presumably such discrepencies arise from the specific composition and physiological state of the microplankton in the system. For example, if phytoplankton are an important

population component and are growing rapidly, one might expect a marked stimulatory effect of light on phosphorus uptake.

Partitioning of P uptake by total microplankton and bacteria

Considerable attention has been focused on the relative metabolic activities of various components of the microplankton population (Malone, 1971; Faust and Correll, 1976; Yentsch and Ryther, 1959; Berman and Stiller, 1977; Friebelle et al., 1978). Usually, fractionation of the microorganisms has been accomplished by differential filtration (Sheldon and Sutcliffe, 1969) either before or after incubation with radioactive tracers. In this study, I prefiltered samples through 1 μm Nucleopore filters in order to obtain a fraction essentially free of algae and clumped or attached bacteria. The proportion of $^{32}P\text{-}P_i$ taken up by 'single bacteria' compared to incorporation by total microplankton ranged from 11% to 95% (Table 4). For the estuarine waters of Sapelo Island the relative amount of P uptake attributed to "single" bacteria tended to be low, with the exception of one sample from Station 7 on 24 July 1977. For coastal shelf waters, more than 60% of the phosphorus incorporation was associated with the "single" bacteria although lower values were found in Gulf Stream intrusion water (15 m and 20 m on 15 August 1977). Curiously, there were no marked changes in P_i residence times between 5 m and deeper water on this date (Table 2). These data are in agreement with the observation of Harrison et al., (1977) who reported that at least 50% of phosphorus uptake in Coastal Pacific waters was associated with organisms smaller than 1 μm. In the Duplin River and Doboy Sound the relatively greater amounts of phosphorus uptake attributed to attached bacteria could result from the larger amounts of silt and ditrital

matter in the water column. Hansen and Weibe (1978) have also noted a similar trend in the distribution of single and attached (or clumped) heterotrophic bacteria responsible for glucose uptake in these waters. The wide variability of uptake by "single" bacteria relative to the total microplankton which I observed was probably due to rapid local changes in the size distribution spectrum of the planktonic population and detritus. However, it is clear that, especially in pelagic waters, unattached bacteria are often responsible for a large portion of the total phosphorus uptake.

Phosphorus Uptake into "Macromolecules" (10% TCA precipitate)

The kinetics of ^{32}P orthophosphate incorporation into whole cells are often difficult to interpret (Brown and Koonce, 1978) possibly because of multiple uptake mechanisms, (Brown and Button, 1979) rapid cycling of phosphorus compounds within intracellular pools (Taft et al., 1975; Berman and Skyring, 1979), and some release of phosphorus from the cells to the exterior medium (Loan, 1973). I followed the time course of ^{32}P orthophosphate incorporation into a 10% cold TCA precipitate fraction ("macromolecules") and compared this with uptake into unfractionated cells (Figs. 2 & 3). In some experiments, the uptake of ^{32}P orthophosphate into intact cells was fairly linear with time but, despite corrections for non-biological adsorption of isotope, the graphs did not extrapolate to zero, suggesting that initially there was a faster uptake rate than that measured subsequently. For other experimental runs, the time course of phosphorus uptake into cells more closely followed a hyperbolic pattern. In this study the uptake of ^{32}P orthophosphate into the TCA precipitable fraction was more consistently linear with time, irrespective of the

form of uptake into whole cells. If this consistency proves to be general then measurement of incorporation of ^{32}P into the TCA precipitable fraction may be a useful method to compare the phosphorus status of natural microplankton populations and their response to phosphorus additions.

The ratio of "macromolecular" P to "total cell" P after isotopic equilibrium had been reached (24 hrs) was also measured. The difference between these two quantities represented an acid soluble phosphorus pool, part of which may include the "surplus" P fraction of Fitzgerald and Nelson (1966). "Bacterial" populations (< 1 µm) usually had a relatively greater percentage of P in "macromolecules" (average 75% ± 9%, of total P) than did the total microplankton population (average 62% ±14%, of total P), suggesting that soluble phosphorus pools were usually slightly larger in algae than in bacteria.

The phosphorus fraction precipitable with TCA may also be useful in characterising the physiolocial status of microplankton. Recent work by Algavish (1978) suggests that, for some freshwater algae, the intracellular levels of phosphorus in this fraction are inversely related to the rates of phosphorus uptake and alkaline phosphatase activity of these organisms. A more detailed study of the biochemical components of the TCA precipitable phosphorus pool, their fluctuation and metabolic control in aquatic microorganisms would seem very justified.

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Table 1. Orthophosphate Residence Times and Uptake Rates and Chlorophyll Concentrations in Estuarine Waters*

Station No.	Date	Tidal state	Chlorophyll ($\mu g\ l^{-1}$)	Ambient Pi P (μg at l^{-1})	Residence time (h)	Uptake rate ($\mu g\ P\ l^{-1}h^{-1}$)
1	8 July 1977	High slack	-	15.9 m	12.7	17.4
2	"	"	-	2.2	19.7	1.6
3	"	"	-	2.3	14.4	2.2
4	"	"	-	2.3	18.1	1.8
6	"	"	-	9.9	17.0	8.1
2	27 July 1977	Low slack	2.12	3.7	15.7	3.3
3	"	"	1.86	3.7	17.0	3.0
4	"	"	6.00	3.3	12.7	3.6
5	"	"	14.6	3.9	3.5	15.7
6	"	"	-	7.2	7.6	12.8
6+	"	"	31.80	10.6	3.3	38.6
7+	"	"	78.00	7.1	1.6	62.2
2	28 July 1977	Low slack	0.34	4.0	24.6	2.3
3	"	"	1.66	3.8	38.9	1.4
4	"	"	3.68	3.0	13.6	3.1
5	"	"	0.54	3.2	8.1	5.6
6	"	"	-	5.0	10.0	7.0
2	28 July 1977	High slack	1.32	3.4	-	-
3	"	"	1.87	3.3	11.4	3.9
4	"	"	1.40	3.7	21.9	2.4
5	"	"	1.24	4.0	14.6	3.8
6	"	"	-	3.7	7.8	6.6
7	"	"	2.52	3.3	15.5	3.0

* See Fig. 1 for site locations.

+ In the presence of a bloom of Kryptoperidinium sp.

Table 2. Orthophosphate Residence Times in Coastal Shelf (C.S.) and Gulf Stream Intrusion (G.S.I.) Waters*

Water mass	Date	Depth (m)	Residence Time (h)
C.S.	15 Aug. 1977	5	96
G.S.I.		15	86
G.S.I.		20	105
C.S.	16 Aug. 1977	5	42
C.S.		15	57
C.S.		20	24

*Station location 30° 30' N, 80° 30' W

Table 3. Percentage of Particulate to Total ^{32}P after incubation of natural microplankton

Station No.	Date	Incubation time (h)	Particulate P / Total P (%)
2	8 July	24	55
3	"	24	74
4	"	24	54
6	"	24	27
1	12 July	24	49
7	17 July	24	55
8	22 July	24	42
7	24 July	24	25
7	"	48	34
9	"	24	35
9	"	48	48
9	26 July	48	97
2	27 July	18	29
3	"	18	22
4	"	18	20
5	"	18	47
6 +	"	18	20
6 f	"	18	32
7	"	18	100

+ Kryptoperidinium bloom

Table 4. "Single" Bacterial (< /μm) Uptake of Phosphorus as Percentage of Total Microplankton Uptake.

Station	Date	Tidal state	Incubation (h)	'Bacteria' / Total %
1	12 July	Low slack	24	18
7	17 July	Low slack	24	33
8	22 July	High tide	24	46
7	24 July	High tide	24	95
9	24 July	High tide	24	11
9	26 July	Low tide	24	15
* C.S.	15 August	5 m	24	78
* G.S.I.	15 August	15 m	24	17
G.S.I.	15 August	20 m	24	15
C.S.	16 August	5 m	21	76
C.S.	16 August	15 m	21	61
C.S.	16 August	20 m	21	75

* Station location 30° 30' N; 80° 30' W (C.S. = Coastal shelf water, G.S.I. = Gulf Stream Intrusion).

FIGURE LEGENDS

Fig. 1. Locations of sampling sites on Duplin River and Doboy Sound.

Fig. 2. Time course of ^{32}P orthophosphate uptake into intact cells and cold 10% TCA fraction.

A. Station 1, 13 January 1977. All other samples from 8 July 1977:
B. Station 1, C. Station 4, D. Station 3, E. Station 2,
F. Station 5; Intact cells; 10% cold TCA precipitate O.

Fig. 3. All samples taken on 22 July 1977. A. Station 2, B. Station 3, C. Station 4, D. Station 5, E. Station 5 upstream, F. Station 6 G. Station 6 (*Kryptoperidinium sp.* bloom) Intact cells; 10% cold TCA precipitate O.

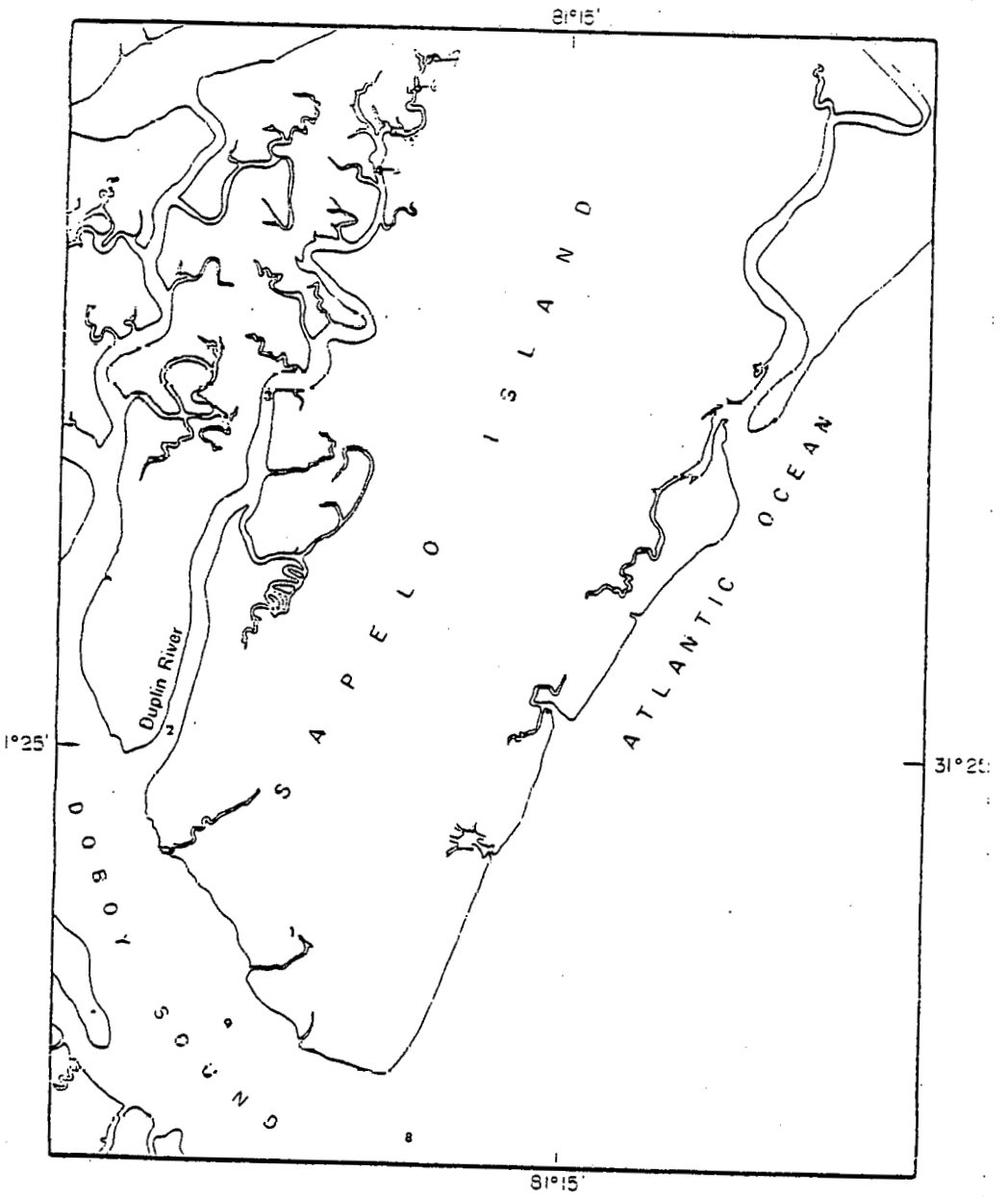
81°15'
SAPELO ISLAND
ATLANTIC OCEAN
Duplin River
1°25'
31°25'
81°15'

Figure 1

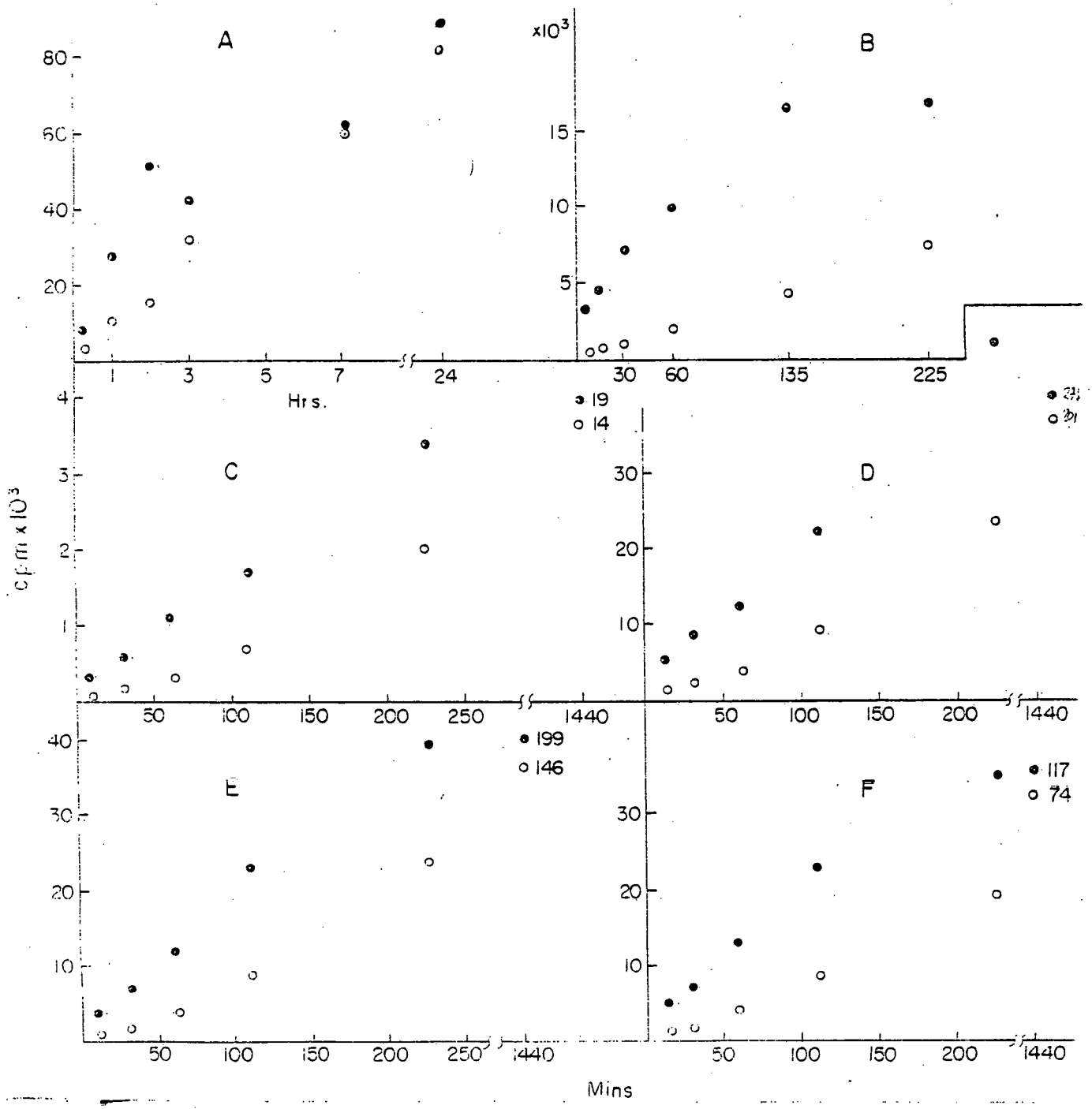

Figure 2

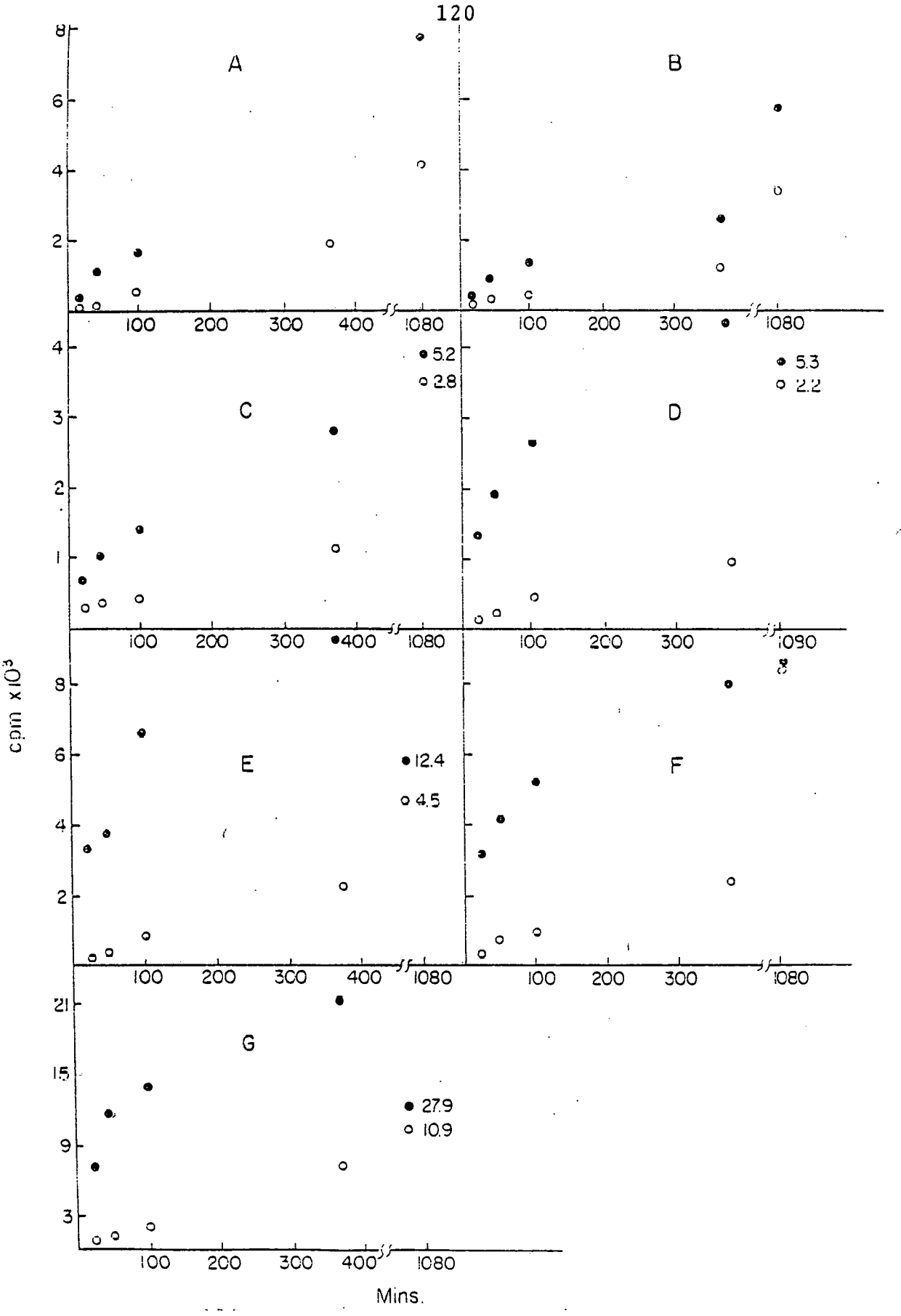

Figure 3

MICROBIAL DISTRIBUTION AND ABUNDANCE IN RESPONSE TO PHYSICAL AND BIOLOGICAL PROCESSES ON THE CONTINENTAL SHELF OF SOUTHEASTERN U. S.

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Abstract

The distribution and abundance of bacteria and phytoplankton on the continental shelf of southeastern U. S. was observed in relation to physical processes. Phytoplankton production was influenced by inputs of water of reduced salinity from the estuaries and by inputs of high salinity, low temperature North Atlantic Central water from the west front of the Gulf Stream. The distribution of chlorophyll suggests that in both cases production is influenced both by inputs of nitrogen and by the enhanced vertical stability associated with the stratification of waters of different densities. The standing stock of bacteria on the inner shelf, 10^6 per ml, is little changed by the influx of water of reduced salinity. On the outer shelf, where the usual standing stock of bacteria is 10^5 per ml, the numbers increase to 10^6 per ml in water above intrusions of Gulf Stream water in which phytoplankton blooms have developed, suggesting that the bacteria respond to both primary and secondary products of phytoplankton production. Adenylate energy charge values in the waters of the southeastern shelf are variable and volatile. At some times values of 0.7-0.8 are widespread over most of the shelf, while at other times values <0.6 are widespread, with localized patches of high values. Both autotroph-dominated and heterotroph-dominated microbial communities show these variations of energy charge.

Introduction

Bacteria and other microorganisms are abundant and responsive components of marine communities. Because of their potentially short doubling times, bacteria may be the first populations to respond to changing physicochemical conditions, and their responses may be indicative of changes which will be manifested later in the populations of phytoplankton and zooplankton. However, the bacteria also respond to biological changes such as rates of production of phytoplankton and zooplankton (Iturriaga, in press), so although their intrinsic growth rates may be greater than those of the phytoplankton and zooplankton, their population dynamics may be closely tied to the latter. Recent studies suggest that production of bacterial biomass may be a significant link in the food chain, rapidly cycling both dissolved and particulate matter back into living biomass (Fuhrman and Azam, 1980, King et al. 1980) and using a large fraction of available organic matter as respiratory substrates (Williams, 1981). In continental shelf waters of southeastern U. S. the bacterial biomass is about equally divided between small (0.2-0.5 μm) bacteria free in the water and larger (1-2 μm) bacteria attached to particles of non-living organic detritus (Hodson et al. 1981). Few bacteria appear to be attached to living plankton. As part of a multidisciplinary study of the southeastern continental shelf we have examined the abundance and distribution of bacteria and some other microbial populations in relation to the hydrographic regime and the biological responses of phytoplankton and zooplankton to it.

The southeastern continental shelf is broad, exceeding 100 km in width near the center of the bight, and comparatively shallow, <100 m at

the shelf break. There is considerable variation, both temporal and spatial, in water chemistry in response to physical and biological processes impinging differentially on various parts of the shelf (Atkinson, 1977; Atkinson et al, 1978; Bishop et al., 1980; Blanton et al. 1981).

Wave-like perturbations (meanders) on the western edge of the Gulf Stream induce upwelling of cold, nutrient-rich North Atlantic Central Water (Lee et al., 1981). Under favorable hydrographic conditions, the upwelled water can intrude along the bottom and displace large amounts of shelf water (Blanton, 1971; Blanton and Pietrafesa, 1978). Particularly favorable conditions occur during summer when shelf water has its lowest density (Atkinson, 1977). Moreover, certain regions of the shelf favor frequent intrusions. The Florida shelf north of Cape Canaveral and the shoals separating the Carolina Embayments induce divergins flow patterns that cause upwelled water at the shelf break to intrude far onto the continental shelf. Such is not the case off Georgia where it has been shown that summer bottom termperatures on the shelf are warmer and less vertically stratified than those off Florida or Carolina Embayments (Blanton et al., 1981).

Meanders occur each 5-10 days (Webster, 1961) and propagate northward at about 30 km/day (Legeckis, 1975). The wave-like nature implies upwelling of nitrate water as the Gulf Stream swings offshore followed by downwelling and some removal of nitrate as the stream approaches the shelf break. Thus the meanders, when considered by themselves, both add nitrogen to and remove nitrogen from the photic zone near the shelf break. Lee et al., (1981), using a termperature-nitrate correlation, have calculated a net onshore transport of nitrogen suggesting that the wave process is assymetrical. Because meanders progress northeastward along the shelf break, the upwelling-down-welling events presumably progress in a similar manner along the outer continental

shelf. As the water rises into the euphotic zone on the upper continental slope, a phytoplankton bloom develops, so that the bloom is well underway when an intrusion comes onto the shelf (Yoder et al., in press).

Most river influence impinging on the southeastern shelf is between Cape Fear and the St. Johns River (30-34°N). Onslow and Raleigh Bays, as well as the Florida coast, have relatively little river input, with water of high salinity near shore. The discharge of 1-5 km^3 of fresh water per month into the coastal zone of the Southeastern U.S. (Atkinson et al., 1978a), interacting with tides having an amplitude of 2-3 m (Blanton, 1980), produces both vertical stratification and frontal features (Blanton and Atkinson, 1978). Blanton (1980) points out that there is a zone of reduced salinity, which covers approximately the first 10-15 km of the shelf. It is bounded offshore by a density discontinuity. This coastal water has a high rate of primary productivity throughout most of the year (Thomas, 1966 Haines and Dunstan, 1975) and supports major fish and shellfish populations. The region of reduced salinity is most often found off the Georgia-South Carolina coast. During autumn, however, southwestward wind stress is well organized over the inner shelf (Weber and Blanton, 1980). Climatological oceanographic data show that lowest coastal salinities are found off the Florida caost during autumn in response to the autumn wind regime (Atkinson et al., in preparation). Since there are no large rivers on the Florida coast except for the St. Johns, reduced salinity water found off Florida in autumn probably originate from the zone of large river input farther north.

Because of its size and variability, there are problems of scale in developing a research program to understand processes on the continental shelf. We began with a series of four cruises consisting of 6 - 8 transects of the shelf between Cape Fear and the St. Johns River with stations every

10 - 15 km. An interdisciplinary group then examined particular hydrographic features of the shelf, such as the influence of the Gulf Stream on the outer shelf, the influence of fresh-water runoff on the shelf, and the regime of coastal fronts and jets near shore. The results of these latter studies have made it possible to understand better the findings of initial surveys, while the surveys provide perspective with respect to the extent of the influence of some of the special hydrographic and biological features. Because this research extends over 6 years, 1975 - 1980, there has been some evolution of both methods and perspectives. Some of the early cruises do not provide all of the data which we might like to have today, but they are useful nevertheless.

Methods

On the 1975-76 cruises, temperature was measured with a mechanical bathythermograph or expendable bathythermographs, and on later cruises with a Plessy CTD system and expendable bathythermographs. Salinity was measured on the 1975-76 cruises by precision salinometer readings of samples from Niskin bottles and after that from corrected readings of the CTD records. Chlorophyll _a_ was measured by the HPLC method of Jacobsen (1978) on 200-1000 ml samples collected on Reeve-Angel 984H glass fiber filters and extracted with 90% acetone. Because exclusion of interfering phaeopigments is complete, the values obtained may be slightly lower, and occasionally much lower, than values based on acid-corrected fluorescence methods (Jacobsen, in press). Adenylate samples were collected on 0.45 μm membrane filters which were plunged into boiling bicarbonate buffer before reaching total dryness. The extracts were quickly mixed with cold tris buffer and frozen for analysis on shore. Adenylate analyses were performed by the method of Chapman et al. (1971) as modified by Karl and Holm-Hansen (1978a). Adenylate energy charge

(EC) ratio (Karl and Holm-Hansen, 1978b) and the ratio of total adenylates to chlorophyll (A/C) were calculated from adenylate and chlorophyll data (Campbell et al. 1979). The A/C ratio indicates the trophic group, autotrophs or heterotrophs, having dominant biomass, thereby indicating the group whose EC ratio is determined. If the A/C ratio is less than three, autotrophs are dominant; if it is greater than ten, heterotrophs are dominant (Ibid.).

On most cruises a Zeiss standard microscope equipped with epifluorescence was used at sea to observe freshly collected material. However, routine counting of bacteria and other microorganisms was done later on shore with samples preserved in cold glutaraldehyde buffered with cacodylic acid and kept at 5<0> C. At various times we used both Sartorius SM, 0.2 μm black filters and Nuclepore 0.2 μm filters stained with Ergalan black (Hobbie et al. 1977). We also used both acridine orange and DAPI (Porter and Feig, 1980) stains. To detect possible bias from the various treatments, a comparison was made of both filter types, using both stains, with slides prepared aboard ship and also in the laboratory after the cruise. In this comparison, as with all of our bacterial counting, 40 fields were counted. Mean counts for each group were compared by analysis of variance followed by f tests. There was no significant difference between counts on Sartorius and Nuclepore filters, nor was there a significant difference between acridine orange counts of slides prepared at sea and ashore. Acridine orange counts were significantly greater than DAPI counts ($p<.001$), and DAPI counts of slides made ashore were significantly greater than those made at sea ($p<.005$). These differences appear to be a result of the light sensitivity of the DAPI stain and can be avoided by processing samples in light. In the comparison, processing on board ship was done in a laboratory with daylight illumination. Most of the counts reported here were done with acridine orange, which we find somewhat

easier to use. DAPI offers advantages when interfering autofluorescent materials are encountered in the samples, but on the southeastern continental shelf we have only rarely found that to be a problem.

Water samples were collected in cleaned Niskin samplers, individually placed on the hydro wire or attached to a rosette. In either case, water samples were drawn immediately after the bottles were brought on deck. Because of the shallow depths involved in this study and the rapidity of removal of water from the samplers, settling of material within the Niskin samplers after closure was not a problem.

Observations

Surveys of the shelf.

During 1975 and 1976 six to eight transects of the shelf, with stations 10-15 km apart, were made on four occasions. Major features of the distribution of microbial biomass and related parameters were seen in these broad surveys. In subsequent years a number of sections of the continental shelf were observed on cruises of R/V BLUE FIN, supplementing both the coverage and the kinds of data collected, such as counts of bacteria and other organisms.

In June 1975, fresh-water runoff onto the continental shelf was high, with a prominent plume off the Savannah River and a lens of water of reduced salinity in mid-shelf off Charleston (Figures 1 and 2). Salinity increased toward Florida and toward Onslow Bay, reflecting the absence of major rivers in those regions. Chlorophyll *a* concentrations were quite high, exceeding 5 mg l^{-1} along the Georgia coast and in the water of reduced salinity in mid-shelf off Charleston. However, chlorophyll *a* maxima were associated with interfaces between water of estuarine and oceanic origin rather than in the

brackish water (Figure 2). At this time the entire shelf was stratified and with a deep chlorophyll maximum. The total-adenylate/chlorophyll a ratio (A/C) was less than four everywhere, indicating that the plankton biomass was dominated by phytoplankton (cf. Campbell et al., 1979). Therefore, the total adenylates measured were largely those of phytoplankton, and indeed there was a high similarity between the distribution of chlorophyll and of adenylates.

In December, 1975, there was less fresh water on the shelf than the previous June, with the $36^{o}/_{oo}$ isohaline running along the mid-shelf region (Figure 3). Chlorophyll concentration was less than half that in June, with maximal values of around 2 mg l^{-1} along the Georgia coast and in the coastal region off Charleston. The latter was not associated with reduced salinity. A curious feature appeared off the St. Marys River (Figure 3) where a tongue of reduced-salinity water extended to the outer shelf and contained high chlorophyll concentrations. Immediately to the north there was another tongue of oceanic water of high salinity with high energy charge which showed A/C ratios greater than four but less than ten, indicating mixed autotrophic-heterotrophic biomass. Other regions of high energy charge were associated with the near-shore chlorophyll maxima and A/C ratios less than four.

In February 1976, salinity was reduced nearly to levels of the previous June, with the $36^{o}/_{oo}$ isohaline near the shelf break except off Florida and in Onslow Bay (Figure 4). Chlorophyll concentrations were lower than in December, with maxima of around one mg l^{-1} along the coast. A/C ratio was sharply divided between regions less than four (autotrophs dominant) and regions greater than ten (heterotrophs dominant). Three regions were dominated by heterotrophs: one off the St. Marys River on the outer shelf at 30° 30'N, a large one in mid shelf and outer shelf off the Charleston-Cape

Fear region at 32-33°N, and a small one off St. Helena Sound. Energy charge was high everywhere, both in the autotrophically and heterotrophically dominated areas. Total adenylates were mostly low, except for high values in heterotrophically dominated regions. Thus the mid-winter condition on the shelf was one of low autotrophic biomass and relatively high heterotrophic biomass with active, healthy populations of both.

In June 1976, the distribution of fresh water on the shelf was much like that in February with slightly less fresh water than the previous June (Figure 5). Chlorophyll concentrations were substantially lower than the previous June, except in coastal water off Georgia and in Long Bay, South Carolina. A/C ratios were less than four in the region of highest chlorophyll off the Georgia coast and were between four and ten elsewhere, except for a patch greater than ten on the outer shelf off the St. Marys River. The latter also had high concentrations of total adenylates. Energy charge was high on the inner shelf, dropping to 7 at the shelf break, with no difference between water where the biomass was autotrophically dominated heterotrophically dominated, or mixed.

After 1976 no surveys of the southeastern continental shelf as a whole were made, but some transects of the shelf, principally between Savannah and St. Augustine, were made each year, mostly by R/V BLUE FIN. While these observations did not provide synoptic views of shelf-wide conditions, they did supplement our information on a range of conditions present on the shelf. Moreover, we took additional kinds of data on some occasions. Counts of microorganisms showed both spatial and seasonal variation. Mean numbers of free bacteria ranged from 10^6 in the first ten km of coastal water to 10^5 on the outer continental shelf. These values varied little from station to station, season to season, or year to year. Attached bacteria were two orders

of magnitude less abundant in all parts of the shelf. The numbers of chroococcoid bluegreen bacteria were generally lower on the southeastern shelf than those reported north of Cape Hatteras (Waterbury et al., 1979). Maximal numbers were seen in winter, but they never constituted a major fraction of the biomass of photoautotrophs. Also characteristic of winter were large numbers of spherical fruiting bodies of fungi or thraustochytrids. They reached a peak in January or February and dominated the biomass on the inner and middle shelf, occurring as far south as Cape Canaveral and extending to the shelf break, with the peak abundance seen on the middle and inner shelf off Savannah.

Gulf Stream Intrusions.

Most intrusions of North Atlantic Central water are subsurface accompanied by characteristic signatures of Gulf Stream meanders and eddies (Lee et al., 1981). Using satellite infrared imagery and XBT surveys, Atkinson and Lee located advancing intrusion events on the outer continental shelf for these studies. Within the limits of what could be accomplished by a single ship, we found it best to establish a transect line ahead of the advancing intrusion and to take a daily series of stations as the event passed the section. Because there was usually some cyclonic motion associated with the intrusions, there was significant but varying movement of water through the section and laterally along it. Therefore, the same water was not sampled from day to day, and sequential events could only be deduced assuming that similar events were occurring at all places as the intrusion event moved through the section. This assumption was not strictly true because there was strong evidence of patchiness. However, the sort of three-dimensional, closely spaced sampling which would be required to follow total water movement

through the course of an event would require the simultaneous use of several ships and many experienced personnel. What we had to do instead was obtain the less intensive observations a number of times in order to extract the common features from imperfect sets of observations.

On April 24, 1978, R/V ISELIN encountered an intrusion already well onto the shelf. A section was taken on that day and the following day, when the cold water was beginning to cascade back over the shelf break. Some aspects of that event have been reported previously (Campbell et al., 1979). The phytoplankton bloom, as delineated by the A/C ratio, extended from the shelf break some 20 km across the shelf, extending to the surface at the shelf break. On the 25th, as cascading began, the bloom enlarged, still extending some 15 km onto the shelf, but now extending at least 15 km beyond the shelf break and down the slope to at least 150 m. Energy charge ratio was not especially high in the bloom, no more than 0.6 on the 24th but reached 0.8 in a small region near the bottom on the outer shelf on the 25th. Because of the low A/C ratio, the energy charge within the bloom area was attributed to phytoplankton exclusively; heterotrophic biomass was too small to influence the ratio.

