Chesapeake Research Consortium, Incorporated

ANNUAL TECHNICAL REPORT

NSF/RANN GRANT G. I. 38973

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NATIONAL SCIENCE FOUNDATION

VOLUME XI

Waste Water:

PHYSICAL, CHEMICAL AND BIOLOGICAL PROCESSES IN RHODE RIVER

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The Johns Hopkins University Smithsonian Institution
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Chesapeake Research Consortium, Incorporated
100 Whitehead Hall
The Johns Hopkins University
Baltimore, Maryland 21218
(301) 366-3300 Extension 766

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PHYSICAL, CHEMICAL AND BIOLOGICAL PROCESSES IN RHODE RIVER

FINAL REPORT

SUBMITTED TO

NSF - RANN

SUSPENDED AND BOTTOM SEDIMENTS OF THE RHODE RIVER

J. W. Pierce
Division of Sedimentology
Smithsonian Institution
Washington, D. C. 20560

Total Direct Budget: $14,567

ABSTRACT

The majority of sediments on the floor of the Rhode River are silty clay. The upper part of each subestuary is covered with poorly sorted material. Sand sized material is restricted to the shallower areas of the estuary where they constitute a lag deposit after winnowing and removal of the fines.

The minerals with the highest exchange capacity are more prominent in the upper part of the estuary, mirroring the results of the suspended material and indicating loss of these minerals from suspension. Oxidizable organic material in the bottom sediments is considerably less (wt/wt) in the bottom sediments than in the suspended phase. Both recycling and utilization within the water column and degradation after deposition could explain the difference.

Background concentration levels in the Rhode River are not out of line with that in other parts of the Bay. Perturbation in concentrations are due to sediment discharge from terrestial areas and biologic productivity. Concentrations generally decrease down-estuary. Organic suspensates peak during the summer season as does total suspensates. Mineral particulates are at their seasonal highest level in the fall. Winter has the lowest seasonal average of all particulates.

At the head of the estuary, the turbidity maximum migrates up-estuary at least 0.5 km during flood tide and down-estuary possibly 1.4 km during ebb. Resuspension of bottom sediments appears to occur during ebb tide. During flood tide, there was no evidence of resuspension. At the present state of knowledge regarding water flux in the upper estuary, it is impossible to calculate sediment transport.

At the mouth of the Rhode River, the turbidity maximum moved about the same distances, during a tidal cycle, as in the upper estuary: up-estuary between 0.5 and 1.2 km; down-estuary about 1.75 km. There was no obvious resuspension of bottom sediments. Attempts to calculate the flux of suspensates, using the exchange coefficients developed for conservative properties, were unsuccessful, resulting in calculated concentrations higher by a factor of 2 than the measured concentrations.

The minerals with the highest cation exchange capacity are most prominent, as suspensates, in the upper estuary. The cation exchange capacity of the estuarine waters is determined by the amount of organic material in the suspended particulates. The total cation exchange capacity is estimated to be 207×10^6 milliequivalents.

Concentrations of the major cations increase downestuary, as would be expected. Fluctuation in Mg may be due to incorporation into chlorophyll. Silicon decreases downestuary but at a more rapid rate than mixing indexes would indicate, suggesting biologic uptake or deposition.

Interpretation of acid extractable ions by acid extracts from the suspensates is hampered by lack of data (one sampling period). Most iron occurs as coatings on the suspensates, which is partially solubilized by acid solutions. Hence, in an acid environment, could be remobilized.

PROJECT OBJECTIVES

At the time of submission of the original proposal, data on the following was guaranteed:

1. Type of bottom sediments in the Rhode River characterized as to sand-silt-clay ratios;
2. Mineralogy of bottom sediments and amount of organic material in bottom sediments at selected points;
3. Mass of material suspended in the Rhode River at different sampling times;
4. Definition of point sources for major inputs of sediment into the system; and
5. Mineral fractionation during suspended transport in the estuary.

Revamping of the objectives of this project to fit the overall objectives of the Waste Water Program caused a grouping of the above objectives into a more general statement. The revised objective was to estimate the distribution of the sediment, discharged by Muddy Creek, in the Rhode River. The above objectives, in part, fulfill the new objective.

INTRODUCTION

The final report covers the results of research conducted during the period 1 June 1973 through 30 September 1974. Results from earlier funding are included, where pertinent, for completeness.

Data, collected during the early stages of this grant, were incorporated into the final report submitted in late 1973 to fulfill requirements of a previous grant. Some of this data will be repeated here.

BOTTOM SEDIMENTS

Methods

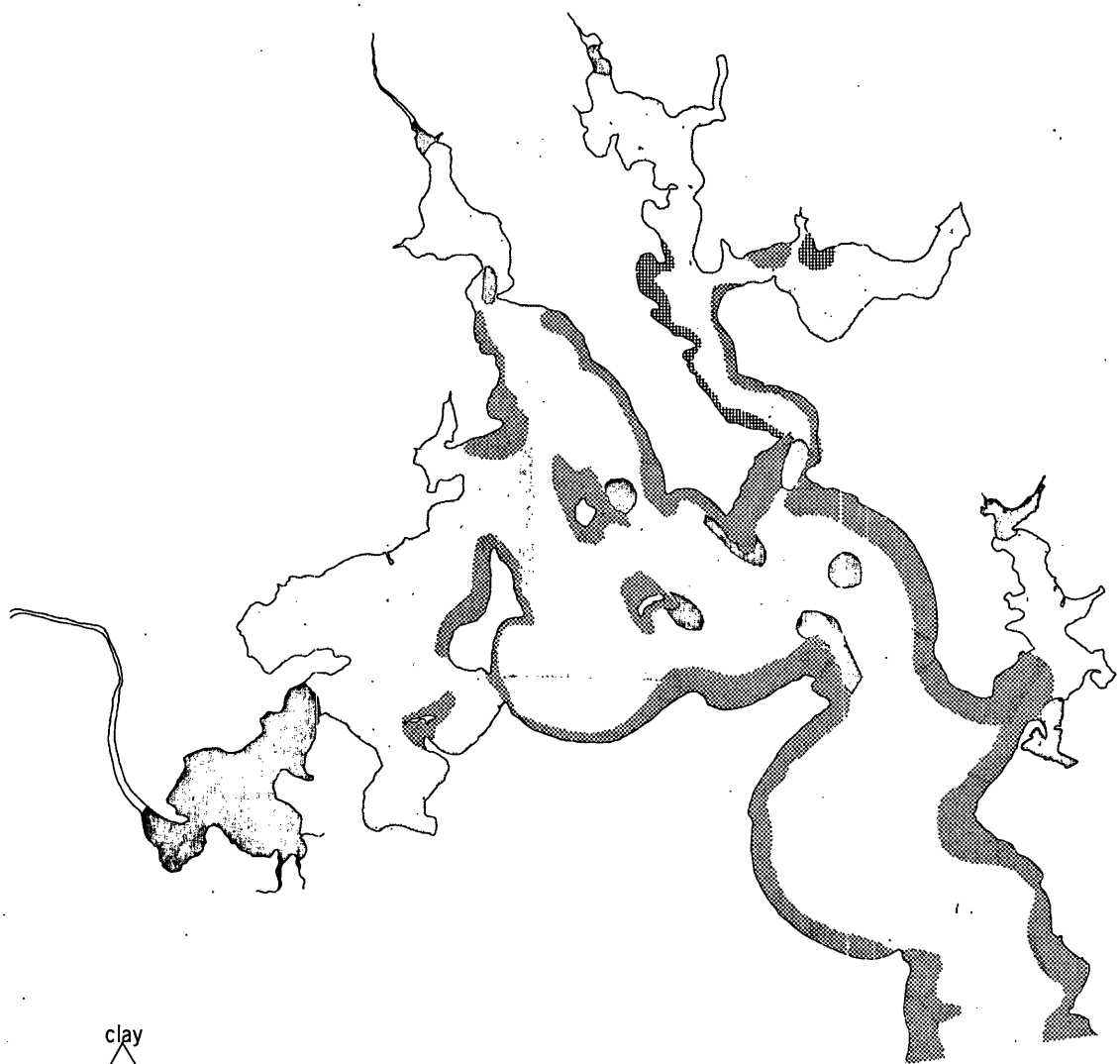
Six hundred seventy four bottom samples were taken with the Ekman dredge in Muddy Creek, Rhode River and West River. All samples were analyzed for amount of sand, salt, and clay by wet sieving and by pipetting (or hydrometer). Percent of oxidizable organics was determined on selected samples by treatment with hydrogen peroxide.

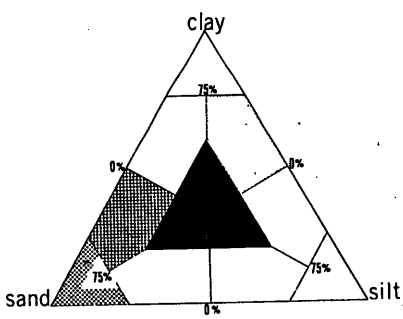
X-ray diffractograms also were made of selected samples to determine mineralogy of the bottom sediments. Diffraction scans were made on the <2 μm and <50 μm size fractions. Untreated and glycolated samples were scanned in all cases; heat treatment was applied to some of the samples.

The relative amount of diffracted intensities of both size fractions were calculated separately and the results recombined to give percent of diffracted intensity for each mineral in the <50 μm fraction. The results are presented as percent of total diffracted intensity for each mineral. This method of presentation is satisfactory for determination of trends. Considering the sad state of quantitative diffraction of natural materials, we feel that this method is better than giving the reader a false sense of securi that the results reported represent actual percentages of the mineral present and therefore can be compared directly to published data.

Grain Size

The majority of bottom sediments in the Rhode River are silty clay (Fig. 1). These sediments range from 25 to 50% silt, greater than 50% clay-sized material, and the remainder consisting of a few sand grains.





RHODE RIVER

BOTTOM SEDIMENT DISTRIBUTION

Fig. 1.

The bottom of the uppermost part of each tributary is covered with a poorly sorted mud consisting of roughly equal parts of sand, silt, and clay. The heterogeneity of all of these deposits is attributed to one of two processes or combination thereof.

Coarser material transported during high fluvial discharge is deposited in the uppermost parts of the estuaries. Some finer material would be deposited at the same time but most would be carried farther down estuary. During periods of normal stream discharge, coarse material is not transported and the finer particles, which are transported, are deposited in the uppermost parts of the estuary. Normally, this would result in layers of coarse and fine material. Burrowing organisms disrupt this layering resulting in a homogenous mixture of heterogenous sizes.

Sand sized material is restricted to shallow areas where the bottom sediments are subjected to wave action and where erosion of the shoreline is occurring. In both cases, the finer fraction of the sediment is removed by suspension transport, leaving behind the coarser material as a lag deposit. Much of the resuspended finer material is redeposited in the deeper areas, now covered by silty clay.

The distribution of bottom sediments strongly indicate that the uppermost estuaries are the areas of maximum deposition. From the distribution of bottom sediments, very little deposition occurs in much of the estuary.

Mineralogy

It should be stressed again here that the results are reported as percent of total diffracted intensity of each mineral, not a percent of mineral present. Thus the data can notbe compared directly to results reported elsewhere as percent of minerals present. This method adequately represents trends without the undue complications of multiplying by various factors.

The bottom sediments are the result of deposition from suspension, reworking and resuspension as well as some minor traction load contributions. Theyresult from introduction of material from many sources, over a long period of time, as apposed to samples of suspended material which represent an instant in time. For the most part, the operation of several processes over a time period result in a smearing of the data with much of the noise smoothed out and the relevant parameters difficult to extract from the data.

Montmorillonite-vermiculite abundance experiences a sudden drop between segment 5 and segment 4 (Table 1,Fig.2). This substantiates the conclusions from the suspended sediment data that much of this type material is lost in the uppermost part of the estuary, in a salinity range of less than 5%. Montmorillonite has higher values than expected at the lower end of the Rhode River. These may be due to erosion of the edges of the estuary, covered by the Talbot Formation which has large amounts of montmorillonite.

Mica shows a general increase down estuary. Surprisingly, quartz appears to show a marked increase where montmorillonite shows a decrease. As the number system is closed, part of this may be sympathetic although it is believed to be,in part, real.

Table 1. Average relative diffraction intensities of minerals in bottom sediments of Rhode River. The values in the table are the percent of total diffraction intensity ascribed to the given minerals. It is NOT the percent of mineral present. To obtain this, multiply the tabulated values by whatever factor desired, sum values, and divide each resulting value by sum.

Mineral \ Segment	5	4	3	2
Montmorillonite Vermiculite	57	40	44	45
Mica	7	8	9	9
Kaolinite	10	19	12	12
Quartz	16	25	30	28
Feldspar	5	1	1	2
Gibbsite	3	4	2	2
Chlorite	2	3	2	3

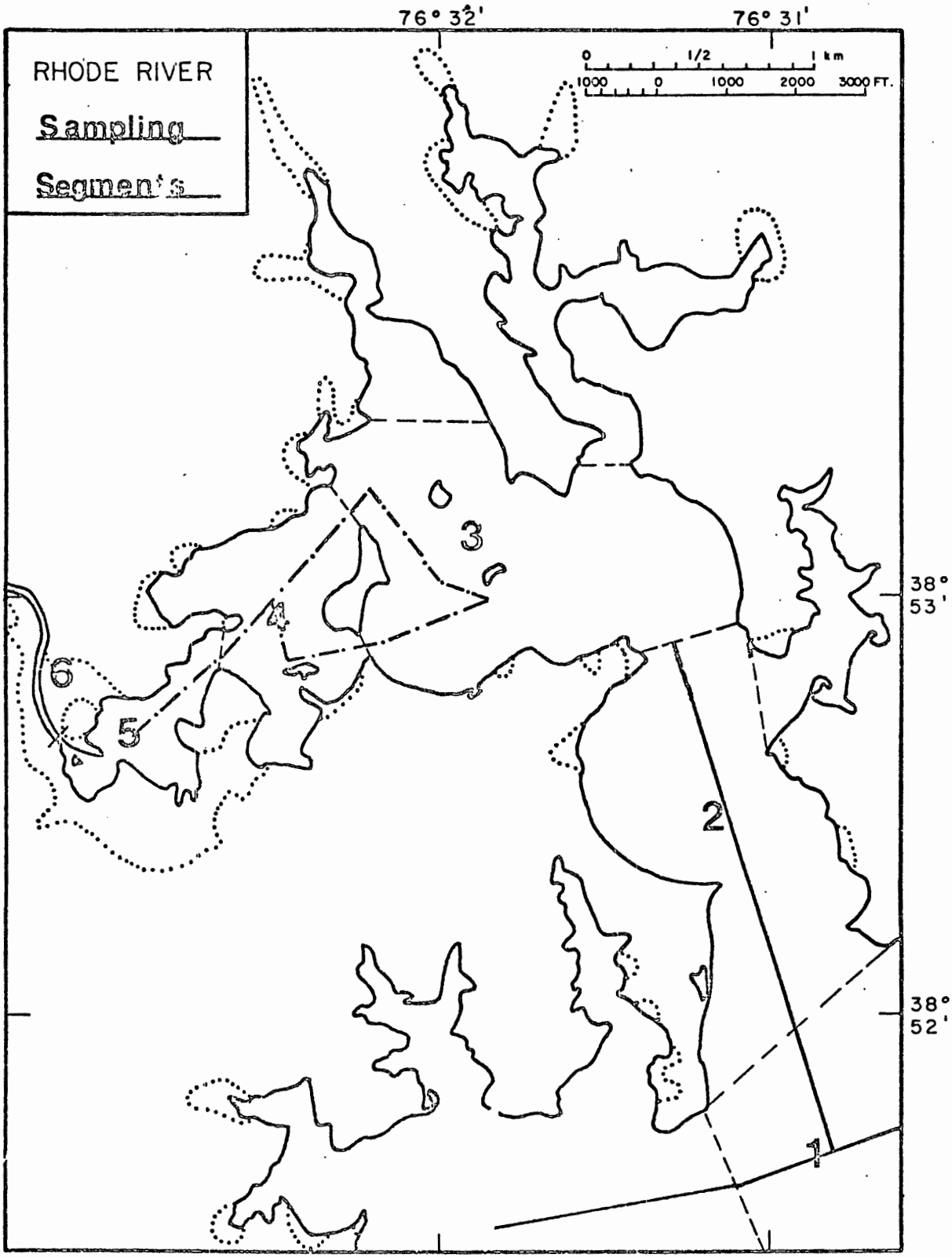


Fig. 2. Location map of Rhode River segments. The track lines occupied during tidal cycle sampling are shown as heavier lines.

Kaolinite shows little change down estuary except for an increase in segment 4. This may be due to contributions from Sellman Creek.

Organic Material

The oxidizable organic matter in the bottom sediments is considerably less than that associated with suspended particulate material (Table 2). There seems to be no apparent correlation between the trends, down-estuary, in the bottom sediments with that in the water column, with respect to percentages which can be misleading. The total amount of material may decrease but because of a large decrease in mineral matter the percentage of organics may increase.

There is a slightly better agreement between the average amount of suspended organic material and the amount in the bottom sediments. Segment 4 has the highest percent of organic material in the bottom and the largest average amount of suspended organic particles; segment 2 has the lowest in both cases.

There are many reasons for the drastic decrease of organic matter between the suspended fraction and the bottom sediments. Two of the more obvious are non-deposition of organic material (recycled within the water column) and degradation after deposition.

Given the propensity of organic material for complexing trace elements, the degradation has important considerations in recycling of the elements from the bottom sediments back into the water column.

Table 2. Comparison of percentage of oxidizable organic material in Rhode River segments. The tabulated percent is an wt/wt basis, due to weight loss by hydrogen peroxide oxidation. Percentage in suspended material based on sampling in August 1972 when the bottom samples were taken.

	% oxidizable organic material		
Segment	Bottom	Suspended %	Quantity mg/l
2	2.01	66.50	9.2
3	3.50	72.14	13.1
4	4.11	51.83	16.2
5	3.16	41.45	15.9

SUSPENDED PARTICULATE MATTER

Methods

Samples were taken from the layer of maximum turbidity by either a modified stream sampler or by a battery-driven centrifugal pump. Samples of 250 ml or 500 ml were stored in volumetric flasks at 3°C until processing began.

Samples were filtered through 47 mm dia., 0.45 millipore HA filters, which were pre-washed and pre-weighed. The filters plus particulates were dried and weighed and concentrations per liter calculated.

The filter was dissolved by multiple washings with acetone, followed by ether, ethanol, and distilled water washes. The organic matter was oxidized with 30% H_2O_2; the remaining solids dried and weighed; and the difference between total particulates and the remaining solids assigned to the oxidizable organic fraction.

The mineral matter was washed with distilled water, saturated with $MgCl_2$, and washed thrice with distilled water. The solids were dispersed in 0.01 ml distilled water by ultrasonic means and placed on a labelled glass slide for x-ray diffraction.

Vertical profiles of light transmission were obtained by use of a Hydroproducts Model 410 transmissometer with a 10 cm pathlength. The light was filtered through a green filter to minimize absorbance by dissolved materials and to reduce the wave length for maximum particle effect.

The values of light transmission were converted to concentration by regressing transmission values on concentrations. The equation derived is

$$C = \frac{0.04234 + \log_{10}(\frac{1}{T})}{0.01478}$$

where C is concentration in mg/l and T is percent transmittance.

Background and Variations in Suspended Particulates

Background

Each area within an estuary has a "natural background" level, below which concentrations of suspended material does not go. Dyer (1972) states that most estuaries have natural background levels of 10-20 mg/1. Schubel (1971) found that such levels in the upper Chesapeake Bay are about 15 mg/1 at the surface increasing to 20 mg/1 at depth at one station. Obviously, this must apply only at one station because he shows other areas where concentrations drop to less than 4 mg/1.

The background levels of total material in the Rhode River are more of the order of the latter figure than 20 mg/1, ranging from 2.02 at the mouth of the Rhode River to 3.89 mg/1 at the upper end. Note that the sum of the organic material and mineral matter does not equal the total concentration.

Segment	Total Concentration mg/1	Organic Concentration mg/1	Mineral Concentration mg/1
5	3.89	1.06	1.6
4	2.84	0.98	1.17
3	2.66	0.6	2.0
2	2.02	0.4	1.5

This results from the fact that the lowest levels recorded for the individual types does not occur at the same time. Suspended organic material is controlled primarily by productivity whose peak does not occur at the same time as maximum sediment discharge from the watershed.

