BACTERIAL BIOMASS AND HETEROTROPHIC POTENTIAL IN THE WATERS OF THE CHESAPEAKE BAY PLUME AND CONTIGUOUS CONTINENTAL SHELF[a]

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SUMMARY

Viable count bacterial numbers in surface water samples collected during June 1980 ranged from a maximum of 190×10^3 MPN (most probable number) $m\ell^{-1}$ at the Bay mouth to a minimum of 7.9×10^3 MPN $m\ell^{-1}$ offshore. Similarly, direct count densities ranged from 1800×10^3 BU (bacterial units) $m\ell^{-1}$ to 24×10^3 BU $m\ell^{-1}$. Heterotrophic potential (V_{max}) was largest at the Bay mouth (0.770 µg glucose $\ell^{-1}h^{-1}$) and lowest offshore (0.057 µg glucose $\ell^{-1}h^{-1}$). Biomass and V_{max} values usually decreased with depth although subsurface maxima were occasionally observed at inshore stations.

Correlation of biomass and heterotrophic potential data with selected hydrographic variables was determined with a non-parametric statistic (Kendall Tau). Results indicated viable counts were positively and significantly correlated with total chlorophyll, temperature, direct count and V_{max} during June 1980; significant negative correlations were obtained with salinity and depth; no correlation was observed for suspended particulates. Calculations of bacterial standing crop are discussed.

INTRODUCTION

Bacterial populations in Chesapeake Bay and contiguous shelf waters are significant to such essential processes as mineralization, nutrient recycling, degradation of pollutants and biomass production. However, our understanding of the dynamic relationships of physical and chemical factors to bacterial biomass and activities in Chesapeake Bay plume waters is limited. The availability of synoptic hydrographic (and remotely sensed physical-chemical) data obtained simultaneously with measurements of microbial biomass and activity presented an opportunity to examine such relationships.

Specific objectives of this study were: (1) to compile seasonal baseline data on bacterial biomass and heterotrophic uptake in the Chesapeake Bay plume

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and contiguous Atlantic Ocean shelf waters, (2) to relate bacterial data to relevant physical-chemical variables also potentially measurable by remote sensing techniques, and (3) to further evaluate and define methodology currently utilized for the measurement of bacterial biomass and heterotrophic activity at sea.

METHODS

Sample Collection

Water samples for the determinations of bacterial density and activity (heterotrophic potential) were collected by hydrocast using Niskin sterile bag samplers at appropriate depths. Samples were processed immediately after collection using aseptic techniques.

Bacterial Viable Count Determinations

Estimates of viable heterotrophic bacteria in Bay and plume waters were obtained using a five tube MPN (most probable number) technique employing a heterotrophic seawater medium. This medium consisted of a 1.0 g ℓ^{-1} peptone, 0.5 g ℓ^{-1} yeast extract, 0.01 g ℓ^{-1} ferric citrate, 0.1 g ℓ^{-1} sodium glycerol phosphate in 1000 mℓ of aged seawater adjusted to the proper salinity prior to autoclaving (121°C for 15 min). Inocula from appropriate serial dilutions of two subsamples from each water sample collected were planted in appropriate tubes and the tubes incubated at ca. 20°C for two weeks. MPN values were calculated using standard tables (1) and the values expressed as MPN mℓ^{-1} seawater.

Direct Bacterial Count

Twenty-mℓ aliquots of each water sample were aseptically transferred to sterile tubes to which 2 mℓ of a 5% glutaraldehyde seawater solution were added as a fixative. Tubes were sealed and immediately refrigerated during the period prior to filtration.