A more complete sequence of events was observed east of the St. Johns River along a section at 30° 20' N which was occupied daily from April 21 to 24, 1979, by R/V GILLISS. The patchy nature of intrusion events was evident. On April 21 rather high chlorophyll concentrations were already present in the intrusion and around its interface with overlying shelf water (Figure 6). On the following day the chlorophyll concentration was significantly lower, again followed by high chlorophyll concentrations on the 23rd. On the 24th the concentration had dropped to that characteristic of normal outer shelf water. The total adenylate values followed a different sequence, being high on the

first day, highest on the second, still high on the third, and very low on the fourth. The difference in periodicity of changes in concentration of chlorophyll and adenylates not only showed the patchy nature of the passing intrusion, but also the characteristic separation of patches of water high in phytoplankton from other patches high in heterotrophic biomass. The differences were also reflected in the A/C ratio, which was highest on April 22. Energy charge ratio rose above normal shelf values, but never exceeded 0.7.

Because the dominance of phytoplankton biomass frequently obscures bacterial biomass when such parameters as ATP or total adenylates are employed, bacteria were counted by acridine orange epifluorescence in a section sampled aboard R/V EASTWARD at 30° 00' N, August 10-12, 1979. An intrusion already extended at least 20 km over the shelf on August 10, and a phytoplankton bloom was commencing over the shelf break at the interface between shelf and intrusion water. Bacterial numbers were maximal in water above the interface, a factor of five higher than normal outer shelf values (Figure 7). Energy charge appeared to be higher in the center of the phytoplankton bloom and in a small region of surface water above the bacterial maximum. Energy charge in the bacterial maximum was about 0.6. On the 11th the phytoplankton bloom expanded, extending 10 km over the shelf and more than 10 km beyond the shelf break. Bacterial numbers did not increase, but the region of elevated bacterial numbers enlarged to encompass the phytoplankton bloom area and a large region above and inshore of it. Energy charge remained low in the phytoplankton bloom and in the region of maximum bacteria, but was high in a small region of surface water above the bacterial maximum and inshore of the phytoplankton bloom. On August 12 the phytoplankton bloom appeared to be starting to cascade down the slope. Bacterial numbers

increased to a maximum of nearly 10^6 ml^{-1}, with the region of maximum bacteria now inshore of the phytoplankton bloom and extending through the entire water column. Energy charge was moderately high in the phytoplankton bloom but low elsewhere. Because of the very high A/C ratio, total adenylates proved on this occasion to be a quite good indicator of the location of maximal bacteria populations throughout the intrusion, being obscured by phytoplankton only in the center of the bloom.

Discussion

Adenylates of Microbial Communities

The use of adenylate analysis to estimate biomass offers the possibility to obtain more data than can be obtained in a reasonable time by microscopy. ATP alone, although commonly used, may be subject to suspicion because ATP is so highly labile within cells that the intracellular pool must turn over thousands of times while a sample is collected and processed (Karl, 1980). Because the total pool of ATP, ADP, and AMP is potentially less subject to short-term fluctuations than is that of ATP alone, we chose to measure total adenylates. Having done so, it is possible to compare the results that would have been obtained by measuring ATP alone with those from total adenylates by examining partial correlations of each with bacterial numbers. Correlation of both total bacteria and free bacteria with ATP was 0.72, while correlation of total and free bacteria with total adenylates was 0.82. The analysis of total adenylates thus offers an improvement, but one which may not be worth the considerable additional time and cost. Although ATP may be labile, we obtained consistent results with the ATP analysis alone, notwithstanding the fact that collection and processing of a sample from sea water takes more than one hour. There is reason to believe that success in extracting ATP from

plankton requires attention to certain details, such as seeing that the filter does not become dry, keeping the temperature of the buffer very near 100C during extraction, keeping the extraction time within narrow limits, and keeping the extract frozen, preferably at -20C, until analysis. Therefore there is a need to validate extraction efficiency and its consistency. Having done so, ATP analysis may be sufficient, if there is no other need for the total adenylate data, such as computation of energy charge ratios.

While the universality of the adenylates in living organisms makes it a useful measure of biomass, it also makes difficult the discrimination of the trophic group to which that biomass belongs. A number of investigators have attemped to circumvent this by microscopic counts of bacteria, phytoplankton, and other groups. However, this is laborious and in doing it one loses a major advantage of adenylate analysis, which is the ease of taking many samples. A number of investigators have used the ratio of ATP to chlorophyll to indicate whether the predominant biomass is autotrophic or heterotrophic. We have used the ratio of total adenylates to chlorophyll a for this purpose and have shown elsewhere (Campbell et al., 1979) that values of the ratio less than four indicate dominance of the biomass by phytoplankton, and values greater than ten indicate dominance by heterotrophs. However, this is a qualitative evaluation, and the ratio cannot be used to assess the exact fractional proportions of autotrophic and heterotrophic biomass. Therefore, one must take the data as they come: sometimes we have information about autotrophs and sometimes about heterotrophs. If the values fall between four and ten, we have information about nothing specific.

The same limitations apply to the interpretation of adenylate energy charge ratios (EC) of the microbial community of continental shelf waters. Using the A/C ratio to indicate whether autotrophic or heterotrophic biomass

is dominant, we have information about the EC of one, the other, or neither, depending on the A/C value. There are other limitations to the interpretation of EC, for Karl (1980) points out that a community EC of 0.6 may represent a mixture of populations having energy charges from 0.4 to 0.9. In fact, we can assume that in any microbial community, autotrophic or heterotrophic, there will be some degree of mixing of active and inactive cells. Therefore, very high or very low EC values provide unequivocal statements about the condition of the community while intermediate values tell us relatively much less. Because other approaches to the evaluation of metabolic state of microbial populations in nature are more laborious and also to some degree equivocal, the EC offers a limited but often useful indication at an acceptable cost. If it can be supplemented by other evidence, that is certainly desirable. In the present study our EC calculations showed that the continental shelf is a region of highly variable metabolic states which occur in unexpected times and places.

Frontal Regime of the Outer Shelf

Throughout the year the dominant influence on productivity of the outer continental shelf is the meandering and eddy formation of the west wall of the Gulf Stream, periodically transporting cold, nitrate-rich water onto the shelf. Atkinson et al. (1978b) have showed that nitrate concentration in intruding water is correlated with temperature. Therefore, new intrusions of North Atlantic Central water have a characteristic nitrate-temperature relationship, and the length of time that cold water has been stranded on the shelf can be estimated from its nitrate depletion. The response of phytoplankton to intrusions of the nitrate-rich water is very rapid (Yoder et al., in press). Usually a bloom is evident on the first day of intrusion over the shelf.

While the response of bacteria is as immediate as is that of phytoplankton, their response appears to be somewhat more complex. By using the A/C ratio in conjunction with total adenylates and epifluorescence counts of natural bacteria populations, the abundance of bacteria can be seen to increase not only in the intrusion itself but more strikingly in the waters above and inshore of the intrusion. Thus the growth of bacteria appears respond to the soluble and particulate by-products of both primary and secondary production. A partial correlation analysis of data on several cruises (EASTWARD, August, 1979; ISELIN, November, 1979; BLUE FIN, November, 1979; October, 1980) shows a correlation of total bacteria and free bacteria with chlorophyll ($r^2 = 0.60$). Therefore, bacteria do respond to primary production. However, the highest concentrations of bacteria occur above or inshore of the intruded water and the associated phytoplankton bloom. Numbers of attached bacteria also show a partial correlation of 0.44 with those of free bacteria, suggesting that both free and attached bacteria respond to excretory and fecal products of grazing zooplankton. These effects are seen where the concentrations of zooplankton are highest. That there is a separation of chlorophyll maxima and bacterial maxima is evident not only from counts of bacteria but also from the distribution of the A/C ratio. Bacterial biomass maxima are typically associated with an intrusion but are never in it.

Bacterial numbers in the vicinity of intrusions are less variable than are changes in chlorophyll concentration. Chlorophyll increases by at least two orders of magnitude, while bacteria increase by at most one order of magnitude. This may be because substrates available to bacteria are not linearly related to increase in standing stocks of phytoplankton, or it bacterial numbers may be limited by grazers. Outside of the blooms with in intrusions, bacterial numbers appear to be remarkably uniform over great

expanses of the outer continental shelf, so either there is a very uniform and steady supply of growth substrates or, more likely,there is a refuge density below which grazers find it difficult or energetically inefficient to remove bacteria from the water. Experimental evidence suggests that pelagic tunicates cannot have a significant impact on bacterial numbers, even in the most dense swarms (King et al., 1980). The potential impact of protozoan grazers is less well known and is more likely to impose an upper limit.

In Liverpool Bay, Floodgate et al. (1981) found both bacteria and phytoplankton were more abundant at a persistent front and inshore of it than elsewhere, but the maximal populations of bacteria and phytoplankton were separated in time, the bacterial maximum occurring in winter and phytoplankton maximum in spring. Floodgate et al. postulated that the bacteria were most productive at the time of maximal river outflow, while the phytoplankton were most productive in the spring period of high insolation and water stability. That is admittedly a quite different situation from the one described here, but the observation of a separation of phytoplankton and bacterial maxima in both cases suggests that it is a widespread occurrence. In the case of Gulf Stream intrusions it is unlikely that the bacteria are influenced by terrigenous materials. Floodgate et al. found that the concentration of urea in the water was higher at the front and that it reflected a greater biomass and metabolic activity of zooplankton there. In the case of the intrusions of Gulf Stream water, it seems possible that the release of dissolved organic materials, such as urea, by zooplankton may be the source of growth substrate for the observed bacterial maxima. We agree with Floodgate et al. that utilization of dissolved organic materials released by phytoplankton also is a significant factor in the increased bacterial numbers. Whether that release

is a linear function of photosynthesis or a highly variable fraction of the fixed carbon is a subject of debate (Smith and Wiebe, 1976). Therefore, we cannot be certain whether or not the greatest rate of release of dissolved organic compounds by phytoplankton will coincide with the greatest concentration of chlorophyll a.

Coastal Water.

Much of the fresh water runoff from South Carolina and Georgia rivers is confined within a front, some 10-20 km offshore. Water of reduced salinity inside the front is deflected southward by a barsclinic pressure gradient (Blanton, 1982). Although there would appear to be an excess of nutrients for phytoplankton production in the coastal water, there is strong evidence of high production at the interface of coastal water of reduced salinity with shelf water (Figure 2), suggesting some nutrient deficiency in the coastal water. This relationship did not show clearly in the earlier work of Thomas (1966), who postulated that light limited to photosynthesis in the inner coastal zone. It is important, however, that Thomas found the critical depth to be below the sea bottom at all times in the first 13 km of the continental shelf. At 13 km critical depth appeared to be near the bottom in winter. Therefore, primary production should be strongly positive in the first 10 km and less so seaward in the absence of thermohaline stratification. Indeed, this is what studies of photosynthesis have shown (Thomas, 1966; Haines and Dunstan, 1975) although the importance of critical depth as a controlling factor does not seem to have been singled out. Haines (1975) suggested that the supply of nitrogen is limiting. On the inner shelf there probably is active recycling from the bottom, as well as terrigenous inputs. On the middle shelf recycling will be less effective during periods of

stratification, but these are the only periods when critical depth is below the mixed layer. Therefore, most production is at the interface of the nitrogen-rich lower layer and the upper layer which may provide some other limiting nutrient such as silicate, while limiting vertical mixing of the phytoplankton. Production near shore may also be enhanced by water stability at times of thermohaline stratification there, notably in the winter-spring period of high river flow.

Because of the proximity of the bottom in the coastal portion of the shelf, interactions between the bottom and the water column are significant, not only in the regeneration of phosphate and ammonia but also in resuspension of organic detritus which has settled out of the water and the input of excretory and fecal material from benthic populations, all provide additional substrates for both free and attached bacteria and other microbial populations. Bacteria are more abundant, and their mean size is larger, in the coastal zone than in the waters of the outer shelf. While on the outer shelf free bacteria are about 10^5 per ml, reaching 10^6 per ml above intrusions, the normal abundance of bacteria in the coastal zone is 10^6 per ml. Taking their larger size into account, there is probably two orders of magnitude more bacterial biomass per unit volume in the coasta water than in outer shelf water. Attached bacteria are also more abundant in the coastal zone and they are associated with flocculent material which is likely fecalin origin. Their numbers are always lower than those of free bacteria by about two orders of magnitude, but because of their much larger size, they constitute as much as half of the bacterial biomass and probably account for half of the bacterial production (Hodson et al., 1981). The greater number of bacteria in the coastal zone, together with their greater mean size, raises questions about the factors limiting bacterial populations in continental

shelf waters. It does not seem reasonable that the larger bacteria, which are also an order of magnitude more abundant, are less vulnerable to grazing than are the lesser numbers of smaller organisms offshore. Both substrate supply and grazing pressure may be significant determinants of bacterial population size in these continental shelf waters. However, a definitive test must involve more attention to protozoan grazing in all parts of the shelf waters.

Coastal-oceanic interactions

Factors controlling biological processes on the southeastern continental shelf are complex, and there are conflicting accounts of these processes in the literature. Turner at al. (1979) postulated that productivity on the entire shelf is driven by nutrient effluents from the coastal salt marshes, while Bishop et al. (1981) stated that nutrients of terrestrial origin are isolated from the middle and outer shelf by coastal salinity fronts. Estimates of the rate of flushing of water from the northern, Onslow Bay section of the shelf (Blanton and Pietrafesa, 1978) and from the remaining part of the shelf south of Cape Fear (Atkinson et al. (1978a) are in approximate agreement at 2-4 months. Atkinson et al. (1978a) present evidence that much of the flushing is driven by the Gulf Stream which entrains filaments of water of reduced salinity. Evidence from the present study tends to confirm this, indicating that intrusions interact with regional wind fields to displace bodies of water from the coastal zone to the middle or outer shelf, where they are then entrained. Blanton (1982) predicts that this should occur off Florida, and there is possible indication of it in Figures 3 and 5, in which the high chlorophyll concentration of the coastal water hooks offshore off Fernandina or Jacksonville.

Both the intrusions of North Atlantic Central water from the Gulf Stream and plumes of water of reduced salinity from the coastal zone contribute to the supply of nitrogen and phosphorus to shelf waters. Haines (1975) and the 1975-76 data of this study show plumes and bodies of water of reduced salinity extending from the coastal zone into the midshelf region (Figures 1 and 2). Haines's data show that those bodies are enriched in nitrate and phosphate. The previous studies of intrusions of North Atlantic Central water (Atkinson, 1977; Atkinson et al., 1978a and 1978b; Atkinson et al., 1980; Blanton et al., 1981; Yoder et al., in press) and the present data, show that there is a significant input of nitrate by intrusions, and that the input has a marked effect on phytoplankton on the outer continental shelf. This effect takes the form of rapid, 2-4 day pulses in phytoplankton production, and because the intruded water is stratified and lies on the bottom, neither the nitrate nor the resulting bloom is seen at the surface. We have found, however, that subsurface blooms are accompanied by increased populations of bacteria in the surface water, resulting in regions of surface water with heterotrophically dominated biomass. There are also regions of heterotrophically dominated biomass on the inner shelf, but they occur in water of reduced salinity. Therefore, the presence of heterotrophically dominated biomass on the outer shelf, associated with oceanic salinity, is indicative of the immediate influence of Gulf Stream intrusions.

Atkinson et al. (1978b) showed that Gulf Stream intrusions potentially supply more new nitrate to the southeastern continental shelf than do all other sources, but the fate of that nitrate, and production derived from it, depends on the locations within the southeastern shelf and the time of year. Blanton et al. (1981) showed that intrusion frequency, indicated by reduced water temperature, is greatest off norther Florida, with maximal intensity

during summer. A lesser maximum occurred in Onslow Bay. Gulf Stream nitrate has a marked effect on the productivity of the continental shelf of northern Florida and Onslow and Raleigh Bays. It has much less impact on the shelf off Georgia. Moreover, the supply of nitrate, and possibly silicate or other elements, from rivers is condined within a density front along the Georgia coast and reaches the middle or outer shelf only after moving south to Florida. Therefore, we see a highly productive coastal zone off Georgia but a remarkably unproductive middle shelf, since it receives little fertilization from either the land on the Gulf Stream. At times the body of water of reduced salinity may extend across the middle shelf, as it did in June, 1975 (Figures 1 and 1), and the maximum productivity, which lies on the shoreward side of the front, is near the middle of the shelf. However, during most of the year, when there is less fresh water input to the shelf, the front is not more than 20 km offshore, and the highest productivity lies inside it.

The region of near-shore productivity is consistently seen in all surveys of the shelf, both the present and previous date. Regions of high productivity immediately attributable to intrusions of cold, nitrate-rich Gulf Stream water are less frequently encountered. Of all of the surveys which have been conducted on the southeastern continental shelf of the U. S., two encountered cold bottom water on the shelf. Bishop et al. (1980) encountered cold water on the outer shelf off Savannah. One of the GILL cruises also encountered cold water on the shelf (Anderson et al., 1956). However, Turner et al. (1979), Haines (1975), Atkinson et al. (1978a), and our present 1975-76 observations found no cold bottom water on the shelf.

We have discussed nutrient enrichment as if nitrogen and phosphorus were the only potential limiting factors. While there is evidence that nitrogen in particular is very important in stimulating phytoplankton production on both

the inner and outer parts of the continental shelf, there is reason to believe that other factors may also influence it. Atkinson et al. (1978b) found that phytoplankton production was greatest at the interface of a nitrate-rich Gulf Stream intrusion with silicate-rich coastal water. Our 1975-76 observations also show maximum phytoplankton populations at the interface between coastal water and underlying shelf water. Therefore, two factors, one in the coastal water and another in the shelf water or ocean water, may act synergistically to enhance production. This may be another essential element, such as silicon, or it may be a chelating factor in the dissolved organic matter (Wood, 1980).

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Figure Captions

Figure 1. Distribution of near-surface temperature, salinity, chlorophyll a, and total adenylates on the continental shelf of southeastern U. S., June 11-17, 1975. Cape Fear is at the upper right, the Savannah River is at 32°, and the St. Johns River (Jacksonville, Florida) is at the lower left. The dotted line is the shelf break.

Figure 2. Distribution of salinity (left) and chlorophyll a (right) along sections across the continental shelf of southeastern U. S., June 11-17, 1975. The sections from top to bottom are Charleston (32° 30'N), Wassaw (31° 55'N), Doboy (31° 30'N), St. Simons (31° 10'N), Fernandina (30° 45'N), and Jacksonville (30° 25'N).

Figure 3. Distribution of near-surface temperature, salinity, chlorophyll a, total adenylates, adenylate/chlorophyll ratio, and energy charge ratio on the continental shelf of southeastern U. S., December 1-7, 1975.

Figure 4. Distribution of near-surface temperature, salinity, chlorophyll a, total adenylates, adenylate/chlorophyll ratio, and energy charge ratio on the continental shelf of southeastern U. S., February 8-15, 1976.

Figure 5. Distribution of near-surface temperature, salinity, chlorophyll a, total adenylates, adenylate/chlorophyll ratio, and energy charge ratio on the continental shelf of southeastern U. S., June 7-11, 1976.

Figure 6. Distribution of salinity, temperature, chlorophyll a, total adenylates, adenylate/chlorophyll ratio, and energy charge ratio on a section of the continental shelf of southeastern U. S. at 30° 20' N, April 21-24, 1979.

Figure 7. Distribution of parameters of microbial biomass and activity and related physical and chemical parameters along a section of the continental shelf of southeastern U. S. at 30° 00' N, August 10-12, 1979.

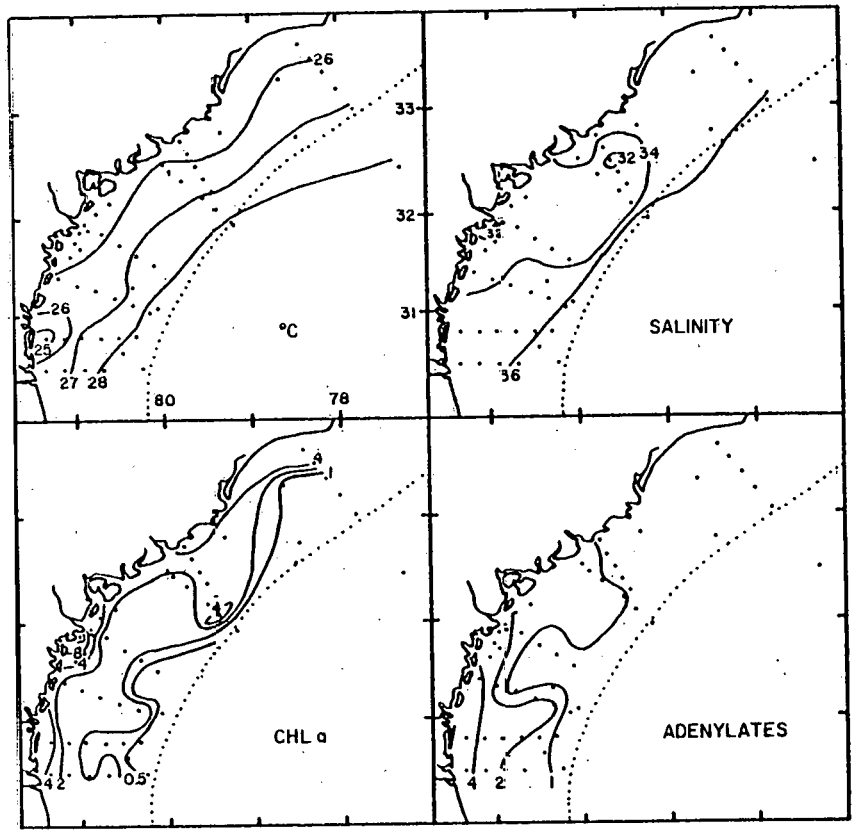


FIGURE 1

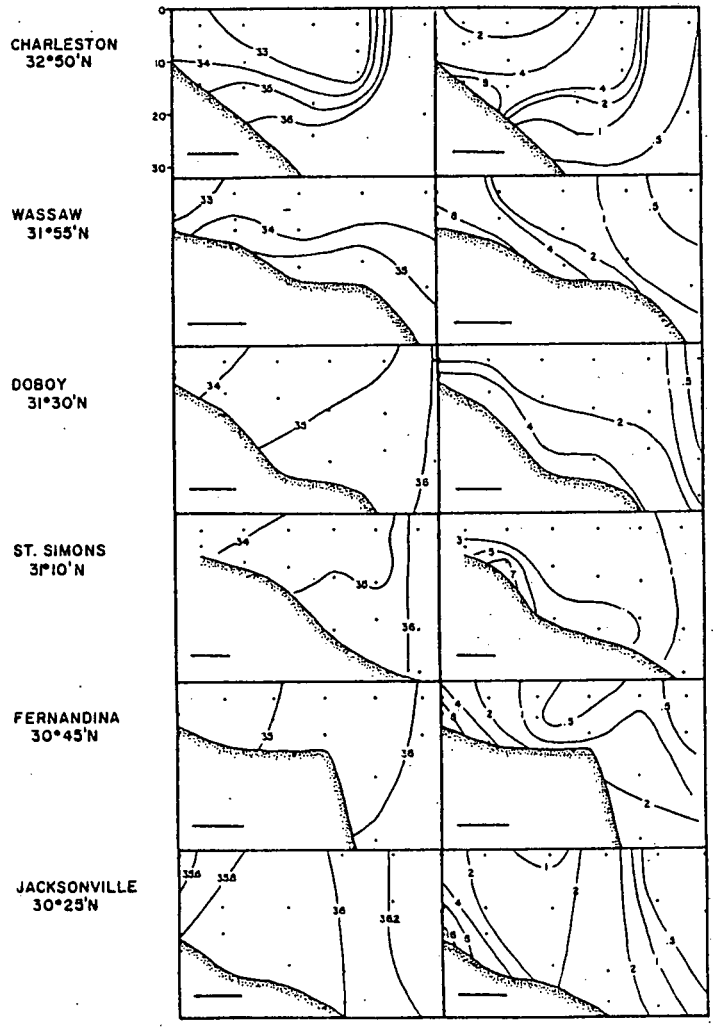


FIGURE 2

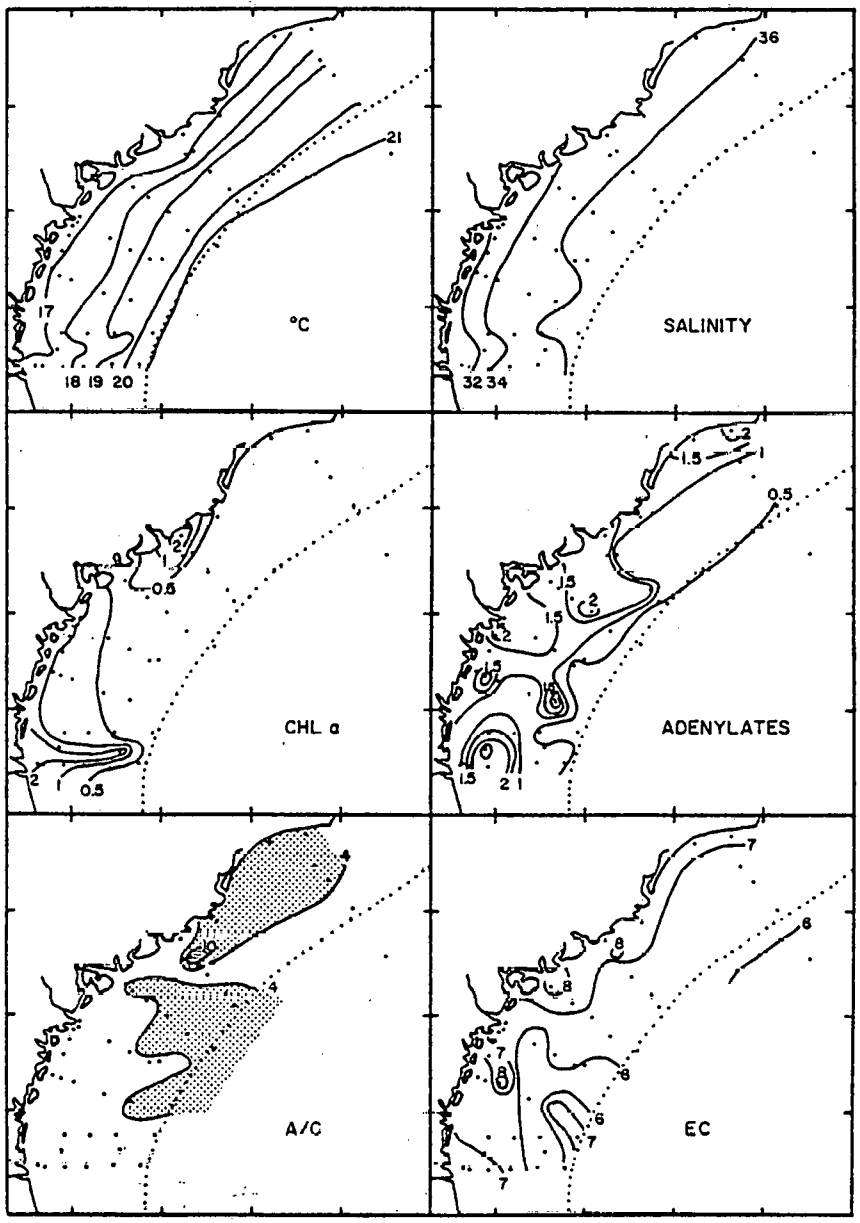
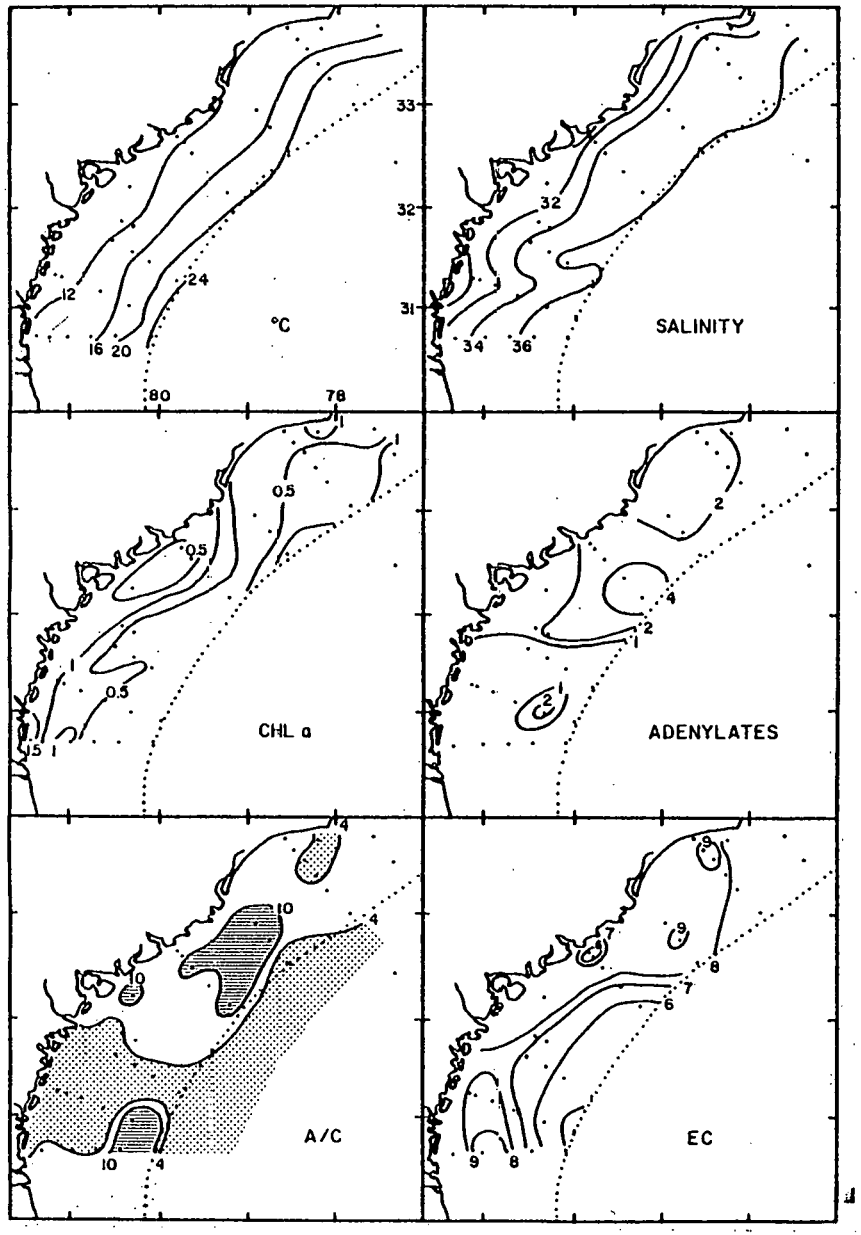


FIGURE 3



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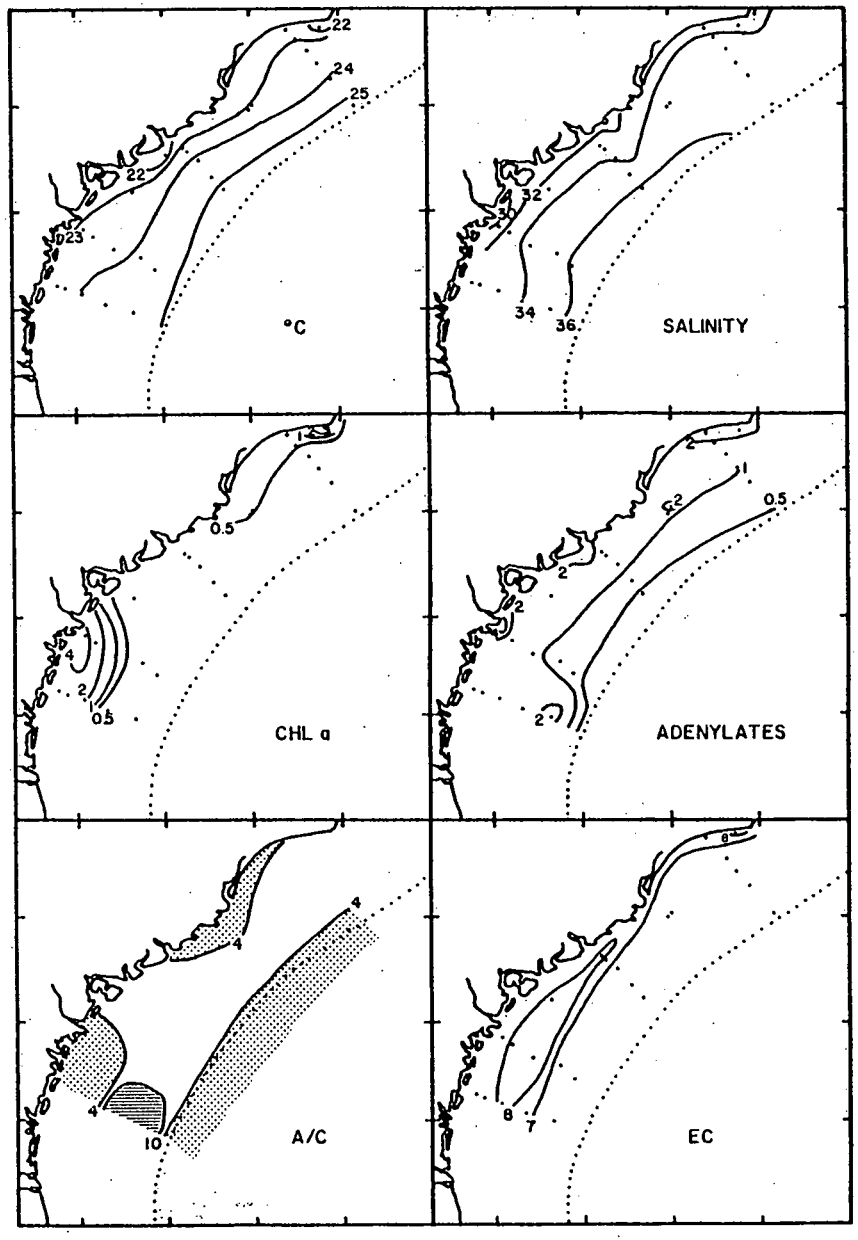

FIGURE 5

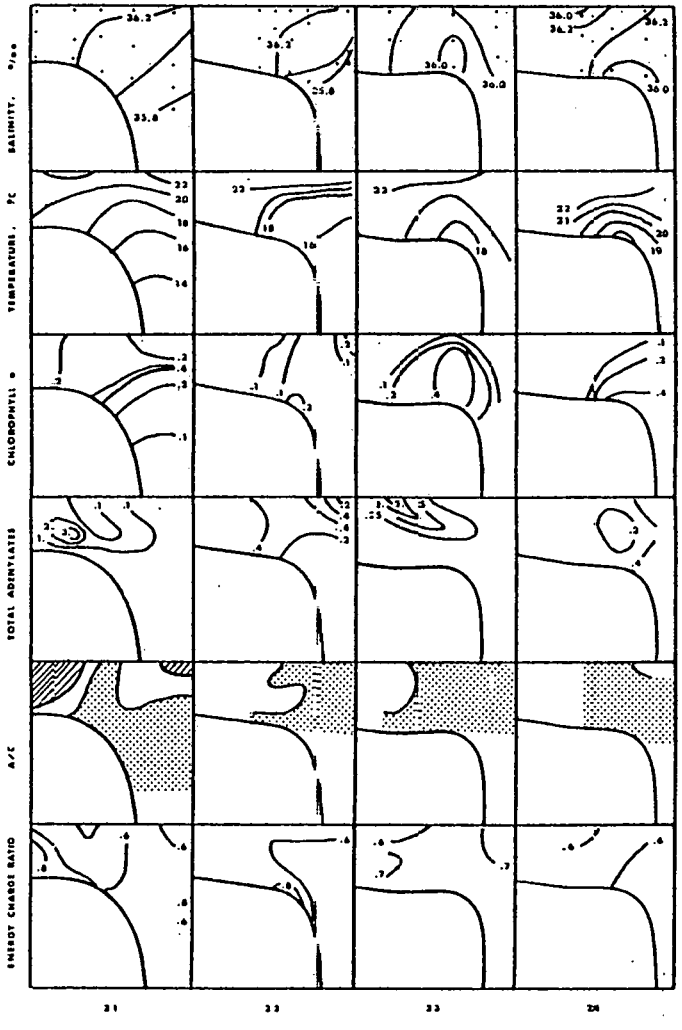

FIGURE 6

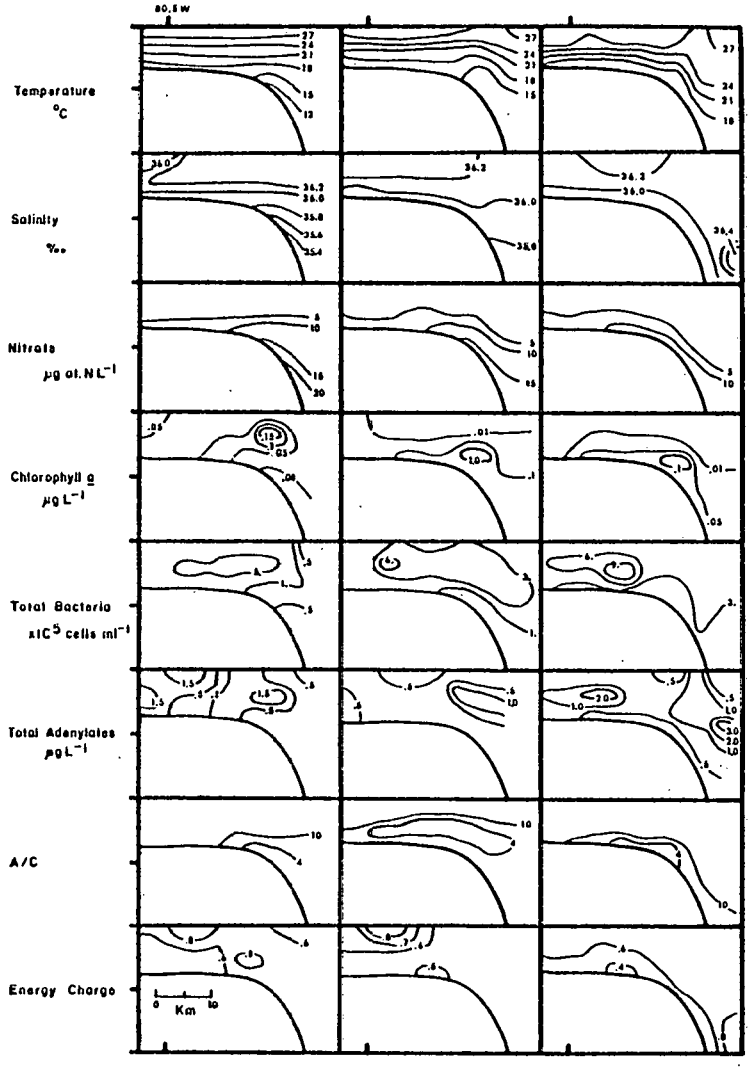

FIGURE 7

Phytoplankton and Bacterioplankton Responses to a Gulf Stream Intrusion

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Abstract

Introduction

Recently, heterotrophic microbial populations have been shown to be quantitatively important members of the pelagic marine food web of continental shelf ecosystems (Fuhrman and Azam, 1980). Marine bacteria, initially perceived as of little importance in the food chain (based on low biomass estimates by plate counts), are now viewed as quantitatively important members of a food web (Landry, 1977; Sieburth, 1977). Parsons and Strickland (1962) first demonstrated that marine bacterioplankton rapidly assimilated glucose from seawater. Subsequent studies have demonstrated that marine bacteria can utilize nanomolar concentrations of labile organic compounds (Wright and Hobbie, 1965; Vaccaro et al., 1968; Andrews and Williams, 1971; Wright, 1973; Wright and Shah, 1975). The labile organic compounds are released from several sources (Wangersky, 1978), but there are two major sources in pelagic marine ecosystems. Photoautotrophic populations release significant quantities of photosynthate (Nalewajko, 1978; Herbland, 1975; Hellebust, 1974; Thomas, 1971; Choi, 1972; Fogg et al., 1965). Heterotrophic populations also enrich the system with labile organic compounds by excretion and defecation (Webb and Johannes, 1967; Cowey and Corner, 1966). The rapid assimilation and mineralization of labile organics by heterotrophic bacteria is a potentially important pathway of nutrient cycling in pelagic marine ecosystems. Hollibaugh et al. (1980) found that marine bacteria rapidly converted organic nitrogen compounds (primary amines) to inorganic nitrogen that could be utilized by photoautotrophs.