Lowest concentration of total material and of the organic fraction decreases down-estuary. Concentrations of mineral matter shows a decrease between segment 5 and 4, where we believe much of the material contributed by Muddy Creek is deposited. It increases again to segment 3 which we attribute to erosion of the islands and shorelines (a large part of segment 3 has a cliffed shoreline).

Variation in Suspended Load

Perturbations occur in the load of suspended particulates giving rise to increases in the load above the background levels. Not only are there estuary-wide changes but differences occur at individual stations over short time intervals. Seasonal changes would seem to be relevant to discussion rather than the fluctuations over a short time span as the latter could be considered noise imposed on the significant trends.

Segment 3 consistently has the greatest mass of suspended material (Table 3). This results from a combination of concentrations that are higher than segment 2 coupled with the largest volume of water of any segment. Segment 2 contains the next largest mass of suspensates followed by 4 and 5. In each case, the ranking of the segments is due solely to the water volume, not differences in concentration of suspensates.

Segment 5 has the highest average concentration of suspended particulates, followed either by 4 or 3, depending on the season. The upper two segments, are affected greatly by changing values of organic

Table 3.

Season		Winter	Spring	Summer	Fall
Segment					
2	Mass	3.45	6.86	8.33	6.64
	Ave. Conc.	9.87	19.63	23.83	18.99
		27	49	31	57
3	Mass	4.32	7.31	10.01	7.98
	Ave. Conc.	11.52	19.52	26.72	21.30
		--	92	24	52
4	Mass	0.76	1.42	2.50	2.49
	Ave. Conc.	9.20	17.13	30.12	29.99
		54	69	46	57
5	Mass	0.15	0.83	2.25	0.39
	Ave. Conc.	11.17	61.15	165.45	28.80
		--	96	31	37

Mass = $X10^4$kg

Conc. = mg/l

matter, due to either productivity or loss from marshes, as well as sediment discharge from the streams. The lower two segments are less affected unless a sample happens to be taken in a bloom.

The total load peaks during the summer season in all segments. This peak is due to large amounts of organic matter. Mineral solids attain their greatest seasonal load during the fall. The winter season has the lowest concentrations of all material.

Tidal Cycle Sampling

Two turbidity maxima have been persistently present in the Rhode River during the two years of data gathering on suspended particulate matter. One exists near the mouth of the Rhode River (Segment 2); the other, where Muddy Creek opens into the Rhode River (Segment 5). Both areas exhibit marked similarities in areal changes and bottom bathymetry. In both cases, the immediate down estuary segment has a much greater surface area and there is also a decreasing depth up-estuary; both circumstances indicate a marked volume change between segments.

Continuous sampling was conducted over approximately one tidal cycle at each of these two areas. The purpose was to examine the movement of the near-bottom turbid layer with changes in the tidal cycle. Also, if possible, given the limits of the box model, to estimate the amount of material in the turbidity maxima that has been resuspended from the bottom. Both purposes have application to criteria for siting of waste water plants and to estuarine management in particular.

As pointed out by Helz and Huggett (1974), sediments are highly enriched in trace metals for a few miles downstream of treatment plants. The overlying waters, on the other hand, are enriched in metals only in the immediate vicinity of the outfall. They also point out that there is some remobilization of metals into the water column from the bottom sediments. Recently, it has been reported that a very turbid near-bottom layer in Bordeaux harbor was greatly enriched in trace metals.

Thus, resuspension of bottom sediments could release lare amounts of metals (or their complexes) at a much greater rate than biologic activity

or ionic diffusion. The movement of the turbid layers could also affect the distribution of metals in the estuary.

Upper Estuary

This area is one of high sedimentation rates for much of the material being transported down Muddy Creek, the source of suspended inorganic material in the Rhode River.

Sampling was conducted from 0822 until 1620, 20 November 1973; low tide was at 0633 and high tide at 1331. Sampling covered Rhode River segments 4 and 5 and part of 3 (Fig. 2).

The turbidity maximum moved up estuary, during flood tide, about 0.5 km. Increased turbidity levels extended slightly farther (Fig. 3). At the change of tide, the turbidity maximum moved down estuary with increases in readings occurring atleast 1.4 km away two hours after the change in tide.

There appears to be some resuspension of bottom sediments during ebb tide as indicated by turbid layers within the water column down-current from two sills. In both cases, the increased turbidity layers are surrounded by clearer water. The turbid layers are approximately at sill depth (Fig. 3). In one case, temperature-salinity data, taken concurrently with the turbidity readings, suggest a very slight pycnocline associated with the turbid layer. In the second case, no density enterfaces were present.

Resuspension during flood tide is less evident from the profile. Any resuspended material would be incorporated into the turbidity maximum

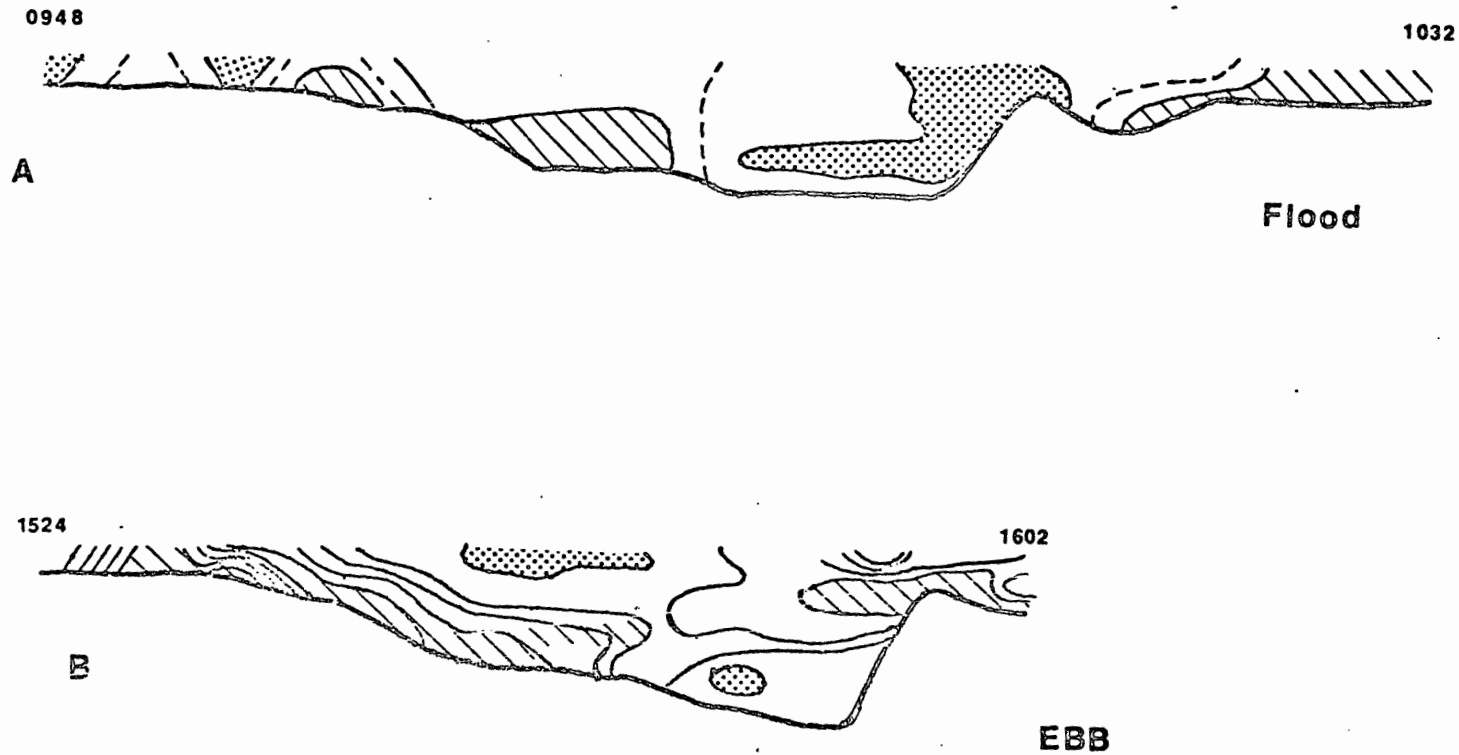


Fig. 3. Profiles of turbidity at upper end of estuary, 20 November 1973. The upper profile (A) returns to starting point; the lower does not. High turbidity shown by hatching; low by dots. See Fig. for station locality.

and would not be present as separate entities within the water column. Concentrations of suspended material did not increase from low to high tide. Thus, any resuspension was insufficient to overcome the intrusion of less turbid water.

Attempts were made to calculate the concentrations of the suspension resulting from mixing of the water in segments 4 and 5 and segments 3 and 4 using initial concentrations and volume transports. Exchange between segments 3 and 4 were taken from volume II, Interim Progress Report of February 1974. Those for segments 4 and 5 from the current measurements made by personnel from Chesapeake Bay Institute in August and September 1973.

The calculated concentrations did not agree at all well with the observed values. In all cases, the calculated concentrations were higher than the observed. From this, one of two conclusions can be drawn. We have insufficient data to calculate exchange rates or the rate of sedimentation is greater than the rate of resuspension during flood tide. It would seem that the weak link is our knowledge of exchange rates. Intuitively it hardly seems likely that the sedimentation rates during maximum currents will exceed the loss from suspension, by deposition, over the same time span.

Selection of certain data for suspended sediment concentrations coupled judicious selection of volume transport between segments 4 and 5 will give calculated values that indicate some resuspension, but not much, in the uppermost part of the estuary. We must conclude that resuspension by tidal currents is insignificant in this part of the estuary.

Lower Rhode River

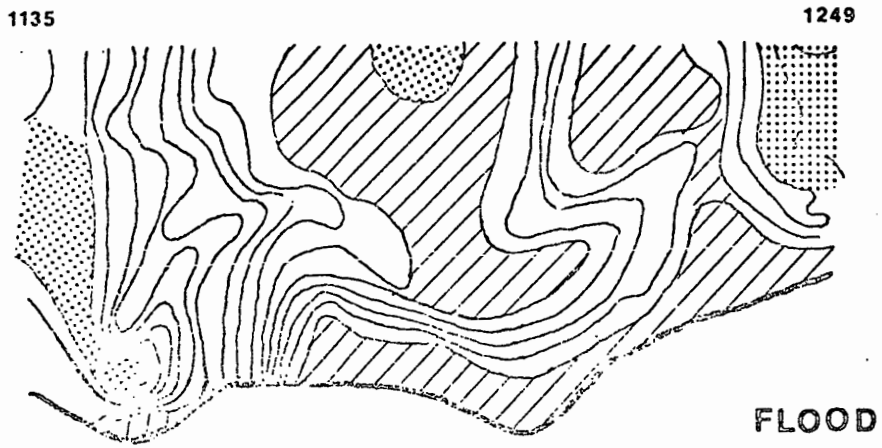
Sampling had also shown the existence of a rather persistent turbidity maximum near the mouth of the Rhode River. Although depths and volumes are much greater than the upper part of the estuary, there is a similarity in the two areas in that there is a shallowing as well as a decrease in surface area.

Sampling was conducted in segments 1, 2, and 3 of the Rhode River as well as the adjacent part of the Bay on 30 May 1974 (Fig. 2). Sampling started at 0851 (EDT) and ended at 1930 (EDT). Low tides were at 0742 and 1921; high tide, at 1305.

A near-bottom turbidity maximum was present in the area throughout the sampling (Fig. 4). Concentrations of suspended material are one-fourth again to twice as much as the overlying water. The turbidity maximum moved up-estuary at least 0.5 km and possibly as much as 1.2 km during flood tide. It moved out of the Rhode River into Chesapeake Bay on ebb tide, migrating a distance of possibly 1.75 km (Fig. 4).

There are no obvious resuspension fingers on the profiles. Quite often the turbid layer is isolated from overlying waters by a strong density gradient. Such a gradient is not always present and the turbidity maximum does not appear to be completely isolated from clearer water, even though strong concentration gradients for suspended material may be present.

Movement of the water masses can be followed by observing the entrance of the less turbid water from the Bay on flood tide and the exit of the more turbid waters of the Rhode River on ebb tide (Fig. 4). The change in concentration values would suggest that water from beyond segment 1 enters the Rhode during flood tide rather than being limited to transfer



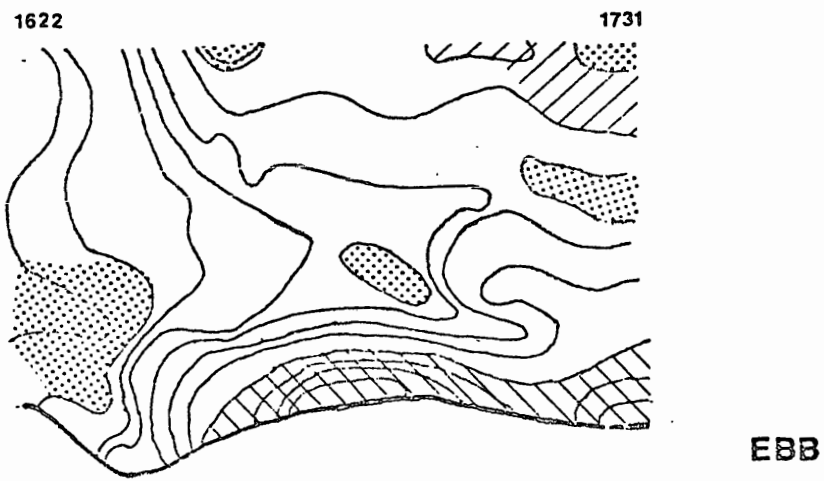


Fig. 4. Turbidity profiles at mouth of Rhode River, 30 May 1974. Higher turbidity shown by hatching; low by dots.

from segment 1 as is presumed in the box model. Determination of volume transport from concentrations of suspended particulates is fraught with problems, at best.

On the other hand, one should be able to determine flux of suspended material from one segment to another using the exchange coefficients developed for conservative properties (salinity). Attempts to do this for segments 2 and 3 found that the calculated concentrations are higher than those actually measured, by approximately a factor of 2. The same problem was encountered in attempting to calculate flux in the upper part of the estuary.

Discussion

The problems encountered in attempting to determine suspended sediment flux from current measurements or exchange coefficients should not be passed off lightly. One should be able to arrive at a figure that is relatively close and in the right direction, when some values for resuspension and deposition are included. The values calculated here are meaningless unless an extremely high rate of sedimentation and no resuspension are assumed.

It would seem that a model should include parameters capable of handling the flux of suspended particulate material if resuspension and deposition are omitted. Considering the propensity of suspended particles to be associated with metals, some chlorinated hydrocarbons, bacteria, and some nutrients, one must wonder how the pathways of these substances can be predicted if the models will not work for prediction of suspended material.

Mineral Fractionation

Certain minerals will be lost, through deposition, before others during suspension transport. The controlling variable for settling velocity of a particle through a liquid is the mass of the coherent settling unit. This unit may be a single particle or an aggregate of several particles of differing composition. Mass in turn is controlled by the volume and density of the unit. Larger particles will settle early, as well as the denser particles. One should be able to predict the most probable size of a mineral in an area and, knowing the density, design a sequence for mineral fall out. Unfortunately, much of the clay-size material occurs in aggregates with a much larger mass than would be predicted from the size of the individual particles.

Fractionation during transport controls the ability of the suspended mineral suite to adsorb materials from the water and will dictate where materials adsorbed on the particle will be located on the estuarine floor. Some minerals are more prone than others to scavenge metals or organic compounds from the water. The ability to adsorb materials from the water is dependent upon unsatisfied charges in the mineral lattice and the existence of broken bands on the edges of the structure.

Attention will be focused here primarily on the minerals montmorillonite and vermiculite for several reasons. The two minerals can be distinguished only with difficulty. Both have large cation exchange capacity, being about equal in the range of 80 to 150 meq/100g (Grim, 1968). Both occur suspended in the estuarine waters. Bacteria prefer montmorillonite as

suspended substrates for colonization over kaolinite and illite according to experiments conducted in the Rhode River by Maria Faust.

It should be pointed out here that what we refer to as montmorillonite is really montmorillonite and vermiculite. For scientific purposes, it is necessary to distinguish the two. For applied purposes, it does not seem worthwhile to do so because of their similarity in exchange capacity.

Montmorillonite shows a general decrease in percent of total diffracted intensity down-estuary (Table 4), indicating a progressive loss from the suspended suite, from segment 6 through 4. Segment 3 has an increase and then a decrease occurs to segments 1 and 2. Examination of the data from the individual stations strongly suggests that the increase in segment 3 is due to contributions of montmorillonite from Sellman Creek and from erosion of the islands. Without these contributions, montmorillonite would, on the average, account for about 13% of the total diffracted intensity in segment 3.

Other minerals show a general increase down-estuary.

It is difficult to comprehend what 20% of the total diffracted intensity implies for any given mineral. Each mineral diffracts x-rays differently, depending on chemical composition, structure, and degree of crystallinity. The estimated percentage of the mineral is given in parentheses in Table 4. Conversion was through use of factors developed for the Deep Ocean Drilling project (Bader et al, 1970).

A few comments should be directed here toward cation exchange capacity of the suspended material. Exchange capacity of the suspended material in the Rhode River has not been done but has been estimated from the amount of ions in the HCl extractable fraction (see section on major ions).

Table 4. Percent of total diffracted intensity attributable to more prominent minerals. MV = montmorillonite-vermiculite; M = mica, illite, glauconite; K/C = kaolinite/chlorite; G = gibbsite; Q = quartz; Fldsp = feldspars. To obtain percentage of any mineral, multiply by appropriate factor. Estimated percentage of each mineral in brackets.

Mineral → / Segment ↓	MV	M	K/C	G	Q	Fldsp
6	23 (31)	9 (23)	15 (16)	3 (3)	47 (21)	3 (6)
5	20 (24)	14 (35)	18 (17)	1 (1)	43 (17)	4 (6)
4	14 (15)	23 (49)	17 (15)	1 (1)	40 (15)	5 (6)
3	20 (20)	23 (47)	16 (13)	2 (1)	35 (12)	4 (6)
2	13 (13)	26 (53)	18 (14)	1 (1)	36 (12)	6 (7)
1	13 (17)	12 (34)	14 (15)	0	56 (26)	5 (8)

If the factors for converting diffracted intensity of the minerals to percent are corrected, we can use the amount of each mineral present plus organic matter to estimate the total exchange capacity of each segment or the estuary. Cation exchange capacities for the various minerals and organic matter are from Carroll (1959). The estimated exchange capacity of the suspended material is 207 X 10^6 meq (Table 5). A very large part of this capacity is due to organic matter: Ninety-two percent in segments 2 and 3; 85 and 74 percent in segments 4 and 5, respectively.

Despite using an extremely low value of 170 meq./100 g, the exchange capacity of organic matter over-shadows the capacities of the minerals. Using a larger value would only increase the percentage that could be allocated to organic matter.

Table 5. Mass of mineral and organic matter suspended in Rhode River and estimated cation exchange capacity of suspended material.

Segment	Mass of Mineral X 10^6g	Mass of organic material X 10^6g	Cation exch. capacity X 10^6 meq.
2	22.8	44.7	82.9
3	21.0	53.9	99.7
4	7.9	7.9	15.8
5	5.1	3.7	8.5
		ε	207

SIGNIFICANCE TO THE WASTE-WATER PROGRAM

This project, by itself, has little significance to siting of waste-water plants. Mineral solids are not part of the effluent and, in most cases, solids are removed from the effluent. Thus, the project can be evaluated only in the context of the overall program and the interaction of suspended particulates with other materials, either suspended or dissolved, in the water column.

One of the projects of the waste-water program was segmintation of the Bay and its tributaries so that criteria developed in one segment could be applied to all similar segments. The Rhode River has been divided into 5 segments. Insofar as the type and quantity of suspensates, only the boundary between segments 5-4 and 4-3 seem to be valid boundaries. The boundaries between 3-2 and 2-1 appear to be arbitrary since our data strongly suggests no change at the boundaries but a transition from segment 3 into the Bay proper.

Segmentation is based on conservative properties. These properties are, by nature and definition, maintained and can be used for mixing and dilution studies. They do not interact with materials in the effluent nor those that arise as a result of the dissolved substances in the effluent.

The conservative properties can be used for developing siting criteria if dilution and mixing appear to be the best way to reduce the concentrations of deleterious substances emanating from the waste-water plant. If, on the hand, removal of the substances from the water column, then suspensates and other non-conservative properties may be a more valid criteria.

Siting would be preferable in areas with high concentrations of suspended material, having large exchange coefficients, if removal from the water column is preferred. For the Bay area, this will occur in the uppermost parts of the estuaries where there is a relatively high discharge of solids from the streams.