Direct counts were processed using the basic technique of Hobbie et al. (2) with some modifications. Five- or ten-mℓ aliquots of water samples were filtered through stained (Irgalan Black) Nuclepore^{R} filters (0.2 µm, 25 mm dia.) at reduced pressure (100 mm Hg). Cells were then washed by filtration with 10 mℓ of a 0.2% solution of sodium metabisulfite (aldehyde block) in distilled water. Several mℓ of sterile distilled water were then placed on the filter followed by 200 µℓ of the fluorescent dye proflavin (0.033% in distilled water). Staining was for 5 min followed by a 10-mℓ wash with distilled water. Filters were removed upon dryness, cleared with non-fluorescing immersion oil on a standard microscope slide, covered with a #1 coverslip and stored under refrigeration pending examination by epifluorescence. Using this methodology, relatively stable high contrast images without rapid bleaching were routinely obtained. All solutions and washes were filtered through 0.2-µm membrane

filters immediately prior to processing.

Cells were counted using a Zeiss Standard microscope equipped with an epi-fluorescence illuminator for FITC fluorescence (exciter filter KP 490, beam splitter FT 510 and barrier LP 520). Only recognizable bacterial cells were counted from 49 randomly chosen fields within a known area of an ocular grid. Count values were corrected for area, sample volume, dilution factors and expressed as bacterial units (BU) $m\ell^{-1}$. Replicate counts of randomly chosen samples as well as procedural blanks were performed.

Heterotrophic Potential

Heterotrophic potential or V_{max} (glucose) was determined by incubating replicate 10 $m\ell$-aliquots of each water sample with uniformly labeled ^{14}C-glucose (250-360 mCi mmole^{-1}) at final concentrations of 37.5, 75.0, 187.5 and 370.5 $\mu g\ \ell^{-1}$ in the dark at ambient water temperatures for 3 h. Control and incubated samples were inactivated by the addition of 0.1 $m\ell$ of 2% buffered formalin. ^{14}C-labeled particulate fractions were collected on cellulose acetate filters (Millipore^R EHWP 0.5 μm), the filters placed in 4.0-$m\ell$ mini-vials (Wheaton) to which 3.5 $m\ell$ of Aquasol II (New England Nuclear) was added. Counting was carried out at 88-91% efficiency using a liquid scintillation counter with external standardization (Beckman LS-150). The calculation of V_{max} (glucose) using linear regression analysis had an r value of 0.9 or greater for at least 3 of the 4 substrate concentrations used. No provision was made to trap and measure respired ^{14}C-CO_2 during the incubation period. Therefore, calculated V_{max} values represent only that portion of labeled substrate in particulate form and are minimum estimates of substrate uptake.

RESULTS

Data for viable, direct bacterial counts, V_{max} (glucose) and relevant physical-chemical measurements are compiled in Tables 1 and 2. Locations of stations are shown in Figure 1.

Viable bacterial count densities were consistently smaller than corresponding direct count densities. Viable counts in surface waters ranged from a maximum of 190×10^3 MPN $m\ell^{-1}$ at the Bay mouth to a minimum of 7.9×10^3 MPN $m\ell^{-1}$ in offshore waters. Similarly, direct count densities ranged from 1800×10^3 BU $m\ell^{-1}$ to a minimum of 24×10^3 BU $m\ell^{-1}$ offshore. Mean viable count densities were approximately 10x smaller than direct count densities (Table 2). Such a relationship is considered usual since direct counting techniques enumerate all cells present, including active, dead, and dormant cells and cells metabolically incapable of a positive response in the heterotroph medium employed. Furthermore, correction for positive bias inherent in the MPN technique would reduce the viable counts and thus increase the differential between direct and viable MPN counts.

Although a detailed quantitative analysis was not made, the majority of bacteria (80-90%) appeared as free-living cells and were not attached to

particulates. Analysis of direct count samples revealed possibilities for enumeration and identification of heterotrophic and photosynthetic flagellates and algae and the presence of sometimes abundant coccoid cells somewhat larger than bacteria. Cells resembling the latter have been reported to be coccoid cyanobacteria (3); however, the decay of natural fluorescence in stored samples prevented definitive identification. Therefore, the direct counting epifluorescence procedure will be most useful for the identification and quantification of microorganisms (other than bacteria) if preparations are processed and examined on shipboard before the naturally fluorescing photopigments decay.