The high degree of interaction and coupling, that appears to exist between photoautotrophic and heterotrophic components of the microbial community, has not been adequately documented in nature. Most studies have examined fragments of the microbial food web, but few have simultaneously addressed both photoautotrophic and heterotrophic components of the microbial community. Recent studies (Fuhrman and Azam, 1980; Fuhrman et al., 1980; Floodgate et al., 1981) have suggested that photosynthesis and bacterial activity are tightly coupled in the pelagic marine food web of continental shelves. We have investigated the degree of interaction of phytoplankton and bacterioplankton by simultaneous analysis of their abundance and dynamics during an intrusion of Gulf Stream water onto the continental shelf.

Intrusion of Gulf Stream water on the continental shelf of the southeastern United States provides a natural system, whereby the interactions and couplings of photoautotrophs and heterotrophic microbial populations can be studied in response to nutrient enrichment (Atkinson et al., 1978). Gulf Stream meanders or spinoff eddies occur on the shelf from Cape Canaveral and Cape Hatteras, at a rate of 6 events per months (3 meanders, 3 spinoff eddies on the average), with an average duration on the shelf of approximately 5 days (Lee et al., in press). Thus, some region along the continental shelf is constantly being affected by an intrusion event. Filament dimensions of spinoff eddies are 100-200 km in length and 35-40 km in width (Lee et al., in press). Atkinson and Pietrafesa (1980) determined the volume of an intrusion, stranded on the shelf, to be 84 km^{-3}. The movement of water, on the shelf by Gulf Stream intrusions, provides the majority of NO_3 and PO_4 to the shelf (Dunstan and Atkinson, 1976; Lee et al., in press).

Most recent estimates of nitrogen input, to the continental shelf ecosystem of the southeastern United States, are in the order of 66,000 tons yr^{-1}. This input of "new" nitrogen can theoreticaly support an annual phytoplankton carbon production of 38 to 77 g C m^{-2} yr^{-1} without nitrogen recycling (Lee et al., in press). Gulf Stream intrusions (the advective movements of deeper cold Gulf Stream water on to the shelf) are the major physical factors operating as, what Dunstan and Atkinson (1976) termed, a "batch type" nutrient enrichment to the continental shelf of the southeastern United States.

Atkinson et al. (1978) demonstrated that the advection of cold nutrient rich Gulf Stream water on the continental shelf of the southeastern United States initiates subsurface phytoplankton blooms. The intruded water contains a microbial community, but its biomass or cell numbers is one to two orders of magnitude lower than the populations found in the shelf waters. Following the time course of an intrusion provides a means of examining phytoplankton and bacterioplankton populations, under conditions of nutrient enrichment.

Materials and Methods

Hydrographic observations of an intrusion of subsurface Gulf Stream water was made, 7-12 August 1979, on the continental shelf off St. Augustine, Florida, at 30° north latitude. A biological station position was defined by daily transects across the shelf by temperature, salinity and nitrate. Chlorophyll a (Jacobsen, 1978), total adenylates (Karl and Holm-Hansen, 1978) and bacterial numbers by acridine orange epifluorescence direct counts (Hobbie et al., 1977), were also measured.

A position in the center of the intruded water was occupied daily from 8 to 11 August. Water samples, in the overlying shelf water, in the thermocline and within the intrusion, were collected with clean 7L PVC Niskin water samplers. Photosynthesis was measured by following the 6-hour ^{14}C uptake of 10 μC $NaH^{14}CO_3$, free of organics, by 125 ml samples in a deck incubator. Illumination intensities were dark, 100%, 12%, and 1.5% incident sunlight, attenuated by neutral density filters. Photosynthetically active radiation (400-700 nm) was measured with a Li-Cor 2π sensor during the incubation period. Total active radiation was determined by integration of the analog output recorded on a strip chart recorder. After incubation, 5 ml aliquots of unfiltered water and 0.45 μm filtrate, from each sample, were processed by a modification of the acid bubbling technique of Schindler et al. (1972). Postincubation size fractionation was done to determine the size of photosynthetically active populations. The carbon label retained on the particulate fraction was determined by filtering 25 ml aliquots through a 10 μm Nuclepore filter or 0.45 μm Millipore filter. Filters were placed in glass scintillation vials. 1.5 ml of 4N HCl was added to remove any ^{14}C not incorporated into organic carbon (Lean and Burnison, 1978). After 30 min., 10 ml of water-compatible liquid scintillation cocktail (Scintiverse) was added. Samples were counted in a liquid scintillation counter using channels ratio method to correct for variable quench.

Relative heterotrophic activity of the microbial community was evaluated by following the uptake of ^{14}C- or tritium-labeled glucose, ATP, glycine, glyoxylate and the ^{14}C-labeled organic exudate, obtained from an axenic culture of Cylindrotheca fusiformis, incubated with $NaH^{14}CO_3$. Killed controls (5% formalin final volume) were used to

monitor nonbiological uptake or other potential losses of substrate. A 50 ml aliquot of seawater was incubated in the dark with 0.1-0.5 μCi of labeled substrate, containing natural or elevated carrier concentrations. The length of incubation was 2 to 4 hours, which maintained linear uptake of a fraction of the added substrate. At the end of the incubation period, samples and controls were filtered through 0.2 μm Nuclepore membranes, rinsed with filtered sea water, and placed in scintillation vials with 10 ml of liquid scintillation cocktail. Size fractionation was done following the method of Azam and Hodson (1977). Respiratory loss of ^{14}C was measured by trapping CO_2 in an ethanolamine-soaked wick, by acidifying 25 ml aliquot of the incubation mixture. Trapping efficiency of liberated CO_2 was 98-100%. The parameters of uptake kinetics were determined by the techniques outlined by Wright and Hobbie (1965).

Results

A detailed description of transects for the study period is presented elsewhere (Pomeroy et al. manuscript). Table 1 presents the biological station data.

The low nitrate concentrations found on 8 August at the center station indicated that the upwelled water had been on the shelf for some time (Table 1). Chlorophyll and total adenylates were higher at and within the intrusion. The adenylate/chlorophyll ratio (Campbell et al., 1979) indicated that heterotrophs dominated most of the water column. The community energy charge values were uniform throughout the water column. On 9 August slightly higher nitrate conentrations were found

within the intruded water mass. Chlorophyll and adenylate were higher within the intrusion. Heterotrophs dominated upper waters and the intrusion interface but autotrophs were dominant at the bottom of the intruded water. The energy charge values were highest at the intrusion interface. On 10 and 11 August 1979 the photoautotrophic populations dominated the intrusion. Chlorophyll and adenylate concentrations were 1 to 2 orders of magnitude greater than the overlying shelf waters. The adenylate/chlorophyll ratio shows the shift to photoautotrophic dominance at and within the intruded water.

The increases in active chlorophyll a, found at the thermocline and within the intruded water, were accompanied by even greater changes in photosynthetic rates (Table 2). Photosynthetic rates, at the thermocline or below it, were higher at the low light levels. Full sunlight inhibited ^{14}C uptake by phytoplankton taken from the intruded waters. Extracellular photosynthetic release products were found in the newly upwelled water on 10 August. On 11 August, photosynthesis at the thermocline remained high, but was stimulated by full sunlight. Phytoplankton populations in the overlying shelf water were not fixing more carbon per unit of active chlorophyll a than the populations at or below the thermocline. Phytoplankton populations in the intruded water were photoadapted to the low light levels during 8-10 August. However, phytoplankton found at the thermocline 11 August (1.0 $\mu g\ l^{-1}$ active chlorophyll a), were not efficiently utilizing incident radiation as in previous days.

Heterotrophic activity of the bacterioplankton closely paralleled the photosynthetic responses of the phytoplankton. Glucose

turnover times, on 8 August in the overlying waters, were representative of the bacterioplankton populations on the outer shelf (Table 3). On 9 August surface turnover time decreased, but at the thermocline of the newly intruded water, turnover time increased dramatically for both glucose and ATP. Attached bacteria assimilated approximately 50% of the labeled substrate. Heterotrophic turnover times for glucose and ATP at the thermocline decreased dramatically on 10 August, but within the intruded water, turnover time was still slow (T_T > 1000 h). Heterotrophic metabolism in the intruded water on 11 August decreased, indicating either colonization or mobilization of active heterotrophic populations. On all days sampled, the overlying shelf waters maintained turnover times of 80-180 h. Turnover times at the interface and within the intruded water fluctuated by 2 orders of magnitude, in response to the movement of the nutrient water onto the shelf. Bacterioplankton responses during this study appeared to be coupled with the phytoplankton responses.

The percentage of free bacterial activity determined by size fractionation did not vary greatly (47.0 to 74.0% free bacteria) with position in the water column or day of the study. The percentage of free bacteria, determined by epifluorescence direct counts, however, was significantly different from the free bacterial activity determined by size fractionation (P>F .001, P>F .005).

Natural seston was collected by reverse-flow concentration (Pomeroy and Johannes, 1966) with 1.0 μm Nuclepore membranes, and scanning electron micrographs were prepared (Jacobsen, 1981). Large rod shaped bacteria were found attached to phytoplankton (Figure 10, A. B

and C) and in fecal aggregates (D). Bacteria attached to fecal aggregates were considerably more abundant than those attached to algae. Fecal aggregates counted ranged 1.4 to 3.5 x 10^3 ml^{-1}. The free coccoid or minibacteria ($\bar{x}$ diameter = 0.21 μm) were small in relation to the larger attached bacteria ($\bar{x}$ length = 1.18 μm, $\bar{x}$ diameter = 0.46 μm). The free coccoid forms did, however, comprise the numerically dominant fraction of the bacterioplankton. Calculation of the surface area and volume of both forms of bacteria revealed that the attached bacteria, although only a small numerical fraction of the total bacterioplankton population, comprised 50% of the total biovolume.

The attached bacteria possess more than one order to magnitude greater biomass and cell surface area per cell than the minibacteria. Assuming that both the attached rods and free coccoid bacteria have the same number of active transport sites per unit of membrane surface area, the attached forms should, based on the numbers obtained by acridine orange direct counts, have approximately one half of the total number of transport sites of the total bacterial population. This explains why attached bacteria assimilated 1/4 to 1/2 of substrate.

Although glyoxylate and glycine are intermediate products of photosynthesis and likely are among the substances released by phytoplankton (Halldal and Holmen, 1979), C. fusiformis release products were taken up considerably faster by the bacterioplankton than either glycine or glyoxylate (Figure 2). A glucose uptake time course, done on 8 August with water from the thermocline, was slightly higher than the glyoxylate values. Glycine uptake kinetics, examined on 9 August, yielded turnover times of 42 h and 57 h for the overlying waters and at

the thermocline of the intruded waters.

Discussion

The perceived sequence of microbial responses to a Gulf Stream intrusion may be more complex than presented in this study. The Lagrangian approach, attempted in this study, was hampered by the physical properties and transient behavior of Gulf Stream intrusions. Although an intrusion of Gulf Stream water was found at 30°N and a center station determined daily as to the longitudinal position during the study period, we were unable to follow the intruded water on its latitudinal axis. The intruded water found on the shelf, in addition to cross shelf movement, has an along shelf movement in a northerly direction. Lee et al. (in press) found that the northward velocity of intruded water averages 40 cm sec^{-1}. At this rate of northward movement, the intruded water would be displaced approximately 35 km daily to the north. Further complications in accurately following a particle of water arise from the cyclonic movement of the water. The dynamics found at the center station may, therefore, be the result of varying size patches of microbial activity and abundance. A more accurate analysis of the microbial patch size and assessment of microbial responses can only be done by a multi-ship or, preferably, a ship-real time remote sensing operation. Satellite thermal imagery has proven useful in defining areas of intruded bottom water (Lee et al., in press). Aircraft ocean color scaning (Kim et al., 1980) has been done to define a phytoplankton bloom resulting from a spinoff eddy (Yoder et al., in press), but the remote sensing portion of the study did not provide real time analysis of the phytoplankton patches.

The phytoplankton populations in this study responded to the intrusion by a rapid increase in biomass (chlorophyll a) and photosynthetic activity. Estimated integral daily production on 11 August, for example, reached 1.3 g C m^{-2} day^{-1}. These values are approximately an order of magnitude greater than previously published values (Haines and Dunstan, 1975) for the outer shelf of the southeastern United States. Haines and Dunstan (1975) conducted a statistical survey of shelf particulate primary production from five transects, across the shelf in September, December, April and May. Water samples were collected based to 5 light intensities from an extinction coefficient determined by Secchi disc measurements. Lee et al. (in press) and Yoder et al. (in press) believe that intrusions of Gulf Stream water on the outer shelf are responsible for considerably higher primary production than determined by previous estimates. Lee et al. (in press) contend that past sampling strategies have not been adequate to resolve such an event-dominated primary production process. Accurate yearly production rates for the continental shelf are, however, difficult to obtain even though we are now beginning to define the epsiodic nature and patchiness of the intrusion events on the shelf. Completion of seasonal comparisons of microbial processes and primary production, analyzed in the context of the physical processes occurring on the shelf, should demonstrate that the continental shelf ecosystem of the southeastern United States is considerably more productive than previously reported.

Photoautotrophic populations, by increasing light capturing efficiency, maximize photosynthesis (Prèzelin and Sweeney, 1978; Prèzelin and Alberte, 1978; Falkowski and Owen, 1978; Perry et al.,

1981) and growth at low light levels (10% or less of surface light). Photoadaption, by the phytoplankton populations in the intruded waters, allows the population to better maintain or increase in population size under the intensive grazing found in intrusions. Anderson (1969) found that photoautotrophic populations, in the chlorophyll maxima off the coast of Oregon, contained larger amounts of chlorophyll per cell than the surface populations. Positive daily primary production values were found in that system down to 0.1% of surface light levels. The photoautotrophic populations at the thermocline, 8-10 August, were adapted to low light levels, based on calculated assimilation numbers.

Release rates of photosynthetic products found at the thermocline were maximal on 10 and 11 August. These were, however, not the largest percentages of total photosynthesis found during the study. Measurement of extracellular release rates in aquatic ecosystems is rather imprecise. Actively metabolizing bacterioplankton populations may assimilate algal extracellular release products as they are being released. Wiebe and Smith (1977) found that marine bacterial populations assimilate significant quanitities of algal extracellular release products on the same time scale as normal ^{14}C incubation times. Nalewajko et al. (1980) found that a mixed alga-bacteria culture contained significantly more particulate ^{14}C label than the algal culture alone during a 6 h incubation. The true rate of algal release products may be several times higher than the measured filtrate values obtained. Thus the lack of release products found at the beginning of the study may represent the rapid assimilation of release products by bacterioplankton populations and not the absence of extracellular release products. The results in ths study indicate that, depending on

the day and position a sample is taken, routinely measured release rates will be highly variable. The controversy surrounding the importance or unimportance of algal extracellular release products in pelagic marine ecosystems (Sharp, 1977) may be resolved if simultaneous assessments of heterotrophic activity are made with photosynthesis measurements. The particulate ^{14}C fixation obtained may represent ^{14}C present in heterotrophic bacteria obtained via release of photosynthate by photoautotrophs. The amounts of photosynthetic dissolved release products found will then be dependent on bacterial rates of assimilation.

A number of difficulties still exist with the measurement of heterotrophic activity in aquatic ecosystems. The methodology has not been standardized (Gocke, 1977a). Several problems of technique must be addressed (Thompson and Hamilton, 1974), and in some cases, the basic assumptons of the kinetic approach are not always realized (Wright, 1973). One area of concern is that a large number of substrates have been utilized to estimate heterotrophic activity, but few comparisons have been made between substrates to determine if there is any effect of substrate choice on the rate of heterotrophic activity measured. The results presented in Figure 2 convincingly demonstrate that uptake of a single substrate parallels the uptake of the C. fusiformis extracellular release products, but that the absolute magnitude of carbon assimilation is considerably less. Use of single-substrate determinations may provide sound information on bacterial utilization of that particular compound, but the total flux of carbon (photosynthate) to bacterioplankton will be greatly underestimated.

Attempts have been made to utilize extracellular release products, obtained from an axenic algal culture, as a standard substrate for determination of heterotrophic potential (Bell and Mitchell, 1972; Bell, 1980; Bell and Sakshaug, 1980). Algal extracellular release products, as a standard substrate, offer few advantages over single substrate assays. The proportions and composition of algal release products vary considerably, based on growth state, nutrient limitation and light intensity. In addition, the substrate mixture found in nature is a combination of algal release products, heterotrophic release products and cellular pools from dying cells or fecal material. Attention should be focused on the utilization of a few substrates that can define growth or various metabolic processes. Use of thymidine (Fuhrman and Azam, 1980) incorporation, for example, estimates the rate of bacterial deoxyribonucleic acid synthesis, thus providing a measure of growth. Determination of the major constituents of algal release products is required to define the appropriate substrates for assessment of protein synthesis or total carbon assimilation by bacterial populations. The flow of energy between phytoplankton and bacterioplankton in pelagic marine ecosystems needs to be examined further.

The attachment of bacteria to particles in pelagic marine ecosystems has been proposed as a mechanism for rapid bacterial growth and activity (Jannasch, 1970; Seki, 1972). Literature values of bacterial numbers or metabolic activity of attached versus non-attached forms range from 0 to 100%, depending on the technique used. The results of this study have indicated that the attached forms, although not numerically dominant, do assimilate a significant fraction of the

dissolved organic matter. The per cell activity (calculated by the method of Wright, 1978) of the attached forms was approximately six fold greater than the free coccoid forms for dissolved adenosine triphosphate in this study (Hodson et al., 1981). Other studies have demonstrated that the metablic activity of bacteria in natural water does not correlate with total numbers of bacteria, as revealed by direct counts (Hobbie, 1979). Hobbie and Wright (1968) and Gocke (1977b) found that the V_{max} for single substrates exhibited a 50,000-fold change, while bacterial numbers changed only 10-fold in unpolluted natural waters. These results suggest that the measured heterotrophic activity, found by use of single substrates, may represent the percentage of membrane transport sites able to transport that particular compound at a point in time.

Examination of the surface area and biovolume of the coccoid and rod forms found, as free and attached bacteria, can provide some answers to the difference in activity, as measured by filter size fractionation techniques. The biovolume of individual attached bacteria in this intrusion study was more than one order of magnitude greater than that of the free coccoid forms. This would lead one to believe that the small free coccoid forms were in a dormant or semi-dormant metabolic state, based on the higher numbers and the disproportionally low metabolic activity found. However, normalization of the % free bacterial to membrane surface area indicated that there was not a difference in assimilation efficiency between the free coccoid and attached rod forms. Surface area, as opposed to biovolume, was a better parameter for normalization of assimilation rates, since the uptake sites are a surface area (membrane) function. Equating assimilation to

biovolume would provide a 2X bias to the rod shaped forms, since they have a lower surface to volume ratio (10.1) than the free coccoid (18.8) forms. Analysis of bacterial populations, on a unit of membrane surface area in aquatic ecosystems, may provide a better means of analyzing substrate uptake kinetics than previously proposed normalization procedures.

Intrusion events initiate a sequence of events that stimulate changes in abundance and activity of the phytoplankton populations (Atkinson et al., 1978). Phytoplankton populations respond rapidly to increased nitrogen (NO_3) in intruded water (Atkinson et al., 1978), at the thermocline, by increasing biomass (active chlorophyll a) and photosynthetic activity. This study has demonstrated that bacterioplankton populations also undergo changes in activity and abundance during an intrusion. The bacterioplankton responses were coupled with dynamics of the phytoplankton. Heterotrophic bacterial populations, although active in surface populations, initialy have slow rates of turnover at the intrusion interface (T_T > 1000 h). As the thermocline becomes dominated by photoautotrophs, based on H.P. index values, and photosynthate is released, bacterioplankton populations increase in metabolic activity. Heterotrophic activity remains high after photoautotrophic activity declines. Atkinson et al. (1978) stated that phytoplankton activity, in intruded water, declines because of depletion of nitrogen, grazing, or a combination of both.

The decreased turnover time, found in response to the intruded water, represented real increases in metabolic activity and substrate assimilation. Since turnover time is merely the time required for the

labeled substrate to be completely utilized, changes in the in situ substrate concentration and/or bacterial population size can increase or decrease turnover time, without any change in the rate of assimilation of the bacterial. Heterotrophic bacterial populations, found at the thermocline, were apparently increasing rates of assimilation, in response to the intrusion event. Accompanying the decreased turnover times at the thermocline on 10 August, bacterial cell numbers were 3 times less than above the thermocline, and the pool of dissolved labile organic compounds was increasing, based on the photosynthetic release rates found. The combination of these factors strongly indicates the the bacterioplankton were increasing the rate of assimilation of labile organic molecules in response to the photoautotrophic populations.

Phytoplankton and bacterioplankton responses within a Gulf Stream intrusion influence, and are influenced by, changes in the zooplankton community. Doliolid swarms multiply in intruded water by rapid asexual reproduction (Deibel, 1980). Thaliacean swarms, in addition to intially excluding other zooplankters from the intruded water, tend to provide a selective grazing pressure on the photoautotrophs. The high clearance rate (>30 ml zooid^{-1} day^{-1}) of thaliaceans (Deibel, 1980) selects for smaller (<20 μm) photoautotrophs. The small phytoplankton can, because of their more rapid reproductive rates, maintain or increase in biomass over the larger phytoplanktors.

A significant amount of secondary production can be accounted for by thaliacean biomass. Thaliaceans are reported to have carbon assimilation efficiency values of 44-85% (Deibel, 1980), thus efficiently mediating primary production through the food web.

Apparently, during an intrusion thaliacean production is exported from the continental shelf via the Gulf Stream (Deibel, 1980), therefore representing a significant carbon loss to higher levels of the food web of the continental shelf.

Thaliaceans also provide a selective force for heterotrophic populations in intruded waters. Egestion of fecal aggregates by thaliaceans provides sites for heterotrophic microbial activity. Pomeroy and Deibel (1980) demonstrated a sequence of heterotrophic responses to thaliacean fecal aggregates. Freshly produced aggregates were rapidly colonized by bacteria which grew and multiplied in the aggregate. The increased bacterial biomass of the aggregate was then grazed by ciliates. In this study, the large rod-shaped bacteria attached to particles (mainly fecal aggregates) were actively assimilating dissolved organic compounds.

Dunstan and Atkinson (1976) first hypothesized that Gulf Stream intrusions operated as "batch" type production system for the phytoplankton populations on the continental shelf of the southeastern United States. This study demonstrated that phytoplankton and bacterioplankton populations increased in abundance and activity in response to the intruded water. The bacterioplankton response was coupled to the increased phytoplankton activity.

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List of Figures

Figure 1 Scanning electron micrograph of seston concentrated by reverse flow filtration.

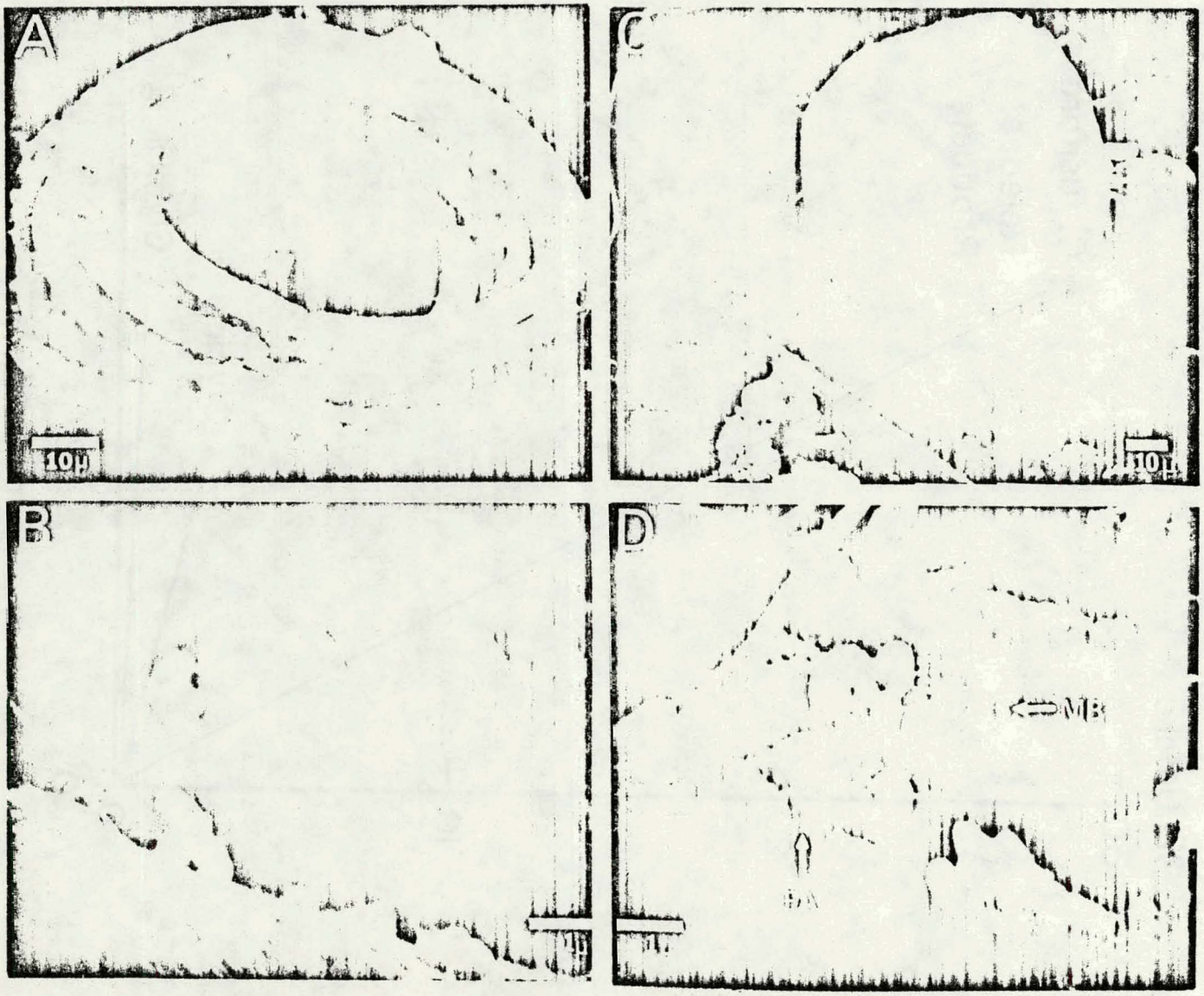
A) chain forming photoautotroph containing numerous attached bacteria.

B) enlargement of A, showing the attached rod shaped bacteria.

C) large diatom surrounded by smaller photoautotrophs and fecal aggregates.

D) Enlargement of C showing the attached rod shaped bacteria in a fecal aggregate (FA) and free coccoid minibacteria (MB).

Figure 2 Assimilation time course of samples taken from the intrusion thermocline on 11 August 1979.


A
10μ
C
10μ
B
D
MB

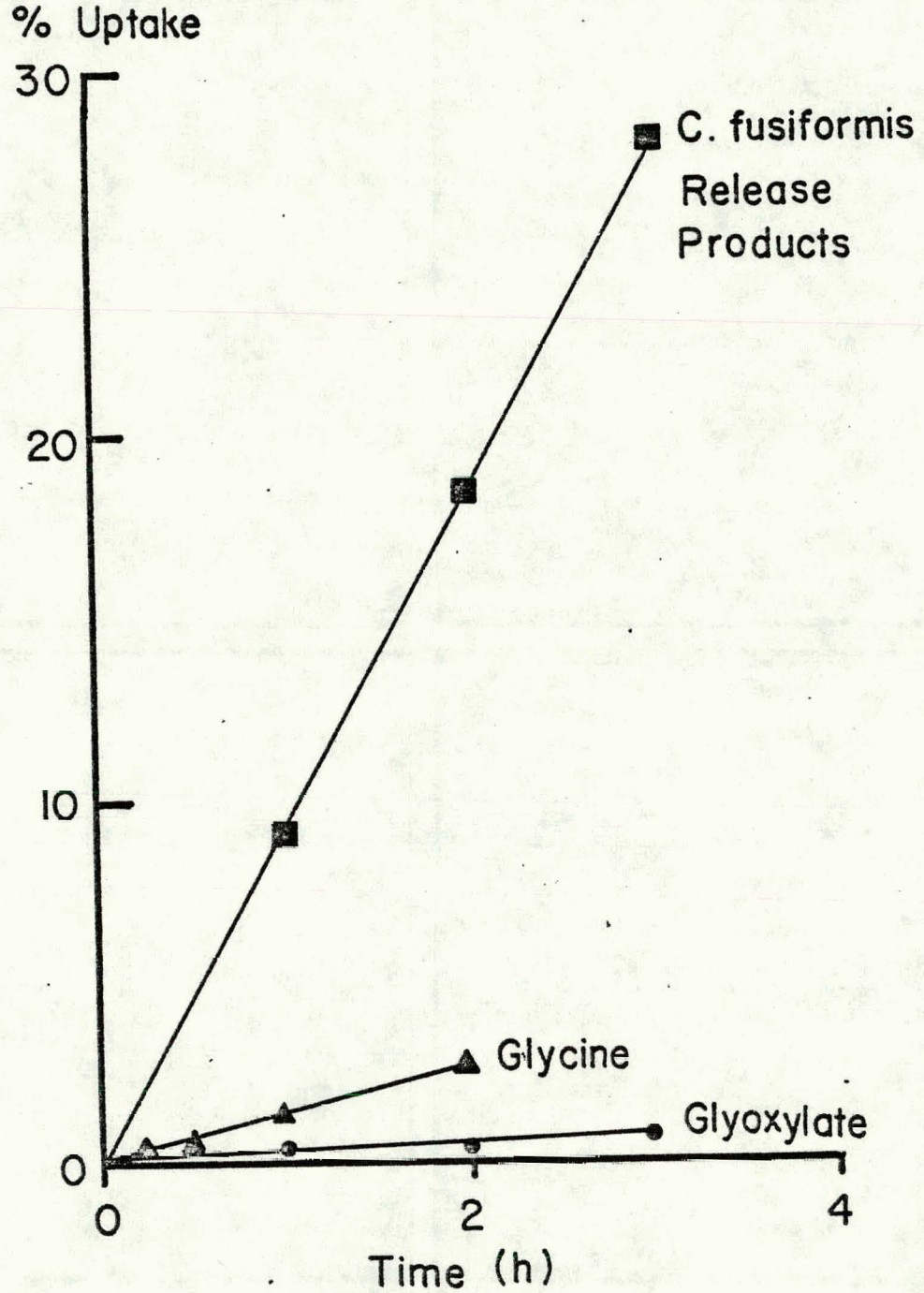

% Uptake
30
20
10
0
0
2
4
Time (h)
C. fusiformis
Release
Products
Glycine
Glyoxylate

TABLE 1. Temperature, salinity, nitrate chlorophyll a (Chla), Adenosine Triphosphate (ATP), total adenylates (AXP), Energy Charge (E.C.) and adenylate to chlorophyll ratio (A/C) at the center station 8-11 August 1979.