It is obvious that a decision to locate a plant in such an area generally precludes the use of such areas for other activities. Essentially, fishing and recreational use would be prohibited.

MAJOR CATIONS IN SUSPENDED PARTICULATES

J. W. Pierce and Tung L. Wu
Smithsonian Institution

INTRODUCTION

Most of this study was supported by funds from the Smithsonian Institution, with a minor amount from the NSF-RANN money available to Dr. J. W. Pierce.

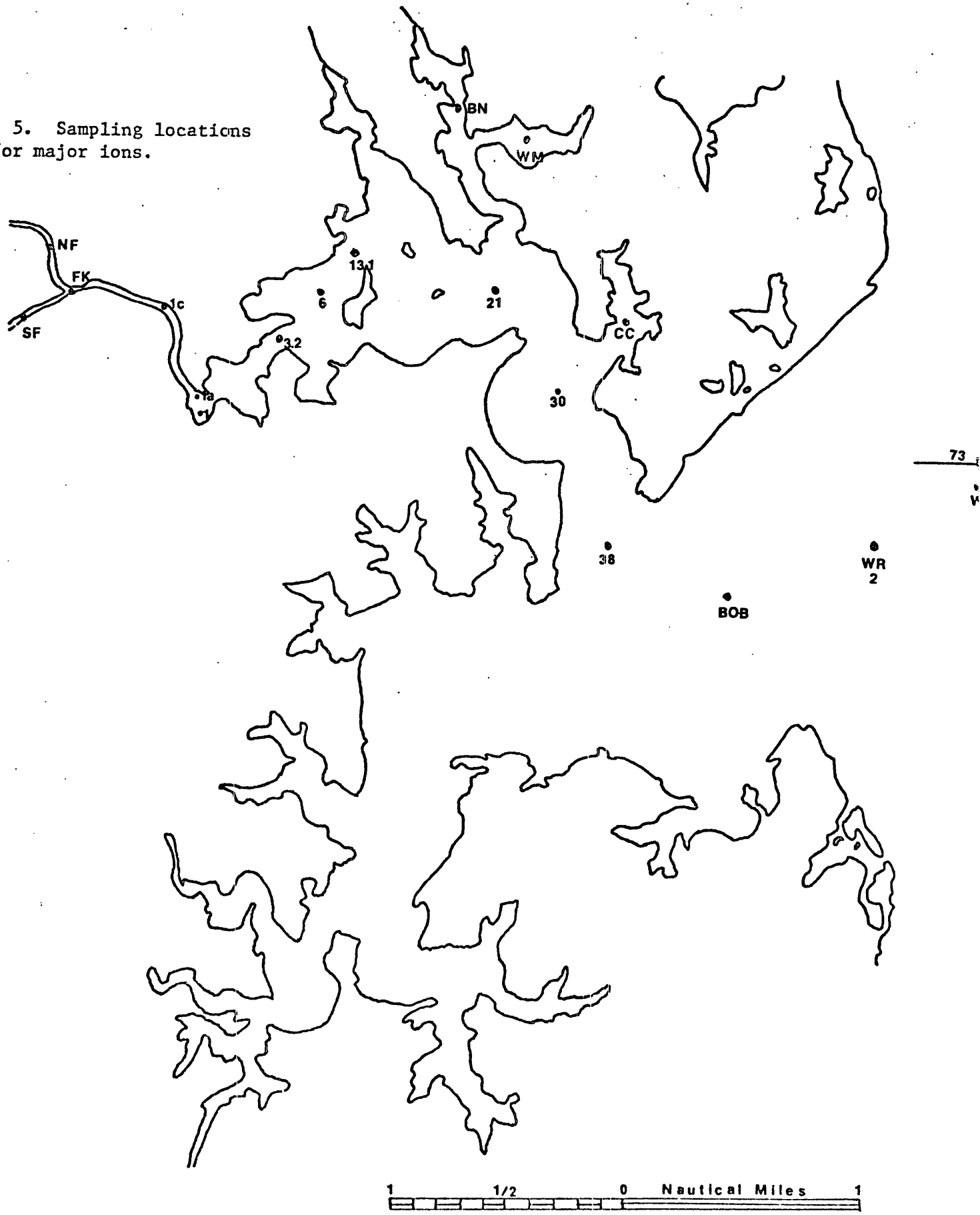
The study deals with the major cations plus iron and silicon in the Rhode River-Muddy Creek fluvial-estuarine system. These elements are important in understanding physico-chemical processes in a natural estuarine system because they are always present in large amounts; hence the analyses are easy. In an estuarine system, none are considered limiting factors in biologic processes.

EXPERIMENTAL

Sample Collection

Water samples from the surface layer and from the maximum turbidity zone were pumped into plastic containers at each station (Fig. 5). The water was filtered, within 24 hrs, through 47-mm diameter, 0.45 μm diameter Millipore HA filters. Bottom samples were obtained with an Eckman dredge. The central part of each

Fig. 5. Sampling locations for major ions.



dredge haul was taken and the surficial material and that from a depth of 3 cm roughly separated.

Sample Preparation

Two hundred fifty ml of river water were passed through one filter and the filtrate saved for analysis of dissolved major ions. The particulates on the filter were rinsed three times with 5 ml of distilled water. Duplicate samples of the solids in each sample were prepared: one for analysis on the total material and the other for elements in the different fractions.

Bottom sediment samples were air-dried for about 3 weeks, ground, and sieved through a 1 mm screen.

Total Solids

The filter with the air-dried particulates was heated to 250°C for 10 minutes in a covered, pre-ignited, graphite crucible to char the filter; 100 mg of lithium metaborate added and mixed well with the sample. The sample was ignited at 1000°C for ten minutes and the resulting red-hot, fused sample poured into a polyethylene bottle containing 10 ml of 0.5N HCl solution. Dilution (up to 30 times with distilled water), after dissolution, was occasionally necessary for the subsequent atomic absorption analyses.

Extractable Fractions

A sample of solids from 250 ml of river water was washed with distilled water, air dried, and placed in a 50 ml polypropylene tube. Twenty-five ml of 0.1N HCl were added, the tube centrifuged for 20 minutes at 2000 rpm and set aside for 1 hour. The HCl solution was decanted and set aside for analysis as the HCl extractable fraction.

The filter and particulate remaining in the tube were boiled twice in 2 ml of concentrated, reagent-grade nitric acid, evaporated to about 0.5 ml, diluted with 10 ml distilled water, and boiled for 30 minutes. After cooling, the solution was diluted to 30 ml and set aside for analysis as the HNO_3 extractable fractions and the total solids.

Bottom Sediments

Sediments were air-dried for about three weeks, ground and sieved through 1 mm screens. Twenty mg were weighted accurately into a pre-ignited graphite crucible and 100 mg of lithium metaborate added. The resulting mixture was fused at 1000°C for 10 minutes, the red-hot, fused sample poured into 10 ml 0.5N HCl. Lanthanum nitrate was added to make a 1% solution of $La(NO_3)_3$. This solution was set aside for later analysis.

Analytical Methods

A Jarrell-Ash 82-500 atomic absorption spectrophotometer was used for cation analyses. A Beckman DU spectrophotometer was used for silicon. Dissolved reactive silicate was measured according to Strickland and Parsons (1972) with slight modifications.

RESULTS AND DISCUSSION

Dissolved Ions

Samples for dissolved ions were collected on 18 January, 15 April, and 17 June, 1974.

The concentrations of major cations generally increase down-estuary, as would be expected (Table 6). The increase is primarily a mixing phenomena of fresh water stream discharge and more saline Bay water. Samples taken on 18 January maintain essentially constant ratios in all samples for major ions, nearly the same ratios as for normal marine waters. More variability and departure from fixed ratios are noted for other sampling dates. There is a surprising enrichment in sodium most of the time, with considerable variability.

Magnesium is relatively lower during the warmer months, suggesting uptake by biological processes. It is possible that uptake into chlorophyll may account for some of the change in magnesium. Our other data does not negate or prove this possibility.

Table 6. Concentrations of major dissolved ions in Rhode River. Sampling locations on Figure 5.[1]

Element	Sampl. Date	Concentration (10^3 x μgat/l)																	
		Bay	West River			Rhode River										Muddy Creek			
		73	#1	#2	BOB	38	30	Cadle	21	BN	WM	13.1	6	3.2	1	1a	1c	FK	SF	NF
Na	1-18	186 192	192	186		168			171			143			71	90	93	37	65	62
	4-15				452	231	117	139	117	192	244	583		91.5	39.1			8.7	2.5	26
	6-17	220 150			154 126	214 218	119 227	119 104	117 176	108		161 102	106 192	100	91 69.5		39		17	19.6
K	1-18	4.50 4.61	4.61	4.50		4.29			4.34			3.82			2.35	2.72	2.75	.94	1.83	1.99
	4-15				2.46	2.20	2.26	2.46	2.61	2.52	2.46	2.26		1.70	.72			.20	.20	
	6-17	4.09 4.23			3.20 3.33	3.20	3.33 4.00	3.33 3.20	3.20 1.16	2.82		3.20 3.71	3.09 3.33	2.82	2.33 1.28		2.45		.91	

1. Values in table are for samples from the maximum turbidity zone except where two values are shown. The upper value then is for the surface layer; the lower value for maximum turbidity.

Table 6 - continued

Element	Sampl. Date	Concnetration (10^3 x µgat/l) Bay 73	West River #1	#2	BOB	Rhode River 38	30	Cadle	21	BN	WM	13.1	6	3.2	1	Muddy Creek 1a	1c	FK	SF	NF
Ca	1-18	2.36																		
		2.41	2.41	2.33		2.21			2.18			1.95			1.20	1.39	1.46	.56	.94	1.01
	4-15				2.16	1.92	1.92	1.92	2.16	2.16	2.16	1.92		1.44	.48				.24	
	6-17	3.01			2.89	2.76	2.76	2.45	2.70	2.64		2.76	2.70	2.64	2.07					
		2.64			2.26	2.26	2.64	2.07	2.76			2.07	2.26		2.01		1.52		1.0	.44
Mg	1-18	14.2																		
		14.2	14.2	13.3		13.3			12.5			10.0			6.3	6.7	7.5	.3	4.6	5.0
	4-15				8.68	9.04	8.50	8.84	8.68		8.76	6.94		7.90	3.34			.44	.69	.69
	6-17	10.72			10.7	9.05	10.07	7.94	7.94	8.44		7.94	9.35	8.82	6.65					
		9.80			8.96	8.35	10.10	8.44	9.99			7.94	7.94		5.63		2.90		.83	1.67
Si**	1-18	29.4																		
		29.3	28.3	30.4		32.2	26.6		31.6			41.2			116	88.6	81.8	167.8	138.8	129.5
	4-15				51.6	53.4	59.5	31.4	50.1	48.0	52.8	61.5		157	332			412	456	378
	6-17	33.8			56.8	87.8	83.5	75.4	65.0	102.5		113.5	119	136	183.5					
		32.6			67.4	97.6	45.7	102	47.5			81.2	98		177		189.2		548	310

** Concentrations in µgat/l

Not surprisingly, dissolved silicate undergoes a distinct decrease down-estuary. The source of dissolved silica is the soils of the watershed. The decrease can be the result of utilization by siliceous plankton and removal from the dissolved phase to the particulate phase, in addition to the dilution factor. Crude estimates can be made of the loss by a combination of the two processes but, at the present time, they can not be separated.

By using chlorinity values, a "mixing-index" of fluvial and Bay water can be obtained. Then, by using this mixing index, concentrations of Si that should occur may be calculated. Departures of measured values indicate loss of Si by some process (Table 7).

HCl Extractable Fraction

The ions released by exchange with H+ are assumed to be those in exchangeable positions in the minerals. Carbonates would also be attacked but the low values of Ca indicate that carbonates probably are an insignificant part of the suspended material. Si and Fe are not generally exchangeable ions and their presence may indicate some attack on clays or amorphous material.

The data on exchangeable ions are difficult to interpret (Table 8). Laboratory experiments indicate that Na is not a preferred ion in soil minerals (Marshall, 1964), yet our values show that the HCl fraction is generally higher than the other

Table 7

Calculated and Measured Dissolved Silica Concentration for Rhode River Segment 3.

Date	Mixture Bay / fluvial	Si Conc. 10^3 ugat/l Bay / fluvial	Cale. conc. Si Segment 3 10^3 ugat/l	Measured conc. conc. Si Segment 3 10^3 ugat/l
18 Jan.	.33 / .67	32.2 / 116	88.35	31.6
15 April	.98 / .02	53.4 / 332	58.97	50.1
17 June (surf)	.94 / .06	87.8 / 183.5	93.54	65.0

Table 8
Ions extracted by 0.1N HCl wash from suspended material taken on June 17, 1974.
Given in ugat/l of river water.

Sample	Sample Number	Concentration (ugat/l of river water)					
		Na	K	Ca	Mg	Fe	Si
SF-surface	g1	.2	1.23	.2	2.6	31.5	12.9
mxa. turb.	no						
NF-surface	g4	*	*	*	*	*	*
max. turb.	no						
1c-surface	g5	0	.72	.2	3.1	17.9	7.1
max. turb.	no						
1-surface	g6	1.0	1.25	.2	3.8	15.4	4.5
max. turb.	5	0	.91	0	2.5	12.9	1.3
3.2-surface	g3	3.1	.72	.1	3.1	4.7	5.2
max. turb.	no						
6-surface	g9	12.9	.62	0	1.7	.72	3.9
max. turb.	k	20.2	1.33	.1	2.8	.72	2.6
13.1-surface	g10	2.1	.71	0	2.3	.72	2.2
max. turb.	8	2.1	.92	0	2.6	4.3	2.6
Bear Neck-surf	g11	2.2	.61	0	1.2	.72	2.2
max. turb.	no						
21-surface	g7	2.6	.51	0	1.7	.72	1.1
max. turb.	4	0	.91	0	2.5	.72	6.5
Cadle-surface	B	5.2	1.13	0	2.3	2.5	.7
max. turb.	10	3.8	.62	.3	1.7	.72	.5
30-surface	0	1.0	.82	0	2.0	..72	2.6
max. turb.	9	3.5	.82	.8	4.0	2.15	2.7
38-surface	gk	*	*	*	*	*	*
max. turb.	I	0	1.1	0	2.3	0	3.8
BOB-surface	17	*	*	*	*	*	*
max. turb.	H	4.4	.72	.2	1.7	0	2.0
Bay 73-surface	73	15.7	.82	.0	1.7	.72	8.1
max. turb.	c	1.0	1.2	.1	3.3	3.9	2.7

*Sample Lost

cations becoming more so down estuary. This would substantiate that concentration in solution plays an important part. Data from station 6 appears to be normal for all elements except Na, which is anomalously high. We have no reason to question this value except for its magnitude. It is possible that the sample was not washed sufficiently to remove the salt.

The minimum total adsorbed cations in a sample would be a summation of the major ions in the HCl extractable fraction. From this, the total absorbed ions and exchange capacity may be estimated. Our data (exclusive of sta. 6) show exchange capacities of from 10 to 65 milliequivalents/100 g (Fig. 6). This is slightly higher than that reported for selected eastern streams (Kennedy, 1965) and may be due to the very low values of organic material in Kennedy's samples.

The exchange capacity of the suspended phases is the result of an interaction between the mineral fraction and organic fraction as well as compositional changes within the two fractions. The changing compositions and mineral : organic ratios make the system exceedingly complex.

It should be pointed out that the exchange capacities calculated in a previous section from the mineral and organic content range from 96 to 133 meg/100 g. This is a considerably larger exchange capacity than that estimated in this section.

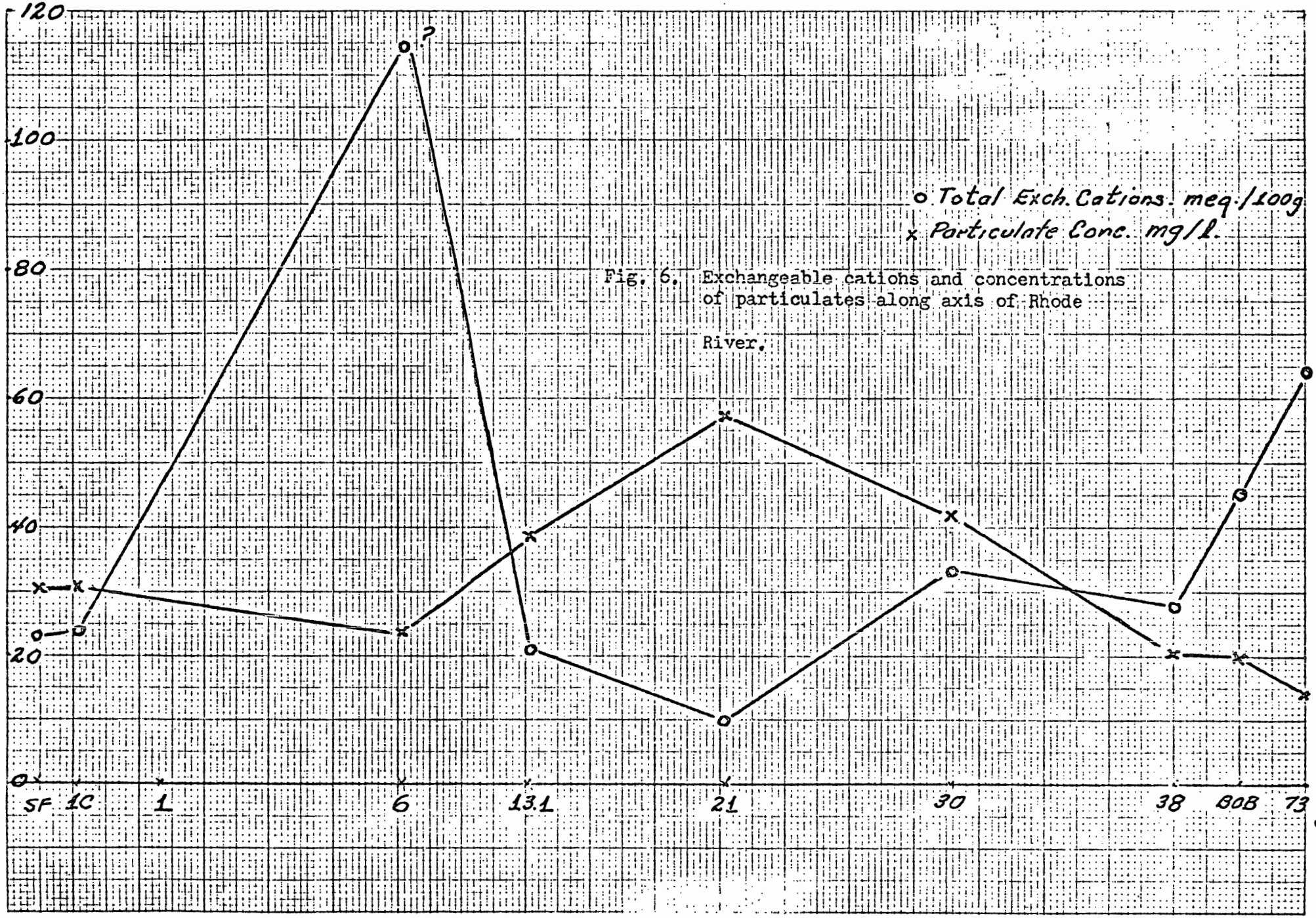


Fig. 6. Exchangeable cations and concentrations of particulates along axis of Rhode River.

Marshall (1964) noted that the exchange capacity of a mineral/organic mixture is always lower, by as much as one-half, than the sum of the capacities of the two components. We could have also used incorrect mineral ratios or exchange capacities of the minerals or organics, since there is a range of capacities.

The HCl extractable fraction showed that some iron is either exchangeable or soluble in dilute HCl. Some of the iron hydroxides, present as coatings, are probably dissolved by the dilute acid.

HNO_3 Extractable Fraction

This technique breaks down the organic material. Some mineral structures may be degraded by this concentrated acid but most of the ions are believed to be associated with organic material. Because of interference, Si could not be determined on this fraction.

Iron has relatively high values and the amounts vary down the estuary (Fig. 7). The iron in both acid extracts is generally higher than the sum of the alkalies-alkaline earths. Fripiat and Gastuche (1973) report that the amount of iron, present as coatings on particles, was ten times the cation exchange capacity of the clays.

Station 6 is low in both acid extracts, most noticeably in the HNO_3 extract. It is also low in total particulate indicating a close association of iron and particulates. This is substantiated by the amount of iron in the non-extractable fraction (Table 10).