Bacterial numbers and V_{max} were generally largest in surface waters and at all depths in the water column for stations closest to the Bay mouth. V_{max} values ranged from a maximum of 0.770 µg glucose $\ell^{-1}h^{-1}$ at the Bay mouth to a minimum of 0.057 µg glucose $\ell^{-1}h^{-1}$ offshore. Figure 1 shows the spatial distribution of bacterial count and V_{max} values contoured for surface (1 m) water. Smaller values were located outside the "plume" and were generally farthest offshore. These spatial distributions are "quasi-synoptic" since the data were collected over a range of tidal and meteorological conditions during a seven day cruise interval.

Biomass and V_{max} values generally decreased with depth although subsurface maxima were occasionally observed at inshore stations. Such values tended to correspond to elevated levels of particulates (Table 1). However, it was not clear if these elevated levels were due to suspension of sediment through bottoming of the sampler or cable weight during rolling, turbulence generated by the vessel, or to an actual subsurface turbidity maximum.

Non-parametric correlation analyses (4) of microbial data with selected hydrographic measurements were performed (Table 3). Viable count data were significantly correlated with direct counts and V_{max}. V_{max} was significantly correlated with both viable and direct counts. Viable counts were positively and significantly correlated with chlorophyll concentrations and temperature, negatively and significantly correlated with salinity and depth, but not correlated with suspended particulates. Absolute values of the Kendall Tau statistic are not directly comparable with correlation coefficients derived using other statistics and indicate only relative degrees of correlation or correspondence.

Table 4 indicates the relationship of sampling depth to arithmetic means of microbiological data for each depth. Both mean numbers of viable saprophytic bacteria and direct counts decreased with depth. Proportionately, the decrease in mean V_{max} at the greatest depth was closer to the decrease in mean direct count than to mean viable count. Thus, values of V_{max} and direct count at depths greater than 15 m were approximately 50% of the surface values while mean viable count was 19% of the surface.

DISCUSSION

Despite inherent limitations associated with quasi-synoptic chemical and biological sampling of a large and dynamically complex estuarine-shelf system

such as the Chesapeake Bay plume, non-parametric correlation analyses of microbial and selected hydrographic variables revealed statistically significant relationships. Furthermore, the significant correspondence of microbial variables with plume hydrographic characteristics provided (at least during this cruise) a means for detection and spatial location of plume waters using microbiological measurements.

Highly significant values of Tau ($\alpha \leq 0.001$) were obtained for viable bacterial counts with direct bacterial counts and V_{max}. Significant negative correlations of these microbial parameters with salinity and depth indicated surface or lower salinity plume water contained the largest bacterial biomass and the most active cells. This association also appeared as a significant positive correlation of microbial parameters with water temperature. A significant negative correlation of salinity with temperature suggested a stratified hydrographic regime typical of the summer period. Lack of significant correlation of microbial data with suspended particulates may have been related to the presence of subsurface suspended solids maxima or to the relatively small variation in the suspended solids data set.

Microbial analyses of Chesapeake Bay plume waters revealed a highly active population of saprophytic bacteria. Both bacterial standing crop (direct or viable count) and V_{max} activity measurements were significantly greater in surface plume waters compared with the colder shelf water. Saprophytic bacterial populations are known to require relatively high levels of natural or pollutant-derived organic solutes which must be present in Bay plume waters.