Date	Temp C°	Salinity ‰	Nitrate µg at.N l^{-1}	Chl a µg l^{-1}	ATP µg l^{-1}	AXP µg l^{-1}	E.C.	A/C
8 August								
Above	28.4	36.00	0.00	0.010	.192	0.468	.62	46
Thermocline	23.2	36.22	1.62	0.030	.240	0.629	.64	18
Below	18.3	36.29	6.60	0.122	.292	0.764	.62	6
9 August								
Above	28.6	36.30	0.00	0.010	.369	1.052	.64	104
Thermocline	22.7	36.29	1.96	0.030	.828	1.153	.81	37
Below	18.2	36.08	10.06	0.050	.062	0.151	.62	3
10 August								
Above	28.0	36.38	0.06	0.005	.176	0.437	.59	93
Thermocline	23.5	36.03	0.00	0.179	.992	1.978	.63	11
Below	14.1	35.92	14.46	0.010	.036	0.055	.64	5
11 August 79								
Above	28.2	36.29	0.00	0.025	.164	0.397	.59	16
Thermocline	22.0	36.27	3.95	1.003	.474	1.417	.58	1
Below	17.2	36.01	8.83	0.233	.744	1.036	.78	4

TABLE 2. Daily photosynthesis measurements ($\mu gC\ l^{-1}\ h^{-1}$), corrected for dark bottle, for center station of Gulf Stream intrusion 8 to 11 August 1979. Dissolved release products rate ($\mu gC\ l^{-1}\ h^{-1}$) defined as the labeled organic carbon found in the 0.45μm Millipore filtrate.

	Total Photosynthesis			Dissolved Release Products		
Light level	100%	12%	1.5%	100%	12%	1.5%
8 August						
Above	4.6	3.5	0.2	0	0	0
Thermocline	0	14.4	3.2	0	0	0
Below	0.7	14.4	15.3	0	0	0
9 August						
Above	1.5	1.6	0.7	0	0	0
Thermocline	5.9	14.3	3.9	0	0	0
10 August						
Above	1.3	1.7	0.4	0	0	0
Thermocline	5.8	63.0	14.4	0.7	5.9	1.7
Below	4.6	34.5	33.2	2.2	0.1	0
11 August						
Above	2.9	2.6	0.2	0	0	0
Thermocline	106.2	46.8	26.1	16.0	8.7	4.0
Below	4.4	2.3	1.0	0.3	0	0

TABLE 3. Heterotrophic Metabolism of Glucose and Adenosine Triphosphate in Gulf Stream Intrusion 8-11 August 1979.

Date	Position In Intrusion	Turnover Time Glucose (h) 0.2 μ	0.6 μ	% Free	Turnover Time ATP (h) 0.2 μ	0.6 μ	% Free
8 Aug. 1979							
	above	180	*	*	*	*	*
	thermocline	58	*	*	*	*	*
	below	120	*	*	*	*	*
9 Aug. 1979							
	above	80	308	74.0	347	747	54.0
	thermocline	1820	*	*	4749	10,428	53.0
	below	*	*	*	*	*	*
10 Aug. 1979							
	above	127	240	47.0	156	218	28.0
	thermocline	38	67	59.0	122	317	62.0
	below	1374	3447	60.0	3033	4351	30.0
11 Aug. 1979							
	above	140	*	*	*	*	*
	thermocline	60	160	62.5	*	*	*
	below	870	2250	61.3	*	*	*

*Data not collected

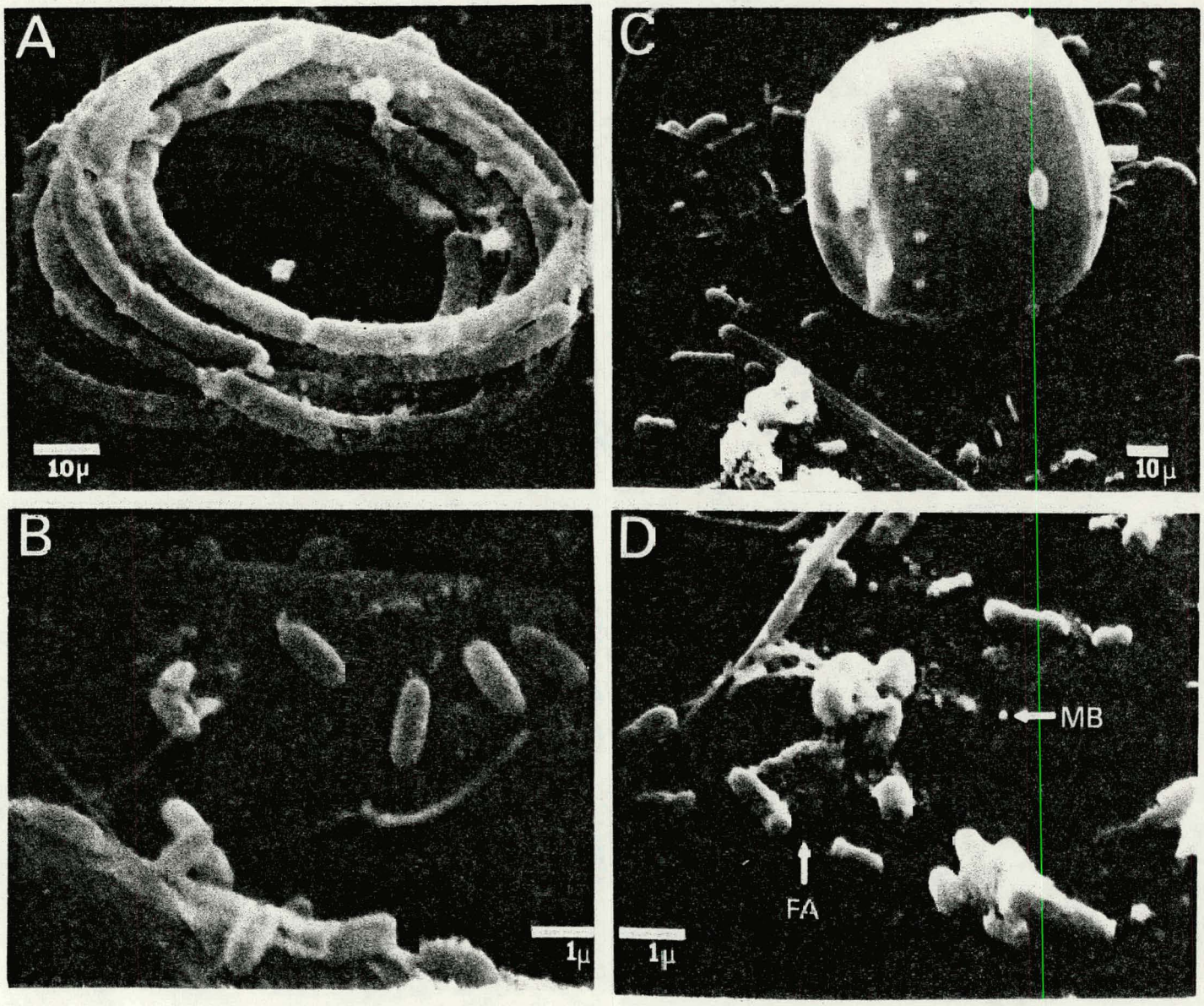
TABLE 4. Comparison of Calculation Methods of Percentage of Free Bacteria by Epifluorescence Direct Counts and Size Fractionation Techniques.

Date	Position in Intrusion	Epifluorescence $x10^5$ cells ml^{-1} Free bacteria	Attached bacteria	% Free	Glucose % Free	ATP % Free
8 Aug.	above	2.6	.02	99.2	*	*
	thermocline	2.9	0.0	100.0	*	*
	below	2.7	.47	85.6	*	*
9 Aug.	above	2.0	.12	94.8	74.0	54.0
	thermocline	4.7	.25	94.9	*	*
	below	0.8	.03	96.6	*	*
10 Aug.	above	2.5	.14	94.5	47.0	28.0
	thermocline	5.3	.16	97.1	59.0	62.0
	below	0.7	0.0	100.0	60.0	30.0
11 Aug.	above	4.4	.03	99.3	*	*
	thermocline	2.1	0.0	100.0	62.5	*
	below	1.9	0.0	100.0	61.3	*

* Data not collected

Analysis of Variance

Comparison	Fs	P>F
Epifluorescence-Glucose % Free	102.1	.001
Epifluorescence-ATP % Free	49.0	.005
Glucose-ATP % Free	5.6	.100


A
10μ
C
10μ
B
1μ
D
MB
FA
1μ

COPPER COMPLEXING CAPACITY ON THE CONTINENTAL SHELF OF THE SOUTHEASTERN UNITED STATES AND IN THE SARGASSO SEA

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ABSTRACT

An ion exchange technique was used to determine the copper complexing capacity (CuCC) of strong organic complexing agents at 21 stations across the continental shelf of the Southeastern United States and in the western Sargasso Sea. The concentration of dissolved organic carbon (DOC) and total particulate material (TPM), two pools of potential complexing agents, was also measured at each station. CuCC ranged from 0.082-1.681 μEq Cu l^{-1} on the inner shelf, from 0.043-0.095 in mid and outer shelf waters, and from < 0.010-0.036 μEq Cu l^{-1} at the Sargasso Sea stations. The correlation between CuCC and both DOC and TPM was highly significant ($\alpha \leq 0.01$). Two synoptic surveys of the distribution of DOC and TPM across the shelf showed that DOC ranges from > 3 mg C l^{-1} nearshore to < 1 mg C l^{-1} offshore and that TPM ranges from > 50 mg l^{-1} nearshore to < 1 mg l^{-1} offshore. Both TPM and DOC are most variable on the inner shelf. These data are consistent with CuCC data which indicate that the CuCC of inner shelf waters was relatively high and very heterogeneous. In contrast, DOC, TPM, and copper complexing capacity are low and nearly invariant at the Sargasso Sea stations. We present a hypothetical model of the distribution of complexing agents in different marine environments and hypothesize that the mechanisms underlying differences between environments relate to differences in the source(s) and nature of complexing agents in each system.

INTRODUCTION

The importance of complexing agents to the mineral nutrition of phytoplankton and other marine organisms has been recognized for more than 20 years (Spencer 1957; Johnston 1962, 1964; Barber and Ryther 1969). Complexing agents have been held responsible for the solubilization of iron and, therefore, its greater biological availability (Lewin and Chen 1971). In contrast, complexing agents are assumed to reduce the biological availability of copper (Cu) and minimize its toxic effect (Lewis et al. 1972; Davey et al. 1973; Pagenkopf et al. 1974; Gnassia Barrelli et al. 1978; Hongve et al. 1980). Experiments with pure cultures of phytoplankton in chemically defined media have demonstrated that copper toxicity is directly correlated with cupric ion concentration and independent of the total Cu concentration (Sunda and Guillard 1976; Anderson and Morel 1978; Murphy et al. 1980). In these experiments, cupric ion (Cu^{++}) concentration was varied in media containing a wide range of total Cu concentrations through the use of artificial complexing agents. When Cu^{++} concentration is calculated for earlier experiments with phytoplankton in defined media, it appears that Cu^{++} is toxic to a number of phytoplankton species in concentrations as low as 10^{-6} μM (Jackson and Morgan 1978). Since Cu concentration in the world ocean typically ranges from 10^{-4} to 10^{-1} μM (Chester and Stoner 1974; Alberts et al. 1976; Boyle et al. 1977; Windom and Smith 1979), complexing agents and other materials affecting the solution chemistry of Cu must maintain the Cu^{++} concentration at sublethal levels.

Copper may exist in particulate, colloidal, and dissolved forms in sea water. In the absence of organic ligands, or particulate and colloidal species, carbonate and hydroxide complexes account for greater than 98% of the inorganic copper in seawater (Zirino and Yamamoto 1972). The Cu^{++} concentration can be

calculated if pH, ionic strength, and the necessary stability constants are known (Stumm and Morgan 1970; Sibley and Morgan 1975; Zirino and Yamamoto 1972). In most natural systems, the presence of organic materials and sorptive surfaces significantly alters speciation and decreases the utility of equilibrium calculations. Analytical difficulties in the measurement of Cu^{++} and Cu associated with naturally occurring ligands has encouraged numerous workers to introduce the "complexation capacity" concept (Davey et al. 1973; Shuman and Woodward 1973; Chau and Chan 1974). Functionally, the Cu complexing capacity of a water sample is the ability of the sample to remove added copper from the free ion pool (Campbell et al. 1977). Analytically, complexation capacity measurements depend on quantitation of the complexing ability of an operationally defined group of ligands. The assumption is made that unknown ligands may be classed into meaningful groups on the basis of the physical properties of their metallo-complexes (e.g., lability to anodic stripping voltammetry (ASV), chelating resins, or ultraviolet radiation). Schemes to determine the concentration of Cu associated with different classes have been proposed as useful ways to address complexing capacity questions in natural systems (Stiff 1971; Batley and Florence 1976). Different analytical procedures measure the Cu chelating capacity of slightly different classes of ligands and there is some overlap in the complexes included in classes defined by different techniques. For example, while there is a small fraction of organic material in seawater which forms ASV-labile complexes not dissociated by Chelex resin (Florence and Batley 1961), most ASV-labile complexes are also labile to chelating resins (Figura and McDuffie 1979).

In this paper, we describe the use of an ion exchange procedure to compare the Cu complexing capacity of strong organic complexing agents in continental

shelf water along the southeastern United States with that of oceanic waters in the Sargasso Sea. We also analyze the relationship between the Cu complexing capacity of this specific group of complexing agents and the concentration of two large heterogeneous pools of potential complexing agents: dissolved organic carbon and total particulate material.

EXPERIMENTAL

Study Area

The continental shelf of the southeastern United States can be divided into three discrete regions on the basis of physiological and biological properties (Bishop et al. 1979). The inner shelf is relatively shallow (15-20 m), well mixed, and very turbid (Blanton and Atkinson 1978). Productivity is high (Bishop et al. 1979) and suspended particulate matter consists primarily of inorganic clay minerals (Oertel 1976). In this zone, potential sources of complexing agents include coastal estuaries and salt marshes, in situ production by phytoplankton and zooplankton, and benthic resuspension. Salinity fronts near the coast restrict transport and result in a patchy midshelf environment where discrete, productive water masses are introduced from the outer shelf or the inner shelf and are separated by oligotrophic midshelf water. These patches of introduced water are likely sources of organic material in the midshelf region since the high concentration of phaeopigments (relative to chlorophyll a) suggest that they are successionally old (Wood 1979). Nutrient levels and production are high on the outer shelf as a result of periodic upwelling of North Atlantic central water induced by westward meanders of the gulfstream (Atkinson 1977; Lee et al., in prep.). Upwelling is discontinuous on the outer shelf; in the absence of upwelling, nutrient levels and primary production are very low, similar to those in the Sargasso Sea.

Field Program

The distribution of dissolved organic carbon (DOC) and particulate material on the continental shelf of the southeastern United States was characterized synoptically on two cruises aboard the R/V Blue Fin (March and July 1979). Complexation capacity measurements were taken in surface waters at five continental shelf stations on the March Blue Fin cruise, in the Sargasso Sea (R/V Pierce), and at two estuarine stations on the Georgia coast. Samples were also taken at the depth of the chlorophyll maximum at six stations in April 1979 (R/V Gillis). Five of these stations were mid-shelf stations in zones of active upwelling; the sixth station (G-74) was a station in Gulfstream waters overlying the shelf. Characterization of the physical oceanography is based on detailed hydrographic sampling and nutrient data (Laslie et al. 1981). Care was taken to minimize sample contamination. In the Sargasso Sea samples were collected from a rubber raft which was rowed $\sim$ 1 km from the ship. A precautionary check for Cu contamination was also made at all stations and, in all cases, total Cu was less than 0.001 µM.

Analytical Methods

DOC samples were filtered through acid washed precombusted Reeve Angel (984H) glass fiber filters into pre-combusted glass filtration flasks. Triplicate DOC samples were sealed in glass ampoules at sea according to Strickland and Parsons (1972). Analysis of the samples was by wet oxidation (Menzel and Vaccaro 1964) using an Oceanography International carbon analyzer. The dry weight of the total particulate fraction and the particulate inorganic fraction were determined by weight before and after combustion by standard methods (Strickland and Parsons 1972).

The ion exchange procedure used to estimate Cu complexation capacity is a modification of that used by Stolzberg and Rosin (1979) and Giesy (1980). Excess Cu^{++} is added to the filtered samples and allowed to equilibrate with available ligands; the sample is then passed through a column packed with Chelex resin. Cu^{++} and Cu associated with weak or rapidly dissociating complexes are removed by the resin and Cu remaining in the sample after chromatography provides a quantitative measure of the Cu chelating capacity of strong ligands remaining in the sample. The procedure has the advantage that complex formation proceeds at seawater pH in a relatively undisturbed sample. However, the procedure also depends on the assumption that essentially all the Cu ligands in the sample are associated with Cu. This involves the reaction: $mCu^{++} + L^{n-} \rightleftharpoons Cu_mL^{(n-2m)^-}$.

The validity of this assumption depends on the relative concentration of Cu^{++} and L^{n-} and on the association/dissociation kinetics of the complexes. Thus, the time allowed for equilibration of the Cu spike with the sample must be appropriate to the rate of complex formation; the flow rate of the sample through the column must be fast enough to prevent dissociation of the complexes while, simultaneously slow enough to prevent breakthrough of Cu^{++}; and finally, the Cu^{++} spike must be large enough to encourage the formation of the copper complex. On the basis of experiments conducted with both estuarine and Sargasso Sea water, we determined that equilibrium between the Cu^{++} spike and the sample occurs within 20 minutes. Extending the equilibration time to as long as 48 hours resulted in no change in the measured complexation capacity of the sample. All chromatography was conducted at flow rates greater than 20 ml cm^{-2} sec^{-1} since slower flow rates resulted in complex dissociation (Fig. 1). There was no apparent Cu complexing capacity in UV-irradiated Sargasso Sea water spiked

with as much as 6.3 µM Cu. Thus, Cu measured in the column eluent from our samples may be assumed to have been associated with organic ligands during chromatography. Samples used for these experiments and all other samples reported here were spiked with 1.57 µM Cu ($CuCl_2$); we found that in both estuarine and Sargasso seawater the complexation capacity of samples spiked with 1.57-4.72 µM Cu was essentially the same and independent of Cu spike concentration. At higher concentrations of copper spike, spike concentration and the observed Cu complexing capacity were directly correlated.

Na-Chelex (BioRad, 100-200 mesh) was used for complexing capacity measurements. The resin was cleaned of metals and trace organics with 2N redistilled HNO_3 and rinsed repeatedly with water (Milli-Q system). The resin was converted to the $NH4^+$ form with redistilled 4N NH_4OH (Riley and Taylor 1968). Polypropylene econo-columns (Biorad) were loaded with approximately 0.4 grams dry weight Chelex immediately before use. At each station, a 500 ml aliquot of filtered sample was passed through the column for pH adjustment. After passage of 200-300 ml seawater (28-36 ppt), eluent pH was ~ 7.8 and remained stable during the passage of as much as 2 l additional seawater. At each station, a replicate 500 ml sample was spiked with 50 µl $CuCl_2$ solution (1.57 µM final concentration). Samples were equilibrated for 4 hours in the light at room temperature, transferred to the ion exchange reservoir, and chromatography begun. An all-plastic gravity feed chromatography stand was used; flow rate was regulated by a Teflon-in-glass stopcock. After the first 50 ml of sample passed through the column, flow rate and pH were measured. Two 110 ml aliquots were then collected in new, clean (as described by Moody and Lindstrom 1977) polypropylene bottles. After acidification to ph< 2.0 with

redistilled concentrated HCl and frozen until analysis. Flow rate and eluent pH were also determined at the end of chromatography. In no instance did the eluent pH change by more than 0.4 pH units during sample collection nor did the flow rate fall below 20 ml cm^{-2} sec^{-1}.

The concentration of Cu in the column eluent was determined by flame atomic absorption spectroscopy of samples which were preconcentrated with ammonium pyrrolidone dithiocarbamate (APDC) and methyl isobutyl ketone (MIBK; Brooks et al. 1967; Stolzberg 1975). Extractive preconcentration was done in a laminar flow hood using Teflon separatory funnels. The pH of the acidified sample was adjusted to pH 2.5-3.5 using 400 µl 8N NH_4OAc (Chelex cleaned). We evaluated the recovery of the APDC/MIBK extraction procedure throughout the pH range 1.6 to 9.1 using 8N NH_4OAc, and 8N NH_4OAc with 5 M NaOH, as buffers. The optimum pH range for this extraction falls between pH 2.0 and 4.0 (Wood 1980). Five ml 1% APDC and 10 ml MIBK were added to each sample. Contaminants were removed from the APDC solution before use by repeated extraction with MIBK. After 2 minutes vigorous agitation, the sample and APDC/MIBK were allowed to equilibrate for 15 minutes. The inorganic layer was then removed. The standard curve for atomic absorption was obtained from extracts of standards prepared by adding $CuCl_2$ to filtered Gulfstream water. A lower limit of detection between 0.1 and 0.2 µM Cu was obtained. This is equivalent to 0.01 to 0.02 µM Cu in the original sample. Recovery of Cu by a single extraction was > 90% in samples containing less than 0.31 µM Cu and > 80% in samples containing more than 0.31 µM Cu.

RESULTS AND DISCUSSION

Survey of DOC and TPM Distribution

Station locations; total depth; and sample depth, temperature, and salinity for the two 1979 Blue Fin cruises are presented in Table 1. Inner shelf,

midshelf, and outer shelf stations were differentiated on the basis of total station depth and location relative to a halocline occurring approximately 40 km from shore (Fig. 3). DOC and TPM were determined at a total of 17 inner shelf stations, 11 midshelf stations, and 2 outer shelf stations. The distribution of DOC and TPM is shown in Figs. 4 and 5. A clear coastal front of TPM is apparent. TPM ranges from 51.49 to 5.28 mg l^{-1} (dry weight) near the mouth of the Altamaha and Ogeechee Rivers and then decreases rapidly with increasing depth and distance from shore. The 5 mg l^{-1} isobar is approximately 20 km from shore and, at the most offshore stations in the inner shelf, TPM ranged from 1.16 to 4.87 mg l^{-1}. TPM concentrations on the inner shelf were lower in July than in March. In the midshelf, TPM ranged from 0.75 to 1.98 mg l^{-1} and the distribution of TPM was no longer associated with an onshore to offshore gradient. Like TPM concentrations, DOC concentrations were highest in the inner shelf and ranged from 1.61 to 4.40 mg l^{-1} in that region. However, a frontal system corresponding to the steep onshore to offshore gradient in TPM concentration was not evident. Very low DOC concentrations (< 0.75 mg l^{-1}) were observed at both outer and inner shelf stations. The DOC concentration at one outer shelf station was extremely high (3.80 mg l^{-1}), suggesting that upwelling was occurring at the time of sampling or that a patch of coastal water had been transported onto the shelf break.

Copper Complexing Capacity Measurements

Copper complexing capacity (CuCC) measurements were made at 21 stations. On the basis of combined salinity and depth data (Table 2), the stations can be arranged into groups of six inner shelf, 6 midshelf, 3 outer shelf, and 6 oceanic stations. Complexing capacity and the concentration of DOC, TPM, and total inorganic particulates at each station are reported in Table 3.

There was no detectable CuCC capacity at five open ocean stations, but a low ($\bar{x}$ = 0.036 μEq Cu l^{-1}) CuCC was detected at station P-5. This station was located approximately 10 km from Bermuda and CuCC at this station was slightly lower, but of the same magnitude as that observed at mid- and outer shelf stations. There was no systematic difference between the CuCC at midshelf and outer shelf stations, and mean CuCC ranged from 0.043 to 0.11 μEq Cu l^{-1} in these environments. All midshelf samples were associated with upwelling events which extended from the shelf break to the 40-50 m contour (Laslie et al. 1981). Stations G-116 and G-136 were sampled near the end of an upwelling period. Stations G-180, G-199, and G-226 represent a time series of samples taken in conjunction with a study of a second upwelling event which began on April 27. These stations were all held at the same geographic location as station G-136 and all samples were taken at the depth of the chlorophyll maximum. Station G-245 was held 10 km north of the time series station at the end of the second upwelling period. CuCC at station G-166 (0.095 μEq Cu l^{-1}) was 50% higher than that observed at any station during the second upwelling event. Complexation capacity measured at the stations held during the second upwelling event were similar one to another and were suggestive of a time dependent decrease in complexing capacity (Table 3). Mean CuCC observed during the second upwelling event ranged from 0.058 μEq Cu l^{-1} on April 27 to 0.046 μEq Cu l^{-1} on April 30. Outer shelf stations were sampled on three different cruises and the values are similar to those observed at midshelf stations. Station G-74 was sampled in Gulfstream water overlying water which contributed to the April 22 upwelling at station G-106. CuCC measured at BF-15 is relatively high and suggests that the sample may have been taken from upwelling waters. Station P-1, located on the continental slope off of North Carolina, is the

only continental shelf station not located in the Georgia Bight. Copper complexing capacity at this station ($\bar{x}$ = 0.043 μEq Cu l^{-1}), however, was in the midrange of other shelf stations. CuCC at inner shelf stations was extremely variable and ranged from 0.014 to 1.681 μEq Cu l^{-1}. CuCC at four stations was quite high; the highest values were observed at stations BF-5 and BF-10; salinity values at these stations were relatively high for this region on the shelf. The lowest value at an inner shelf station was observed at station SK-1 which was located at the entrance to Wassaw Sound, Ga. CuCC at this station was the lowest detected at any station in this study. CuCC at station Sap-3a was within the range previously given for mid- and outer-continental shelf stations. While this estuarine sample was a mixture of water from two sources, greater than 80% of the water came from the station described as Sap-3 by Wood (1980). Additional data from Sap-3, which was located approximately 1 km off Cabretta Beach (Cabretta Island, Ga.) support the conclusion that the water sampled was of offshore origin. Water at Sap-3 was light green in color and less turbid than the surrounding water. Primary production was extremely low and DOC concentration was an order of magnitude higher than that observed at any other station.

Copper complexing capacity, as measured by the ion exchange technique, quantifies the concentration of one operationally defined subset of the total array of copper complexing agents in the sample. Measurement of DOC and TPM, pools which include a wide array of potential complexing agents, were made at each station (Table 3) to provide a means of relating the complexation capacity measurements to the concentration of other complexing agents. Spearman rank correlation coefficients calculated by combining the data from all 21 stations showed significant correlation between complexation

capacity as measured by the ion exchange technique and the concentration of DOC and TPM (Table 4). While the strongest correlation was between CuCC and DOC, there is considerable deviation from direct proportionality and the high level of correlation appears to be based on the co-occurrence of extreme values. The highest DOC, TPM, and CuCC values occur at inner shelf stations and the lowest values occur at the five oceanic stations. The relationship between CuCC as measured by the ion exchange technique and the concentration of DOC and TPM within each region is very weak and frequently insignificant (Table 4). This is demonstrated graphically in Figure 6 where DOC, TPM, and CuCC are plotted in relation to depth. DOC and TPM levels are similar to those which would have been expected on the basis of the synoptic cruises and, while there is a noticeable depth related decrease in CuCC, DOC, and TPM, there is also a depth-related decrease in the variance of these parameters. Thus, the nearshore environment is best characterized as a very heterogeneous chemical environment in which CuCC ranges over several orders of magnitude. In the mid and outer shelf regions, the concentration of DOC and TPM decreases rapidly with increasing depth and asymptotically approach low baseline values ($\sim$ 0.8 mg l^{-1} for DOC, $\sim$ 1.5 mg l^{-1} for TPM). CuCC values fall in the lower end of the range of complexation capacity values observed in the inner shelf and vary independently of the concentration of DOC and TPM. Data from deep oceanic stations are very similar and vary by less than a factor of two; however, within these limits, the concentration of TPM and DOC and the CuCC appear to vary independently of one another.

Our data are consistent with other data which indicate a high degree of environmental variability in the Cu complexing capacity of aquatic environments (Bender et al. 1970; Chau 1973; Montgomery and Echevarria 1973; Sugai and Healy 1978). The range of complexing capacity observed in freshwater systems is, in fact, considerably greater than we report for estuarine and inner shelf systems. The copper complexing capacity of 23 freshwater systems was investigated in Maine and northern Europe using an ion exchange technique very similar to ours (Giesy 1980; Giesy and Briese 1978). Cu complexation capacity ranged from undetectable levels in a system in Maine and one in Norway to 41 μEq Cu l^{-1} in a Swedish river. There is scant data regarding the Cu complexing capacity of marine environments, but our inner shelf data bracket Smith's (1978) estimate of Cu complexing capacity at the mouth of the Ogeechee River (~ 0.75 μEq Cu l^{-1}) and those reported for the Yarra River estuary (Bluestein and Smith 1978). Abdullah et al. (1976) report a Cu complexation capacity of .001 μEq Cu $^{l-1}$ in Oslofjiord, this is ten times lower than we observed at the inner shelf. The estimated Cu solubilization capacity of highly productive waters in the Peru upwelling was higher and less variable than our CuCC data from similar environments, but these estimates were based on the assumption that the Cu solubilization capacity of natural seawater can be extrapolated from Cu solubilization measurements made on distilled water suspensions of isolated organic material (Kerr and Quinn 1980). Our finding that CuCC is highly variable on the continental shelf of the Southeastern U.S. is further supported by studies of the apparent biological availability of copper to phytoplankton in these environments (Wood 1980, 1982).

A hypothetical model of the distribution of Cu complexation capacity in different marine environments is presented in Figure 7. Both range and modal concentration are important defining characters for each region; range and mode are highest in the inner shelf and lowest in the open ocean. We suspect that the mechanisms underlying these characteristics are related to the source and nature of complexing agents in these systems. Six sources of complexing materials are postulated; all of which are likely to contribute to the pool of complexing agents in nearshore waters and only two of which seem likely sources of complexing agents in offshore waters. Detailed characterization of the thermodynamic, kinetic, and stoichiometric properties of Cu complexing substances from these sources are needed if more predictive models of complexing capacity are to emerge.

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TABLE 1.

Station locations and station data for continental shelf survey

Station Number	Date	Station Location		Station Depth (M)	Surface Temp (°C)	Sample	
						Depth (M)	Salinity (%)
March 1979, R/V Blue Fin							
1	3-27	31°37.8'N	79°52.9'W	43	18.75	3	36.314
2	3-27	31 32.0	79 37.0	120	22.05	1.5	36.21
3	3-27	31 26.6	80 06.3	40	17.30	2	36.37
4	3-28	31 22.7	80 27.9	32	15.12	2	35.02
5	3-28	31 20.3	81 07.2	13	16.40	3	29.67
6	3-28	31 16.7	80 48.4	16	15.95	3	31.19
7	3-28	31 11.7	80 26.1	31	17.90	3	35.38
8	3-28	31 09.7	80 16.7	30	19.40	3	35.94
9	3-28	31 32.0	80 40.0	20	15.70	3	34.42
10	3-28	31 37.5	80 46.5	17	15.40	3	32.55
11	3-28	31 52.3	80 53.2	6	15.10	3	34.39
12	3-28	31 49.2	80 45.2	15	15.60	3	32.27
13	3-28	31 44.7	80 26.8	21	14.10	3	34.39
14	3-29	31 38.5	80 04.5	36	15.40	3	35.62
15	3-29	31 33.1	79 46.1	101	18.5	3	36.32
16	3-29	31 26.2	80 30.0	25	17.0	3	35.56
17	3=29	31 22.5	80 53.6	15	18.1	3	30.28
18	3-29	31 19.6	81 12.6	4	18.4	2	23.17
19	3-29	31 28.2	81 04.7	12	17.6	3	29.91
20	3-29	31 29.2	80 51.0	17	17.3	3	31.58
21	3-29	31 33.3	81 05.0	7	18.0	2.5	29.82
22	3-29	31 43.2	81 02.2	6	16.8	2.5	29.08
July 1979, R/V Blue Fin							
1	7-2	31°51.4'N	80°52.4'W	5	24.5	3	31.26
2	7-2	31 46.8	80 49.3	12	25.0	3	30.74
3	7-2	31 42.2	80 47.0	13	26.0	3	32.23
4	7-2	31 35.6	80 41.7	20	25.0	3	33.48
5	7-2	31 33.9	80 39.9	22	26.5	3	33.71
6	7-2	31 38.0	80 44.8	20	26.0	3	33.13
7	7-2	31 42.5	80 50.9	15	26.0	3	32.10
8	7-2	31 46.7	80 54.4	14	27.5	3	31.04
9	7-2	31 48.3	80 57.7	18	27.0	3	29.11

TABLE 2

Station locations and sample data

Station	Date	Location		Total Depth (M)	Sample		
					Depth (M)	Salinity (ppt)	Temperature (°C)
Estuarine Locations							
Skid-1	7- 1-79	30°54.5' N	80°55.3' W	6	0.3	28.5	-
Sap-3a	10-21-79	31°25.4'	81°12.5'	20	0.3	26.0	24.0
R/V Blue Fin Cruise							
BF-5	3-28-79	31°20.3' N	81°07.2' W	13	3.5	30.17	16.4
BF-10	3-28-79	31°37.5'	80°46.5'	17	3.0	32.54	15.4
BF-15	3-29-79	31°33.1'	79°46.1'	101	3.5	36.31	18.5
BF-18	3-29-79	31°19.6'	81°12.6'	4	2.0	22.93	18.4
BF-21	3-29-79	31°33.3'	81°0.50'	7	3.0	29.84	18.0
R/V Gillis Cruise							
G-74	4-21-79	30°34.0'	80°03.8'	230	5.5	36.00	22.7
G-106	4-22-79	30°47.1'	79°59.9'	75	27.5	35.97	16.7
G-136	4-23-79	30°42.6'	80°05.5'	75	35.0	35.96	-
G-180	4-27-79	30°42.4'	80°05.3'	70	31.0	36.00	19.5
G-226	4-29-79	30°42.9'	80°05.4'	67	25.1	35.92	21.9
G-245	4-30-79	30°43.0'	80°05.1'	77	25.5	36.13	21.5
G-199	4-28-79	30°42.9'	80°05.3'	71	10.0	36.00	23.0
R/V Pierce Cruise							
Prc-1	7-26-79	39°12.0' N	69°17.0' W	2500	0.3	33.89	24.5
Prc-3	7-28-79	36°45'	66°33'	5000	0.3	36.32	25.0
Prc-4	7-30-79	33°21'	65°26'	4500	0.3	36.43	26.4
Prc-5	7-31-79	32°11'	64°59'	50	0.3	36.38	26.0
Prc-6	8- 1-79	29°21'	64°59'	4500	0.3	36.51	27.3
Prc-7	8- 3-79	26°17'	67°10'	5000	0.3	36.48	28.0
Prc-8	8- 4-79	25°52'	71°06'	5300	0.3	36.41	28.0

TABLE 3

Copper complexing capacity, concentration of dissolved organic carbon, and the concentration of suspended particulate material at continental shelf stations and in the Sargasso Sea

Station	Cu Complexing Capacity $\mu Eq\ l^{-1}$		Dissolved Organic $mg\ ml^{-1}$ (n=3)	Suspended Particulate Matter ($mg\ l^{-1}$)	
	A[1]	B		Total	Inorganic
Inner Shelf					
Sk-1	0.016	0.012	-	-	-
Sap 3A	0.097	0.066	-	-	-
BF-5	1.493	1.868	4.41 ± 0.2	3.08	1.15
BF-10	1.509	1.509	2.1 ± 0.2	2.4	1.2
BF-18	0.335	0.364	3.2 ± 0.0	51.5	16.9
BF-21	0.194	0.250	3.5 ± 1.0	14.6	9.6
Midshelf					
G-106	0.111	0.079	1.2 ± 0.4	3.1	1.7
G-136	0.074	0.062	1.2 ± 0.6	2.9	1.7
G-180	0.063	0.052	1.0	2.9	1.7
G-199	0.065	0.039	0.9 ± 0.1	2.8	1.5
G-226	0.051	0.059	1.0	2.9	2.2
G-245	0.043	0.049	1.1 ± 0.2	3.3	2.1
Outer Shelf					
BF-15	0.147	0.074	<0.7	1.4	0.6
G-74C	0.047	0.043	1.1 ± 0.3	7.0	3.9
PRC-1	0.047	0.039	1.7 ± 0.2	3.1	2.8
Oceanic					
P-3	ND	ND	0.8 ± 0.1	1.8	1.7
P-4	ND	ND	1.1 ± 0.4	1.0	0.9
P-5	0.036	-	1.3 ± 0.1	1.9	1.7
P-6	ND	ND	1.0 ± 0.1	1.4	1.3
P-7	ND	ND	0.8 ± 0.1	1.4	1.2
P-8	ND	ND	<0.7	1.5	1.3

[1]A and B represent replicate samples.