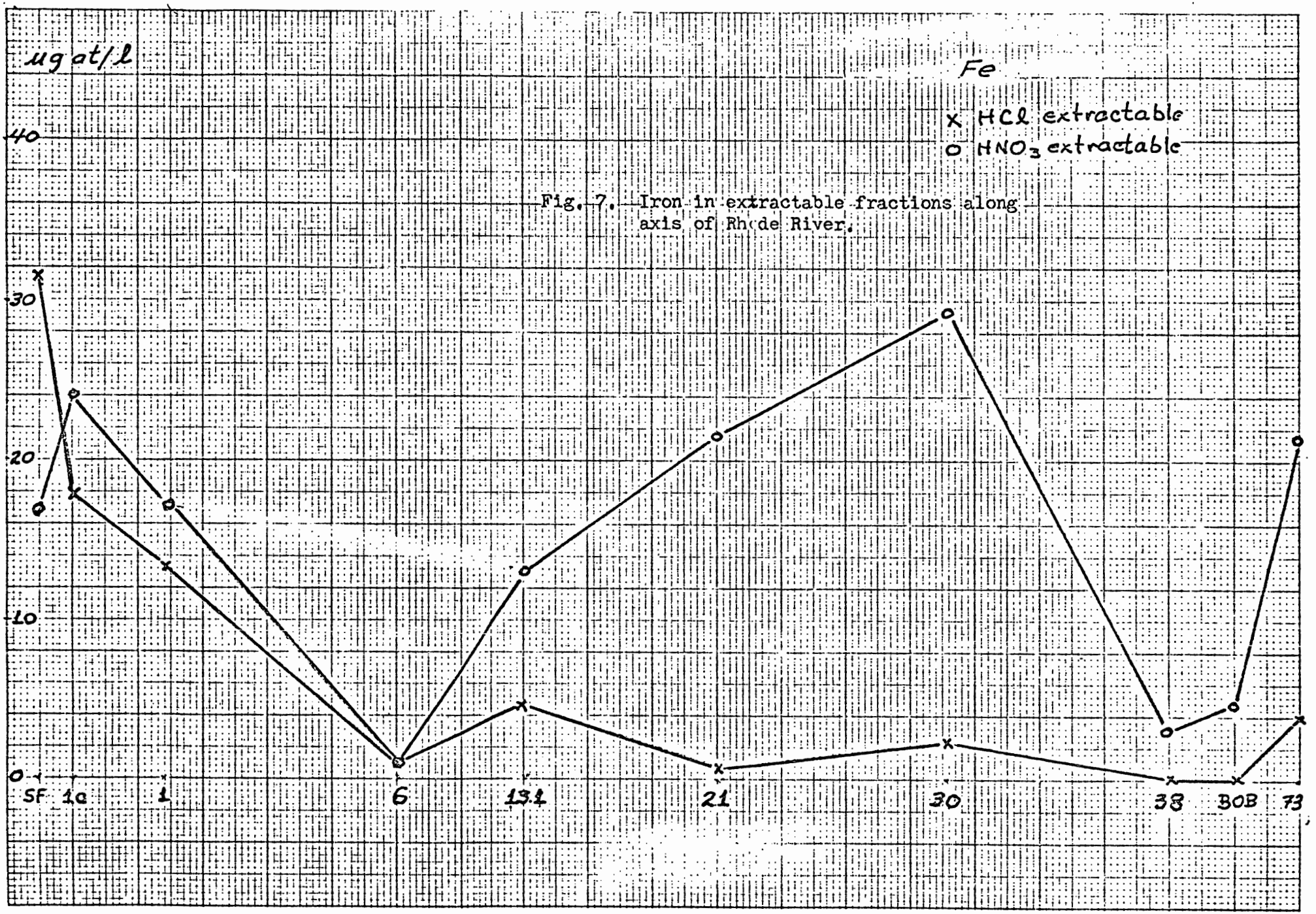


Fig. 7. Iron in extractable fractions along axis of Rhode River.

Table 9

Concentration of ions extracted by conc. HNO_3 from suspended material taken on June 17, 1974. Given in ugat/l.

Sample	Sample Number	Concentration (ugat/l of river water)					
		Na	K	Ca	Mg	Fe	Si
SF-surface	g1	1.0	2.46	0	2.0	16.6	-
max. turb.	no						
NF-surface	g4	*	*	*	*	21.4	-
max. turb.	no						
1c-surface	g5	0	2.46	0	1.5	24.1	-
max. turb.	no						
1-surface	g6	.7	*	0	3.0	18.7	-
max. turb.	5	1.0	3.23	0	3.5	16.6	-
3.2-surface	g3	1.0	2.28	0	2.0	14.0	-
max. turb.	no						
6-surface	g9	1.0	*	0	1.0	3.2	-
max. turb.	k	1.5	.72	0	1.0	.7	-
13.1-surface	g10	.5	1.54	0	1.0	3.2	-
max. turb.	8	.5	3.08	0	1.5	12.9	-
Bear Neck-surf	g11	0	*	0	1.0	3.2	-
max. turb.	nð						
21-surface	g7	.5	.62	0	1.0	3.7	-
max. turb.	4	.5	4.60	0	4.2	21.4	-
Cadle-surface	B	1.2	1.85	0	1.7	13.4	-
max. turb.	10	2.5	1.54	0	1.2	.5	-
30-surface	0	.5	1.68	0	1.0	6.5	-
max. turb.	9	2.0	6.16	0	3.5	29.0	-
38-surface	gk	*	*	*	*	*	-
max. turb.	I	1.2	.76	0	.5	3.2	-
BOB-surface	17	*	*	*	*	*	-
max. turb.	H	.5	1.23	0	1.0	4.3	-
Bay 73-surface	73	1.0	1.84	0	1.2	3.7	
max. turb.	c	.5	4.62	0	3.0	21.4	-

*Sample lost; - no data

Table 10

Concentration of ions in mineral fraction of 1 liter of water taken on June 17, 1974. Concentration obtained by difference between total sample and extracts

Sample	Sample Number	Concentration (ugat/l of river water)					
		Na	K	Ca	Mg	Fe	Si(*)
SF-surface	g1	.75	4.96	1.04	3.82	1.8	125.1
max. turb.	no						
NF-surface	g4	-	9.2(@)	1.35(@)	10.3(@)	36.4(@)	159.0
max. turb.	no						
lc-surface	g5	9.77	3.06	.81	2.70	2.0	51.5
max. turb.	no						
1-surface	g6	5.12	6.21(@)	.70	.70	0	97.0
max. turb.	5	0	0	.45	0	0	145.2
3.2-surface	g3	27.61	7.9	.80	7.1	0	105.3
max. turb.	no						
6-surface	g9	27.31	3.53(@)	1.01	3.12	2.73	31.1
max. turb.	k	0	0	.35	0	2.02	24.2
13.1-surface	g10	12.05	0	1.24	2.32	1.73	27.4
max. turb.	8	5.2	5.76	.45	4.33	.70	126.9
Bear Neck-surf.	g11	9.50	1.0(@)	.56	.79	1.53	21.4
max. turb.	no						
21-surface	g7	6.73	1.17	.56	.76	2.23	35.3
max. turb.	4	14.15	6.0	.45	2.68	0	140.0
Cadle-surface	B	3.94	0	.56	0	0	47.3
max. turb.	10	1.01	0	.04	0	1.83	23.1
30-surface	0	9.25	0.	.34	0	0	26.5
max. turb.	9	7.22	10.32	0	5.65	0	229.3
38-surface	gk	-	1.15(@)	.11	1.87(@)	3.04(@)	23.0
max. turb.	I	11.48	0	.11	0	.25	26.4
BOB-surface	17	-	.34(@)	.11	.94(@)	.64(@)	6.7
max. turb.	H	4.93	0	0	0	0	28
Bay 73-surface	73	0	0	0	0	0	1.85
max. turb.	c	14.13	6.88	.46	4.95	0	161.3

- no data

(@) greater then true value because of unknown amounts in other fractions.

(*) greater then true value. Includes both mineral and HNO_3 extractable

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STANDING CROP, REFLECTANCE, AND NET CO_2 EXCHANGE IN THREE SALT MARSH COMMUNITIES

(Project No. 20)

Bert G. Drake, Principal Investigator

Smithsonian Institution

Radiation Biology Laboratory

12441 Parklawn Drive, Rockville, Md. 20852

Total Budget: $ 30,252

Personnel

Dr. Michael Read	Research Associate
Vete Clements	Technical Assistants
Roger Ford	
Tom Roman	
Douglas Hayes	

PROJECT OBJECTIVES

A. To measure net CO_2 exchange (net primary productivity and respiration) in m^2 sections of marsh communities of Kirkpatrick Marsh for selected weekly intervals during a two year period.

B. To measure net photosynthesis in leaves of individual species during measurements of net CO_2 exchange of marsh communities in order to partition net CO_2 exchange into net photosynthesis and respiration.

C. To measure the standing crop biomass by clipping in communities in which net CO_2 exchange is being studied.

ABSTRACT

Standing crop biomass, net carbon exchange, and a non-destructive radiometric technique for estimating green standing crop were studied in Kirkpatrick Marsh during the summers of 1973 and 1974. Peak of growth in three communities of salt marsh vegetation, dominated by the sedge Scirpus olneyi; the shrub Iva frutescens, or the grass mixture Spartina patens - Distichlis spicata occurred during mid to late August. Net standing crop of green biomass in these communities at peak of growth was between approximately 300 and 600 g m^{-2}.

It was shown that in at least one community type, the sedge, the ratio of the reflectance of far red (775-825 nm) to red (655-705 nm) can be used to predict the standing crop biomass of the community during the first two months of growth with nearly the same accuracy as can the clipping method.

The measurement of net CO_2 exchange over sections of marsh community was successfully carried out. Two marsh communities (the sedge and the grass) have an estimated quantum efficiency of .04 (moles carbon assimilated/ moles quanta absorbed). This is approximately 2/3 the quantum efficiency of stands of corn.

I. Standing Crop Biomass

During the past year we made weekly measurements of green standing crop in several stands of the three most common communities in Kirkpatrick Marsh. These three communities will be identified in this report as being either that dominated by the shrub Iva frutescens, the sedge Scirpus olneyi, or the two grasses Spartina patens and Distichlis spicata. Each sample consisted of five randomly selected, circular, quadrats of 1/2 or 1/4 square meter area. The total standing crop was clipped, green plants separated from brown dead plants, and samples dried at 65° for 3 days. Preliminary experiments showed that this drying time was sufficient to give constant dry weight of the samples at this temperature.

A representative sample of each community with respect to the relative proportions of the biomass of each of the most important species is given in Table I along with the relative proportions of live and dead plant material.

The growth curves of each community as indicated by standing crop of green biomass are shown in figures 1, 2, and 3. Data points are means of 5 samples; representative standard deviations are also plotted. Open circles (0) are data collected in 1974 and closed circles (●) are from 1973.

Peak of green standing crop in the shrub and sedge communities occurred in mid to late August but in the grass community it occurred during early October.

Maximum green standing crop biomass (g m^{-2}) is approximately 375 for the sedge community; 580 for the grass mixture; and nearly 600 for the shrub community. The standing crop and root biomass of two communities during winter is given in Table II.

II. Green Standing Crop Biomass Estimated from Measurements of Reflectance of Red (655-705 nm) and Far red (775-825 nm) Radiation.

As vegetation greens up in the spring, the reflectance of red radiation decreases (due to chlorophyll absorption) and reflectance of far red increases (Fig. 4 taken from Pearson, 1973). We wanted to know whether annual changes in reflectance correlate with changes in standing crop of green biomass and if so, could this correlation be used to estimate standing crop of green plant biomass from measurements of reflectance?

Two silicon photo-diodes were equipped with interference filters. The spectral response of the photo-diodes and transmission characteristics of the filters are shown in figure 5 along with a spectral curve of solar radiation. Figure 6 is a scheme of the radiometer, digital read-out, and details of the measuring method. The apparent incident radiation in both red and far red was taken as the mean of irradiances from a white plate suspended beneath the radiometer before and after each reading. Relative

TABLE I. A representative sample of the relative proportions of important species with respect to biomass in each of the three communities studied

Sedge (*Scirpus olneyi*, *Distichlis spicata*, and *Spartina patens*) Date 7-23-74

	Total Dead ($g\ m^{-2}$)	Total Live ($g\ m^{-2}$)	Live + Dead ($g\ m^{-2}$)	% Dead	% Live	% Live *Scirpus olneyi*	% Live *Spartina patens* and *Distichlis spicata*
	368	276	644	57	43	51	49
	436	352	788	55	45	50	50
	308	260	568	54	46	57	43
	284	440	724	39	61	40	60
	368	296	664	55	45	61	39
$\bar{x}$	353	325	674	52	48	52	48

Shrub (*Iva frutescens* and other species) Date 7-24-74

	Total Dead ($g\ m^{-2}$)	Total Live ($g\ m^{-2}$)	Live + Dead ($g\ m^{-2}$)	% Dead	% Live	% Live *Iva frutescens*	% Live *Scirpus olneyi*, *Spartina patens* and *Distichlis spicata*
	98	508	606	16	84	59	41
	12	300	312	4	96	53	47
	244	476	720	34	66	82	18
	150	436	586	26	74	74	26
	-	552	-	-	-	62	38
$\bar{x}$	126	454	556	20	80	66	34

Grass (*Spartina patens* and *Distichlis spicata*) Date 8-01-74

	Total Dead ($g\ m^{-2}$)	Total Live ($g\ m^{-2}$)	Live + Dead ($g\ m^{-2}$)	% Dead	% Live
	260	236	496	52	48
	256	236	492	52	48
	300	204	504	60	40
	400	184	584	68	32
	424	280	704	60	40
$\bar{x}$	328	228	556	58	42

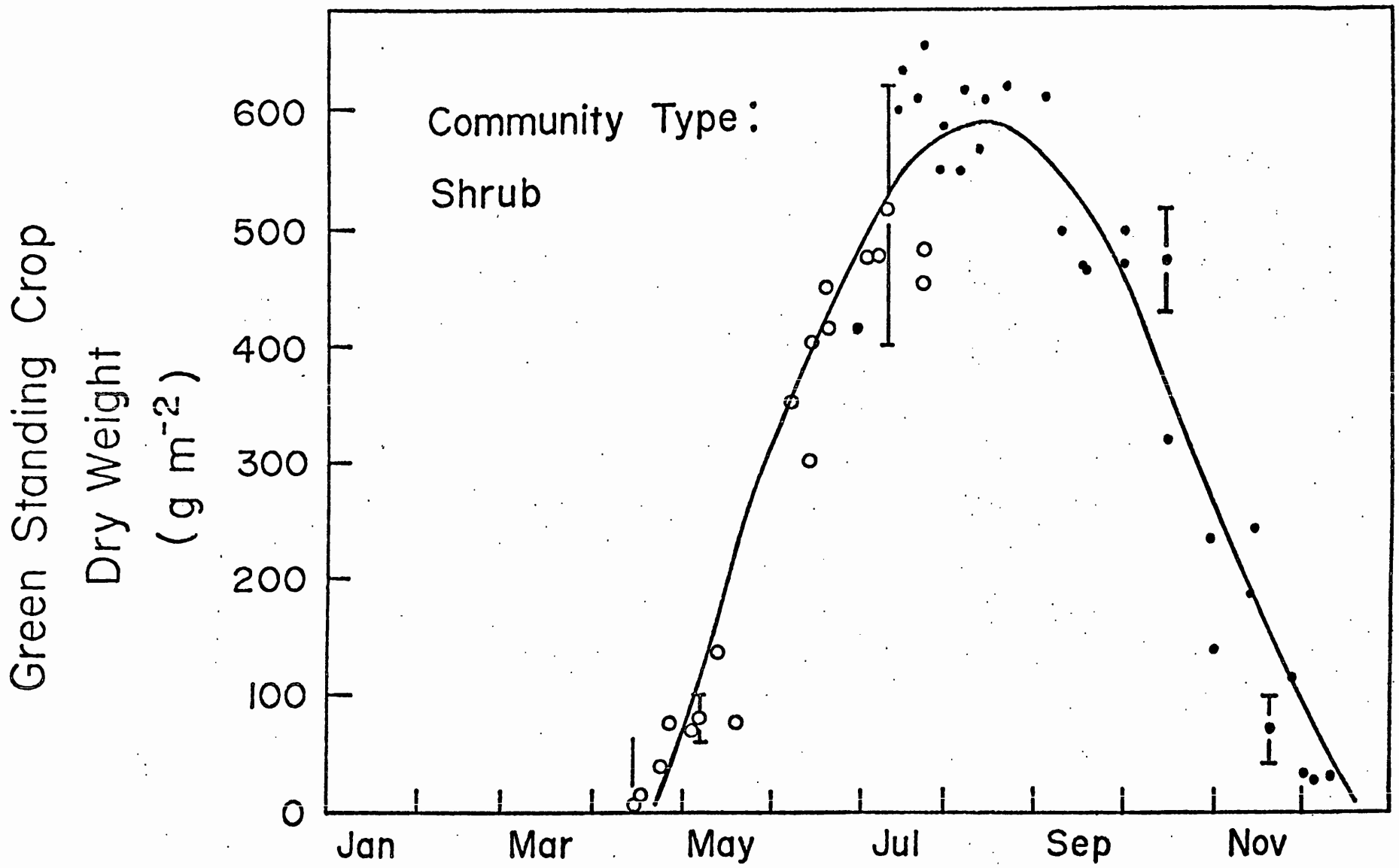


Figure 1: Green Standing Crop Biomass for the Shrub Community (Iva frutescens). Open circles (0) are taken from 1974, closed circles (●) are from 1973.

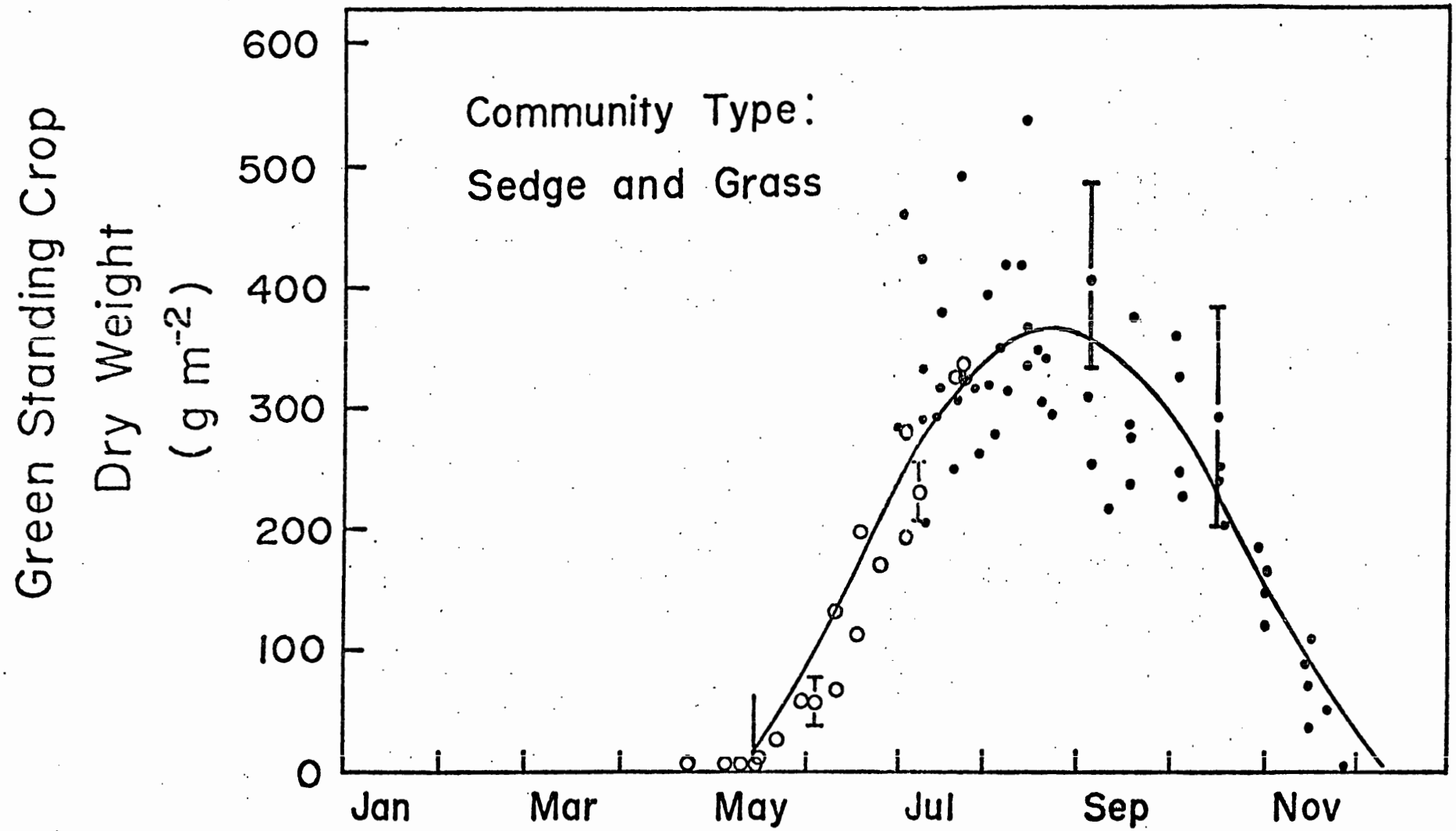


Figure 2: Green Standing Crop Biomass for Sedge (Scirpus olneyi) and the Grass Mixture (Spartina patens and Distichlis spicata). Open circles (0) data for 1974, Closed circles (●) are 1973.