Actual bacterial biomass may be approximated on a weight basis from direct count data using an average cell volume of 0.06 μm^3 (5) and assuming a specific gravity of 1.0. Values shown in Table 5 for mean bacterial densities correspond to direct count density contours shown in Figure 1. The distribution of biomass (and V_{max}) with respect to proximity to the Bay mouth was qualitatively similar to that measured in Kiel Fjord and Bight waters in Germany (6). Mean surface bacterial biomass corresponded to 0.8% of the mean total suspended particulate load within the contour of maximum direct count density. By comparison, if one assumes that chlorophyll concentration may be converted to cellular carbon using an average weight ratio of 60:1 for carbon:chlorophyll (5), the same mean bacterial biomass was approximately equivalent to 4% of the phytoplankton standing crop. Although these estimates are extremely rough, they do suggest the instantaneous standing crop of bacterial biomass in plume waters was not insubstantial as a food source for potential consumers such as heterotrophic flagellates. An estimate of the true flux of bacterial protoplasm as a carbon and energy source to shelf waters is not possible owing to the unavailability of information on the net flux of bacterial biomass from the Bay or seasonal bacterial growth rates during transition from Bay to shelf waters. Finally, although the effect of streamflow volume into the Bay on bacterial productivity and net transport is unknown, it is probable that significantly lower streamflow volumes such as those encountered in 1980 would reduce bacterial biomass production.

In summary, Chesapeake Bay plume waters supported high levels of active saprophytic marine bacteria. These bacteria not only convert nutrients and organic matter into bacterial protoplasm, but appear to be a significant food source of unknown dimension for microorganisms such as heterotrophic flagellates and others.

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Table 1. Biomass and V_{max} data and selected physical-chemical parameters used for Kendall Tau calculations. Chesapeake Bay Plume Experiment II.

NOAA Station No.	Depth m	Viable Count MPNx10^3 mℓ^{-1}	Direct Count BUx10^3 mℓ^{-1}	V_{max} µg glucose $\ell^{-1}h^{-1}$	Salinity o/oo	Temperature °C	Total Chlorophyll µg ℓ^{-1}	Suspended particulates mg ℓ^{-1}
800	1	190	1800	0.681	21.63	22.30	3.41	1.3
	7	120	1900	0.663	21.98	22.00	2.08	2.0
801	1	160	1100	0.590	26.0	20.20	2.73	3.8
	5	80	1500	0.425	27.73	20.20	2.21	1.3
	10	56	1300	-	30.48	19.50	2.57	1.4
	13	100	540	0.535	31.09	19.20	2.89	1.6
69	1	140	870	0.691	27.48	20.50	7.62	2.0
	5	110	700	0.612	28.05	19.70	7.75	3.2
	10	60	380	0.980	31.38	18.20	2.41	5.0
802	1	150	1000	0.737	25.49	20.80	4.32	1.3
	5	23	180	-	28.38	18.30	2.22	0.7
	10	23	110	0.192	31.96	17.80	1.91	2.7
	15	20	210	-	31.92	17.40	1.84	3.7
	17	25	200	0.256	32.18	16.80	1.52	1.4
803	1	150	270	0.351	29.02	20.40	1.62	2.2
	5	82	560	-	31.50	19.80	1.68	0.1
	10	57	390	0.245	32.19	18.90	1.57	0.8
804	1	31	320	0.207	32.15	18.70	1.44	0.3
	5	51	170	-	32.15	18.60	1.55	0.5
	10	48	170	0.221	32.15	18.60	1.32	0.5
	15	33	170	0.305	32.26	18.50	2.86	0.7
805	1	190	980	0.770	25.97	21.00	2.57	1.4
	5	23	120	-	28.06	18.20	2.57	1.2
	10	56	290	0.851	33.97	16.80	2.25	2.0

Table 1 (continued)