TABLE 4.

Spearman Rank Correlation coefficients for copper complexation capacity measurements with related parameters

	All Stations Combined	Inner Shelf Stations	~~Outer~~ Other Shelf Stations
Depth	-0.72***	0.14	-0.37
Salinity	-0.72***	0.54	0.27
DOC	0.75***	0.20	0.12
TPM	0.57**	-0.60	-0.60
PIM	0.14	-0.74	-0.76*

*** Significant at .001 level.

** Significant at .01 level.

* Significant at .05 level.

Figure Captions

Figure 1. Copper complexing capacity (CuCC) as a function of sample flow rate through the ion exchange column. CuCC is expressed as a proportion of the maximum observed value.

Figure 2. The relationship between copper ion spike and estimated copper complexing capacity (CuCC). Dashed line = estuarine samples; solid line = Sargasso Sea sample; dotted line = UV irradiated Sargasso Sea sample.

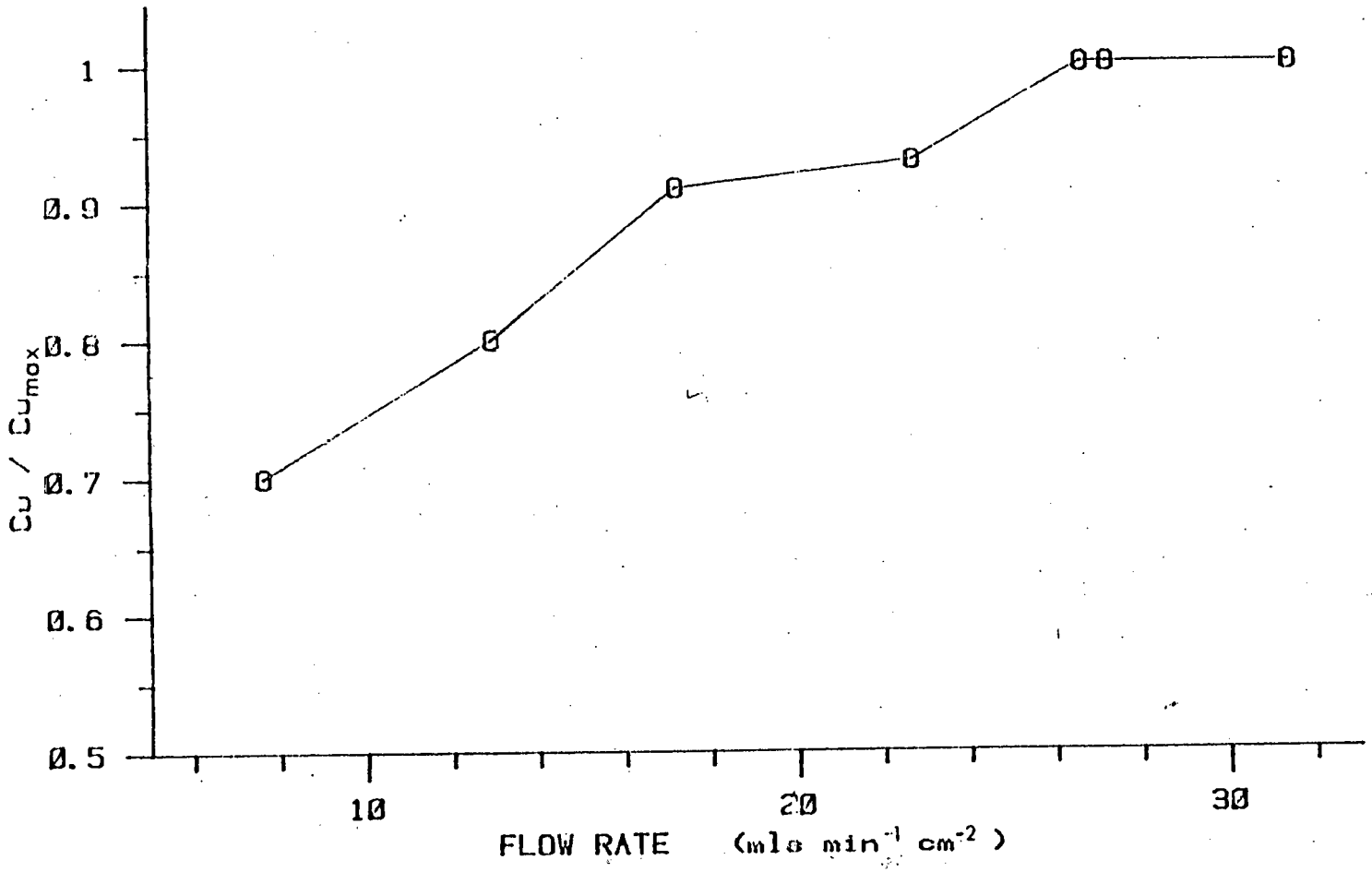
Figure 3. Surface salinity on the continental shelf of the Southeastern United States. ● = March, 1979, station locations; ○ = July, 1979, station locations.

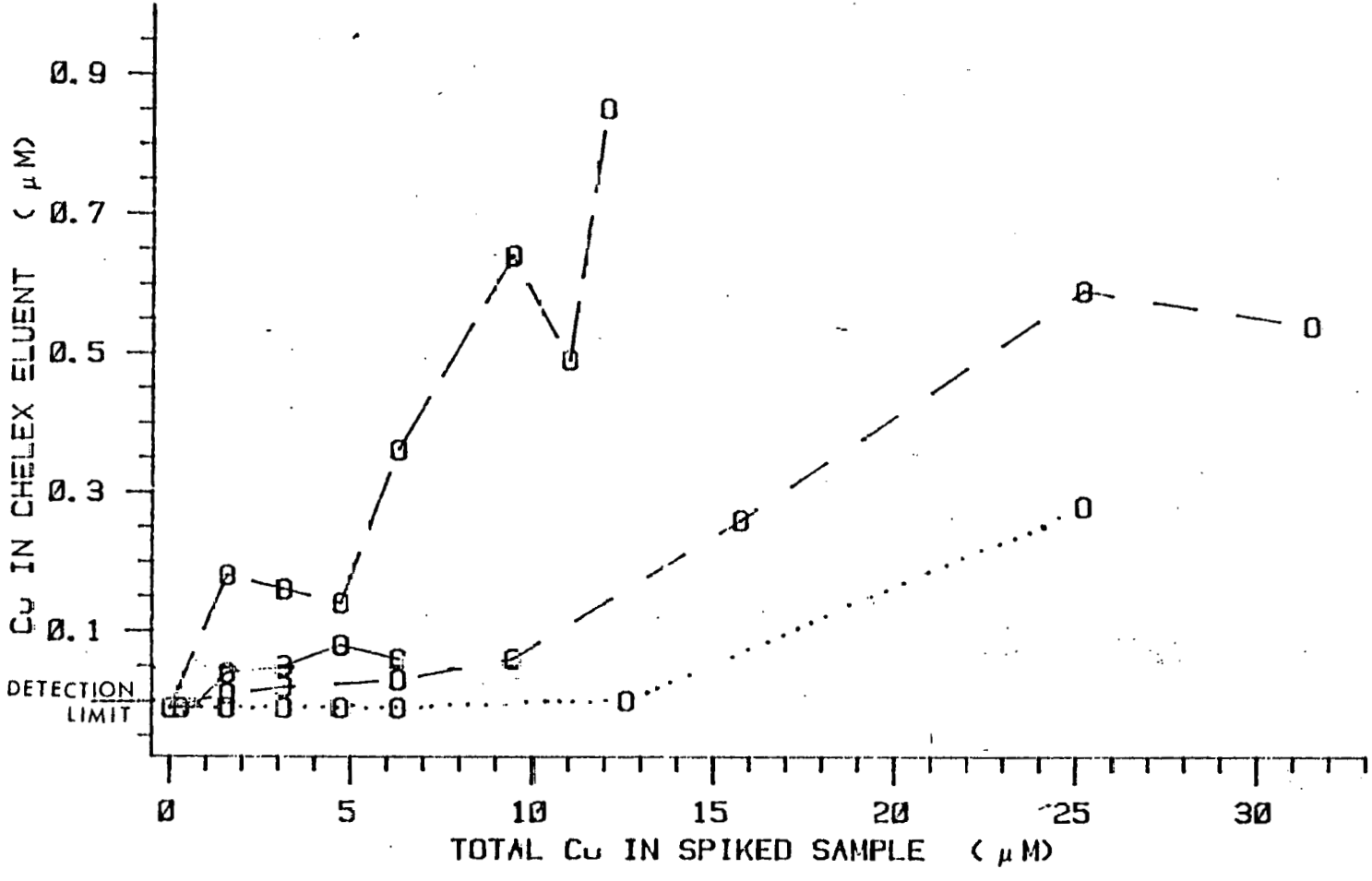
Figure 4. Dissolved organic carbon (DOC) concentration in surface waters of the continental shelf of the southeastern United States in March, 1979. ● station locations. Inset shows DOC concentration in July, 1979.

Figure 5. The distribution of total particulate material (TPM) in surface waters of the continental shelf of the southeastern United States. ● - March, 1979, station locations; ○ = July, 1979, station locations.

Figure 6. Copper complexation capacity (CuCC) compared to the distribution of dissolved organic carbon (DOC) and total particulate material (TPM) as a logarithmic function of depth. Station P-5, located in shallow water near Bermuda, is shown with a circle.

Figure 7. Hypothetical model of the distribution of copper complexing capacity in marine environments. Lower bar graph shows principal sources of complexing agents for each system. Solid line = oceanic systems; dotted line = mid- and outer shelf systems; dashed line = inner shelf systems and estuaries.


1
0.9
0.8
0.7
0.6
0.5
Cu / Cumax
10
20
30
FLOW RATE (mls min-1 cm-2)


0.9
0.7
0.5
0.3
0.1
Cu IN CHELEX ELUENT (μM)
DETECTION LIMIT
0
5
10
15
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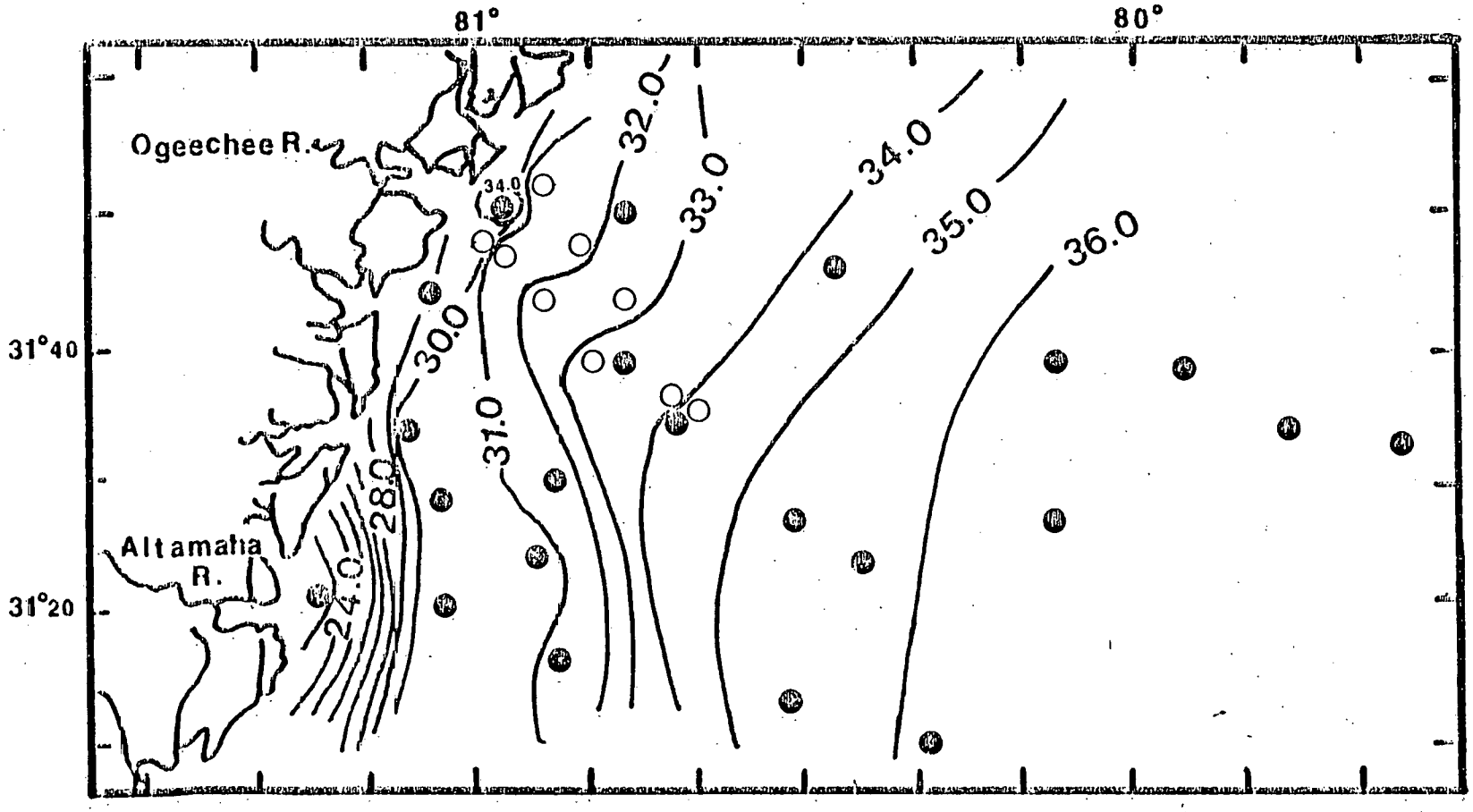
25
30
TOTAL Cu IN SPIKED SAMPLE (μM)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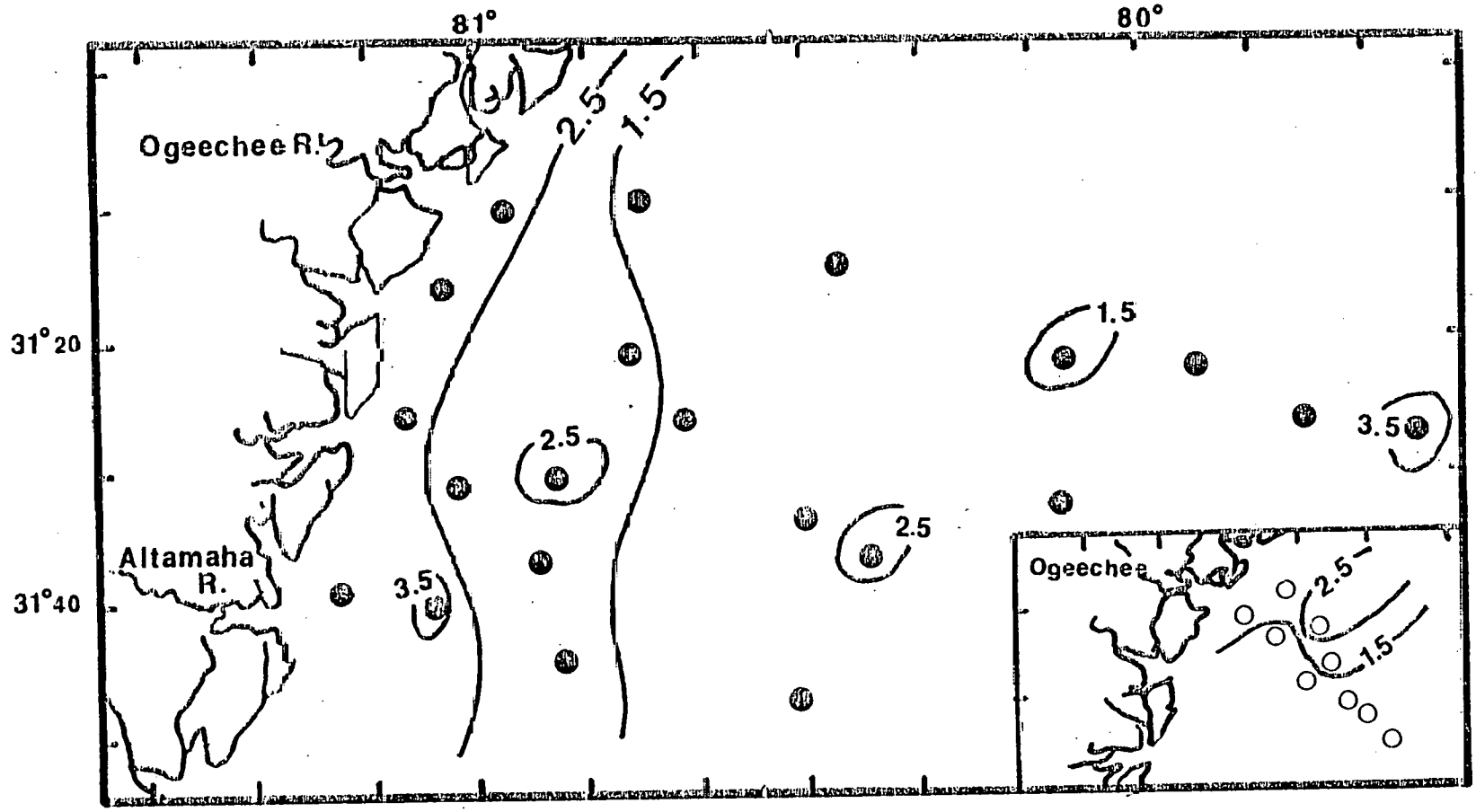
80°
31°40
31°20
Ogeechee R.
Altamaha R.
34.0
32.0
33.0
34.0
35.0
36.0
30.0
31.0
28.0
24.0


81°
80°
31°20
31°40
Ogeechee R.
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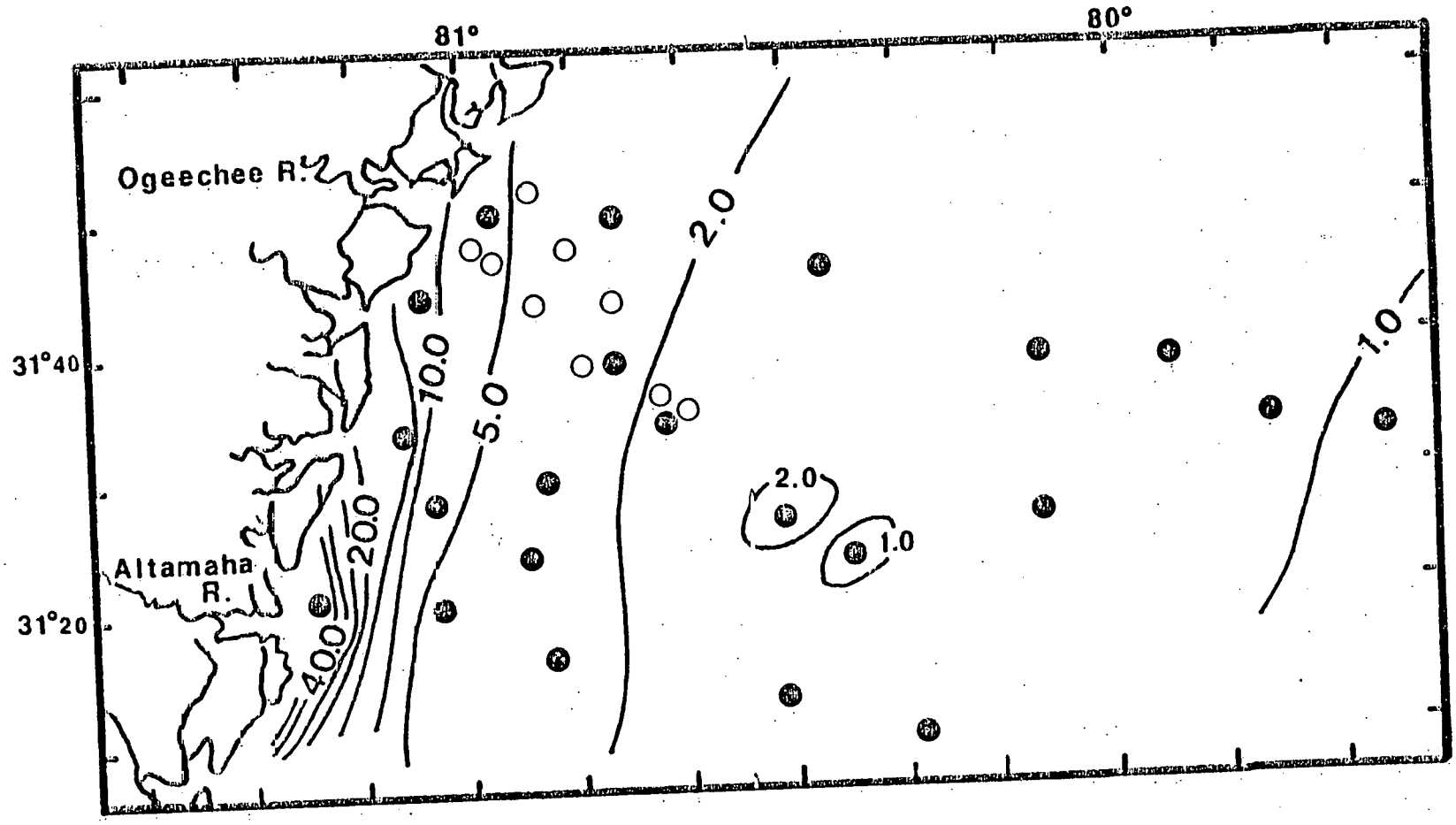
3.5
2.5
2.5
1.5


81°
80°
31°40
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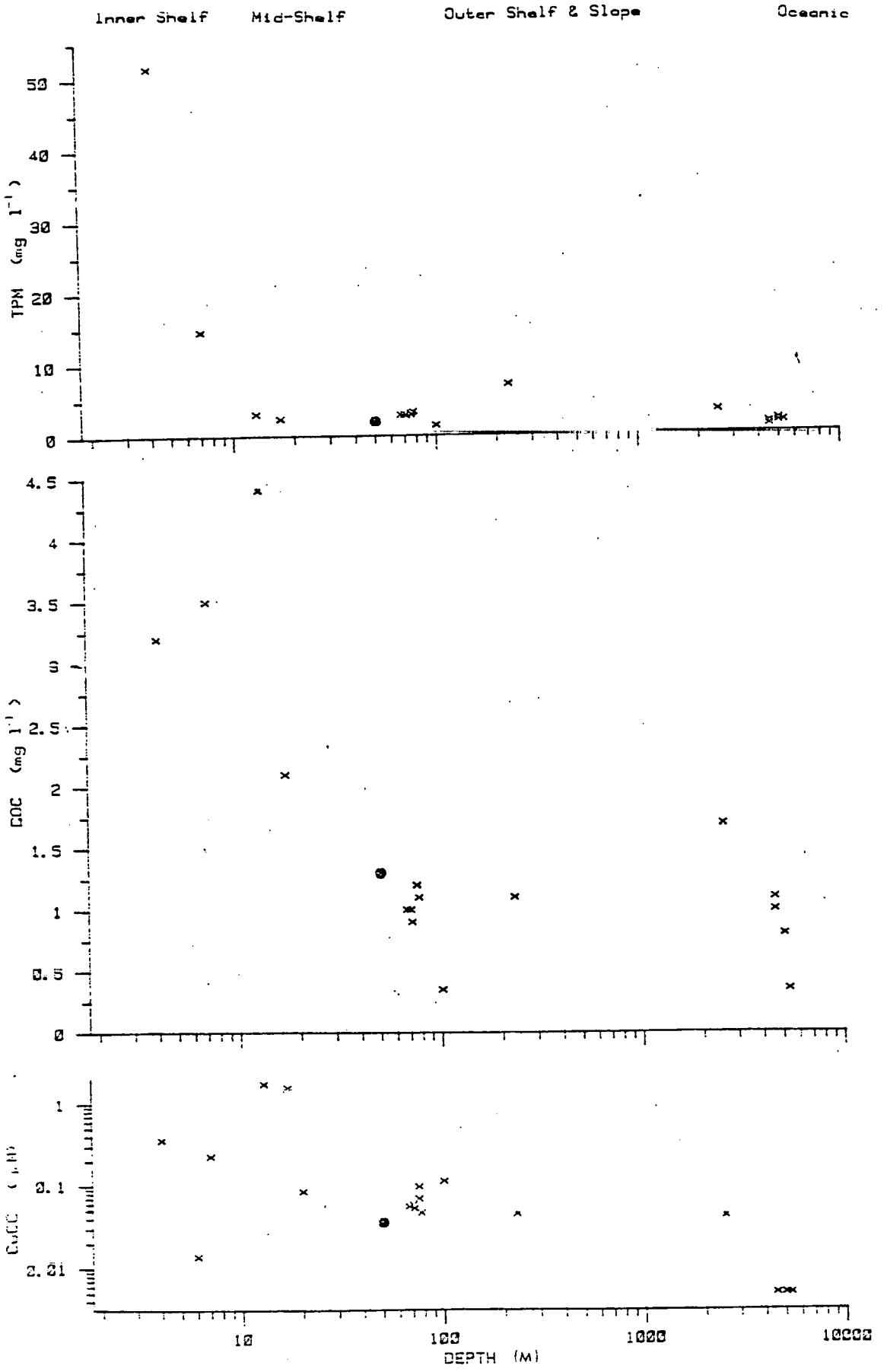
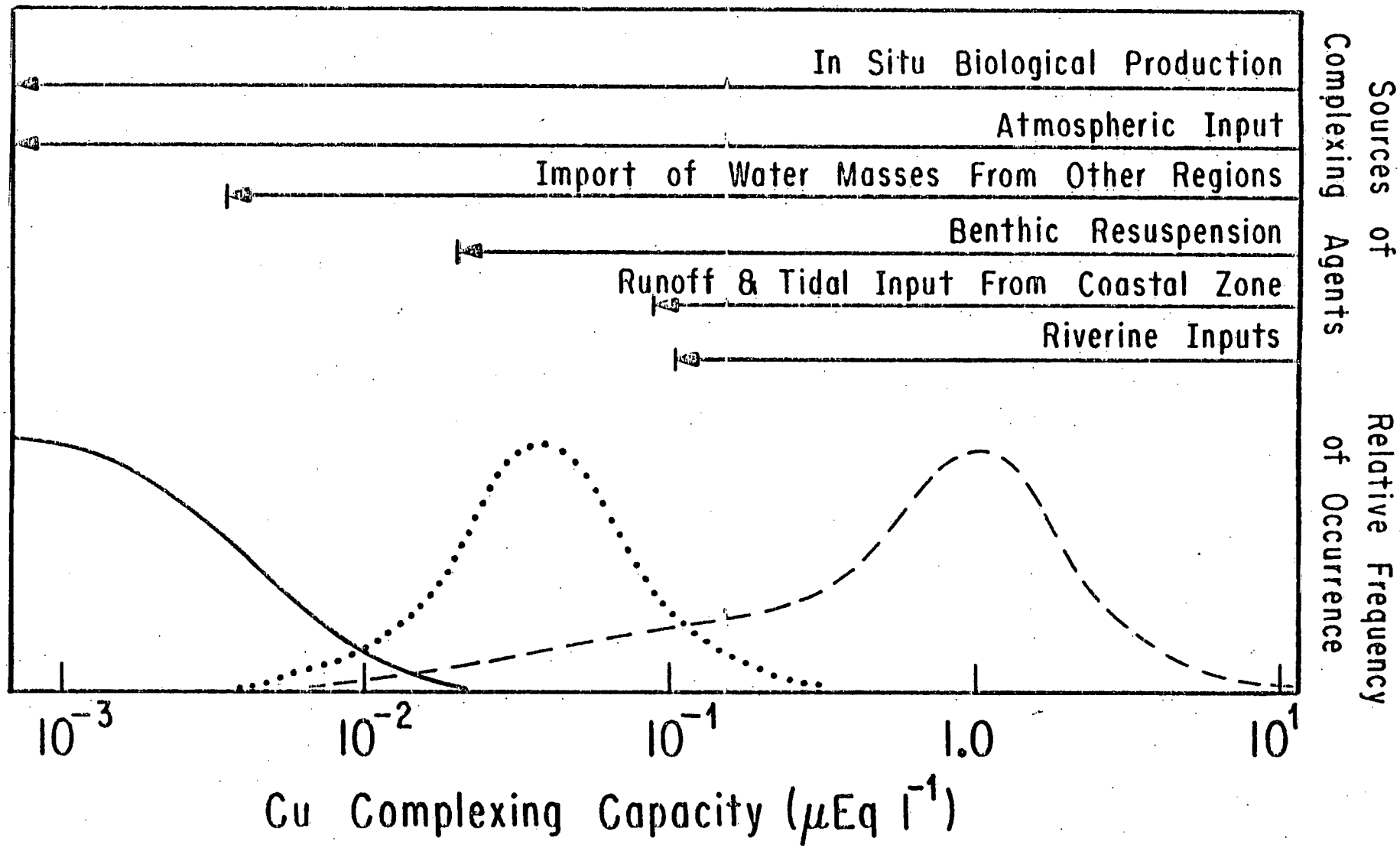
2.0
2.0
1.0
1.0


Inner Shelf
Mid-Shelf
Outer Shelf & Slope
Oceanic
TPM (mg l⁻¹)
50
40
30
20
10
0
DOC (mg l⁻¹)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1
0.1
0.01
10
100
1000
10000
DEPTH (M)


In Situ Biological Production
Atmospheric Input
Import of Water Masses From Other Regions
Benthic Resuspension
Runoff & Tidal Input From Coastal Zone
Riverine Inputs
Sources of Complexing Agents
Relative Frequency of Occurrence
10^{-3}
10^{-2}
10^{-1}
1.0
10^{1}
Cu Complexing Capacity (μEq l^{-1})

AN EXPERIMENTAL COMPARISON OF THE ROLES OF MICROFLAGELLATES AND CILIATES IN MARINE MINERAL CYCLES[1]

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[1] This work was supported by U.S. Department of Energy Contract DE-AS09-76EV00639

ABSTRACT

A study was undertaken to compare the effects of a marine heterotrophic microflagellate (Rhyncomonas sp.) and of a marine ciliate (Uronema, sp.) on phosphorus dynamics in bacterial cultures. The ciliates effected two orders of magnitude reduction in bacterial numbers and a more than two fold decrease in bound phosphorus when compared to a control bacterial culture. The flagellates had no discernable effect on either bacterial numbers or bound phosphorus levels. The turnover rates of phosphorus on a unit biomass basis were shown to be equal in all cultures. The implications of these results in relation to current concepts of the role of protozoans (particularly microflagellates) in marine mineral cycles and energy fluxes are discussed.

INTRODUCTION

There now seems to be little argument among a diverse group of investigators that bacteria play the lead among a cast of microbial characters whose joint actions are essential to the utilization of non-living organic matter and to mineral regeneration in marine ecosystems (Pomeroy 1974; 1975; Landry 1977; Wangersky 1977; Sieburth 1979). The debate appears to center on the details and significance of specific interactions between detritus (both particulate and dissolved), bacteria, and bacterial predators rather than on questions of the fundamental importance of the microbial system as a whole.

It is now widely believed that bactivorous protozoans are major mediators of control on marine bacterial populations, and, as such, have a significant influence on detrital food chains and in mineral cycles (Hamilton and Preslan 1970; Bick 1973; Sieburth 1976; Fenchel and JΦrgensen 1977). This belief is based largely on work with ciliates. The marked effect that ciliate protozoans have on bacterial numbers and other system parameters in sewage treatment plants is well documented (Curds 1973, 1977). Several studies have suggested that marine and freshwater ciliates play an essential role in mineral regeneration (Johannes 1965; Barsdate et al. 1974; Buechler and Dillon 1974).

Citing the inverse correlation between body size and metabolic rate, Johannes (1968) postulated that heterotrophic microflagellates might play an even larger role in mineral cycles than do the larger ciliates which he studied. Considering this and the frequency with which microflagellates were observed in nature, others have suggested that microflagellates may be integral links in marine energy and nutrient fluxes (Lightheart 1969; Pomeroy and Johannes 1968; Fenchel 1977; King et al. 1980).

In spite of the continuing speculation concerning the importance of small, colorless, free-living flagellates in ecological systems, ecologists have made little effort to learn more about them. This reluctance has likely been derived from the small size and typically delicate nature of these sorts of organisms, as such features render them difficult to identify in the field and to manipulate experimentally.

Among the numerous taxonomic groups which contain organisms fitting the vague descriptions often tendered by ecologists, the Kinetoplastida are a group which contains genera that are often cited by ecologists (Fenchel and Jørgensen 1977; Lightheart 1969) and about which a growing body of organismal data is being accumulated by protozoologists (Burzell 1973; Swale 1973; Eyden 1977; Vickerman 1978). The purpose of this study was to experimentally compare the imapct of a microflagellate (Rhyncomonas sp., Kinetoplastida) with the impact of a small ciliate (Uronema sp., Hymenostomatida) on phosphorus levels in marine bacteria cultures in an effort to assess the validity of the speculation concerning the role of microflagellates in marine ecosystems.

MATERIALS AND METHODS

Isolation and Identification of Protozoans

The flagellates used in this study were collected at 21° 25' N, 70° 15' W during cruise E1C74 of the R/V EASTWARD in January, 1974. A few drops of seawater collected in a PVC Niskin bottle were added to culture bottles containing sterile Pan-Mede medium. The medium contained Pan-Mede (10mg/100ml) in artificial seawater (Gold & Baren, 1966) which was Tris-buffered (3g/l, pH 6.5 before autoclaving). Innoculated bottles were returned to the laboratory, incubated in the dark at 22° C and monitored for the growth of heterotrophic microflagellates. Upon discovery, the flagellates were transferred along with the bacterial flora by micropipet to fresh Pan-Mede medium and were maintained on this type of medium under the stated incubation conditions for the duration of the study.

The organism chosen appeared to be a member of the genus Rhyncomonas and was subjected to both light and electron microscopic observations. Light micrographs of living cells were made using a Zeiss Photomicroscope II equipped with both phase contrast and Nomarski interference contrast optics as well as an electronic flash. For electron microscopic examination, cells were collected for fixation by centrifugation (5000 rpm for 15 min.). The resulting pellet was resuspended for 1 hour in 2 percent glutaraldehyde buffered with 0.1 m sodium cacodylate. The fixed cells were pelleted, washed three times with buffer, resuspended, and pelleted again before treatment with 1 percent osmium tetroxide in 0.1 m sodium cacodylate for 30 minutes. The glutaraldehyde fixative was made up in the artificial seawater used in the growth medium. All other fixatives and buffers were made up in distilled water. After fixation, the pellet fragments were dehydrated with ethyl alcohol and embedded in epon-araldite. Sections were made using a Reichert

OmU2 ultramicrotome using glass knives. The sections were stained with uranyl actate and Reynolds lead citrate prior to examination in a Phillips 200 electron microscope operated at 80 kv.

The ciliates used in these experiments were isolated in July, 1977 from a Sparina alterniflora infusion. Dead Spartina stems were collected from the marsh surface at Sapelo Island, Georgia, and immediately transported to Athens where they were placed in culture flasks containing lettuce extract medium. The lettuce extract was prepared by boiling one head of oven-dried lettuce in one liter of distilled water. The resulting extract was filtered through a 0.4 μm cellulose acetate filter and stored frozen. The lettuce extract medium contained 1 ml lettuce extract/l and 7.35 mg glutamic acid/l in the artificial seawater based used for the Pan-Mede medium. After three days incubation, the cultures contained large numbers of microflagellates and ciliates. The ciliates chosen for study were removed by micropipet to fresh cultures and incubated in the dark at 22° C. It was determined that the ciliates could be maintained on the Pan-Mede medium used for stock cultures of microflagellates and that medium was adapted.