Figure 3: Green standing crop biomass in the grass community, a mixture of Spartina patens and Distichlis spicata. Open circles (0) data taken in 1974, closed circles (●) data taken in 1973.

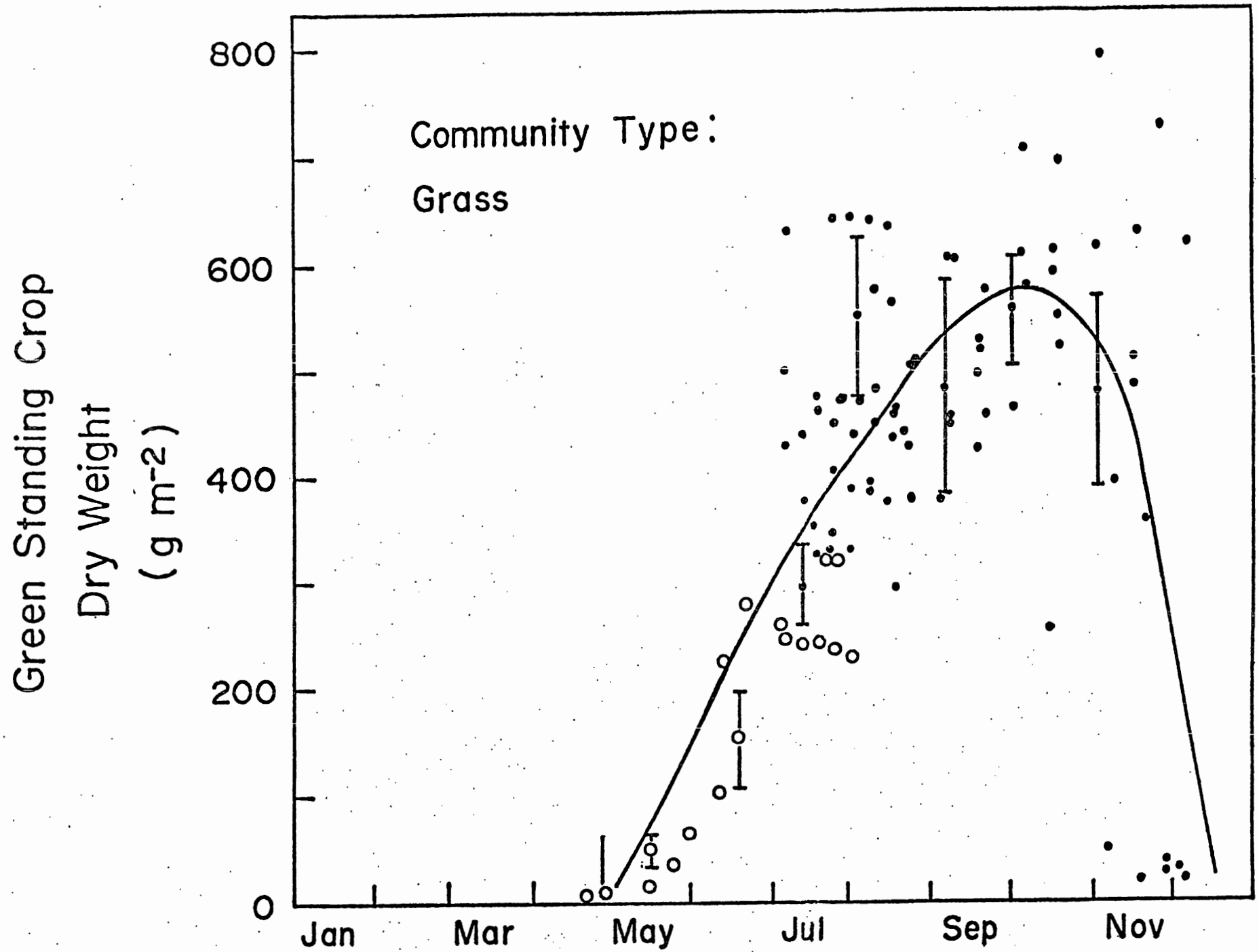

Community Type:
Grass
Green Standing Crop
Dry Weight
(g m-2)
800
600
400
200
0
Jan
Mar
May
Jul
Sep
Nov

Table II. Biomass above and below ground during winter 1974 for two communities in Kirkpatrich Marsh.

Community Type	Above Ground g m^{-2}	Below Ground g m^{-2}	
Grass (*Spartina patens* and *Distichlis spicata*)	1100	37,000	(to 28 cm depth)
Sedge (*Scirpus olneyi*, *Spartina patens*, and *Distichlis spicata*)	1000	23,000	(to 71 cm depth)

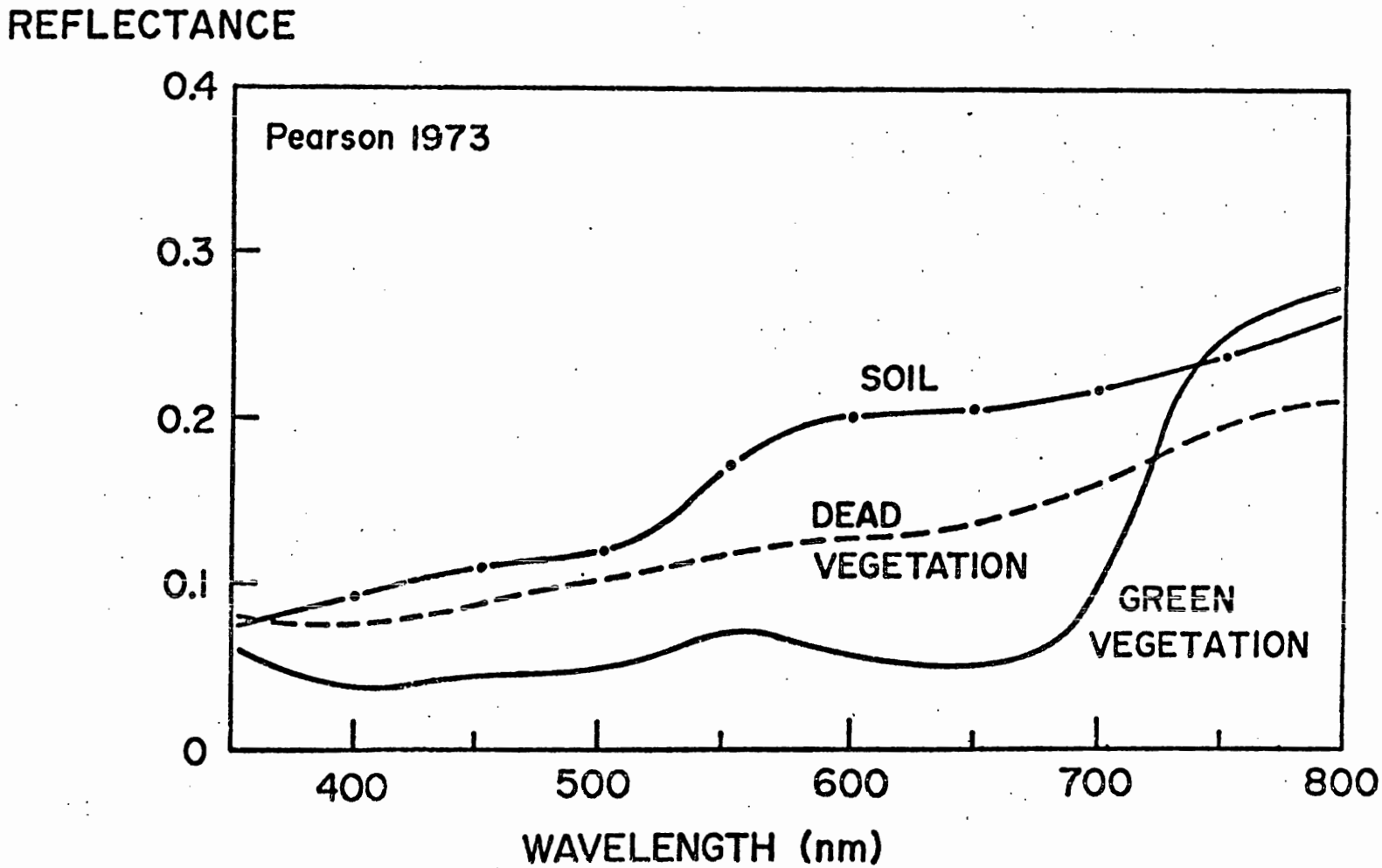


Figure 4: Spectral reflectance of soil, dead, and canopy of green vegetation (Bouteloua gracilis).

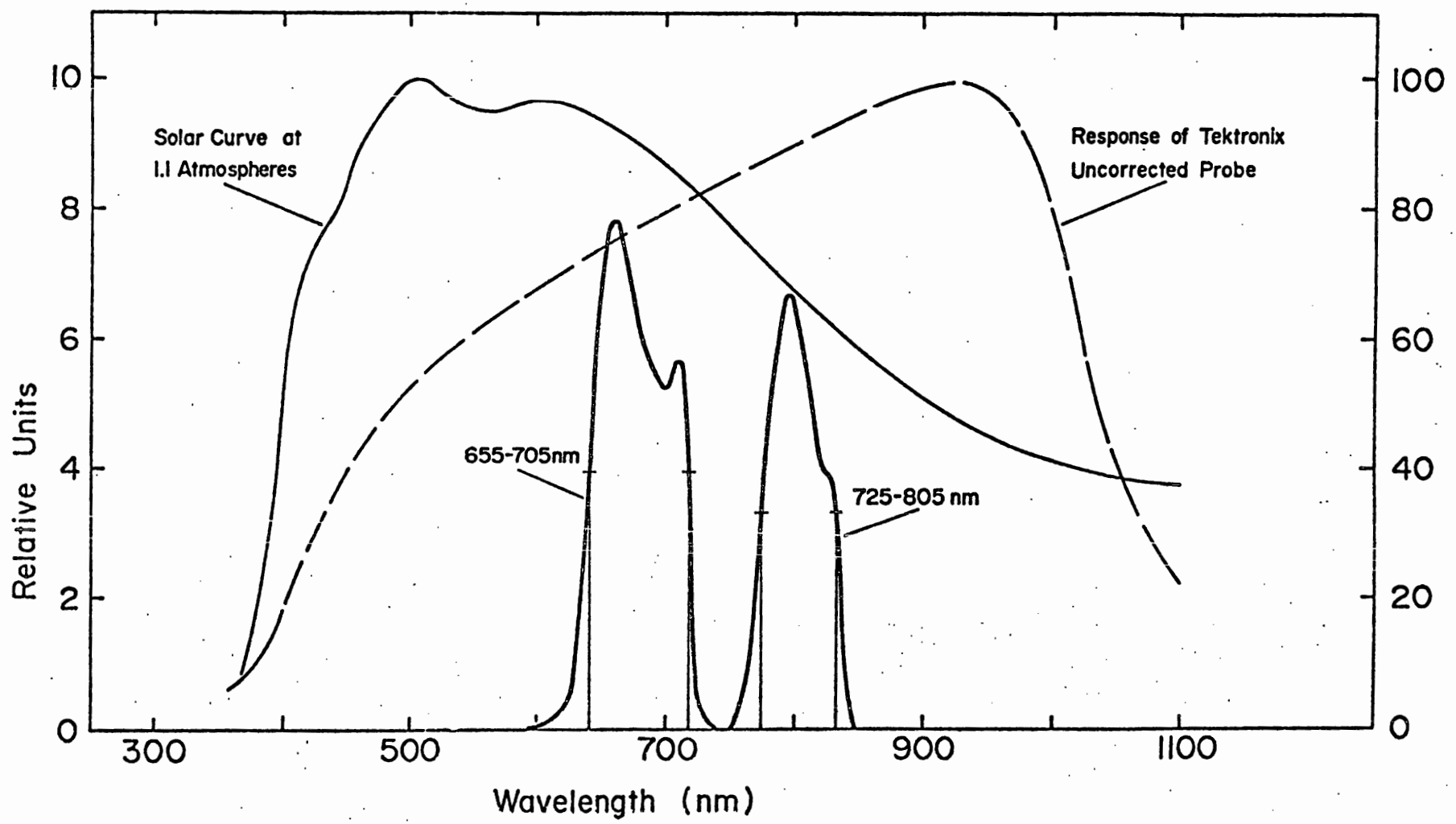
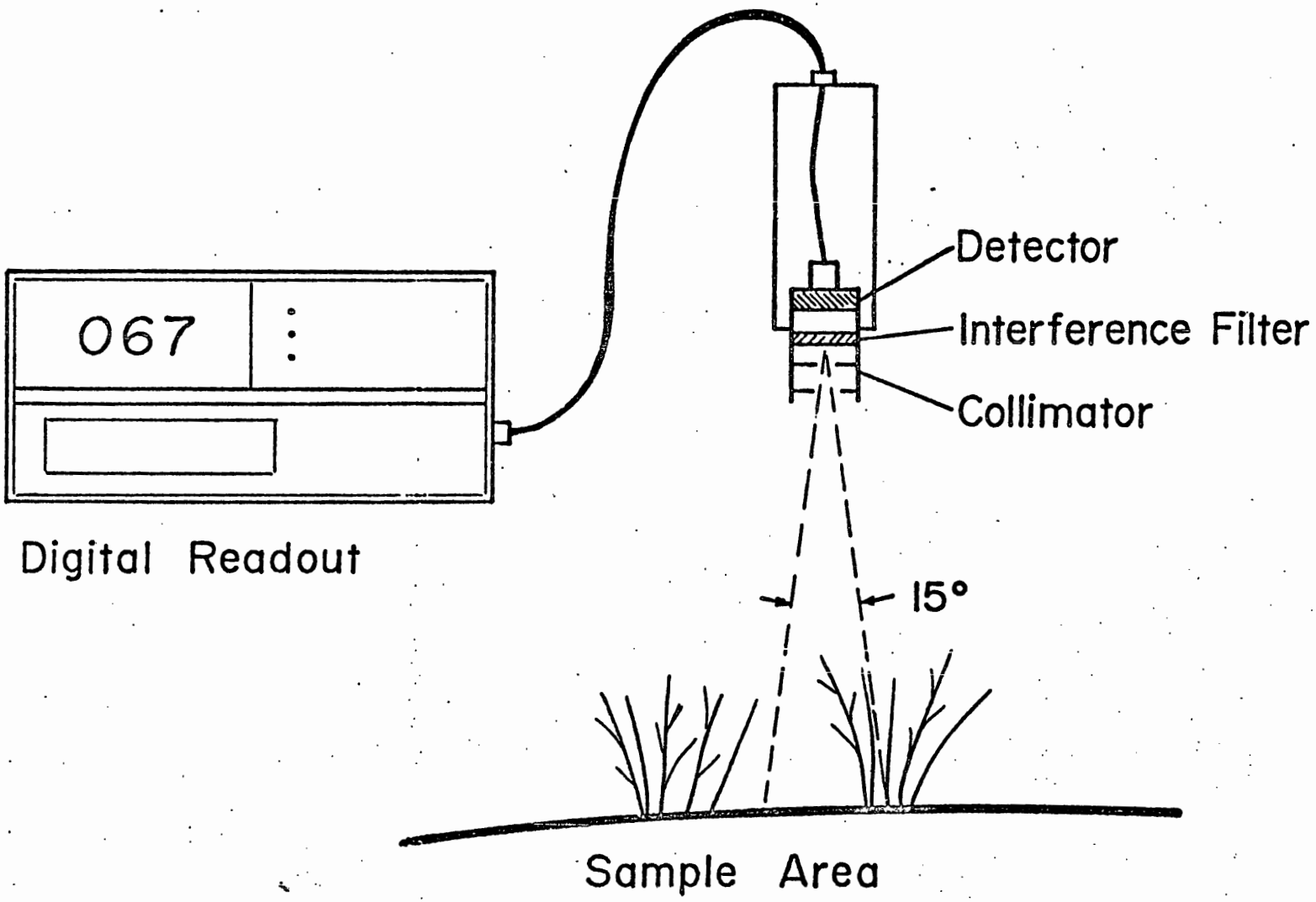


Figure 5; Transmission curves for the interference filters, response of the uncorrected silicon photo diode, and solar spectral curve.

Figure 6: Schematic of one of the two radiometers.



$$\text{Relative Reflectance } (\lambda_1 - \lambda_2) = \frac{I(\lambda_1 - \lambda_2) \text{ Reflected from Vegetation}}{I(\lambda_1 - \lambda_2) \text{ Reflected from Standard}}$$

reflectance is defined as the ratio of the radiation reflected from the vegetation to that which is reflected from the white plate.

$$R_{(\lambda_1-\lambda_2)} = \frac{I_{(\lambda_1-\lambda_2)} \text{ reflected from vegetation}}{I_{(\lambda_1\ \lambda_2)} \text{ reflected from standard}}$$

Measurements of reflectance were made on both cloudy and clear days but always between 1000 and 1400 hrs and only on days when incident radiant intensity was not changing rapidly.

Figure 7 shows results of an experiment in which green vegetation was removed randomly from a plot and reflectance was measured before each removal. The results are plotted as the inverse of reflectance of red radiation $R^{-1}_{(655\text{-}705\ nm)}$ against the measured standing crop of green biomass. Linear regressions were plotted through the data points and are given on the graphs along with correlation coefficients for the line through the data points. Results are shown for an experiment with the grass community, one with sedge, and one with the shrub. Data for the ratio of reflectance of far red to red are shown in figure 8 for the same experiment. This ratio is useful since it may be determined without the need for a measurement of the incident radiation and thus simplify measurement as well as eliminate another source of variation and error.

In several such experiments we found very high correlation coefficients between standing crop and reflectance. These results show that changes in reflectance coincide with changes in the amount of green material being viewed by the radiometer. However, for this technique to be useful, it is necessary to show that such changes in reflectance can be observed during seasonal changes in green standing crop biomass, and that a calibration of the instrument with live plant material is possible.

We measured reflectance of 5 random samples in stands of the three plant types, sedge, grass and shrub. After reflectance had been measured total plant material was collected and dried at 65° as outlined above in section I. In figures 9, 10, and 11 we show means of the 5 reflectances and means of dry weight of 5 samples plotted against the time of collection. Data collection was started in July 1973 and carried out weekly for each stand through July 1974. Data for 1973 is closed circles (●) and for 1974 open circles (○).

The results show seasonal changes in reflectance that coincide with changes in standing crop of green biomass. Linear regressions plotted through the data points for reflectance data collected before and after the onset of growth in spring intersect very close to the time of onset of growth in four of the six graphs.