NOAA Station No.	Depth m	Viable Count MPNx10^3 mℓ^{-1}	Direct Count BUx10^3 mℓ^{-1}	V_{max} µg glucose $\ell^{-1}h^{-1}$	Salinity o/oo	Temperature °C	Total Chlorophyll µg ℓ^{-1}	Suspended particulates mg ℓ^{-1}
70	1	36	200	0.404	26.55	21.40	1.75	0.4
	5	23	240	-	27.16	17.50	1.85	1.2
	10	7.2	130	0.209	31.69	15.40	1.65	1.8
	13	6.4	220	0.222	32.21	14.80	1.51	0.4
806	1	23	130	0.189	29.58	20.00	0.66	1.2
	5	28	79	-	30.72	18.80	0.51	0.6
	10	12	120	0.103	32.16	17.50	0.68	1.0
	15	33	180	0.077	32.26	17.40	1.97	2.8
807	1	7.9	24	0.057	31.60	19.40	0.51	0.4
	5	19	44	0.043	31.60	19.35	0.51	0.2
	10	7.7	57	-	32.03	19.00	0.58	0.2
	15	9	400	0.723*	32.40	14.40	1.3	0.4
808	1	28	460	0.365	29.44	20.00	1.57	0.8
	5	40	500	-	29.41	18.30	1.53	1.0
	10	36	390	0.710	31.96	14.45	5.27	1.2
809	1	41	480	0.484	27.34	21.00	1.46	0.6
	5	56	140	-	27.36	20.80	1.18	0.4
	10	9.5	98	-	30.77	15.00	2.29	2.0
	15	14	260	0.211	31.71	13.80	2.29	0.8
810	1	9.5	57	0.164	30.08	20.20	0.80	1.4
	6	27	77	-	30.09	20.20	0.80	0.8
	12	64	180	0.240	31.28	14.50	1.22	0.4
	18	18	220	0.203	32.78	13.30	3.62	0.2
811	1	9.5	140	-	31.87	20.10	0.71	1.6
	7	18	74	-	31.92	19.20	0.40	2.2
	14	6	76	-	32.31	15.10	0.72	3.8
	21	8	160	-	33.12	12.80	2.07	6.4

Table 1 (concluded)

NOAA Station No.	Depth m	Viable Count MPNx10^3 mℓ^{-1}	Direct Count BUx10^3 mℓ^{-1}	V_{max} µg glucose $\ell^{-1}h^{-1}$	Salinity o/oo	Temperature °C	Total Chlorophyll µg ℓ^{-1}	Suspended particulates mg ℓ^{-1}
813	1	15	83	-	30.42	20.20	0.42	1.2
	6	9	130	-	30.69	19.90	0.35	1.8
	12	6.4	85	-	31.92	19.00	0.46	0.2
	18	3.3	21	-	32.97	12.20	0.86	2.2
812	1	41	110	0.294	28.68	22.00	0.63	1.4
	5	40	96	-	28.90	20.75	0.87	6.2
	10	20	72	0.164	29.65	19.30	0.87	2.0
	15	4.9	340	-	31.75	13.60	2.03	4.0
	20	-	270	0.146	32.54	13.20	2.88	0.6
71	1	25	76	0.189	29.75	21.00	0.70	0.4
	6	46	300	0.189	29.83	20.35	0.78	0.2
	12	46	290	0.252	30.30	19.40	0.98	0.4
814	1	42	510	0.219	29.80	21.20	0.68	0.8
	5	110	460	0.465	29.82	21.00	0.68	3.2
	10	95	780	0.714	30.87	16.40	0.92	0.8

*sand in sample, value discounted

Table 2. Statistical analysis of hydrographic and microbiological parameters.

VARIABLE	N	MEAN	STD DEV	MEDIAN	MINIMUM	MAXIMUM
DEPTH, M	66	7.65	5.66	6.00	1.0	21.0
VIABLE COUNT, MPN $m\ell^{-1}$	65	46901.54	47107.42	28000.00	3300.0	190000.0
DIRECT COUNT, BU $m\ell^{-1}$	66	366333.33	407183.92	210000.00	24000.0	1900000.0
VMAX, µg glucose $\ell^{-1}h^{-1}$	42	0.37	0.24	0.25	0.0	1.0
SALINITY, o/oo	66	30.22	2.47	30.82	21.6	34.0
TEMPERATURE, °C	66	18.42	2.55	19.10	12.2	22.3
CHLOROPHYLL, µg ℓ^{-1}	66	1.80	1.44	1.54	0.3	7.8
SUSPENDED SOLIDS, mg ℓ^{-1}	66	1.53	1.38	1.20	0.1	6.4

Table 3. Values of non-parametric Kendall Tau correlation coefficient calculated for biomass and V_{max} data against selected physical and chemical parameters.