For identification, the ciliates were examined live using the same light microscopic techniques as were applied to the flagellates. In addition, the ciliates were fixed and subjected to the Chaton-Lwoff silver impregnation technique (MacKinnon and Hawes 1961).

Phosphorus Tracer Studies

In order to compare the effects of flagellates and ciliates on phosphorus distribution and dynamics, four cultures were used. One was innoculated with Rhyncomonas culture, one with Uronema culture and two were innoculated with the bacterial flora present in the protozoan cultures. To insure that the

same bacterial flora was present in both the flagellate and ciliate cultures, bacteria were removed from both stock cultures prior to the experiment. They were grown in mixed culture and then used to innoculate both protozoan stock culture lines which were used in the experiment.

Each culture contained 800 ml of the lettuce extract medium described previously in a 1000 ml Wheaton Celstir^{R} tissue culture flask. Preliminary experiments proved that this medium allowed for better labeling of the organisms than various concentrations of the Pan-Mede medium. Organisms were introduced by adding three drops of the appropriate innoculum to each flask. All four cultures were labeled with 5 μCi of $^{33}PO_4$ (carrier-free) at the time of innoculation with organisms.

The experiment was carried out at room temperature with each flask resting on a magnetic stirring device that had been adjusted to the minimum speed required to maintain rotation of the magnetic stir bars in the flasks.

Beginning one hour after innoculation and at 19, 30, 54, and 78 hours after innoculation, duplicate 5 ml aliquots of each culture were removed from the flasks and filtered through 0.4 μm polycarbonate filters. The filters were dipped in a 2 percent solution of Wafos 6 TD (Phillip A. Hunt Chemical Corp.) prior to being used. Wafos 6 TD is a detergent which reduces the hydrophobic nature of the filters (Bowden, 1977). Following filtration, the filters were rinsed by passing 2 ml of artificial seawater through them before they were removed from their holders and placed in glass scintillation vials.

One hour after innoculation and on a daily basis thereafter, 5 ml aliquots from both protozoan cultures and from one bacterial culture were removed, mixed with 5 ml of 4 percent glutaraldehyde in 0.2 sodium cacodylate in artificial seawater, and stored under refrigeration. Microscopic counts of protozoans and bacteria were made on these samples using the epifluorescence technique of Daly and Hobbie (1975).

At 93.5 hours after innoculation, one of the bacteria cultures was poisoned by adding 3 ml of 50 percent formalin. All cultures were then labeled with 5.8 μCi of $^{32}PO_4$ as a tracer of phosphorus turnover in the systems.

At the end of the experiment, 1 ml of Soluene^{R} was added to each vial containing a filter. After the filter had dissolved, 15 ml of Insta-Gel^{R} scintillation fluid was added to each vial and the samples counted in a Packard Tri-Carb liquid scintillation counter for 20 minutes or 20,000 counts. For samples collected prior to the addition of $^{32}PO_4$, ^{33}P counts were converted to disentegrations per minute (dpm) by dividing by the counting efficiency. Subsequent to the addition of $^{32}PO4$, dpm for both isotopes were calculated using the simultaneous equations described by Kobayashi and Maudsley (1970).

RESULTS

Identification and Morphology of Protozoans

The flagellate studied here closely fits both the description of Rhyncomonas metabolita given by Burzell (1973) and that of R. nasuta given by Swale (1973). The most distinguishing feature of Rhyncomonas is the proboscis, a trunk-like structure which extends from the anterior end of the cell. The organism itself is oval in shape with dimensions of approximately 2-4 x 4-9 micrometers. The organism is highly flattened and preferentially orients itself with a particular surface towards the substrate upon which it creeps. Rhyncomonas possesses two flagella of unequal length that arise near the base of the proboscis. The shorter of the two flagella is directed anteriorly and is obscured by the proboscis during light microscopic observations. The larger flagellum is 2-3 times the length of the cell and is directed posteriorly (Figure 1).

A transmission electron micrograph (Figure 2) through the dorsoventral plane in the region of attachment of the proboscis reveals several significant features of the Rhyncomonas cell. The proboscis contains a membrane delimited lumen. The ventral wall of the proboscis has at least three overlapping rows of single microtubules as well as a row of what appear to be doublet microtubules which lie next to the outer membrane. Burzell (1973) considers the proboscis to be a feeding appendage and has described a long (12-18) row of microtubules that runs in an oblique path down the dorsal wall of the sturcture. The microtubules which are located closest to the lumen in Figure 2 may be the ends of the long row. The protrusion of a portion of the proboscis membrane away from the cell is likely an artifact since not every proboscis had such a bulge. These organisms are extremely delicate and similar bulges were seen in various positions in several cells.

The cytopharynx with two rows of microtubules associated with it is located near the surface of the cell and likely extends from the proboscis in a posterior direction. The nucleus and mitochondrion are evident in Figure 2. The mitochondrion is probably a unitary structure as it is in other kinetoplastids. The identity of the large cytoplasmic inclusions of smooth, relatively electron transparent, appearance is not known. Of several sections examined, this was the only one which contained such bodies.

Vacuoles containing bacteria occupy a large portion of Figure 2. Such vacuoles are common features of these cells and the bacteria contained therein (Figure 3) are obviously similar to bacteria outside of the cell. Wiebe and Chapman (1968) have described the fine structure of marine bacteria and the salient features of bacterial morphology including the cell wall, nuclear region, and ribonucleoprotein particles are plainly visible in many of the bacteria within the vacuoles. While not conclusive, this indicates a phagotrophic habit as the mode of nutrition for Rhyncomonas.

The ciliate chosen for study is a member of the genus Uronema. The elongate, ovoid body is some 30 μm long. The anterior pole is devoid of cilia while the posterior pole bears a single long cilium. The mouth bears a small undulating membrane with the adoral membranelles being inconspicuous. The macronucleus is spherical. The contractile vacuole is located posteriorly (Figures 4 and 5). Based on the descriptions of Bick (1972) and Kudo (1954) this organism is probably Uronema marinum.

Phosphorus Dynamics

Bacterial Numbers

The bacteria in all cultures, after an initial lag phase, rapidly increased in numbers so that a maximum was reached after approximately 19 hours (Figure 6). These numbers were maintained in the bacterial culture and in the Rhyncomonas cultures. In the ciliate culture, a drastic reduction in bacterial numbers occurred after 44 hours so that by 68 hours after innoculation, there existed an order of magnitude difference between the number of bacteria in the Rhynocomonas and bacterial cultures.

Protozoan Numbers

The initial increases in both ciliate and flagellate numbers (Figure 7) in their respective cultures were apparent 44 hours subsequent to innoculation with maximum levels being reached after 68 hours. A sharp decline in ciliate numbers occurred approximately 2 days after the maximum was reached or 5 days after innoculation. A lesser decline occurred in the Rhyncomonas culture 4 days after attainment of the maximum. It should be noted that the increase in ciliate numbers and the decrease in bacterial numbers in the ciliate culture occurred simultaneously. The Rhyncomonas population had no apparent effect on the bacterial population in their culture.

Tracer Dynamics

The amount of $^{33}PO_4$ associated with the particulate fractions of the cultures mirrors the growth of the bacteria. In each culture, there was a rapid increase in the incorporated $^{33}PO4$, with the maximum being reached at 19 hours, coincident with the attainment of maximum bacterial numbers (Figures 8, 9, 10, and 11). Thereafter, a slow decrease occurred in the bacterial and Rhyncomonas cultures so that at the end of the experiment, 25% and 20%, respectively, of the maximum $^{33}PO4$ occurred with the largest rate of decline occurring during the same time interval as the sharp decline in bacterial numbers. The $^{33}PO_4$ associated with particulate material in the Rhyncomonas culture was approximately 60% of that associated with particulate in the bacterial culture. At the end of the experiment, the amount of $^{33}PO_4$ incorporated in the ciliate culture was 40% of that incorporated in the bacterial culture. After the initial rapid decline in incorporated $^{33}PO_4$ in the ciliate culture, there was a slow decline over the course of the experiment which appeared similar to the slow loss in Rhyncomonas and bacteria cultures. The net result was that, by the end of the experiment, 20-25% of the incorporated $^{33}PO_4$ in the ciliate culture remained.

Upon the addition of $^{32}PO_4$ to the cultures, there was a generally steady uptake in all cultures except the poisoned control. The amount of $^{32}PO_4$ taken up in the ciliate culture was noticeably less than in the other two cultures probably as a result of the lower bacterial numbers in that culture.

If one looks at the fraction of the isotopes incorporated into the organisms (the sum of the fraction of ^{32}P taken up and the sum of the fraction ^{33}P taken up), something in excess of 50% of the isotopes were bound in organisms in the bacteria and Rhyncomonas cultures (Figures 12 and 13); whereas, close to 20% of the isotopes were bound in the Uronema cultures (Figure 14).

In order to determine if the turnover rates per unit biomass of the three experimental cultures were different, a ratio T was calculated. This was calculated as:

$$T = \frac{^{32}P \text{ uptake}}{^{33}P \text{ uptake}}$$

where uptake is calculated as:

$$\frac{\text{dpm incorporated}}{\text{dpm added}}$$

This ratio was used for two reasons. First, it expresses the rate of ^{32}P uptake as a function of biomass (the amount of ^{33}P taken up) and secondly, it cancels differences in biomass between the cultures. The graphs of the T ratios for the three experimental cultures plus the poisoned control are given in Figure 15.

An analysis of covariance was performed on the T ratios of the three experimental cultures in an effort to determine if there were differences in the slopes (rates of uptake per unit biomass) of those three lines. The F test for differences between the slopes was not significant ($P = .05$), indicating that the behavior of the systems per unit biomass was not different and that the differences in incorporated phosphorus levels between the cultures was likely due to biomass differences.

Discussion

These experiments leave current concepts concerning the role of heterotrophic microflagellates in pelagic marine nutrient and energy fluxes unsubstantiated. Events in the ciliate culture (reduction of bacterial numbers and bound phosphorus) were consistent with the results of past experiments (Johannes 1965; Barsdate et al. 1974; Buechler and Dillon 1974)

and the resulting theory concerning the role of protozoa in marine ecosystems (Pomeroy 1974). Under identical experimental conditions, Rhyncomonas had no discernible effect upon either bacterial numbers or the level of phosphorus in the water. This demonstrates that, in an ecological sense as well as taxonomically, microflagellates are something other than scaled down, more metabolically active versions of the ciliate model.

Finding that Rhyncomonas had little impact upon the parameters measured was not totally unexpected. Johannes (1965) and Curds (1973) have both commented that flagellates do not affect bacterial numbers in different systems to the same degree as do ciliates. If one looks carefully at collections of data showing the relationships of weight specific biological rates to body size for animals (Fenchel 1974), it becomes apparent that a general trend does exist; but that comparisons of protozoans to, say, arthropods may be quite more confidently undertaken than may be comparisons of flagellates and ciliates. There are diverse physiological and biochemical capacities found among protozoans which are important to the ecological roles of the organisms, and there may be inherent differences in metabolic rates which cannot be explained by differences in body size (Stout 1980).

Johannes (1965), comparing phosphorus regeneration by an unidentified microflagellate and by two species of Euplotes, found that the microflagellate effected no more phosphorus release than did the ciliates even though the flagellates were present in far (at least three orders of magnitude) numbers. In the experiment reported here, the flagellates and ciliates were present in equal numbers in the early phases of the experiment with the ciliates being an order of magnitude lower in number in the latter stages. If we assume that the shapes of the cells can be approximated by oblate spheroids, we can calculate that a Uronema cell is roughly 2 orders of magnitude larger than a

Rhyncomonas cell. Johannes' (1968) calculations indicate that a microflagellate should regenerate phosphorus about 10 times faster per unit body weight than does a ciliate. At that rate, the ciliates should have been some 10 times more active than the flagellates in the early stages of the experiment, with the activities being about equal in the latter stages. The ciliates in fact removed 100 times more bacteria than did the flagellates. In an often overlooked disclaimer, Johannes (1968) noted that his speculations concerning microflagellates were based on rather daring extrapolations of the existing data. There is little to indicate that microflagellates prey on bacteria with an efficiency even approaching that of ciliates. If phosphorus regeneration depends on bacterial predation, ciliates may be more important instruments than are microflagellates.

Since the data presented here indicate an undetectable utilization of bacteria as food by Rhyncomonas, a discussion of the nutritional mode of these protozoans is required. It is, of course, well known that various protozoans, which are naturally phagotrophic, may be cultured axenically. These cultivation techniques should be recognized for what they are: useful tools in the study of organismal physiology and biochemistry. An axenic culture is an extremely unnatural ecological setting for a protozoan. Although authors sometimes make passing comments concerning utilization of dissolved substrates by flagellates and competition of bacteria and flagellates for substrates (Curds 1973), it now seems popular among protozoan ecologists to accept the idea that most colorless protozoans are phagotrophic in nature (Stout 1973, Bamforth 1973, Fenchel and Jorgensen 1977). Hass and Webb (1979) were unable to demonstrate osmotrophy in any of the microflagellates that they studied. There is little doubt that Rhyncomonas itself is bactivorous. The micrographs presented here show bacteria in food vacuoles. These bacteria appear

identical to those outside the cell, making for circumstantial evidence that they were ingested. Specialists on the free-living kinetoplastids argue that the group as a whole is bactivorous (Eeckhout 1973; Brooker 1971; Eyden 1977). Furthermore, both Burzell (1973) and Swale (1973), who have conducted extensive investigations of Rhyncomonas, agree that bactivory is the nutritional mode of this genus. Finally, for the purpose of this study, the question of osmotrophy as a habit of Rhyncomonas, is irrelevent since the goal was to compare the effect of the microflagellate on bacterial populations to the effect of ciliates on the same. It is possible to make this comparison without determining the mechanism involved.

An important question on the function of bacteria in marine ecosystems concerns their role as either active or passive agents in mineral regeneration. Pomeroy (1974) considers bacteria to be net consumers of minerals; meaning that without bacterial predation, bacterially-bound minerals are inaccessible to other components of the ecosystem. In Wangersky's (1977) concept of oceanic mineral regeneration, bacteria are thought to be net producers of available minerals. This study indicates that bacteria are consumers of phosphorus, but the controversy on this point is far from resolved. At this point, prudence requires the statement that the role of bacteria in mineral regeneration at a given time and place must surely vary with the metabolic rates and patterns of the populations present. Predation and the nature of the limiting substrates are two environmental factors which are likely to strongly influence bacteria and their metabolic processes.

There is some debate concerning the mechanism whereby protozoans increase phosphorus turnover in marine eco systems. Johannes (1965) and Buechler and Dillon (1974) demonstrated that ciliates ingest bacteria and excrete phosphorus. These authors suggest that protozoa directly effect a higher

turnover of phosphorus by ingesting and excreting bacterial phosphorus which will remain bound in the absence of protozoans. Barsdate et al. (1974), while agreeing that ciliates increase phosphorus turnover in systems, argue that the direct excretion of phosphorus which the ciliates somehow induce in the bacteria themselves. In the experiment described here, the rate of uptake of phosphorus per unit biomass was no greater in the ciliate culture than in the flagellate and bacteria cultures. This indicates that individual bacteria were no more active under heavily-grazed conditions than they were under either lightly or ungrazed conditions (and thus supports the proposition that direct excretion of minerals by protozoans is the significant mechanism of mineral regeneration).

Hunt et al. (1977) have proposed that bacteria in carbon limited systems respond differently to predation than when they are in nitrogen limited systems. In their modeling exercise, they found that return of nutrients by predators did not stimulate nutrient uptake by bacteria under carbon limited conditions whereas nutrient return by predators did increase nutrient uptake under nutrient limited conditions. Hunt et al. explain the data of Barsdate et al. by saying that in that nitrogen-limited system, the lowering of bacterial biomass and the release of nitrogen by the predators caused an increase in the growth rate which resulted in an increased turnover of the non-limiting, nutrient phosphorus.

It is reasonable to believe that the bacteria in this study were carbon-limited. Upon innoculation of the cultures, their numbers increased until all energy yielding substrates were depleted from the medium after which the populations declined slowly in all but the ciliate culture. The ciliates effected a dramatic reduction in bacterial numbers and increased the level of dissolved phosphorus, probably through excretion. But, since the bacteria

were carbon-limited, little additional growth with a resultant demand for phosphorus was possible. Both bacterial numbers and phosphorus uptake rates remained low while the unbound phosphorus levels remained high. Stated simply, this shows that ciliates function in mineral cycles by making minerals available. The fate of those minerals depends upon the capacity for their utilization by other organisms. In a system such as the one described here, changing the level of predation and/or other limiting factors could change the action of the bacteria with regard to phosphorus.

There is widespread belief that protozoan or other grazers stimulate bacterial activity. Johannes (1968) postulated that this was accomplished by the grazers maintaining the bacterial populations below the carrying capacity of the environment. In other words, the bacterial populations were held below levels where density dependent factors restrict bacterial growth. Curds (1977) has pointed out that such a process does not necessarily mean that total bacterial activity will be greater in a grazed system since total bacterial activity is a product of growth rate and biomass.

Fenchel (1977) has described grazed detrital systems which decompose more rapidly than do ungrazed systems. Enrichment with phosphorus speeds up decomposition in both grazed and ungrazed systems. Grazed systems deplete the phosphorus in the water to as low or lower levels than do ungrazed systems. Decomposition in these systems appears to be phosphorus limited but the protozoans do not appear to enhance bacterial activity by regeneration of phosphorus. Since Fenchel did not look at turnover rates of phosphorus, it remains possible that the protozoans do cause more rapid regeneration of phosphorus which was masked in this instance by a corresponding increase in uptake rate. The situation described by Fenchel is one in which total bacterial activity appears to be greater in grazed as opposed to ungrazed systems.

Whether bacteria are active or passive agents of mineral flux depends on the particular element under observation and on the metabolic rates and requirements at that point in time of the population. The effect of bacterial predators on mineral turnover can be either direct or indirect depending also on the mineral in question and its concentration relative to the metabolic requirements and rates of the community. The differences of opinion on the role of bacteria and their predators in mineral cycles have arisen largely as a result of differences in the systems studied.

The major contribution of this study is that is casts considerable doubt on the validity of using the behavior of ciliates to draw conclusions concerning microflagellates. In recognition of this, it should be emphasized that while this study may indicate that heterotrophic microflagellates do not play a major role in energy and nutrient fluxes within pelagic marine ecosystems, it by no means proves this conclusively. As always in studies of this type it is impossible to clearly discern the relationship of an organism's behavior in culture to its behavior in nature. In addition, Rhyncomonas sp. may not be representative of all microflagellates. Ecologists use the term "microflagellate" to describe any small flagellate, and the most cursory of glances through a taxonomic key reveals that such organisms exist in a diverse group of taxonomic divisions. It remains possible that the ecological functions of "microflagellates" are as diverse as their taxonomy. This study also does not rule out a significant role for microflagellates in the whole range of marine ecosystems. Fenchel (1977) has data which indicates a significant role for microflagellates, particularly attached forms, in sediment systems.

The role of bacterial predators in marine mineral cycles is a complex issue with the mechanisms of action and the magnitudes of the effects varying

with the type of predator and the system in question. Rejection of microflagellates as agents of major mineral flux does not consign them to ecological exile. The ubiquity of their numbers and other evidence still indicates a central role for microflagellates in the function of pelagic marine ecosystems (Sieburth et al. 1978). Since protozoans, like other organisms, exhibit morphological, physiological, and behavioral characteristics bestowing fitness to their habitats (Bamforth 1981), understanding microflagellates should tell us something of these ecosystems. It has been demonstrated that small flagellates are indicative of early succession in terrestrial soils and sewage-polluted streams (Bamforth 1973). Thus we might speculate that the protozoan community is held in early successional stages in much of the ocean. Bamforth (1980) has divided terrestrial protozoans into "r" and "K" groups according to the MacArthur-Wilson concept. The "r" group, consisting of small, rapidly growing, forms is favored in areas where conditions allowing microbial growth occur intermittently and for short durations (hours or days). The fact that microflagellates are seen by oceanographers may tell us that conditions favorable for heterotrophic growth occur sporadically and for a short duration (on a protozoan time scale) in the ocean's economy and that the incapacity for overwhelming exploitation of resources is a successful strategy in that realm.

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Fig 1

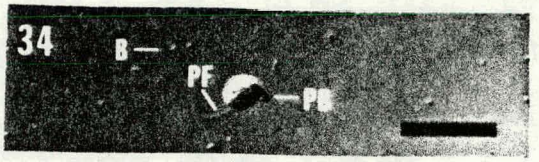
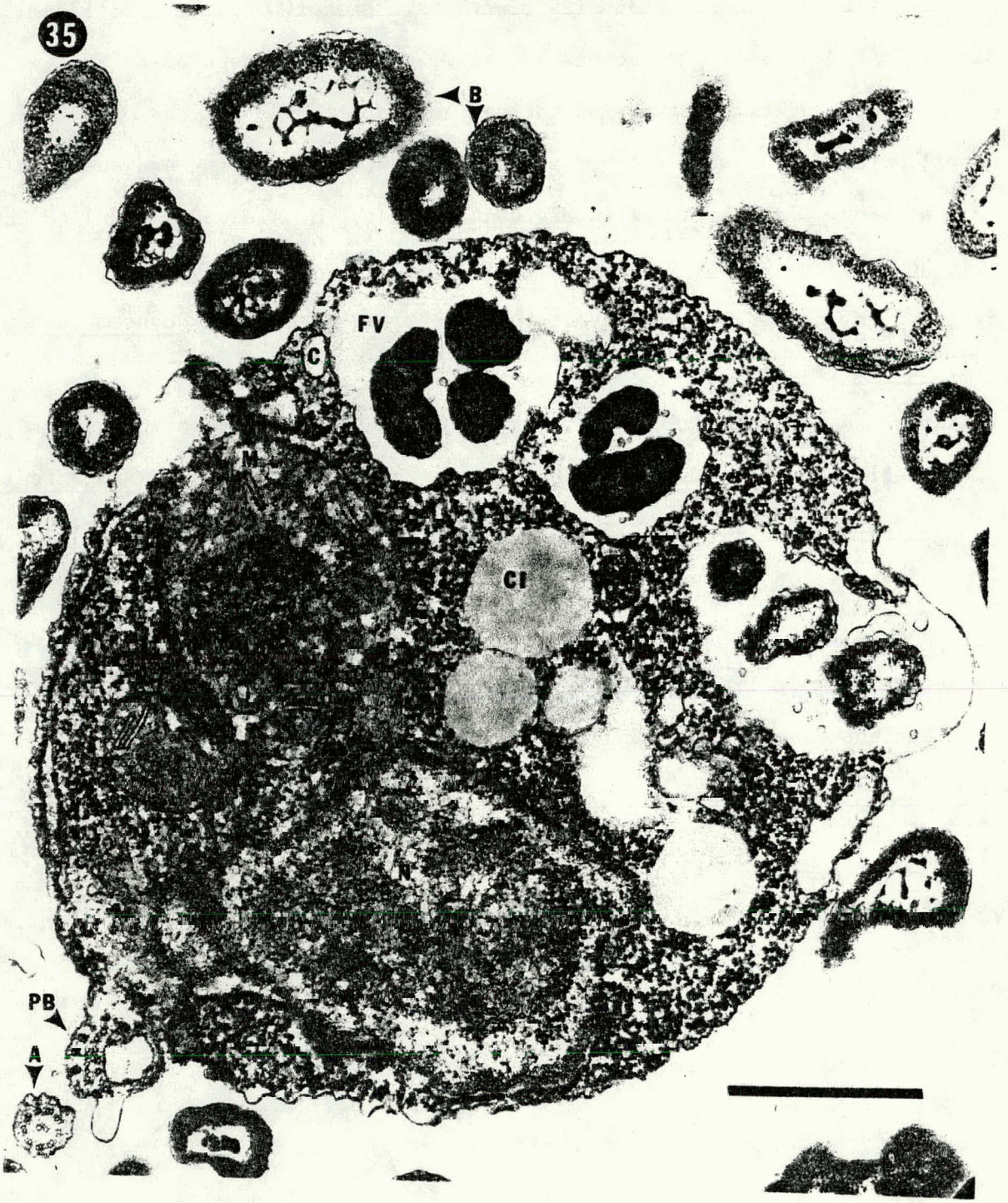


Fig 2



1

Figure 34. Nomarski interference contrast micrograph of a Rhyncomonas culture. The small flagellate is readily identified by its flattened, oval shape, its proboscis (PB), and its trailing posterior flagellum (PF). The flagellate is surrounded by bacteria (B). X1250. Bar = 10um.

2

Figure 35. Transmission electron micrograph of a cross section in the dorsoventral plane of Rhyncomonas. To be seen are the proboscis (PB), the anterior flagellum (A), the cytopharynx (C), the mitochondrion (M), the nucleus (N), food vacuoles (FV), and bacteria (B) in the culture. The identity of the translucent cellular inclusions (CI) is unknown. X30,850. Bar = 1um.

x 30,900

Fig 3

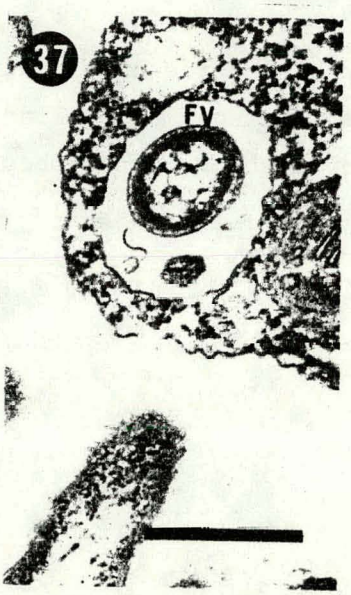


Fig 4

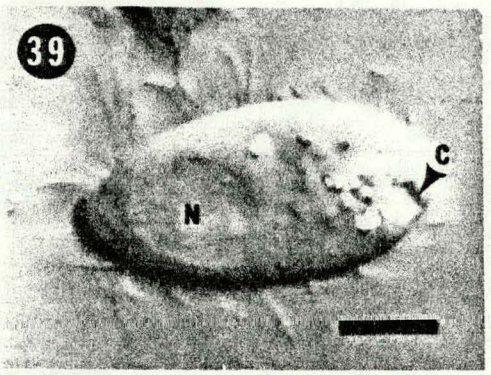
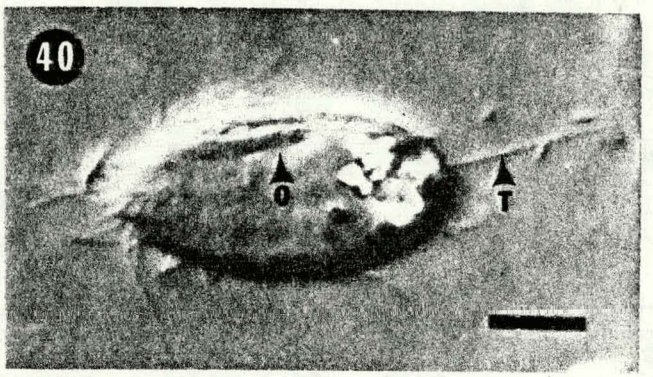
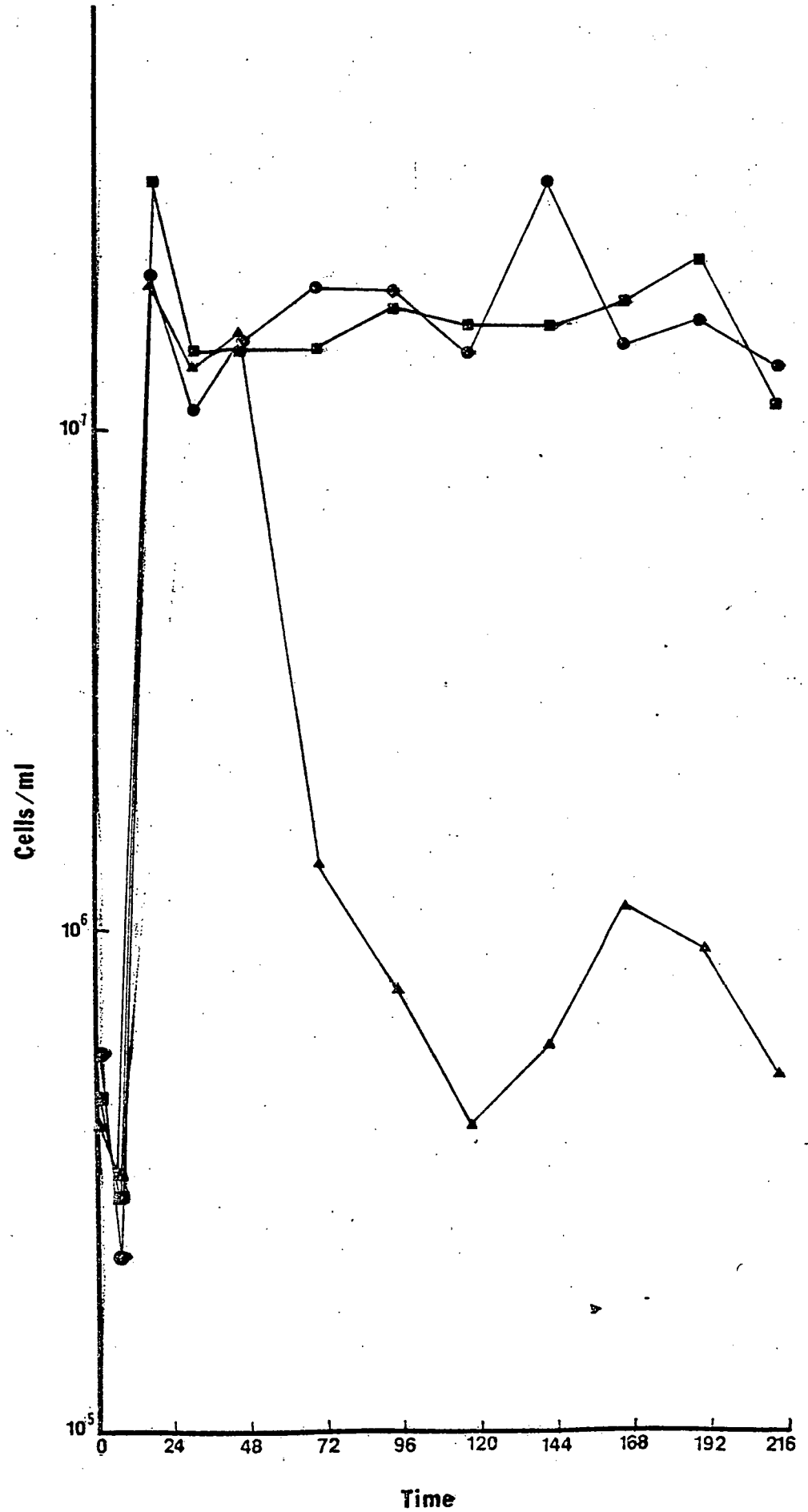


Fig 5




Cells/ml
10^7
10^6
10^5
0
24
48
72
96
120
144
168
192
216
Time

~~Figure 36. Higher magnification of the proboscis in Figure 35. The proboscis (PB) contains a well-defined lumen and an array of microtubules. A, anterior flagellum. X110,000. Bar = 0.25um.~~

3
Figure ~~37~~. Section cut through a food vacuole (FV) of a Rhyncomonas cell. The bacterium contained in the vacuole is close in appearance to those outside the cell. X41,400. Bar = 0.5um.

~~Figure 38. Longitudinal section showing the attachment of the two flagella (F) of Rhyncomonas. X54,900. Bar = 0.5um.~~

4
Figure ~~39~~. Nomarski interference contrast micrograph of the ciliate, Uronema marinum. Note the posterior contractile vacuole (C), the anterior nucleus (N), and the absence of cilia on the anterior pole of the cell. X1250. Bar = 10um.

5
Figure ~~40~~. Nomarski interference contrast micrograph of Uronema showing the oral apparatus (O) and the single, long, caudal cilium (T). X1250. Bar = 10um.

Figure 61. Numbers of bacteria in experimental cultures. ● bacterial culture; ■ flagellate culture; ▲ ciliate culture.

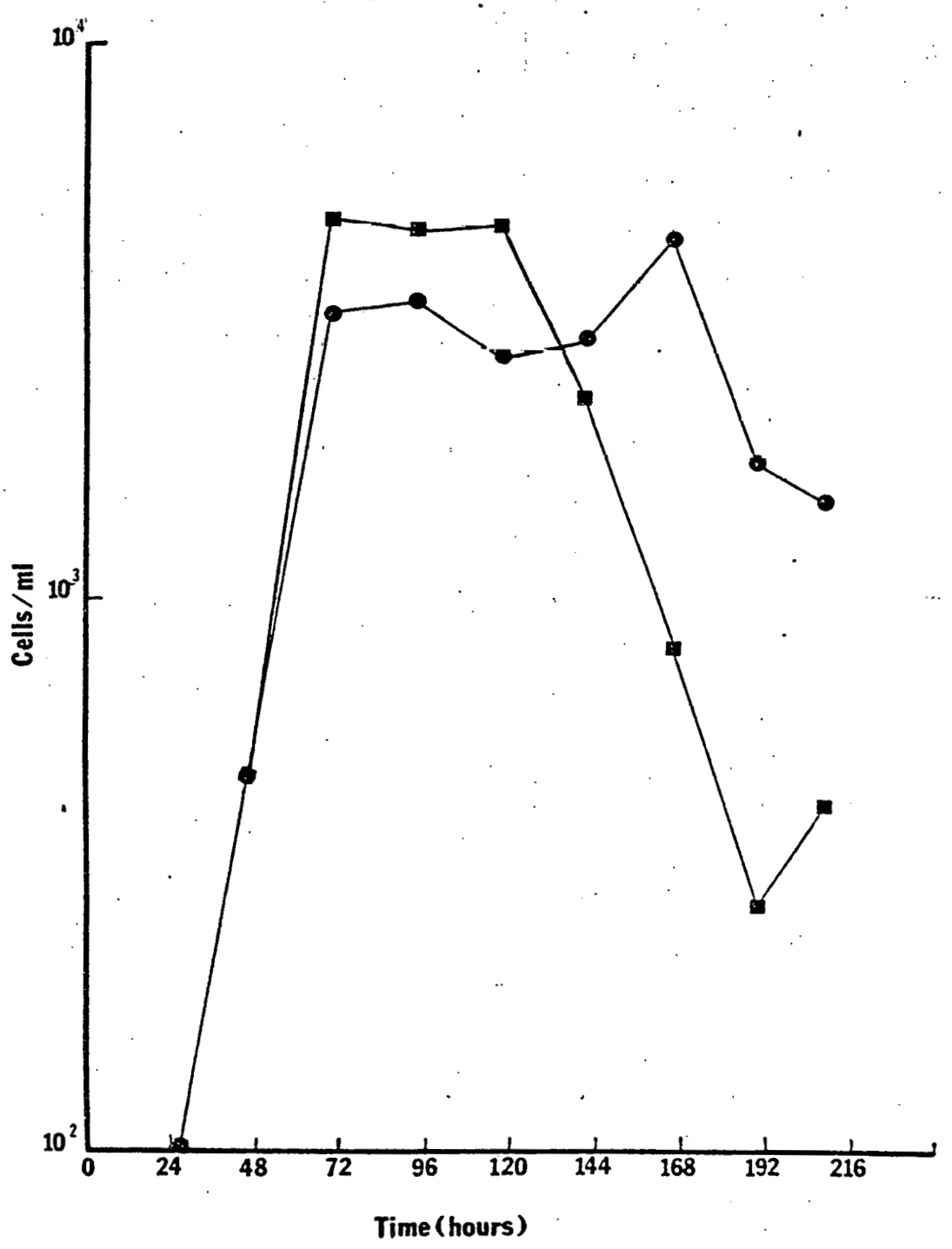

10^4
10^3
10^2
Cells/ml
0
24
48
72
96
120
144
168
192
216
Time (hours)

Figure 42. Protozoan numbers versus time in flagellate and ciliate cultures. ● flagellates; ■ ciliates.