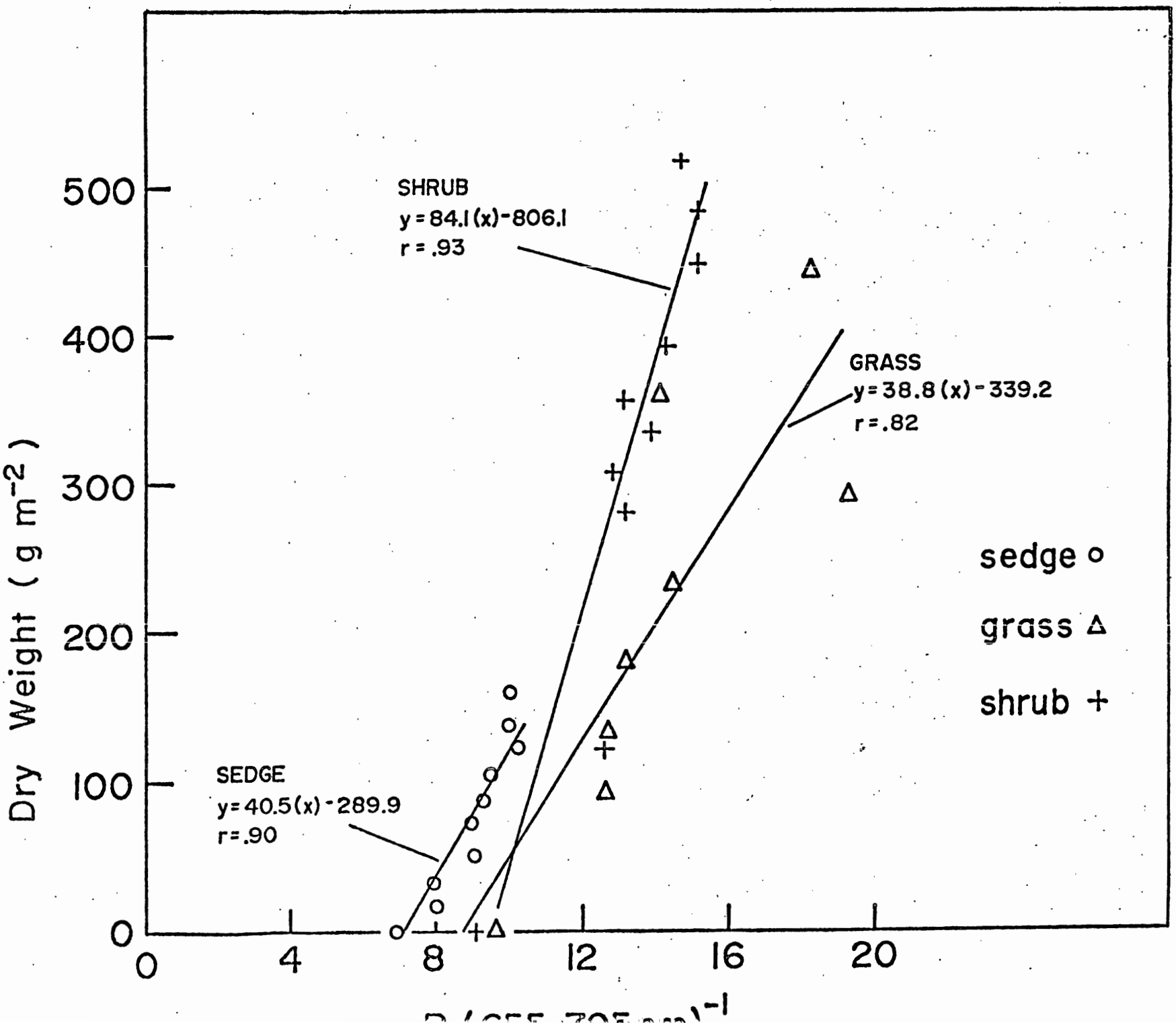
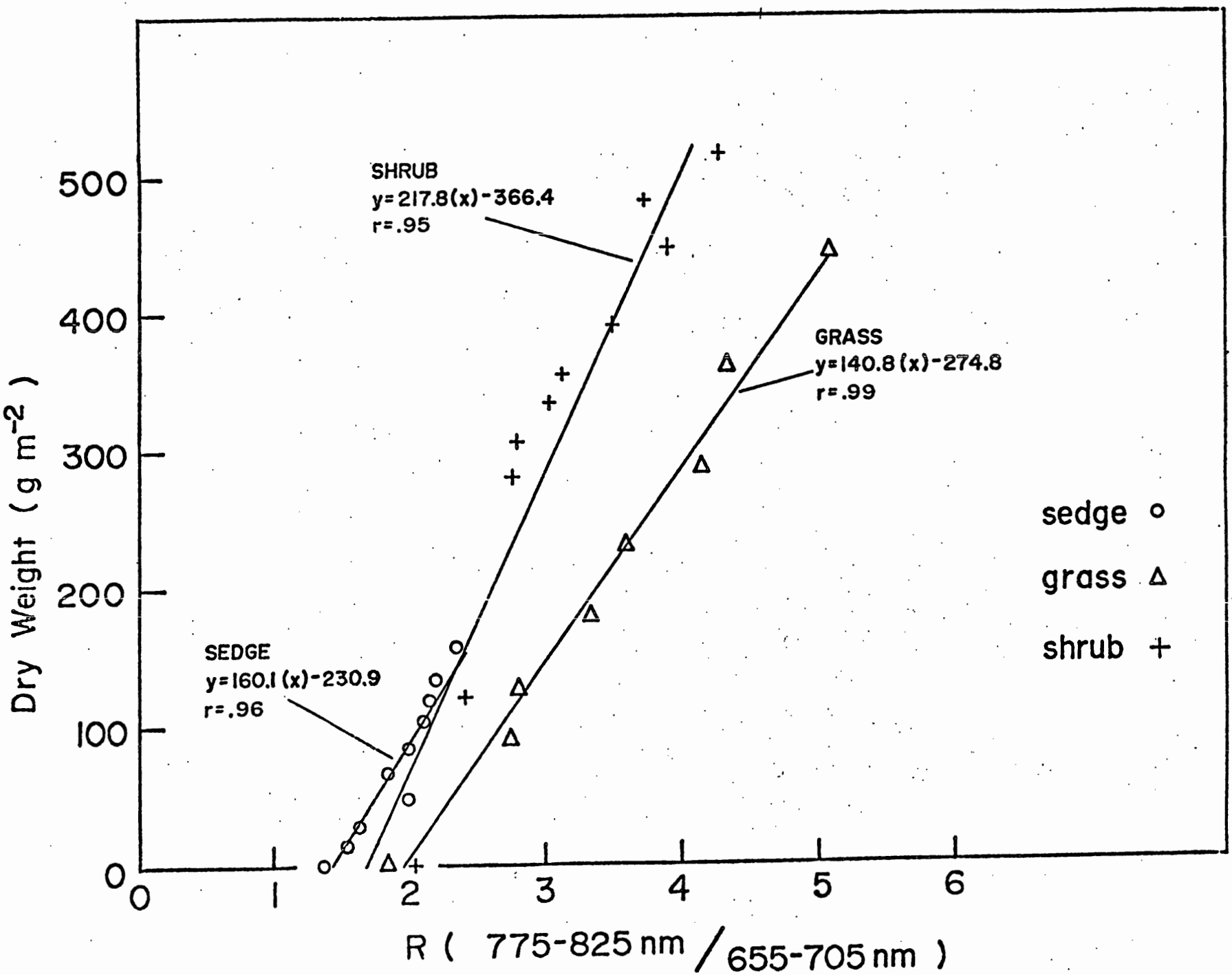

SHRUB
y = 84.1 (x) - 806.1
r = .93
GRASS
y = 38.8 (x) - 339.2
r = .82
SEDGE
y = 40.5 (x) - 289.9
r = .90
sedge o
grass Δ
shrub +
Dry Weight (g m-2)
0
100
200
300
400
500
0
4
8
12
16
20

Figure 7: Dry weight of green standing crop within a circular quadrat plotted opposite the inverse of reflectance of red radiation for the quadrat. Each set of data is a single experiment from a single quadrat.

Figure 8: Dry weight of green standing crop within a circular quadrat plotted opposite the ratio of reflectance of far red to red.


SHRUB
y=217.8(x)-366.4
r=.95
GRASS
y=140.8(x)-274.8
r=.99
SEDGE
y=160.1(x)-230.9
r=.96
sedge o
grass Δ
shrub +
Dry Weight (g m-2)
R (775-825 nm / 655-705 nm)
0
100
200
300
400
500
1
2
3
4
5
6

Correlations between standing crop of individual samples and the reflectance measured on each of the samples were computed. Several different methods of reducing the reflectance data were tried but the best correlations were obtained when the ratio of far red to red was plotted against standing crop. These results are shown in figure 12.

There is good correlation between the ratio of reflectance of far red to red radiation from an individual sample of the sedge community and dry weight of green standing crop biomass. Correlations for similar data for the grass community are also high but there is very little correlation between standing crop and reflectance in the shrub. When we compared the estimate of shrub community reflectance as measured by the mean of ten random measurements of reflectance with an estimate of community standing crop as measured by the mean of 5 random clipping samples of the shrub community, somewhat better correlations were obtained. Further processing of the data may yield a refinement in our approach to the raw data.

A test of this technique in estimating the community standing crop was made. Ten random measurements of reflectance in a community were taken. From the same community 5 random quadrats were selected and the green standing crop was measured by clipping. This procedure was repeated many times during the growing season. Reflectance data were converted to dry weight estimates using the linear regression data in figure 12. Then, the community standing crop estimated by clipping (0) and by reflectance (0) are shown in figure 13. A non-linear regression is plotted through the data points that were determined by clipping, and the correspondence between the standing crop of the communities estimated by reflectance and the standing crop of the community estimated by clipping can be seen.

Summary of Reflectance Data

There are clearly seasonal changes in reflectance that correlate with changes in green standing crop biomass of the three communities we have studied. In at least one case, the sedge community, we found that the ratio of reflected far red to red radiation could predict the standing crop of the community nearly as well as would clipping measurements. This method should receive further study, particularly with respect to the morphological basis for changes in far red reflectance, optimal band-widths for measurement of reflectance, and a simplified method of calibration.

IV. Net CO_2 Exchange in Sections of These Salt Marsh Communities.

Standing crop biomass cannot be used as a measure of the annual carbon balance of salt marsh communities because standing crop (including root matter) is not the amount of carbon that is eventually made available to consumers either on the marsh or in the estuary. In the salt marsh

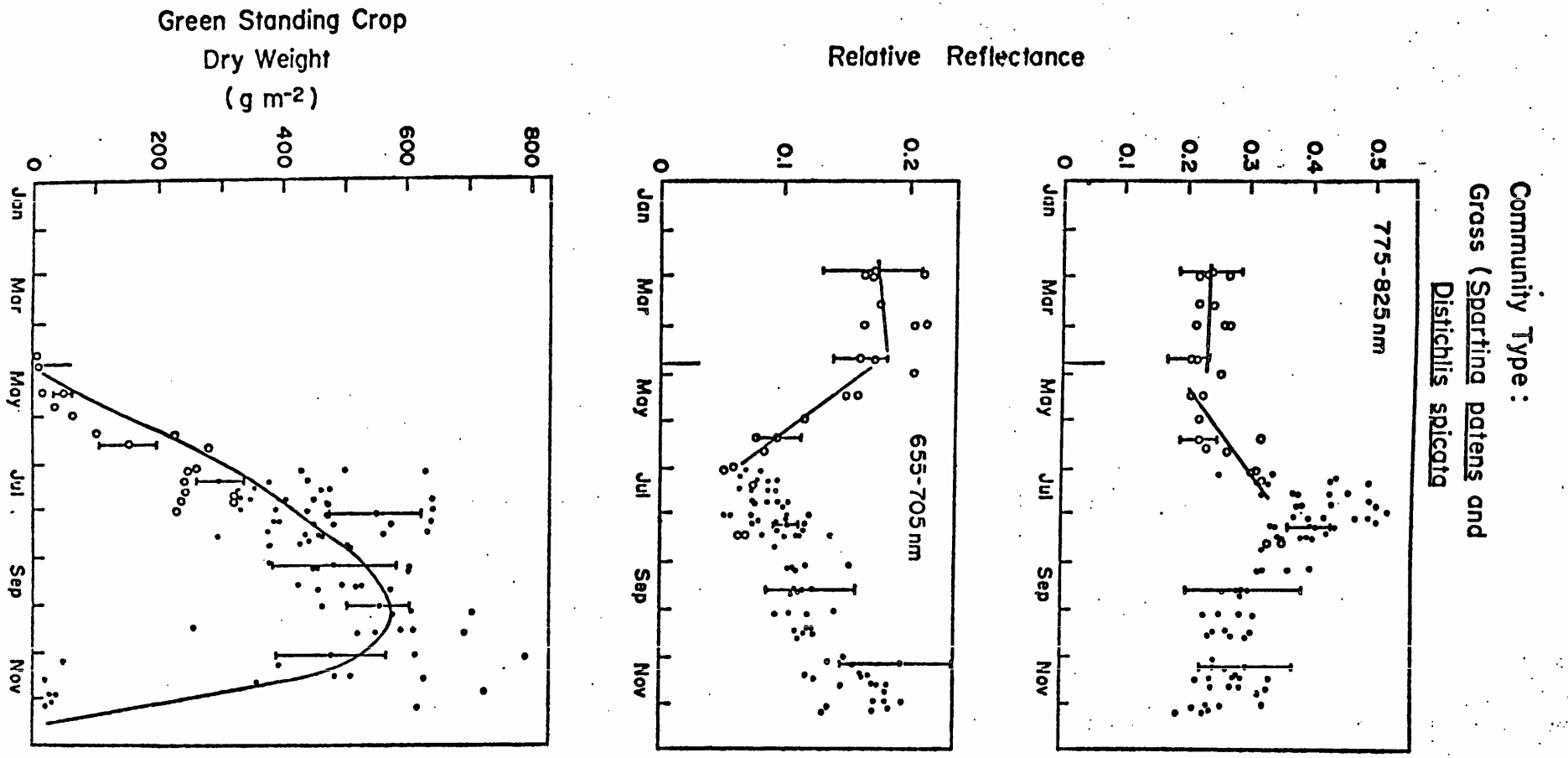


Figure 9: Green standing crop biomass, and reflectance of red (655-705 nm) and far red (775-825 nm) from grass community, a mixture of Spartina patens and Distichlis spicata. Open circles (0) are data from 1974 and closed circles (●) are data from 1973. Note different scales for red and far red.

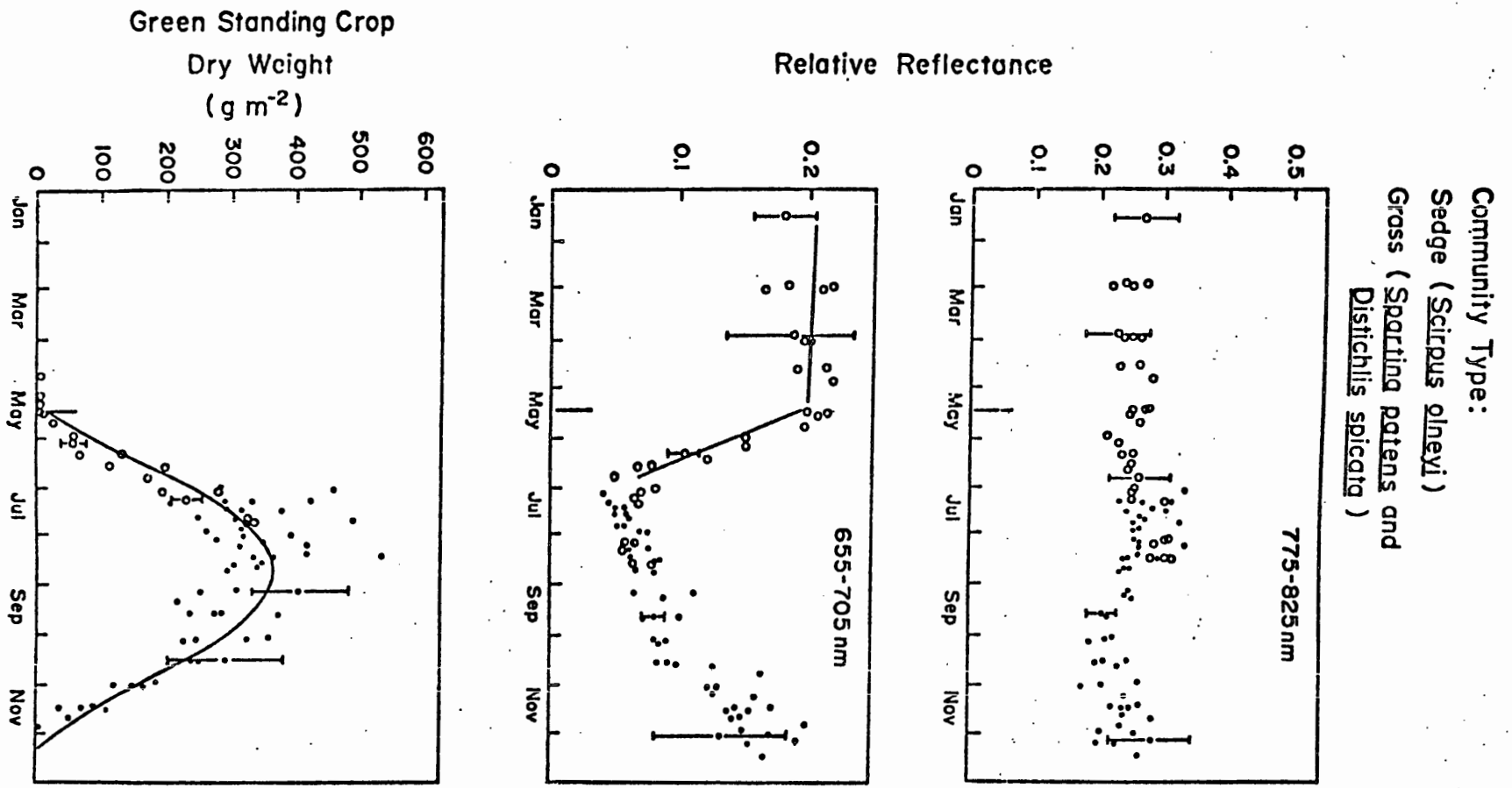


Figure 10: Green standing crop biomass, and reflectance of red (655-705 nm) and far red (775-825 nm) from the sedge community, a mixture of the sedge Scirpus olneyi and the two grasses Spartina patens and Distichlis spicata. Open circles (0) are data from 1974 and closed circles (●) are data from 1973. Note different scales for red and far red.

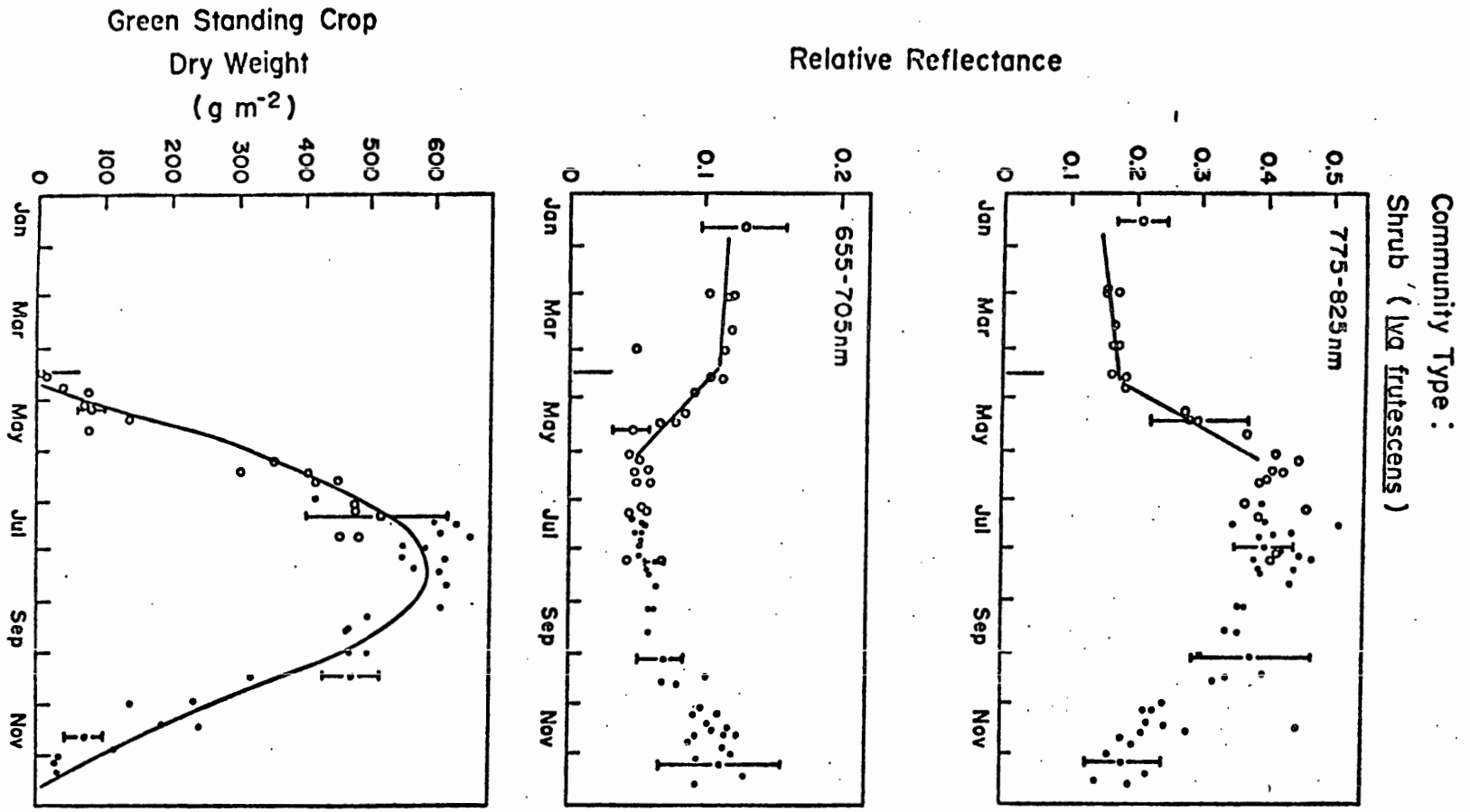


Figure 11: Standing crop of green biomass and reflectance of red (655-705 nm) and far red (775-825 nm) from the shrub community, dominated by Iva frutescens. Open circles (0) are data from 1974, and closed circles (●) are data from 1973. Note different scales for red and far red reflectance.