	DEPTH	VIABLE	DIRECT	VMAX	SAL	TEMP	CHLOR	SS
DEPTH	1.00000[a]	-0.30595	-0.05199	-0.11746	0.57184	-0.63975	0.12728	0.05973
	0.0000[b]	0.0008	0.5646	0.3106	0.0001	0.0001	0.1580	0.5151
	66[c]	65	66	42	66	66	66	66
VIABLE COUNT	-0.30595	1.00000	0.48273	0.58477	-0.39748	0.38225	0.28488	0.01240
	0.0008	0.0000	0.0000	0.0000	0.0000	0.0000	0.0009	0.8871
	65	65	65	41	65	65	65	65
DIRECT COUNT	-0.05199	0.48273	1.00000	0.57494	-0.20094	0.08253	0.43076	0.07243
	0.5646	0.0000	0.0000	0.0000	0.0178	0.3324	0.0000	0.4019
	66	65	66	42	66	66	66	66
VMAX	-0.11746	0.58477	0.57494	1.00000	-0.31389	0.19472	0.41521	0.30512
	0.3106	0.0000	0.0000	0.0000	0.0035	0.0717	0.0001	0.0055
	42	41	42	42	42	42	42	42
SALINITY	0.57194	-0.39748	-0.20094	-0.31389	1.00000	-0.59488	-0.08206	-0.05836
	0.0001	0.0000	0.0178	0.0035	0.0000	0.0001	0.3326	0.4983
	66	64	66	42	66	66	66	66
TEMPERATURE	-0.63975	0.38225	0.08253	0.19472	-0.59488	1.00000	-0.12718	-0.03315
	0.0001	0.0000	0.3324	0.0717	0.0001	0.0000	0.1348	0.7016
	66	65	66	42	66	66	66	66
CHLOROPHYLL	0.12728	0.28488	0.43076	0.41521	-0.08206	-0.12718	1.00000	0.17632
	0.1580	0.0009	0.0000	0.0001	0.3326	0.1348	0.0000	0.0411
	66	65	66	42	66	66	66	66
SUSPENDED SOLIDS	0.05973	0.01240	0.07243	0.30512	-0.05836	-0.03315	0.17632	1.00000
	0.5151	0.8871	0.4019	0.0055	0.4983	0.7016	0.0411	0.0000
	66	65	66	42	66	66	66	66

[a]Kendall Tau correlation coefficient
[b]probability of obtaining value randomly
[c]sample size

Table 4. Mean values of selected microbiological and hydrographic variables for depths indicated.

	1 m	5 m	10 m	15 m	>15 m
Viable Count, MPN $m\ell^{-1}$	71605	52692	37492	26083	13575
Direct Count, BU $m\ell^{-1}$	478333	368384	329769	245916	212000
Vmax, µg glucose $\ell^{-1}h^{-1}$	0.40	0.39	0.44	0.26	0.20
Chlorophyll, µg ℓ^{-1}	1.87	1.93	1.87	1.67	2.19
Suspended Solids, mg ℓ^{-1}	1.25	1.52	1.65	1.60	2.16

Table 5. Estimated bacterial biomass calculated from direct count densities of the surface waters (1 m) as shown in Figure 1.

Density Contour	Mean Direct Count, BUx10^3 $m\ell^{-1}$	Estimated Mean Biomass, Wet, µg $m\ell^{-1}$	Estimated Mean Biomass, Dry, µg $m\ell^{-1}$
6.0-6.5	1300	0.078	0.016
5.5-5.9	500	0.030	0.006
5.0-5.4	160	0.0096	0.0019
4.5-4.9	79	0.0047	0.0009
4.0-4.4	25	0.0015	0.0003