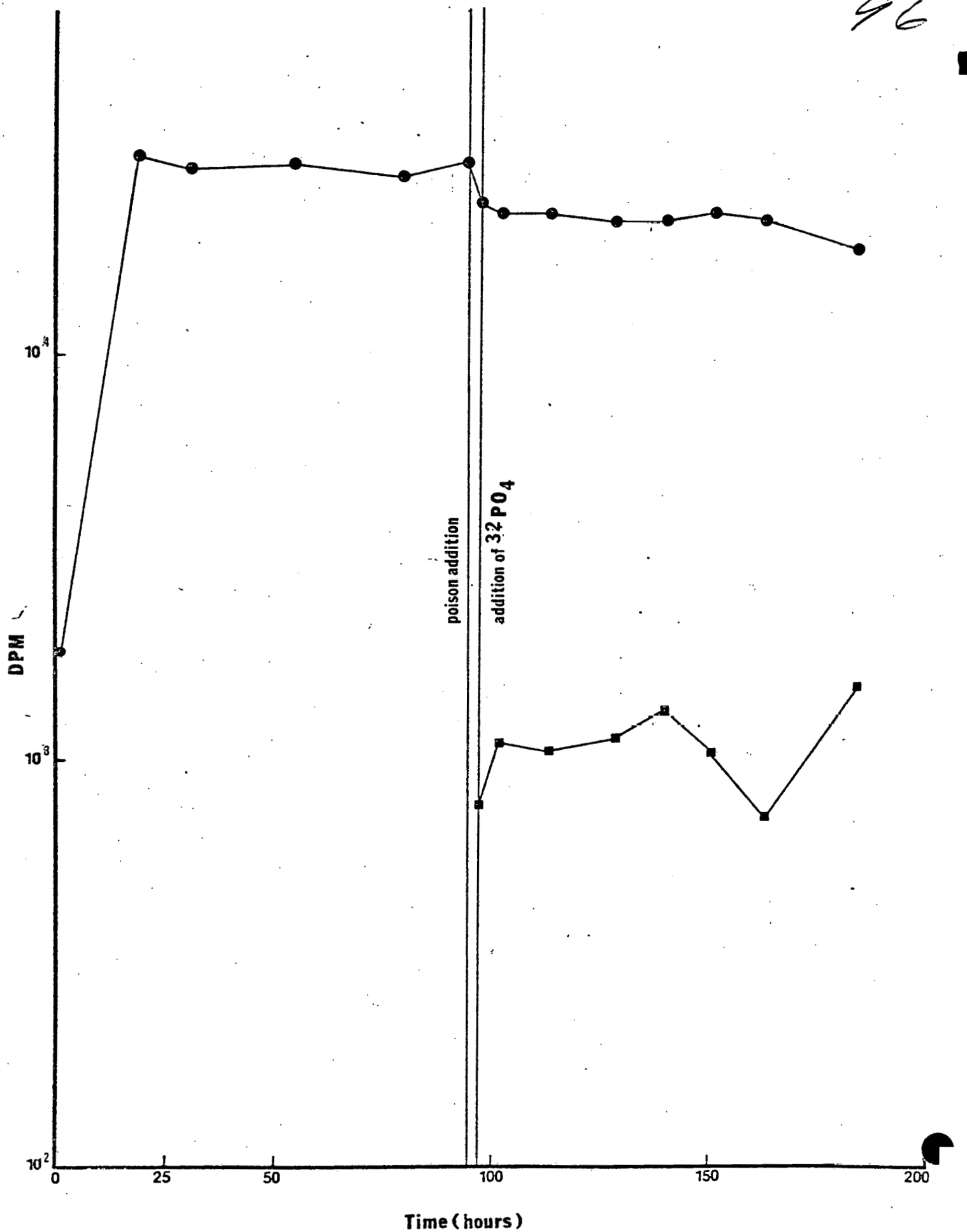
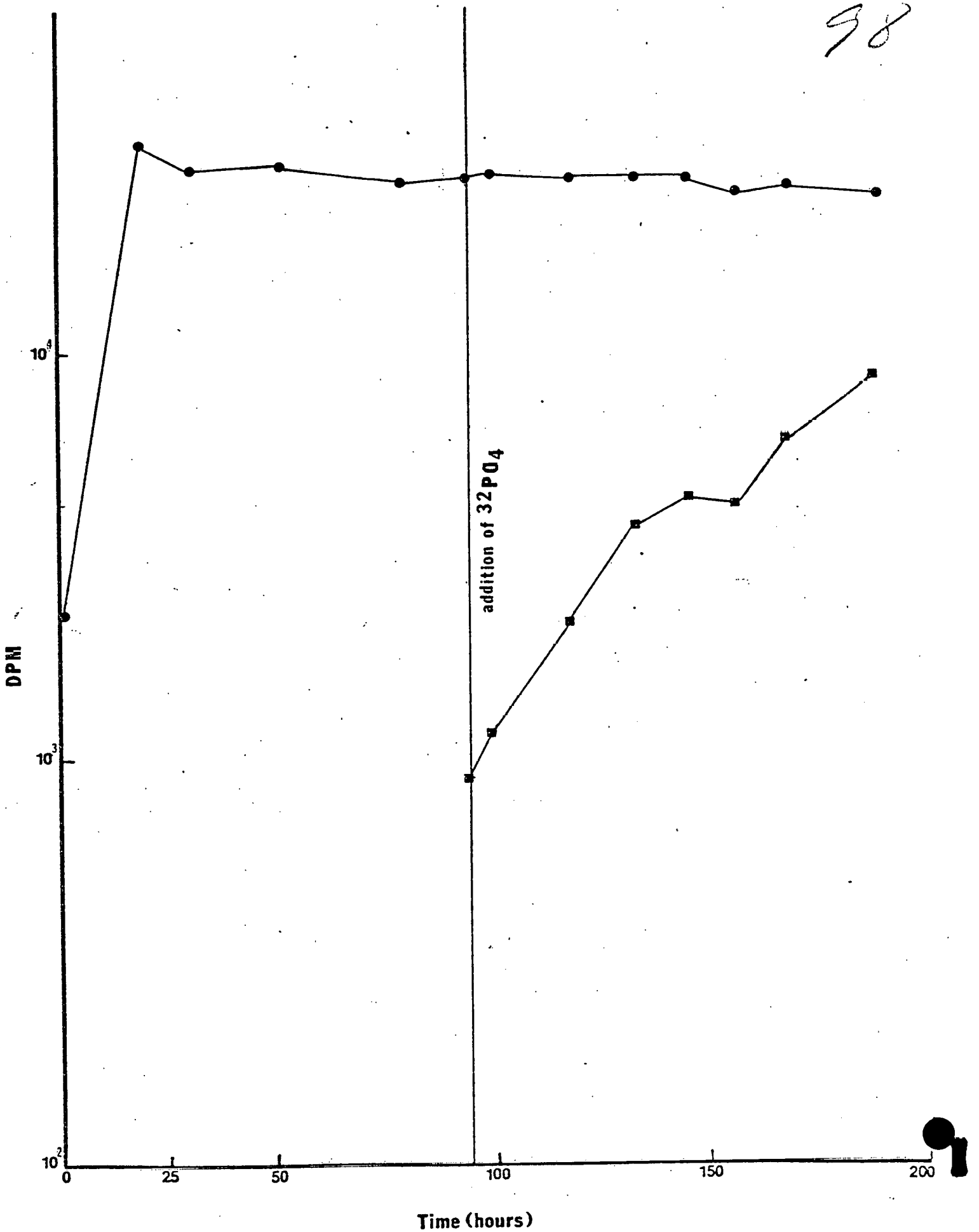

DPM
10^4
10^3
10^2
poison addition
addition of $^{32}PO_4$
0
25
50
100
150
200
Time (hours)

Figure 8. Uptake of isotopes in poisoned control culture. ● ^{33}P; ■ ^{32}P.


DPM
10^4
10^3
10^2
addition of $^{32}PO_4$
0
25
50
100
150
200
Time (hours)

9

Figure 4. Uptake of isotopes in the bacterial culture. DPM = disentegrations per minute; ● ^{33}P; ■ ^{32}P.

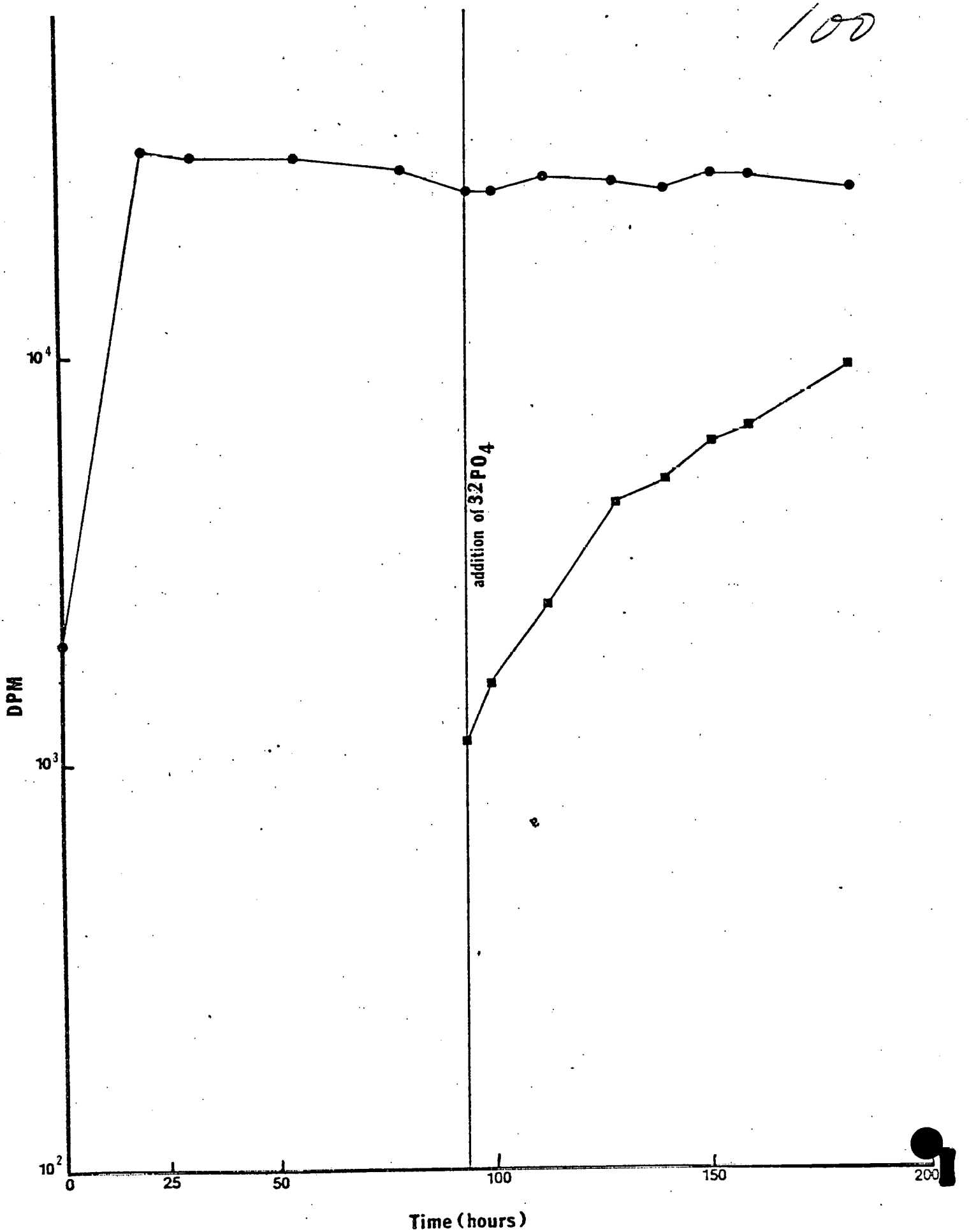

DPM
10^4
10^3
10^2
addition of $^{32}PO_4$
0
25
50
100
150
200
Time (hours)

Figure 10. Uptake of isotopes in the flagellate culture. DPM = disentegrations per minute; ● ^{33}P; ■ ^{32}P.

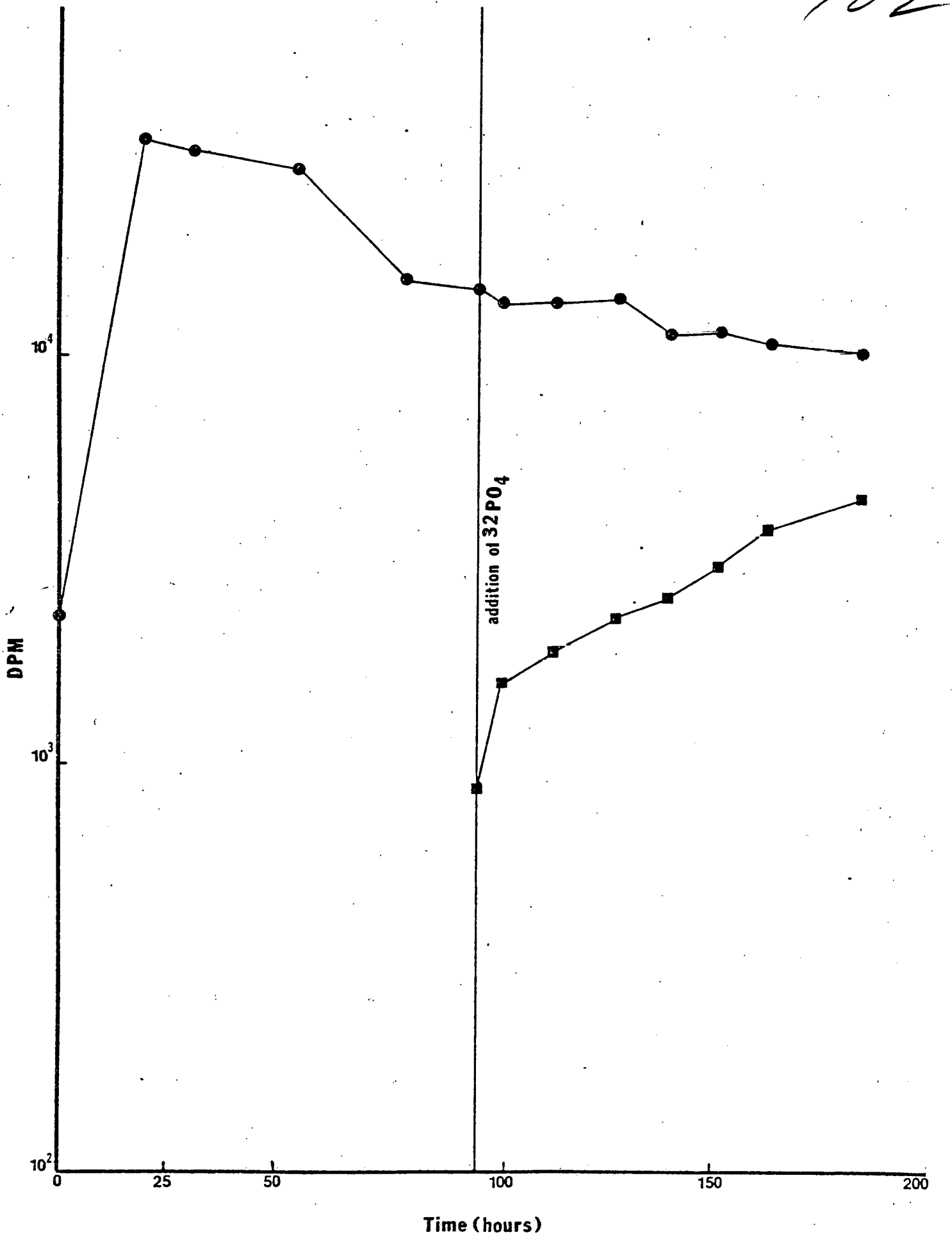

DPM
10^4
10^3
10^2
addition of $^{32}PO_4$
0
25
50
100
150
200
Time (hours)

Figure 11. Uptake of isotopes in the ciliate culture. DPM = disentegrations per minute; ● ^{33}P; ■ ^{32}P.

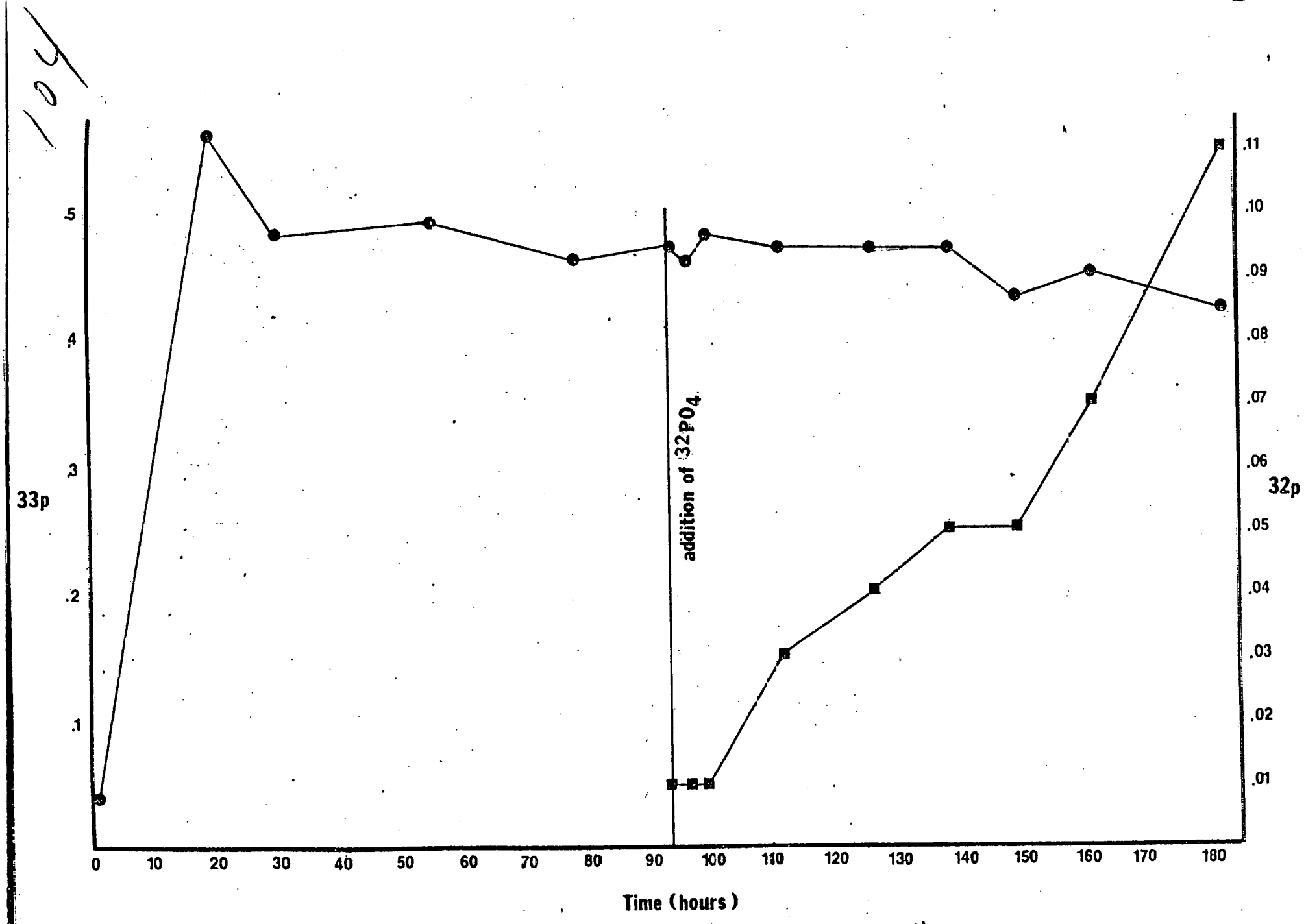

33p
32p
addition of $^{32}PO_4$
Time (hours)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
.1
.2
.3
.4
.5
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
.11

Figure 12. Fraction of isotopes incorporated in the bacterial culture. ● ^{33}P; ■ ^{32}P.

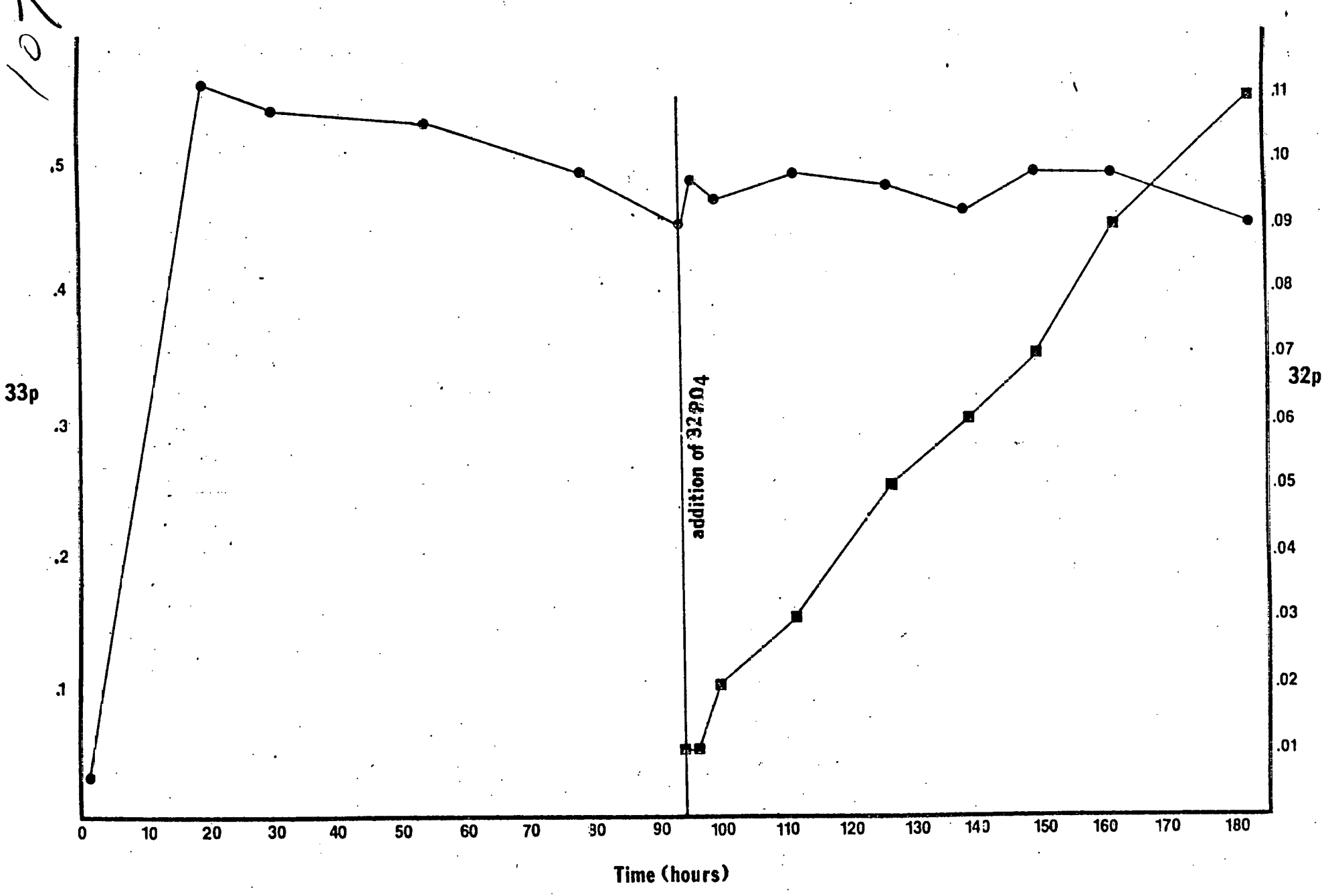

33p
32p
addition of 32PO4
Time (hours)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
.1
.2
.3
.4
.5
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
.11

Figure 13. Fraction of isotopes incorporated in the flagellate culture. ● ^{33}P; ■ ^{32}P.

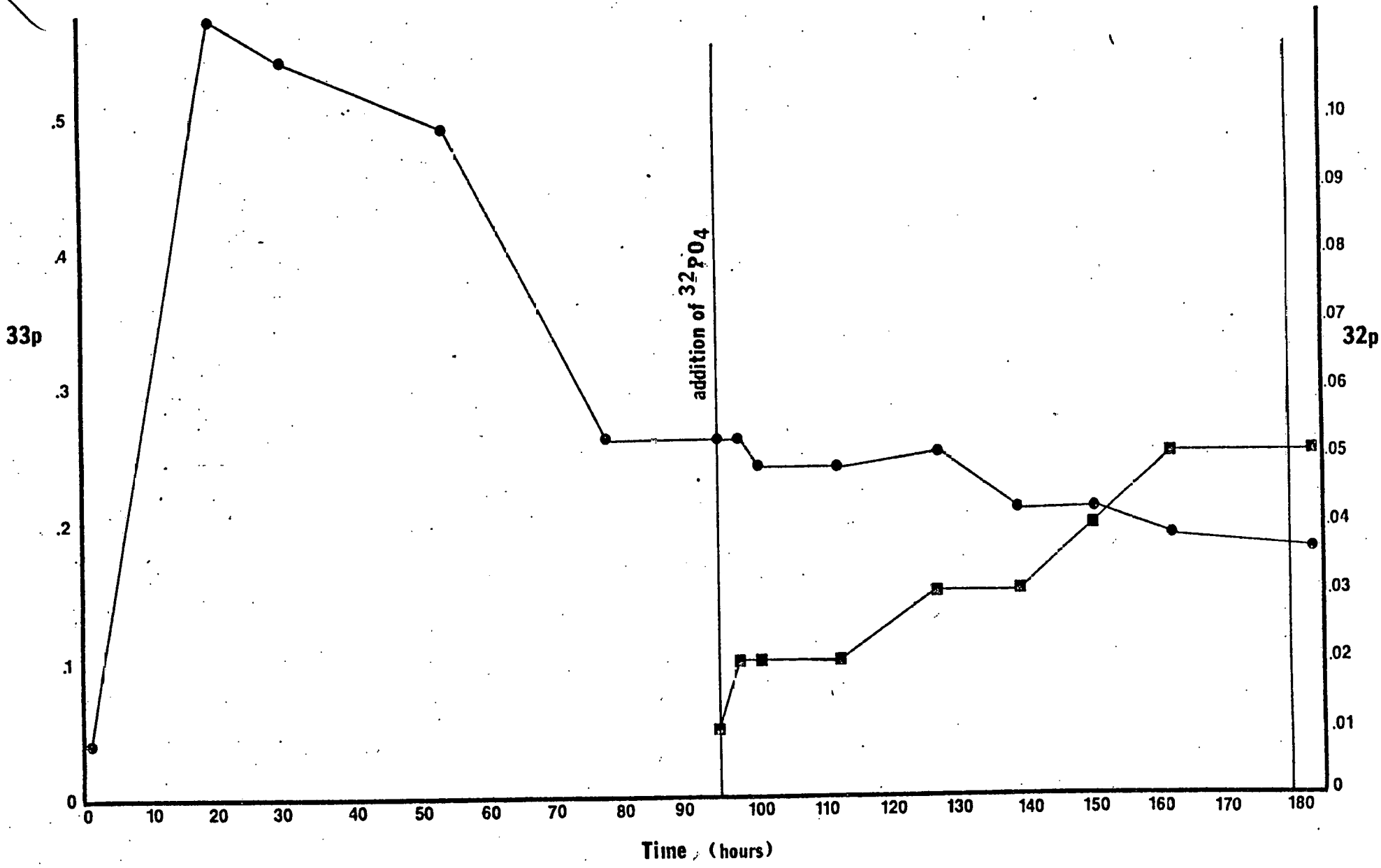

33p
32p
addition of $^{32}PO_4$
Time (hours)
0
.1
.2
.3
.4
.5
.01
.02
.03
.04
.05
.06
.07
.08
.09
.10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180

Figure 14. Fraction of isotopes incorporated in the ciliate culture.
● ^{33}P, ■ ^{32}P.

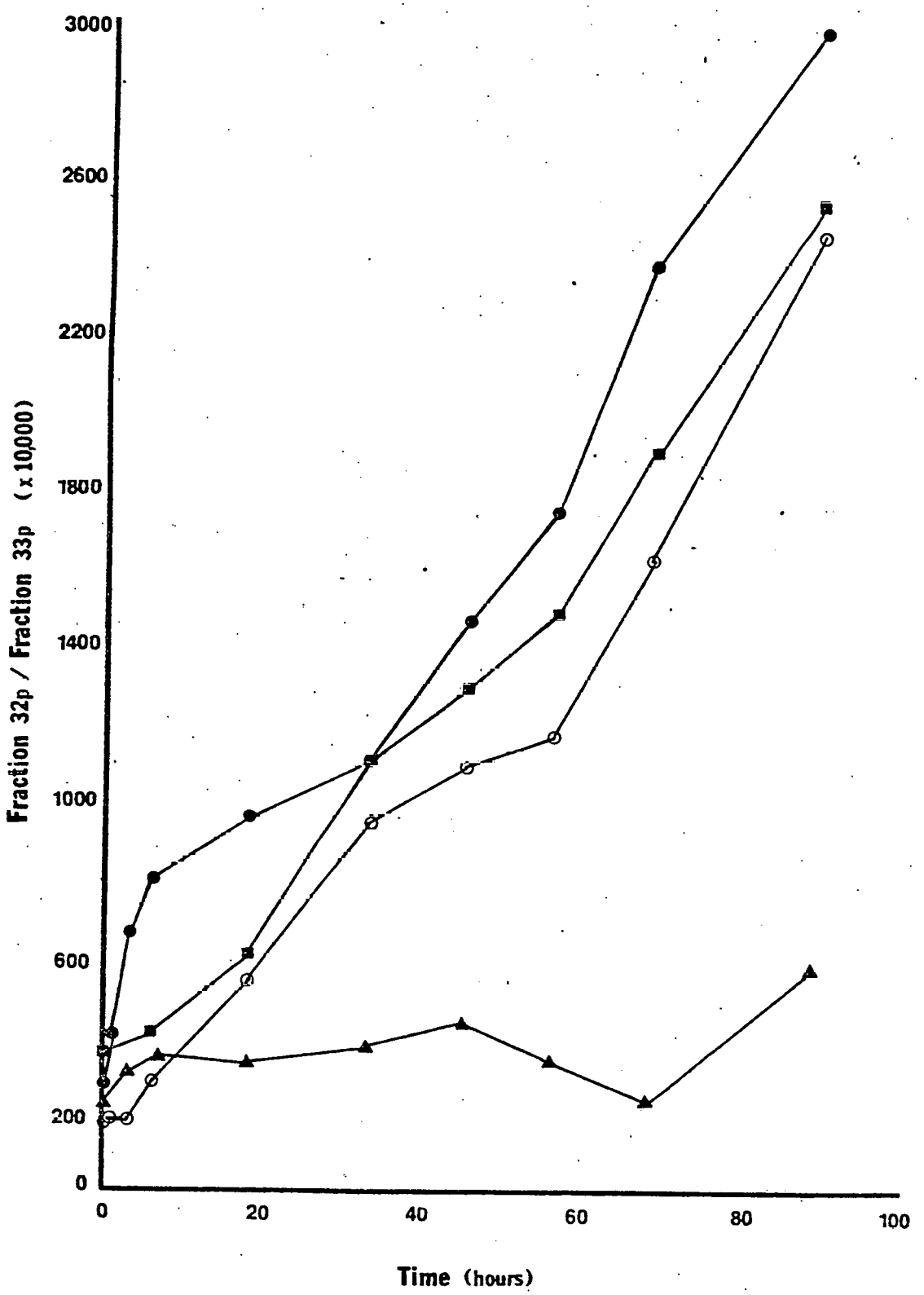

3000
2600
2200
1800
1400
1000
600
200
0
Fraction 32p / Fraction 33p (x 10,000)
0
20
40
60
80
100
Time (hours)

Figure 15. Graph of the ratios of the fractions of isotopes incorporated in the cultures. ▲ poisoned control; o bacterial culture; ■ flagellate culture; ● ciliate culture.

MICROBIAL PROCESSES IN THE SEA: DIVERSITY IN NATURE AND SCIENCE

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Abstract

Microbial processes pervade nearly all aspects of marine ecology and biogeochemistry. Chroococcoid cyanobacteria are significant primary producers in the sea, and protozoans may be significant consumers not only of primary products of photosynthesis but of allochthonous inputs of terrestrial origin, such as organic particles and volatile organic materials transported to the sea by the atmosphere. Secondary production of bacteria is quantitatively significant, but food chains originating with bacterial secondary production are not well understood. Most organic matter is transformed by bacteria, and mostly in the upper 100 meters of the ocean, where both zooplankton and bacteria are active. The relation of bacteria to feces is well known, but their role in the transformation of organic aggregates and sea snow is not understood. Part of the difficulty lies in a lack of consensus about the nature and origin of particulate matter in sea water. Dissolved organic carbon is utilized by both free-living and attached bacteria, but measurements of heterotrophic uptake yield varying results, depending on the substrate chosen. While microorganisms are versatile and ubiquitous decomposers, their failures may be as important as their successes. The conditions under which organic matter is accumulated in sediments and ultimately transformed into methane and petroleum must be such that bacteria are unable to intercept it.

Introduction

Marine microbial ecology is, as its name implies, an interdisciplinary pursuit which draws not from two but from several disciplines, creating a unique and diversified field for research. The results of this interaction of disciplines have had some impact on ecological theory. Recent advances in marine microbial ecology have made the trophic level concept almost meaningless, because microorganisms enter food webs at many trophic levels simultaneously. Studies of microorganisms have contributed to the demise of the belief in a standard "ecological efficiency" of 10 per cent per trophic level, and they have done violence to the concept of a pyramid of numbers, or biomass, in natural communities. At the same time, recent developments in microbial ecology have been influenced by ecological concepts and methods, and many of the classical methods of microbiology have been replaced in marine studies by ones which are more like the methods of other ecologists. The recent results have brought the realization that microbial activities in the ocean biome are greater quantitatively and more varied than marine ecologists had realized.

Bacteria are potentially so fast growing and so responsive to changes in their environment that the microbial populations cultured from isolates taken from the sea sometimes bear little resemblance to the populations actually living there. Laboratory cultures typically are highly enriched, while the ocean, except for some potentially important microenvironments such as fecal pellets, is impoverished in many essential nutrients. When organisms which are adapted to such poverty are presented with the riches of the laboratory-culture environment, some change form and growth state dramatically while others simply die from the excess (Morita, 1980; Wiebe and Pomeroy, 1972). We are still defining the range of natural environmental conditions of

marine microorganisms, but it is apparent that many free-living marine bacteria live in and are physiologically adapted to conditions of extreme scarcity of resources.

The counting of bacteria in sea water has become successful and relatively easy with the development of fluorescent stains and epifluorescence microscopy. Because many marine bacteria do not grow in enriched media, culture methods lead to an underestimation of the numbers and biomass of bacteria in ocean water. They also tell little about growth state or metabolic activity. A number of approaches are being tried to discriminate among activity states of naturally occurring populations of marine bacteria. Often what the ecologist wishes to know is the rate of production or the metabolic rate, and several promising methods have been introduced recently.

Controversy has arisen over a number of significant roles bacteria may play in marine ecosystems. Not only do they degrade many refractory substrates which might otherwise accumulate, but they also compete very effectively for labile substrates. Therefore, they may be significant links in marine food chains. Questions about these roles are bringing marine microbial ecology into more direct contact with other areas of marine ecology. Marine ecologists are recognizing that they must consider the roles and effects of microorganisms if they are to understand marine food webs and trophic relationships. At the same time marine microbiologists are recognizing that microbial activities are interrelated with those of the eukaryotes at many levels and cannot be considered in total isolation. That recognition is reflected in the makeup of this conference.