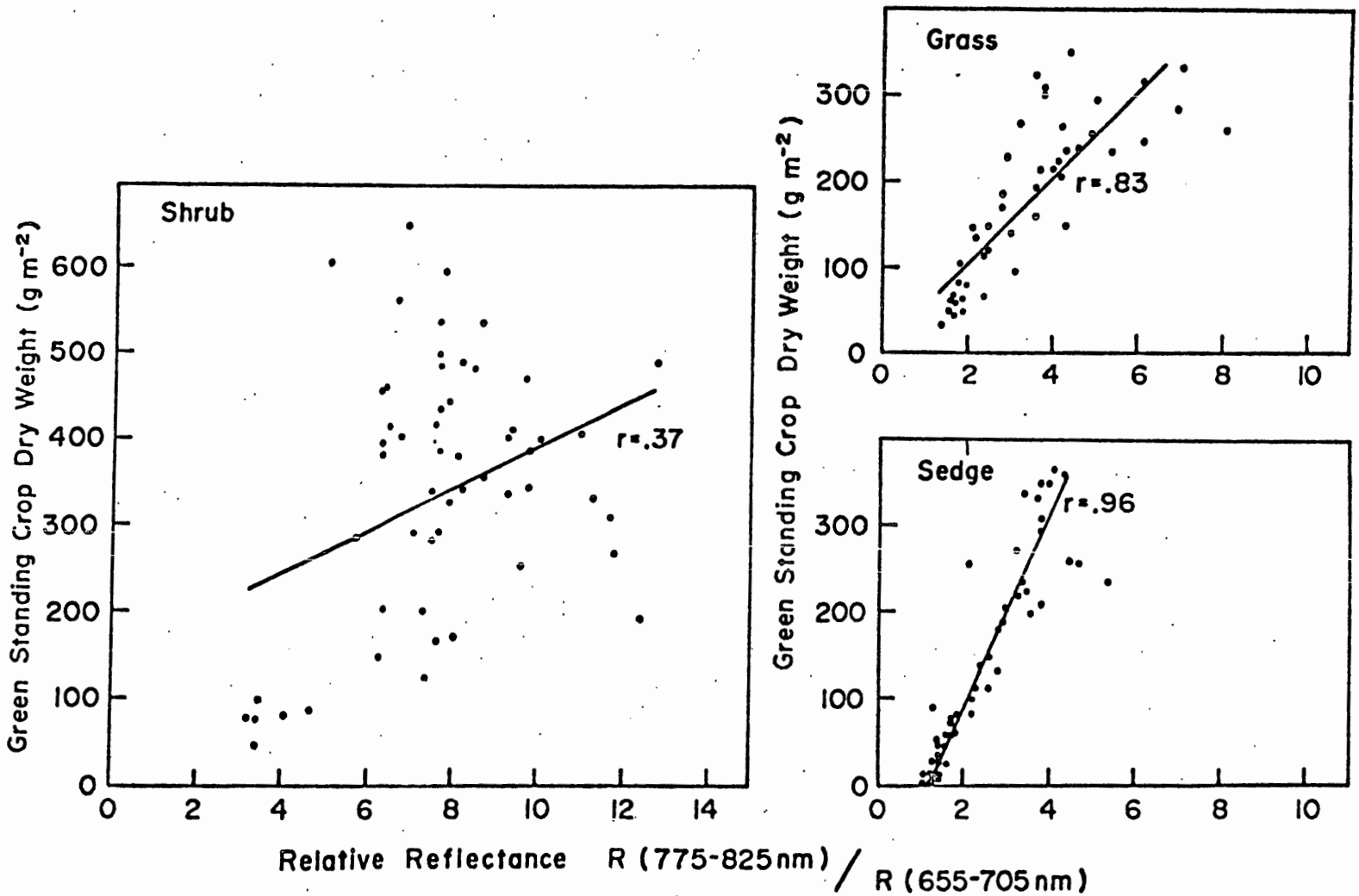
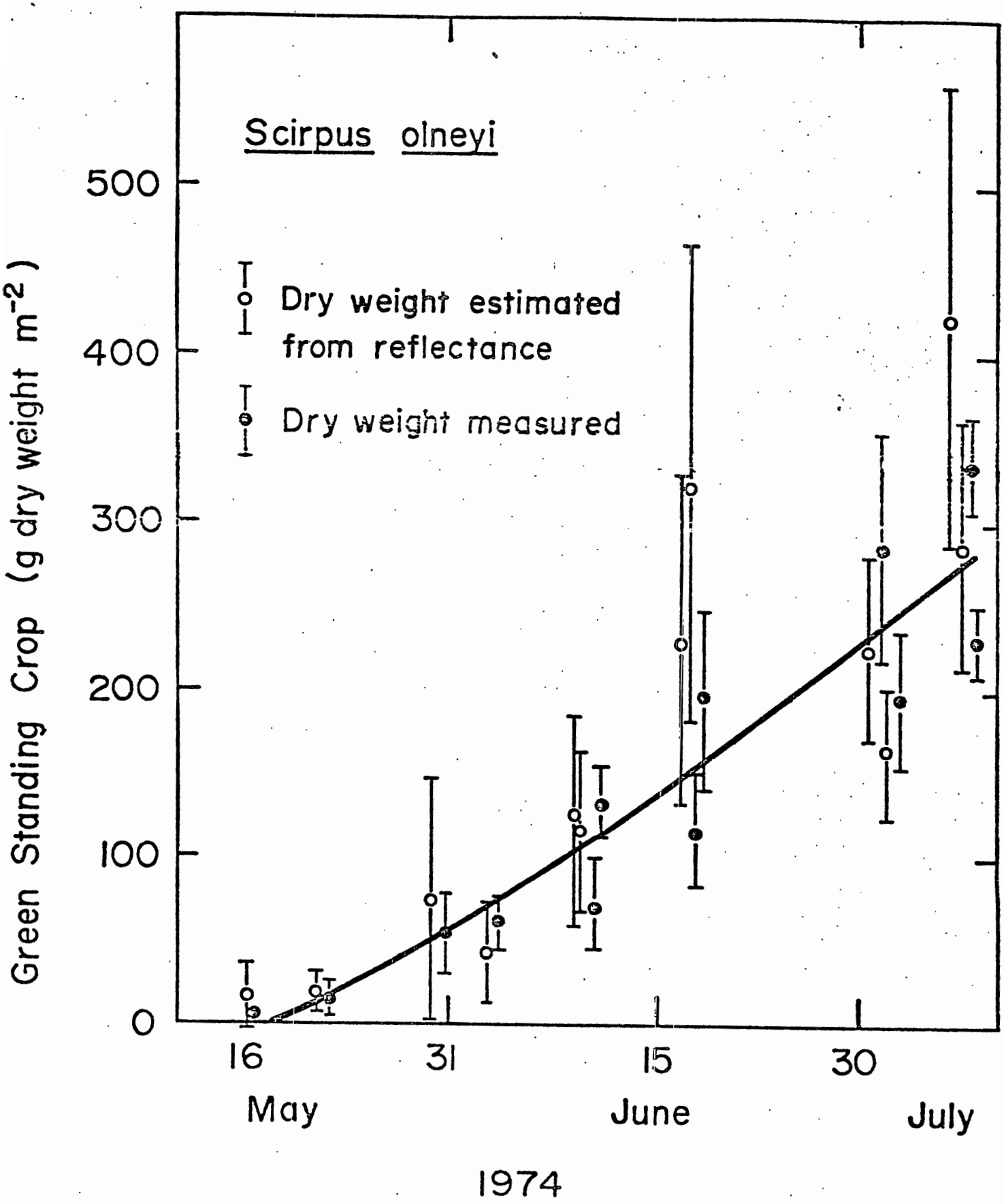


Figure 12: Green standing crop plotted opposite reflectance ratios (far red to red) for the three community types.

Figure 13: The annual course of green standing crop as determined by clippings of green plant material (closed circles (●)) and by reflectance (open circles (○)). 10 random measurements of reflectance and the linear regression in figure 12 for the sedge community were used to compute the standing crop.


Scirpus olneyi
Dry weight estimated from reflectance
Dry weight measured
Green Standing Crop (g dry weight m-2)
500
400
300
200
100
0
16
31
15
30
May
June
July
1974

communities being studied here it is not even possible to estimate annual growth of root matter because of the difficulty in separating live from dead roots. An estimate of the total root matter in two of the three communities studied here shows that there are very large carbon reserves beneath ground (see section 1 above).

Furthermore, there are likely to be algae on the surface of the marsh that can account for a certain amount of carbon assimilated during periods when the higher plants are not active (Pomeroy). In Georgia, the amount of carbon fixed by these plants is estimated to be approximately half as much as is assimilated by higher plants.

However, it is possible to measure accurately the amount of carbon exchanged (as CO_2) across a section of the marsh and theoretically, it should be possible to estimate the annual carbon balance from such measurements. Our approach is to estimate the daily carbon budget; carbon assimilated by the marsh during the photoperiod less that respired during darkness. If the salt marsh system (including the total biota) has a true productivity, that is, if it assimilates more carbon than it uses, then this carbon must either appear as accumulated organic matter or it must be exported from the marsh to the adjacent estuary.

An infra-red gas analysis system was designed for measuring net CO_2 exchange over square meter sections of the communities found in Kirkpatrick Marsh. Two sheds were built on dry ground at the edge of the marsh to house all measuring equipment needed.

Cuvette. A cuvette of 1 m^2 cross-section was made in 3 sections to accommodate different heights of vegetation. A layout of the measuring circuit (omitting many details) is shown in figure 14. The basal section contains the air inlet and outlet ports. The cuvette fit onto an aluminum base frame which had a 14 cm deep apron that was pushed into the marsh. The upper edge of the base frame and each cuvette section is grooved, and the lower edge of each section has a tongue which fits into the groove of the section below. Silicon grease was wiped into the groove to improve the seal. A lid was placed on top of the cuvette, and a seal made using weather strip impregnated with mineral oil. The side panels of the cuvette are 1/8" thick and the lid is 1/4" thick plexiglass. The four corners of the cuvette are glued together and riveted into aluminum angle pieces. On the air inlet and outlet sides of the cuvette a diffuser plate with 1 cm diameter holes was fitted the width and height of the cuvette, 6 cm from the side walls. This served to distribute the air being blown into the cuvette throughout the enclosed space.

Temperature Control. Air was drawn in from 1.8 m above the marsh surface, blown over cooling coils, over heating coils, and into an air duct which carried it to the cuvette. The flexible air duct, 3 m long and 15 cm diameter, was lined with aluminized mylar (Flexaust Co., "Springflex" duct, type V) and instulated with 4 cm of fiberglass matting.

Water was chilled by a 2 HP (2 ton) compressor and pumped from a reservoir to the cooling coils in the heat exchanger a distance of up to 75 m through insulated 3.8 cm ID plastic pipes.

Heat could be added to the air stream by a 1.2 kw heating coil, operation of which was controlled (Electromax III, Leeds and Northrup). The temperature difference between thermocouples inside and outside the cuvette provided the signal for current output of a power supply (Leeds & Northrup) to which the heating coil was connected.

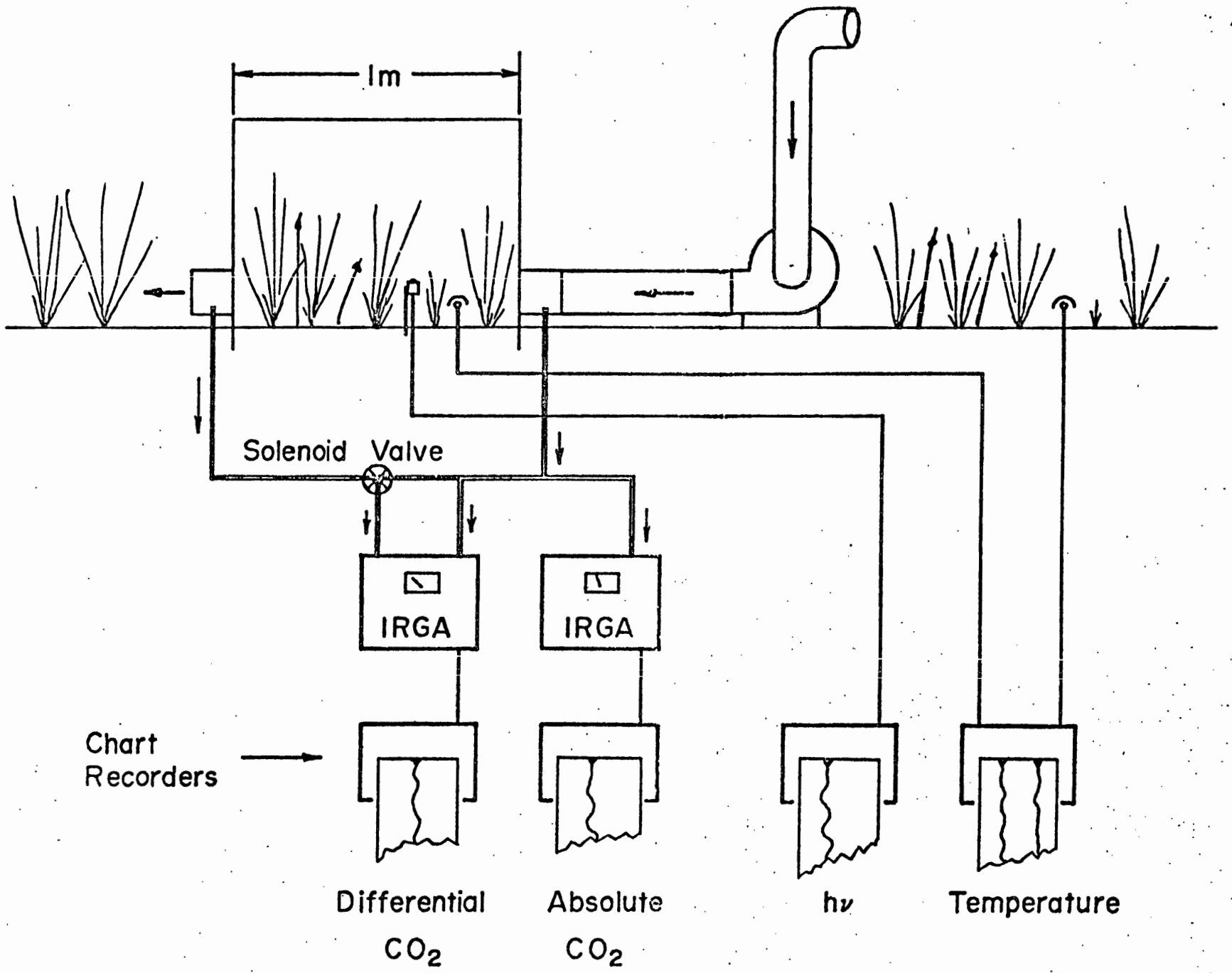
The air-conditioning unit (blower, cooling coils, heating coils) was insulated with fiberglass and placed on a low table to keep it clear of the marsh. The motor for the blower was mounted outside the fan cage.

Temperature inside the cuvette could also be controlled manually without the heater by adjusting the temperature of the water fed to the heat exchanger.

Gas Analysis Circuit (Fig. 14). Air samples were taken at the inlet and outlet of the cuvette and pumped through 1/4" ID polyethylene tubing to the shed. Our gas analyzer (Hartmann & Braun, URAS 2-T) is very sensitive; $\pm$ 10µl CO_2 per liter air full scale. In order to reduce the noise in the signal, a 20 liter flask was placed in line on each sample. This effectively reduced the noise at some loss in sensitivity but caused a lag time between changes in concentration of CO_2 within the cuvette and recording these changes by the gas analyzer of several minutes. The air streams were connected to a water fuse (1.25 M) to maintain constant pressure in the gas analysis circuit. All gas streams passed through an electric condenser to reduce the dew point to a constant 2°C before going to the infrared gas analyzer via flowmeter and diaphragm filters. The gas lines were arranged so that air from the cuvette intake (the reference) passed continuously through the analyzer that could detect absolute CO_2 concentration in the range 0 to 750 µl CO_2 $(1\ air)^{-1}$ and the reference cell of the differential analyzer, set to detect $\pm$ 10 µl CO_2 $(1\ air)^{-1}$. Solenoid valves were placed in the lines and connected to a timer so that periodically air from the reference line would pass through the sample cell of the differential analyzer to establish a zero-line. At all other times, air from the cuvette outlet passed through the sample cell of the differential analyzer. Thus, the CO_2 concentration difference ($\Delta[CO_2]$) across the cuvette was determined.

The flow rate through the cuvette was determined by measuring air velocity with a hot-wire anemometer probe (Hastings Precision Air-Meter, Model B-22). The voltage outputs from both gas analyzer were recorded continuously on strip chart recorders.

Figure 14: A schematic of the open gas analysis system and sampling circuit. Not shown are condenser to remove water vapor from the gas line, heating and cooling coils in the fan housing, flowmeters, etc. By connecting the intake of the fan to the exhaust of the cuvette, the system could be used as a closed system.


1m
Solenoid Valve
IRGA
IRGA
Chart
Recorders
Differential
CO_2
Absolute
CO_2
hν
Temperature

From the out-put of the recorder, the net exchange of carbon is computed as follows:

$$Pn = \Delta mv \cdot R_{CO_2} \cdot F \cdot K_c \cdot A^{-1} \qquad (gC\ m^{-2}\ hr^{-1})$$

where Δ mv is the deflection on the recorder (mv), R_c is the responsivity of the gas analyzer ($\mu l\ CO_2\ 1\ air^{-1}\ mv^{-1}$); F is flow rate across the cuvette (1 air hr^{-1}), k_c is a conversion constant (g CO_2 1 air^{-1})$\cdot$($\mu l\ CO_2^{-1}$ 1 air)$\cdot$ (gC gCO_2^{-1}), A is cross-sectional area (m^2).

Shaded copper-constan thermocouples (24 gauge) were placed at the top of the vegetation both inside and outside the cuvette. A silicon photo-diode (Lambda), measuring photosynthetically active radiation in the waveband 400-700 nm was placed at the surface of the plant canopy inside the cuvette. This sensor was calibrated against Eppley thermopiles with sunlight as radiation source.

CO_2 efflux rates during the day were obtained by covering the cuvette with a light-tight black cover.

Results and Discussion of Net Carbon Exchange Data. Data for a 2 hr period during August 3, 1974 from the recorder traces for net CO_2 exchange (g C m^{-2} hr^{-1}) and for light intensity (moles of quanta m^{-2} hr^{-1}) are shown in figure 15.

Hourly integrations of net carbon exchange and light intensity in the sedge community for two days in August (3rd and 4th) at the peak of growth are plotted in figure 16. In both plots, net CO_2 exchange is closely coupled to light intensity, demonstrating that at least qualitatively, the system can detect changes in CO_2 exchange rates over these large sections of plant communities.

A problem was encountered in measuring net CO_2 exchange at night. It was found that during a rapid change in the ambient CO_2 concentration over the marsh, it was impossible to determine accurately the rate of CO_2 exchange across the cuvette. We found that on the rare night when there was a strong breeze over the marsh, and CO_2 concentrations did not increase above approximately 400 $\mu l l^{-1}$, nor otherwise changed rapidly, it was possible to obtain a steady trace of CO_2 evolution, as for example on the night of August 2nd to 3rd when the wind velocity was of the order of 250 to 400 cm sec^{-1}. However, the following night wind velocity was near 50 to 100 cm sec^{-1} (which is rather low) and CO_2 increased rapidly near the cuvette to a concentration of approximately 450 $\mu l l^{-1}$. It was then possible to interpret the traces of net CO_2 exchange. During the night of August 2nd, when wind velocity near the dock exceeded 200 cm sec^{-1}, ambient CO_2 over the marsh did not go above 400 $\mu l l^{-1}$.