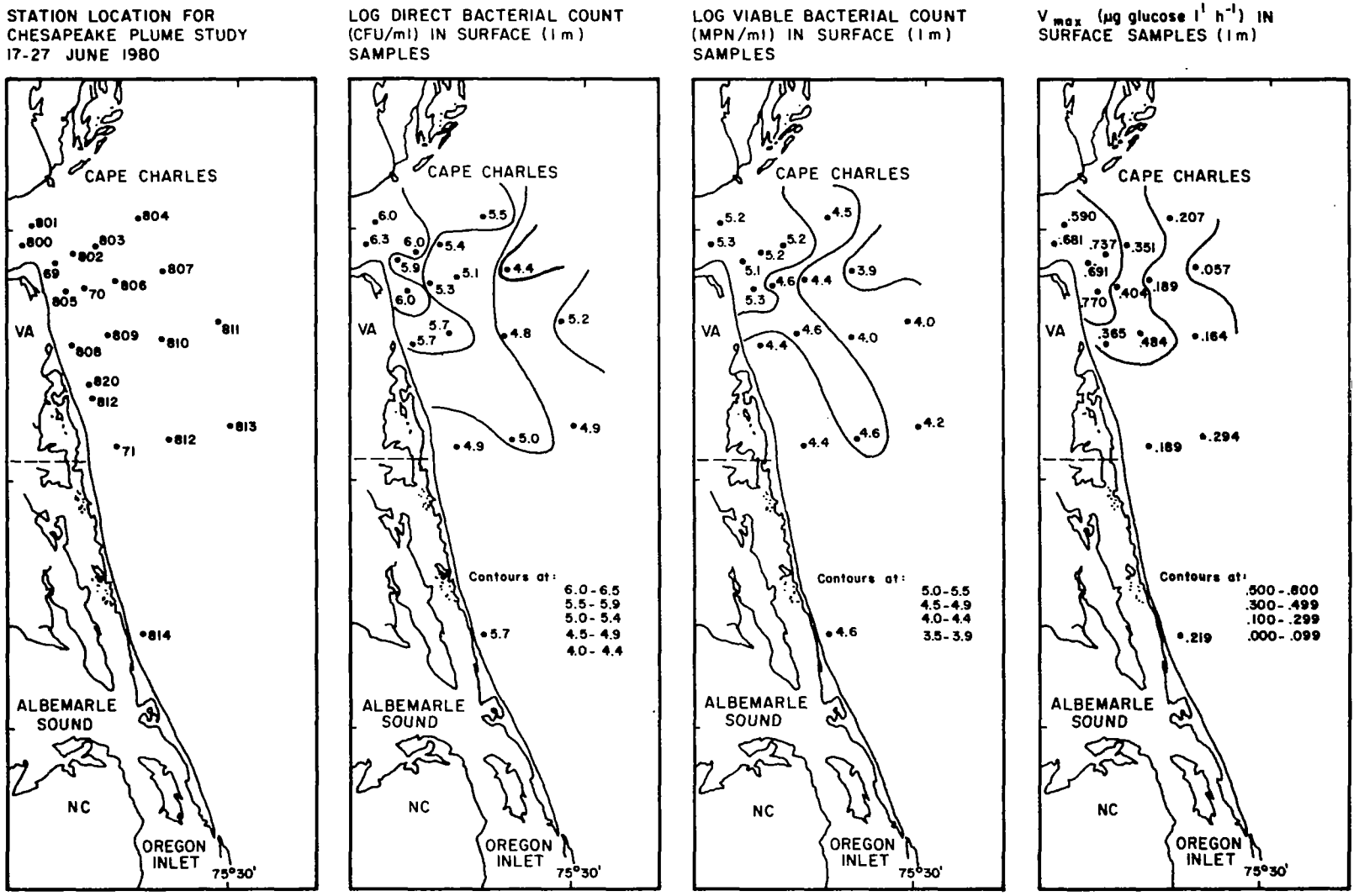

Figure 1. Location charts for observed parameters in the surface waters during Chesapeake Bay Plume Study, 17-27 June 1980. Station Locations/Direct Bacterial Count/Log Viable Bacterial Count/V_{max} (glucose).