Historical Perspective

Marine microbiology, like some of the organisms included in its study, remained small, with a long doubling time, for decades, only to undergo a pleomorphic change into a large, rapidly growing field. The preconditions necessary for such a burst of activity developed long ago, however. Recognition of the global significance of microorganisms goes back more than 50 years (Vernadskii, 1926). A number of early studies implicated microorganisms in the utilization of both particulate and dissolved organic matter in sea water (Waksman et al., 1933; Keys et al., 1935). In spite of this, much of marine microbial ecology remained isolated from other aspects of ecology, an isolation which appears to have been fostered both by microbiologists, whose approach to ecology has been quite distinctive, and by the specialists in higher organisms, who recognized no central role in marine ecosystems for microorganisms. Both groups perceived microorganisms as carrying out the utilization of dilute or refractory substrates but not as serious competitors for the food of higher organisms or the nutrients of higher plants. This view is reflected even in most current textbooks on oceanography. For example, Parsons et al. (1977) devote 4 pages to chemosynthesis and 8 pages to heterotrophic processes, much of the latter an exposition of hyperbolic uptake functions. Since textbooks are usually less than a decade behind the current literature, this is a measure of the rapidity of the change in the perceptions of marine biologists and microbiologists.

The usual experimental method in microbiology involves the isolation of defined organisms and their culture in defined media. A powerful approach, because everything is known and controlled, it is also the procedure against

which all work in microbiology is judged. Many marine microbiologists have been understandably reluctant to involve themselves in direct studies of undefined ocean ecosystem, and of natural populations of bacteria, not all of which can be cultured and described fully at this time. For ecologists this is a familiar problem, one with which they have perhaps learned to live all too comfortably. In place of the defined conditions of the microbiologist or the laboratory chemist, the ecologist tends to fall back upon statistical inferences and order-of-magnitude differences. The significance of observations is less assured, but it is virtually all we have when we directly study the dirty, natural world around us. Some chemists and microbiologists are difficult to persuade that the clean, defined systems they study may not behave in the same way in nature that they do in the culture tube. In the case of the ocean, recent events have demonstrated that the systems we create for the study of marine bacteria are not like the ocean in a number of ways. Most of the ocean is impoverished of both organic and some inorganic substrates. Most of it is very cold, quite dark, and does not support photoautotrophs as such. Moreover, it is not a single, uniform environment. Not only does the water column itself vary, but within it are numerous microhabitats for bacteria: living organisms, non-living particulate matter including feces, the surface film, and the bottom.

Science is a world of ideas, and progress in science is limited ultimately by the emergence of new ideas. Sometimes ideas seem to have a life of their own, emerging over and over until they are accepted by the scientific community at large. Often, however, the emergence and acceptance of ideas is limited by our ability to test them. In the case of marine microbial ecology there have been severe limits in methodology. The usual defined-culture

methods limited our perception and understanding of the real-ocean ecosystems, but those ecosystems were not readily accessible to us as investigators. In recent years an array of new, powerful methods has changed that, and investigators are trying to catch up with technology which offers new insights into marine microbial ecology.

The Ocean's Roles in the Global Cycle of Carbon

Primary Production-- Very small autotrophs are responsible for a substantial fraction of total photosynthesis in the ocean, often more than 90 per cent (Malone, 1980). Chroococcoid cyanobacteria are now known to be ubiquitous and abundant in the ocean (Waterbury et al., 1979; Johnson and Sieburth, 1979). Substantial populations of small autotrophic flagellates of various taxa are globally abundant. It is too early to say whether the flagellates or the cyanobacteria are mainly responsible for photosynthetic activity in the ocean, or whether dominance shifts from one to the other under various environmental conditions. On a global basis organisms <10 μm are responsible for much of the fixation of organic carbon in the ocean, with dominance shifting to larger phytoplankton in some river plumes and upwellings.

Several gaps in our knowledge of primary production remain to be filled. A very important one is verification of the validity of the methods currently in use. There have been many challenges to the ^{14}C method over the years (Ryther, 1956; Odum et al., 1963), but recently these have risen in frequency. A serious criticism is that the incubation time is long relative to the rate of flux of carbon down the food chain. The microcrustacean food chain is usually eliminated from the bottles in which photosynthetic rate is measured, but the protozoan food chain is not. In the absence of much data no concensus

exists at this time regarding the rate at which ciliates and heterotrophic flagellates feed on photoautotrophic microflagellates and cyanobacteria. The few published observations suggest considerable variability. The possibility does exist for substantial cycling of carbon through a protozoan food chain (Figure 1), even in a 4-6 hour experiment (Haas and Webb, 1979; King et al., 1980). DOC released by phytoplankton and organic carbon respired as CO_2 will not be measured. While we do not have direct experimental evidence on which to base a good estimate of the significance of these pathways of carbon flux, we know the biomass of protozoans in the ocean and their metabolic rate are sufficient to warrant serious consideration with respect to this problem. Quantification of this food chain may go a long way toward explaining so-called bottle effects on production of both phytoplankton and bacteria.

Allochthonous Materials-- Input of organic matter from the land to the sea has been the subject of speculation and possible overemphasis, but recent work on the characterization of humates suggests that most marine organic matter originates in the sea. Inputs of particulate organic matter occur, including logs, leaves, and garbage introduced by rivers may be of local importance and may even support certain consumer populations in unique ways, and river-borne allochthonous organic matter may even be significant in the global cycle of carbon. Micro-organic matter moving from the land through the atmosphere may be equally significant, however. Fallout of both inorganic and organic dust of continental origin has been extensively documented (Delaney et al., 1967; Folger, 1970). A number of organisms, fungus hyphae, spores, and freshwater diatoms, are common constituents of dust samples taken over the North Atlantic. I once examined some freshly collected samples of atmospheric particles at Bermuda (Bricker and Prospero, 1969) and was surprised to see

numerous organic particles similar in appearance to the class of organic aggregates called flakes (Riley, 1963; Gordon, 1970a). Of course, these might have been organic aggregates swept into the atmosphere from the sea surface. However, they were collected in mid-summer, at a time of relatively calm seas, and Folger (1970) reports finding only terrigenous organic particles over the North Atlantic. Folger (1970) examined water samples for particles of terrestrial origin and identified mostly organic ones, largely concentrated in what he called organic aggregates. Probably they were largely fecal, but in any case he postulated that they would be ingested and would be removed to the deep water as fecal matter. In this connection it is interesting to note that Gordon (1970b) detected a seasonal variation in particulate organic matter in the North Atlantic, with a winter maximum. Gordon showed that at least 20% of this was readily hydrolyzed by proteolytic enzymes, so presumably it had arrived recently and was subject to future microbial transformation. Since the winter maximum corresponds not with the seasonal peak in surface primary production but with winter winds, the winter pulse of POC may be allochthonous terrestrial organic dust.

Volatile organic materials, such as terpenes and organic sulfides, are moved into the atmosphere from both terrestrial and marine sources. The flux of terpenes was crudely estimated by Went (1966) to be 10^9 tons/year, which is on the order of 10 per cent of global photosynthesis and 1000 times the input of petroleum to the ocean (Morris et al., 1976). This does not include all volatile organic materials analytically recognized today, and it ignores particulate flux. Current interest in the organic sulfides has focused on their oxidation in the upper atmosphere and their impact on the ozone layer. However, some fraction of these materials must also be washed out in rainfall and dryfall over the ocean, where they may be transformed by bacteria. The

observation (Rasmussen and Went, 1965; Went et al., 1967) that a significant flux of volatile organic matter from terrestrial vegetation forms condensation nuclei in the atmosphere has been followed up by a number of investigators. While the nature, source, and residence time of both volatile and particulate organic materials in the atmosphere remains uncertain, a fairly uniform concentration of condensation nuclei has been found over the ocean, on the order of 500/cc (Elliott, 1976; Ketseridis et al., 1976; Eichmann et al., 1979). Their chemical composition is uniform and is compatible with either an origin from plants, both terrestrial and marine, or from anthropogenic sources. Sooner or later most organic fallout is going to be transformed by microorganisms, in soils or in the ocean. Although allochthonous inputs to the sea are probably small compared to phytoplankton photosynthesis, they may not be trivial. Because of their physiochemical nature, they are probably assimilated in the sea by microorganisms. These inputs and the resulting food web are worth further study. Interactions between continental, atmospheric, and oceanic constituents present logistically difficult interdisciplinary problems which often are ignored as a result.

Secondary Production-- The significant gaps in our understanding of primary sources of organic carbon in the ocean are small by comparison with the gaps in our knowledge of secondary production. Many marine biologists believe that secondary production is only the production of microcrustacea, but there is also a very substantial production of microorganisms (Fuhrman and Azam, 1980; Fuhrman et al., 1980). The credibility of microbial production data has suffered, because there is no single, generally accepted method for measuring it in the ocean. Moreover, estimates by the various methods have differed by orders of magnitude. While investigators still do not agree on a single

method, the divergence between results by current methods is narrowing to virtual agreement. The methods of Karl (1979) and Fuhrman and Azam (1980), which are somewhat similar in approach, appear to be in reasonable agreement not only with each other but also with the method of Hagström et al. (1979) which is based on the frequency of dividing cells. Therefore, we can probably have at least as much confidence in the recent data on bacterial production as in the data on primary production. If so, there can be little doubt that bacterial production and also bacterial consumption of organic carbon in the sea is a significant part of the total carbon flux. That proposition is not accepted, however, by many marine biologists. Walsh et al. (1981) claim to have discovered the "missing carbon" in global models to be excess production by phytoplankton on continental shelves, based on simulation modeling which includes no microbial pathways.

In view of all of the uncertainties about the flux of carbon through marine food webs, one is drawn to the conclusion that predictive modeling of the global cycle of carbon really is not possible until there is fundamental agreement on some important biological fluxes -- not only their amount but their very existence. All models suffer from the condensation necessary to keep them within the capacity of computing facilities. As a result, each model tends to emphasize those features of the system which the modeler believes to be significant. If we can ever agree on what those features are, modeling may become really predictive as well as the heueristic device it is today.

Microbial Roles in Carbon Flux-- Bacteria have a generally accepted role in the transformation of particulate organic carbon (POC) to living biomass, but there is little agreement on how they do it. The evidence, such as it is,

comes from observations of particulate material in the water (Wiebe and Pomeroy, 1972) and from microcosm experiments in which bacterial transformations of particulate material of various kinds were observed (Kranck and Milligan, 1980; Tenore, 1977; Tenore et al., 1977; Fenchel, 1970; Herbland, 1975). The reality of these latter observations as representing what happens in the ocean varies from none to considerable, most of them suffering from the effects of excessive concentration of organic substrates.

The origin of particulate matter in the open ocean must be primarily from phytoplankton. Only a small fraction of the particulate matter in ocean water is visually recognizable as algal remains. However, histochemical tests show that most of it reacts like phytoplankton (Gordon, 1970b; Wiebe and Pomeroy, 1972), so the remainder must have passed through some process which transformed phytoplankton or products of phytoplankton into what we see in the ocean and call particulate organic detritus. The most obvious processes are grazing, predation, and defecation.

Particulate material is also formed as organic aggregates (Riley, 1963). Although this term has appeared in many different contexts, originally and as used here this term is limited to particles which form authigenically (de novo) in ocean water, from organic matter derived ultimately from marine photosynthesis. Several mechanisms of authigenic organic particle formation have been confirmed (Johnson, 1976; Johnson and Cooke, 1980; Wheeler, 1974), but there has not been any quantification of the rate of formation of aggregates in the ocean. The relationship of microorganisms to aggregates has been the subject of debate since the concept of aggregate formation emerged. Some investigators have suggested that aggregates form only in the presence of bacteria, either because they are really products of the bacteria, such as slime or other secretions, or because bacteria in some way catalyze aggregate

formation. Neither of these occurs in the case of aggregates produced by bubble collapse, but secondary aggregation of large particles (sea snow) originally produced by bubbling (e. g. Kranck and Milligan, 1980) may involve bacterial processes in the water.

Once formed, aggregates presumably are potential substrates for the growth of bacteria, although here again the observational evidence is slight. Bacteria are reported to be present on some aggregates in sea water and not on others (Pomeroy and Johannes, 1968; Wiebe and Pomeroy, 1972). Observers differ on the frequency of attached bacteria and their significance. There is no experimental evidence showing whether organic aggregates are transformed into bacterial biomass, digested directly by eukaryotes, or both. Presumably they are utilized in the food web in one way or another, because they are rare in deep water >500 m (Wiebe and Pomeroy, 1972). Another possibility is that most continue to aggregate with each other until they become sea snow large enough to sink (Shanks and Trent, 1980).

One of the difficulties with observations of particulate matter in samples of ocean water is the lack of agreement among observers about the nature of the particles they see and describe. Many kinds of particles, including sea snow, have been described as aggregates. Only rarely have particles collected in the water been described as fecal, perhaps because of the widespread belief that all fecal matter is in the form of pellets which fall rapidly to the bottom. Even in the case of the microcrustacea this is not true (Hofmann et al., 1981). The early life history stages of microcrustacea produce very small fecal particles which do not sink rapidly. Other planktonic organisms produce less compacted feces, often lacking a peritrophic membrane. The pelagic tunicates, which sweep from the water the smallest organisms, produce fecal ribbons consisting of a gelatinous matrix in

which boluses of compacted material appear sequentially. The ribbons are fragile, break apart soon after release, and the boluses sink rapidly and fall to the ocean's bottom. They have been collected in sediment traps and unfortunately called fecal pellets (Honjo, 1978; 1980). The boluses are perhaps half of the total fecal material. The remainder of the fecal ribbon disintegrates into small (~50 μm) fragments which remain in the water. They are rapidly colonized by bacteria and subsequently by protozoans (Pomeroy and Deibel, 1980). Over several days they disintegrate into very small fragments which do not appear to be further colonized by bacteria. In the ocean such particles have a significant chance of being reingested, and they may be actively sought by grazers. Certainly they are a potentially good source of food for either grazers or net feeders, containing bacteria, protozoa, and little-digested phytoplankton.

The fecal pellets of copepods are also transformed by bacteria and protozoans (Ferrante and Ptak, 1978; Turner and Ferrante, 1979). Depending on their size and rate of fall through the water, which may be from zero to hundreds of meters per day, fecal pellets in the water column will contain bacterial and protozoan biomass and will be a good source of nutrition for grazers. Considering the scarcity of nutritionally suitable materials in the ocean, it seems probable that there are zooplankton which selectively seek and eat fecal materials. If they do not, they are ignoring a substantial supply of nutrition. Either through direct ingestion or bacterial transformation, most fecal material does not reach the bottom of the ocean (Bishop et al., 1977; 1978; 1980). This does not in any way conflict with the observations of fecal materials in sediment traps in deep water, but it does contradict the implication that all fecal matter falls to the bottom (e. g. Steele, 1974), even in shallow shelves and epicontinental seas.

The efficiency and versatility with which bacteria transform organic substrates makes them different from other components of food webs. Laboratory studies of assimilation efficiency show bacteria to be the most efficient of all organisms (Payne, 1970; Ho and Payne, 1979). From the viewpoint of microbial ecology it is unfortunate that these studies were done at substrate concentrations higher than those ordinarily found in sea water. While efficiency actually seemed to increase with decreasing substrate concentration, the lowest concentrations were still above natural ones, and one would expect efficiency to begin to drop off at some point. Estimates derived from uptake of defined substrates labeled with ^{14}C suggest that efficiency does not drop and is still 70-80 per cent at natural substrate concentrations (Hobbie and Crawford, 1969; Williams, 1970). If this is so, bacterial transformations of dilute or relatively refractory materials in the ocean may be accomplished with much greater efficiency than ecologists ordinarily assume, and more bacterial biomass than expected may be available to consumers.

Studies of heterotrophic uptake have shown that bacteria do utilize dissolved substrates in microgram and even nanogram concentrations (Wright and Hobbie, 1966; Hobbie and Crawford, 1969; Azam and Holm-Hansen, 1973). There may be lower limits below which bacteria do not remove substrates from the water or shift to another more abundant substrate. Experiments which would verify this do not appear to have been done, although they might be rather straightforward. Bacteria do have different lower concentration limits for the uptake of various compounds, for ATP is taken up at concentrations far below the normal concentrations of glucose or individual amino acids.

Uptake experiments involving defined substrates may not reflect the rate of uptake of naturally produced organic compounds. Undefined DOM from

phytoplankton was utilized by bacteria more rapidly than defined substances (Smith and Wiebe, 1976; Wiebe and Smith, 1977). Jacobsen (1981) also found significantly faster uptake of DOM produced by a diatom culture than of defined substrates at comparable concentrations. While there may be some odd compound lurking in the DOM pool which is more significant in the flux of labile DOM than the compounds people have chosen to study, we have no clue to its identity. Perhaps a large number of compounds are released by phytoplankton and the collective uptake of all of them, utilizing many sets of uptake sites, is at least order of magnitude faster than uptake of any one. This hypothesis should be testable with the range of defined substates now at hand.

Free bacteria in the water have no source of nutrition other than dissolved substrates, while attached bacteria presumably are utilizing their particulate environment both as habitat and substrate while utilizing dissolved material as well. Hodson et al. (1981) measured the uptake of labeled ATP by free and attached bacteria, using a 6 μm Nuclepore filter to separate free bacteria from larger particles with attached bacteria. Although there were about 10^3 large, attached bacteria per ml and 10^5 small free bacteria per ml, the two populations took up approximately equal amounts of the labeled substrate. Because of their size difference, when the uptake rates were expressed per unit biomass or per unit bacterial surface area, the two populations were seen to be exposing the same amount of cell surface to the water and presumably had the same number of uptake sites per unit of surface area. The two populations were equally active and had nearly the same biomass. This may not, however, be the case throughout the ocean.

Respiration-- In evaluating bacteria as movers of energy and materials in the ocean, we need to measure the respiratory rate of marine bacteria in their natural state, but it has proven to be difficult. The rate of respiration per unit volume of natural sea water is too small to be measured directly. In estuarine and coastal water, direct measurements are now possible with the high-precision Winkler method (Bryan et al., 1976). Concentration of microorganisms from a large volume of ocean water in order to make respiratory rate measurements possible leads to deactivation or loss of a significant fraction of the activity. In the reverse flow concentration method of Pomeroy and Johannes (1968) probably the free bacteria were largely lost through the filters which were used, leaving the attached bacteria and phytoplankton, which may account for about half of total microbial respiration. Today Nuclepore filters offer a major improvement, but there are still serious questions about the effects of concentration on respiratory rate. Moreover, there are problems in separating the respiration of phytoplankton from that of bacteria. Mechanical separation of the free bacteria probably can be accomplished, but separation of bacteria attached to detritus from the phytoplankton and Protozoa is still impossible. A study of the ratio of adenylates to chlorophyll in ocean water suggests that most of the biomass, most of the time, is phytoplankton (Campbell et al., 1979). It may follow that most of the respiration which is measured in natural sea water is that of phytoplankton. Therefore, we need ultimately not only a sensitive method for measuring respiratory rate in the ocean but also the means to discriminate between the respiration of free bacteria, attached bacteria, phytoplankton, cyanobacteria, and other microorganisms.

Sources and Sinks of Dissolved Organic Carbon-- One of the largest standing stocks of carbon on the planet is that of dissolved organic carbon (DOC) in ocean water, which is rather uniformly distributed throughout the ocean (Menzel and Ryther, 1968), and has a mean radiocarbon age in deep water on the order of 3000 years (Williams et al., 1969). The DOC is relatively refractory. All or some of this material is believed to be transformed by free-living deep-sea bacteria, which are slow-growing psychrophiles, and this is reasonable, in view of the fact that residence time of DOC in the ocean is orders of magnitude shorter than that of Na^+ or Cl. Further clarification of the nature and fate of this material would be worthwhile in view of the size of the DOC pool, although not more than 0.5 per cent of primary production is estimated to enter the DOC pool in deep water (Williams et al., 1969).

In the upper mixed layer of the ocean we find microgram-per-liter quantities of monosaccharides and amino acids and nanogram-per-liter quantities of such metabilites as ATP (Azam and Hodson, 1977). The residence time of these labile materials varies widely (Azam and Holm-Hansen, 1973; Hodson et al., 1981) but is never as long as that of the pool of refractory DOC. We do not know with certainty the source, the rate of production, or the fate of the labile DOC, although it has been generally assumed to be produced by phytoplankton (Fogg, 1971; Thomas, 1971; Nalewajko, 1977). Other secondary sources of DOC may in fact prove to be more significant than primary release from phytoplankton. DOC is produced by zooplankton through excretion and defecation (Pomeroy et al., 1963; Lampert, 1978). Further DOC is produced during the transformation of fecal materials by bacteria and protozoans, most of which takes place in the upper 100 meters of the water column. A study of the food web of the California bight suggests that more DOC originates from zooplankton than from phytoplankton (Fuhrman et al., 1980). A shift of

attention from phytoplankton to zooplankton as producers of DOC may be appropriate, while bacteria should be viewed both as producers and consumers of DOC.

One of the impediments to understanding oceanic DOC is that it has not been described qualitatively, and doing so is a difficult task at present. The refractory DOC which makes up most of the standing stock is presumably humic and fulvic in character, but what is particularly difficult to characterize is the labile material, which is probably diverse in origin and chemistry. However, it constitutes 99 per cent of the DOC flux. Those compounds present in the smallest standing stock and therefore most difficult to find are probably the most significant in terms of short turnover time and high flux rate while the less labile materials tend to accumulate.

The Significance of Decomposition

While no one doubts that marine bacteria are responsible for much decomposition of organic substrates, including DOC, a number of interesting questions remain concerning the long-term global effects of microbial processes, for there are some notable exceptions in decomposer abilities (Alexander, 1980). Synthetic organic materials provide a new and sudden challenge to bacterial versatility, and if bacteria were totally efficient in transforming all substrates, there would be no petroleum, coal, or methane in the sedimentary rocks. Would there also be no oxygen in the atmosphere? Although this is widely believed, the evidence is less than compelling.

Fossil Carbon, Recent Oxygen, and Chroococcoid Photosynthesis-- The orthodox view of the history of the planet is that the atmosphere lacked oxygen until the rise of oxygen-producing photosynthetic organisms. Once

photosynthesis began, according to this view, some reduced carbon was lost to the sediments, excess oxygen was released to the atmosphere, and all of the excess reduced compounds near the surface of the earth, both organic and inorganic, were oxidized. Then, as all exposed reduced materials became oxidized, oxygen began to accumulate in the atmosphere. Such a reasonable and widely accepted paradigm is difficult to challenge, but several investigators have. Van Valen (1971) pointed out that a major source of oxygen is the photolysis of water in the upper atmosphere and the subsequent loss of hydrogen to the solar wind as it streams past the earth. The large requirement for oxygen over the history of the planet, amounting to 1000 times the present standing stock, could have been supplied by the net difference between planetary photosynthesis and respiration, even though it was very small. However, regulation of oxygen in the atmosphere appears to be very weak, and it may have varied by a factor of 10 (Cope and Chaloner 1980). Furthermore, there is evidence that photosynthesis of the oxygen-producing kind evolved only after the oxygen content of the atmosphere had gone well above the Pasteur point (Schwartz and Dayhoff, 1978). We still do not have a really good estimate of the rate of production of oxygen in the upper atmosphere and know even less about the factors which may cause that rate to vary over time. Therefore, we cannot determine whether the biosphere really influences the oxygen content of the present atmosphere significantly. With respect to the early atmosphere, oxygen-producing photosynthesis may have evolved in response to an acute need for reduced carbon compounds at a time when an oxidizing atmosphere and rampant oxidative metabolism made photoautotrophy an essential part of the evolving biosphere. If this were the course of events, probably the pioneers were the small chroococcoid cyanobacteria.

One of the pervasive mysteries associated with the accumulation of petroleum is how this accumulation began and how it escaped bacterial transformation. We look in vain for evidence in the present ocean of the precursors of hydrocarbon concentrations. Because we find so many hydrocarbon deposits of many different ages, is this a rather unusual period in the earth's history? Porter and Robbins (1981) suggest that conditions for accumulation of organic matter occur off California, but this is difficult to perceive. The basins are well oxygenated all the way to bottom. However, the bottom near shore under the Peruvian upwelling region, is anaerobic, or very nearly so, with green sediments. Fecal pellets may be an important constituent, and the high concentration of porphyrins suggested by the color of the sediments is suspicious. No one seems to have looked at them from that viewpoint. However, it is not clear how nearshore sediments on an orogenic coast will be conserved. A piece of the puzzle may still be missing.

The Oligotrophic Ocean-- Little organic matter accumulates on the bottom of the ocean, for what falls has to run the gauntlet of hungry mouths through four kilometers of water, and it has to fall rapidly enough to escape bacterial decomposition. What does fall is transformed by the heterotrophic food web. Considerable layering of activity appears to be orchestrated by the physical regime of solar illumination which promotes both photosynthesis and thermal stratification. Solar penetration is maximal in the blue water of the ocean, but the ocean would be more productive if there were more phytoplankton to intercept the light near the surface of the water, as is the case near shore. Stratification created largely by solar warming appears to have a great influence on the way the ocean works as a biome, and most likely the ocean would be a more productive place if it were more mixed for as we all

know, some of the most productive places are those where water upwells from below the usual depth of the thermocline. Sverdrup (1955) predicted that this would be so, and except for that part of the year when the Antarctic is in darkness and extreme cold, all of the regions of upwelling identified by Sverdrup have proven to be exceptionally productive. However, this productivity requires alternate episodes of upwelling and stability. A continuously mixed ocean is not productive, and there is an optimum mixing intensity (Eppley and Peterson, 1979).

Most of the ocean is stratified however, and the thermocline is a barrier to flux in both directions, although we usually think of it primarily as a barrier to the upwelling of nitrate. Only relatively large (>100 μm) fecal particles and the occasional dead organism go swiftly through the thermocline toward bottom. So the fallout which is so desperately needed at the bottom is diminished both by hungry mouths along the way and by bacteria doing their work both in the upper mixed layer and in the oxygen minimum layer in the thermocline.

Bubnov (1966) and Menzel and Ryther (1968) asserted that the oxygen minimum layers of the major ocean basins are only an artifact of the physical regime, with no significant biological activity except at the point of origin, said to be an upwelling region, where water of reduced dissolved oxygen content produced beneath the productive upwelling slides across the ocean along a constant-density isopleth. The intensity of the oxygen minimum also decreases with distance from the upwelling, an observation which caused the investigators to suggest that all that is happening, once the water leaves its biologically active origin, is gradual diffusive exchange with the adjacent water masses. There is evidence, however, that some distinctive biological activities do occur in oxygen minima. Karl et al. (1976) reported a large

ATP maximum, approaching half the near-surface value, in the oxygen minimum layer of the central North Atlantic, and they assumed that this represented a large population of bacteria. On several cruises in the western North Atlantic and the Caribbean my colleagues and I have found that the number of bacteria in the oxygen minimum layer is greater than that in adjacent water. Moreover, the oxygen minimum layer is the only place in the water column, other than the interior of fecal pellets, where we have seen motile bacteria, a characteristic of an environment of diminished dissolved oxygen (Hobbie et al., 1972). Fellows, Karl, and Knauer (1981) found an increase in RNA synthesis in the oxygen-minimum layer in the northeast Pacific Ocean. These evidences of bacterial biomass and activity in oxygen minima suggest that the layers receive a sufficient source of energy and are sites of biological activity.

Marine Humus-- Litter and humus are important parts of the terrestrial environment where they provide both a habitat and a substrate for microorganisms (Wiegert and Owen 1971). In terrestrial environments litter is produced mostly by direct fall of plant materials from the overstory. In the marine biome most litter is microscopic. Some bloom organisms such as Trichodesmium, which are not readily eaten by most zooplankton, do accumulate and become detritus. Movies of zooplankton feeding by Strickler and Paffenhöfer show that feeding is sometimes inefficient, especially when the food is long chains of diatoms. Predators are also less than perfectly efficient in consuming planktonic prey (Dagg, 1974). So, while Steele's (1974) assumption in his North Sea model that all phytoplankton are eaten by zooplankton certainly is not correct, it is possible that the real value is 80 or 90 per cent, with a large variance. Therefore, the major source of marine

detr[illegible] probably is not scraps of phytoplankton lost by inefficient zoop[illegible]on but is fecal material, primarily from grazers and mucus-net feed[illegible].

[illegible] complex detritus food web is difficult to observe in the real world, beca[illegible]se it is microscopic and highly dispersed. When we concentrate it for obse[illegible]vation, we may get a distorted view of what is really happening. Good obse[illegible]vations are still rare and difficult; laboratory simulations of detritus syst[illegible]ms are more common but probably even more misleading. The ocean is not a hay infusion.

Detritus in the marine biome fills much the same roles it does in terrestrial ones, providing a habitat for microorganisms and small metazoans, a gingerbread house which its inhabitants eat. One of the significant processes in the detritus food web is the regeneration of plant nutrients, especially nitrogen and phosphorus. Both bacteria and protozoans may play a role in this, and the roles vary with the concentration of dissolved oxygen in the microenvironment of the detritus particle. In large fecal pellets there is evidence of anaerobic or reduced oxygen conditions. Under those conditions bacteria do not accumulate polyphosphates (Shapiro, 1967), but release both ammonia and phosphate to the surrounding water. However, in smaller (<100 μm) fecal particles and other detritus, oxygen diffuses to the center of the particle, and motile bacteria are not apparent. Under those conditions bacteria accumulate polyphosphate. They not only use all phosphate from their particulate substrates but take it up from the surrounding water. The phosphate will be released only when protozoans consume the bacteria, releasing excess phosphate. The literature is by no means unanimous or coherent on this point. The postulate that under aerobic conditions metazoans and protozoans excrete significant amounts of phosphate while bacteria do not,

was originally proposed by Pomeroy and Bush (1959), Pomeroy et al. (1963) and Johannes (1964; 1965). Subsequent investigators have both confirmed and denied it. Beuchler and Dillon (1974) confirmed it, using Paramecium cultures. Barsdate et al. (1974) and Fenchel (1977) denied that protozoans play a significant role in nutrient regeneration. This conclusion was based on the use of rather concentrated infusions of sea grass in which anaerobic microenvironments were probable and no retention of phosphate by bacteria would be expected. Moreover, in reporting the results Barsdate et al. seem to confuse turnover time with net flux, the former involving transmembrane exchanges which are irrelevant to the latter. Recently, Kerrick (1981) has shown that the marine flagellate, Bodo, in dilute culture, eats bacteria and excretes phosphate, while the bacteria alone retain it. Most of the work on microbial recycling of nutrients has been done with phosphate. Bacteria do not seem to have a mechanism for accumulating nitrogen other than by excess protein synthesis, so there is a more-or-less steady flux of ammonia being lost from marine bacteria which grow on a nitrogen-sufficient natural organic substrate.

In contrast to many terrestrial biomes, marine humus rarely accumulates in the ocean primarily as DOC. Large litter particles fall rapidly to the bottom, where thay are degraded by the combined action of invertebrates and bacteria (Sieburth and Deitz, 1974). Although degredation by bacteria alone on the ocean bottom is slow (Jannasch et al., 1971; Jannasch and Wirsen, 1973), it still proceeds quite rapidly in the presence of invertebrates which break it up. In this respect the sea bottom is analogous to the forest floor, where the same synergism (or competition?) between invertebrates and bacteria occurs (Janzen, 1977).

Dissolved or colloidal humic acids probably have more influence on the marine environment than is generally appreciated, although their role in the food web is debatable, since they are degraded very slowly. Humates in fresh water play a major role in metal chemistry, both as chelators and as zwitterions, oxidizing and reducing sites. But because of the greater ion strength of sea water, marine humates are different in structure. Nevertheless they probably play a role in marine chemistry, one which does not appear to have been explored as thoroughly as it has been in fresh water. Marine humates are more readily transformed into bacterial biomass than fresh-water humates because of their lower aromaticity. Marine humates lack the lignin fraction with its alkylaromatic esters which are resistant to bacterial transformation (Dereppe et al., 1980). Coastal and estuarine humates are varied in their aromaticity (Hatcher et al. 1980), reflecting diverse origins.

Strategies for Success in the Marine Biome

Any population needs some kind of strategic advantage in order to compete and survive. While a population may overlap with other populations, there is a need for some unique niche dimension which enhances the chance for survival. Bacteria have many potentialities for unique niche development. Bacteria are metabolically flexible and versatile to a greater degree than other organisms. They can transform any organic material, except a few new synthetic compounds, into bacterial biomass. They combine patience with fast response. They can remain for very long periods, variously estimated from tens to millions of years, in resting stages or spores which involve very little expenditure of maintenance energy. Marine bacteria also have shorter lag times and faster doubling rates than any of the eukaryotes and they can compete successfully

with other organisms for transitory supplies of substrates and inorganic nutrients, such as phosphate, nitrate, and ammonia.

Small size offers a number of advantages, and some of the marine bacteria have carried this to its extreme lower limit. Small size carries with it the potential for low maintenance cost for the genome, although whether or not maintenance costs are really low will depend upon the environmental conditions. Small size is also a refuge from predators. While some organisms, particularly the mucus-net feeders, are able to concentrate and eat free-living minibacteria, most have no means of catching them, and it is hardly worth their while to do so. An organism can achieve the same nutrient intake by eating one 2 μm rod attached to a detritus particle that would be obtained by gathering 20 minibacteria from the water. Most metazoans will feed on particles rather than small, free-living bacteria, both because it is easier to do and because the particles are a richer source of bacterial biomass. Relatively few metazoans larger than the protozoans have developed a strategy for eating free-living minibacteria, and probably most protozans preferentially swim from particle to particle, munching on the larger bacteria. Thus the population of minibacteria in the ocean may not be controlled by grazers but only by the availability of substrates, and minibacteria can play a waiting game, statistically safe from enemies until some food comes along. So long as there is a biosphere, some food will come along, and because the bacteria are so versatile, almost anything will suffice.

The bacteria on particles obviously have a different strategy. They change size dramatically in the presence of a suitable substrate, growing from a resting form invisible to the light microscope into a large rod in a very short time, then doubling until a colony is formed on the substrate (Wiebe and

Pomeroy, 1972). These colonies are rapidly found and decimated by protozoans. The particle-colonizing bacteria therefore are switching from a size refuge strategy in their resting state to a rapid multiplication strategy in the presence of a substrate. The statistics of both strategies seem to have been well worked out in an evolutionary sense.

The strategies of marine microorganisms are those which evolve in the face of usually impoverished and highly variable conditions. No group of organisms is more diversified, and so it is appropriate that scientific approaches to the ecology of marine microorganisms have been diverse as well.

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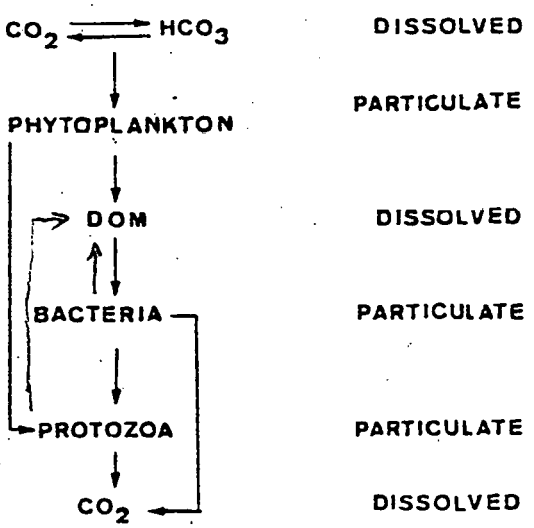
Figure 1. The microbial food chain in a bottle during measurement of photosynthesis by the ^{14}C method. Time to complete the entire pathway is on the order of minutes.


CO_2
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DISSOLVED
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DOM
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PROTOZOA
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CO_2
DISSOLVED