Figure 15: Traces from the recorder showing output from the infrared gas analyzer, photosynthesis, and from the radiometer, irradiance, for a two hour period on the afternoon of August 3, 1974. Hourly integrations were made by planimetering the strip charts.

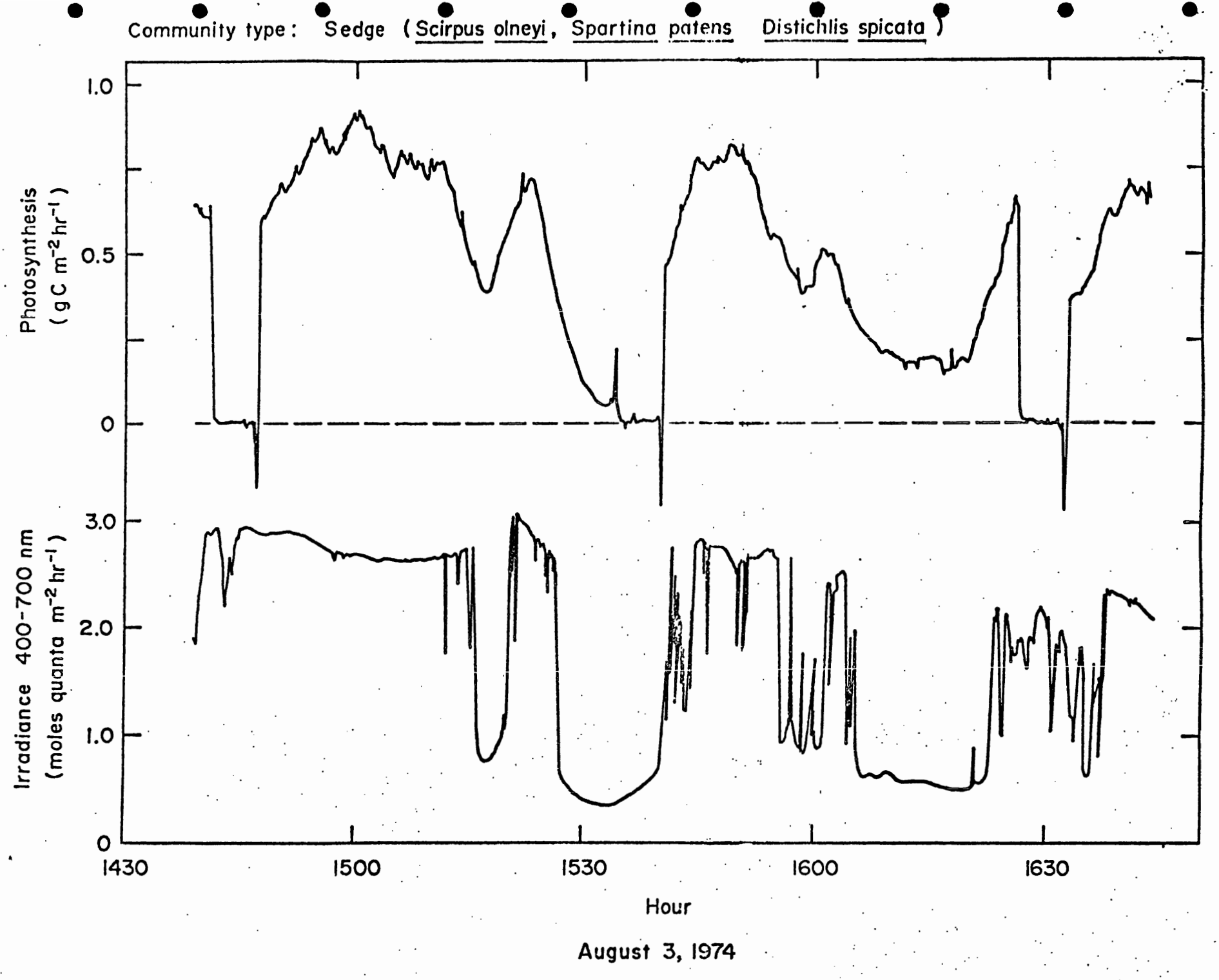
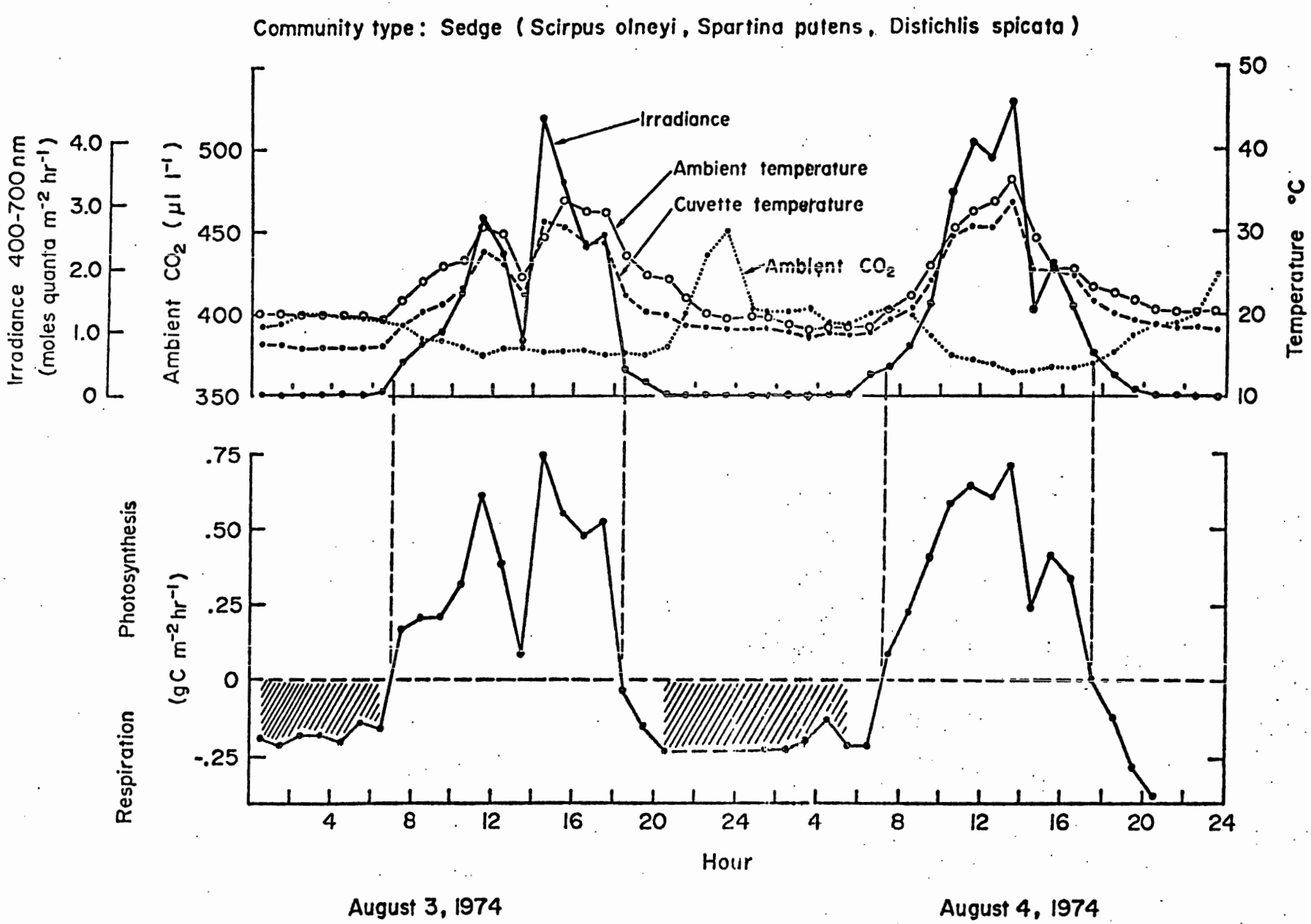

Community type: Sedge (Scirpus olneyi, Spartina patens Distichlis spicata)
Photosynthesis (g C m⁻² hr⁻¹)
1.0
0.5
0
Irradiance 400-700 nm (moles quanta m⁻² hr⁻¹)
3.0
2.0
1.0
0
1430
1500
1530
1600
1630
Hour
August 3, 1974

Figure 16: A 48 hour plot of hourly interactions of net carbon exchange, irradiance, cuvette and ambient temperature, and ambient CO_2 concentration.


Community type: Sedge (Scirpus olneyi , Spartina putens , Distichlis spicata)
Irradiance 400-700 nm (moles quanta m⁻² hr⁻¹)
Ambient CO₂ (µl l⁻¹)
Temperature °C
Irradiance
Ambient temperature
Cuvette temperature
Ambient CO₂
Photosynthesis
Respiration
(gC m⁻² hr⁻¹)
Hour
August 3, 1974
August 4, 1974

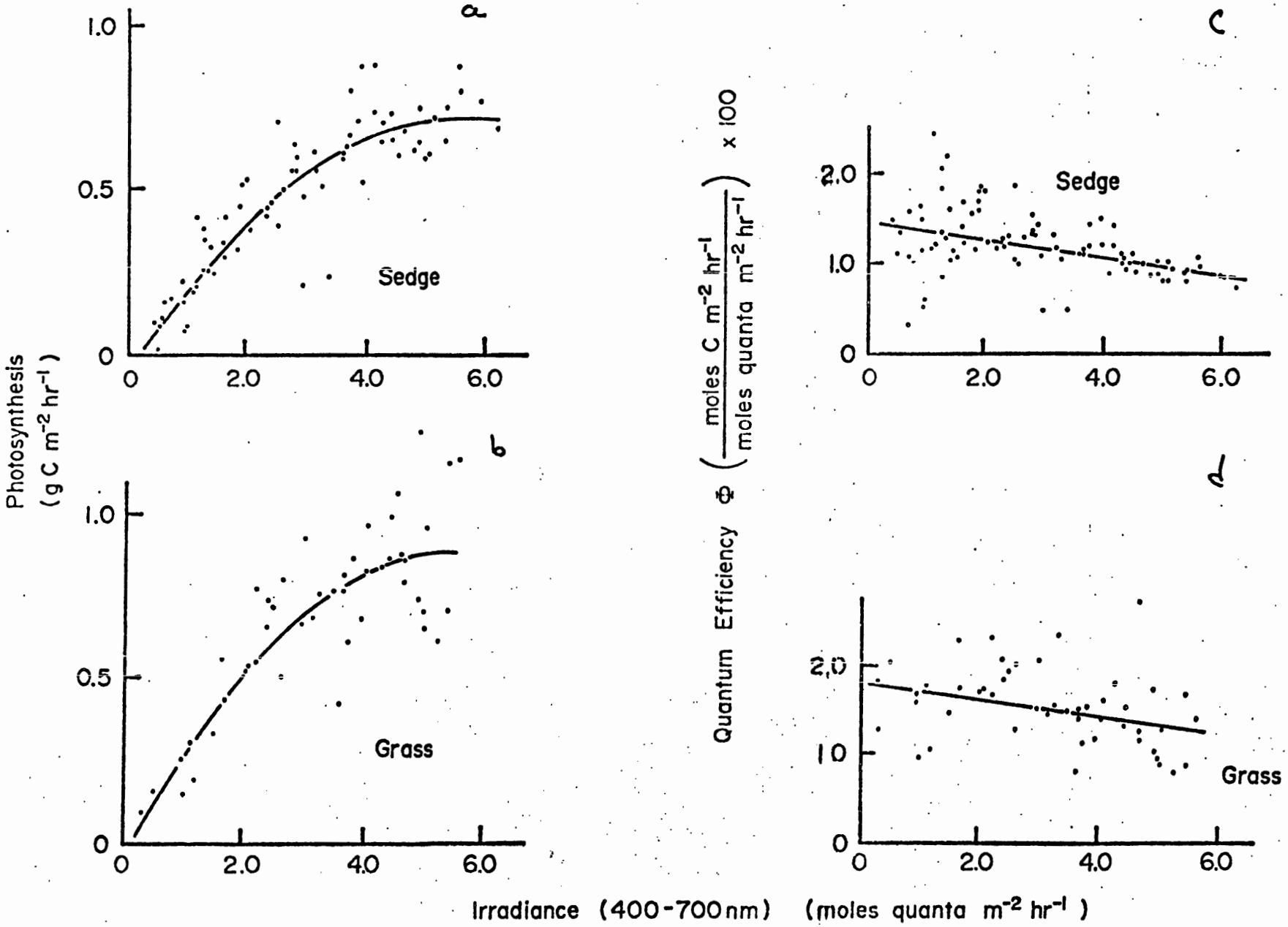


Figure 17: Net photosynthesis (g C m^{-2} hr^{-1}) plotted opposite irradiance (moles quanta m^{-2} hr^{-1}) and minimum quantum efficiency $\left(\frac{\text{moles C m}^{-2}\text{ hr}^{-1}}{\text{moles quantum m}^{-2}\text{ hr}^{-1}}\right)$ plotted against irradiance for the sedge and grass communities.

Hourly integrations for net carbon uptake are plotted against light flux for the two communities (Fig. 17 a,b). Light saturation in both communities occurs at a very high level of light intensity, in this case approximately 4.5 for the sedge and 5.5 moles quanta m^{-2} hr^{-1} for the grass. Maximum solar intensity of radiation between 400 and 700 nm is approximately 7.2 moles quanta m^{-2} hr^{-1} at the latitude of Washington, D.C. during August. Light saturation occurred at 3.2 moles quanta m^{-2} hr^{-1} in potato (Sale, 1974). Uptake of carbon in corn, a C_4 species, was nearly linear up to maximum solar intensity of approximately 7.5 moles quanta m^{-2} hr^{-1} (Lemon, et al.). It is interesting that in the sedge community (approximately 50% C_3 and 50% C_4 species) light saturation occurred at light intensity between that for saturation in potato (C_3) and corn (C_4), but at a lower intensity than saturation in the grass, which is very likely composed totally of C_4 species.

Data for hourly integrations of net CO_2 exchange and light flux were used to compute the minimum quantum efficiency (Φ) of photosynthesis $\frac{\text{(moles carbon } m^{-2} hr^{-1})}{\text{(moles quanta } m^{-2} hr^{-1})}$ and were plotted against irradiance (Fig. 17 c,d). Since absorbance of photosynthetically active radiation in this community is not known, it must be said that the quantum efficiencies (Φ) computed are minimal estimates. If, however, the sedge community absorbs photosynthetically active radiation essentially as does corn, we could expect absorption to be approximately 65% (Lemon, 1966) and our molar efficiency would be increased by approximately 54% (dashed lines in Fig. 17 c,d). Quantum efficiency in corn is approximately 6.4 after correction for respiration.

Respiration in the sedge community can be estimated from figure 16 to be approximately .22 g cm^{-2} hr^{-1}. If this is added to the net carbon uptake by the plants and a figure of 65% absorption used, then the minimal quantum efficiency is approximately .04 or about a third of the best rates obtained in short term laboratory experiments with cultures of algae. Said in another way this means that at low intensities the grass community on our salt marshes requires about 32 quanta of radiation of energy equal to that of yellow light to fix one molecule of CO_2 at very low intensities.

It was the intention in this project to estimate the amount of carbon that could be "produced" for export to the estuary. In order to do this, more extensive measurements are needed than were possible to obtain during the past summer. However, it is hoped that during the coming year, funds will be found to continue this project and that the required data will be forthcoming.

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1. Lemon, E.R. 1966. Aerodynamic studies of CO_2 exchange. In Harvesting the Sun (San Pietro, Greer, and Army eds.) pp. 263-290.

2. Pomeroy, L.R. 1959. Algal productivity in salt marshes of Georgia. Limn. & Ocean. 4(4): 386-397.

3. Sale, P.J.M. 1974. Productivity of vegetable crops in a region of high solar input. III. Carbon balance of potato crops. Aust. J. Plant Physiol. 1: 283-296.

Project Title: Physical, Chemical and Biological Measures of Water Quality in the Rhode River and Adjacent Waters.

Principal Investigator: Robert L. Cory
U.S. Geological Survey
Chesapeake Bay Center
Edgewater, Maryland 21037

Assistants: Martha M. McCullough - May 1972 - June 1973
J. Michael Redding - June 1973 - October 1974

Introduction

The primary objective of participation in the Rhode River Ecosystem study was to contribute useful environmental and biological information. This information was to be used to assist in determining what changes are occurring in the ecosystem, what caused the observed changes and what activities of man, if any, could be specifically related to these observed changes.

The work in the estuary has centered around three primary activities: (I) water quality monitoring, (II) estimates of open water metabolism, and (III) general biological studies of macroinvertebrates and fish. Two additional studies were participated in on a cooperative effort (1) the development and use of low cost oxygen and temperature integrators and (2) a study of geochemistry of the anoxic waters of a deep hole off Bloody Point, Md.

National Science Foundation-RANN funds totaling $46,000 including benefits for the past 32 months were sufficient to support one full time assistant at the GS-5 or 7 level. Purchase of a Martek Portable Water Quality system ($3000.) was the only major equipment purchase with remaining funds being used for miscellaneous project support.

In addition to these funds the U.S. Geological Survey funded this project at about $30,000. per year which included my salary, boat and other project support such as card punching and computer processing. Additional equipment funds from the survey included purchase and installation of a 9 parameter water quality monitor system ($12,000) purchase and maintenance of a 23 ft. cabin cruiser ($11,000) and purchase of a Martek multipoint recorder ($3,000).

Logistic support from the Smithsonian in the form of secretarial services, office and laboratory space, docking facilities and salaries for 2 summer students was an important addition. Total Smithsonian support is estimated to be about valued at $10,000 for the 32 months of RANN funding.

Project Summary

I. Results of water quality monitoring.

Two reports have resulted from this effort and the data have been extensively used by other investigators. For example, Nancy Goff in her University of Maryland master's thesis on Vertical Migration of the phytoplankton began her acknowledgement as follows:

> "The author extends thanks to the United States Geological Survey, in particular, Mr. Robert L. Cory, for furnishing many of the physical data measurements used in this study, and to the Smithsonian Institute Chesapeake Bay Center for Environmental Studies for use of their facilities."

To conserve space only the abstract and figures of the 2 reports are included in this summary.

Water quality in Rhode River at Smithsonian Institution Pier near Annapolis, Maryland, April 1970 through December 1974.

By Robert L. Coryl

J. Michael Redding[2]

Martha M. McCullough[3]

U. S. Geological Survey

Water - Resources Investigations

1972

[1] Oceanographer, U.S. Geological Survey, Chesapeake Bay Center, Edgewater, Md.

[2] Biologist, Smithsonian Institution, Chesapeake Bay Center, Edgewater, Md.

[3] Biologist, Ichthyological Associates, Absecon, N. J.

Abstract

Water temperature, salinity (computed from specific conductance data), turbidity, dissolved oxygen, water level and pH data were collected by a continuously recording water quality monitor located in the Rhode River, at the Smithsonian Institution's pier near Annapolis, Md. from April 1970 through December 1974. Data, as presented in this report, consist of daily maximum and minimum values summarized by week to give weekly averages and extremes.

Temperatures showed an overall range from 0.7 to 32.6°C. The water temperature data indicate successively warmer winters. Salinity ranged from 1.05 to 14.03 parts per thousand. In June 1972, salinity dropped markedly as fresh water from tropical storm Agnes entered the Rhode River from Chesapeake Bay. Most of the fresh water entering upper Chesapeake Bay as a result of tropical storm Agnes came from the Susquehanna River. Turbidity was usually low averaging about 14 Jackson Turbidity Units; however, during spring and early summer of 1972, values averaged about 23 Jackson Turbidity Units. This increase in turbidity was due to the high Susquehanna River flows during that period. Extremes of turbidity ranged from about 5 to 80 Jackson Turbidity Units.

Dissolved oxygen ranged from 0.0 to 19.8 milligrams per liter. Large daily changes in oxygen indicated a high state of biological metabolism. Values of pH ranged from 6.8 to 10.1 and daily changes coincided with oxygen changes. Tide dominated water levels had an overall range of 5.9 feet (1.8 meters) with a mean tidal range of 1.5 feet (0.46 meters).

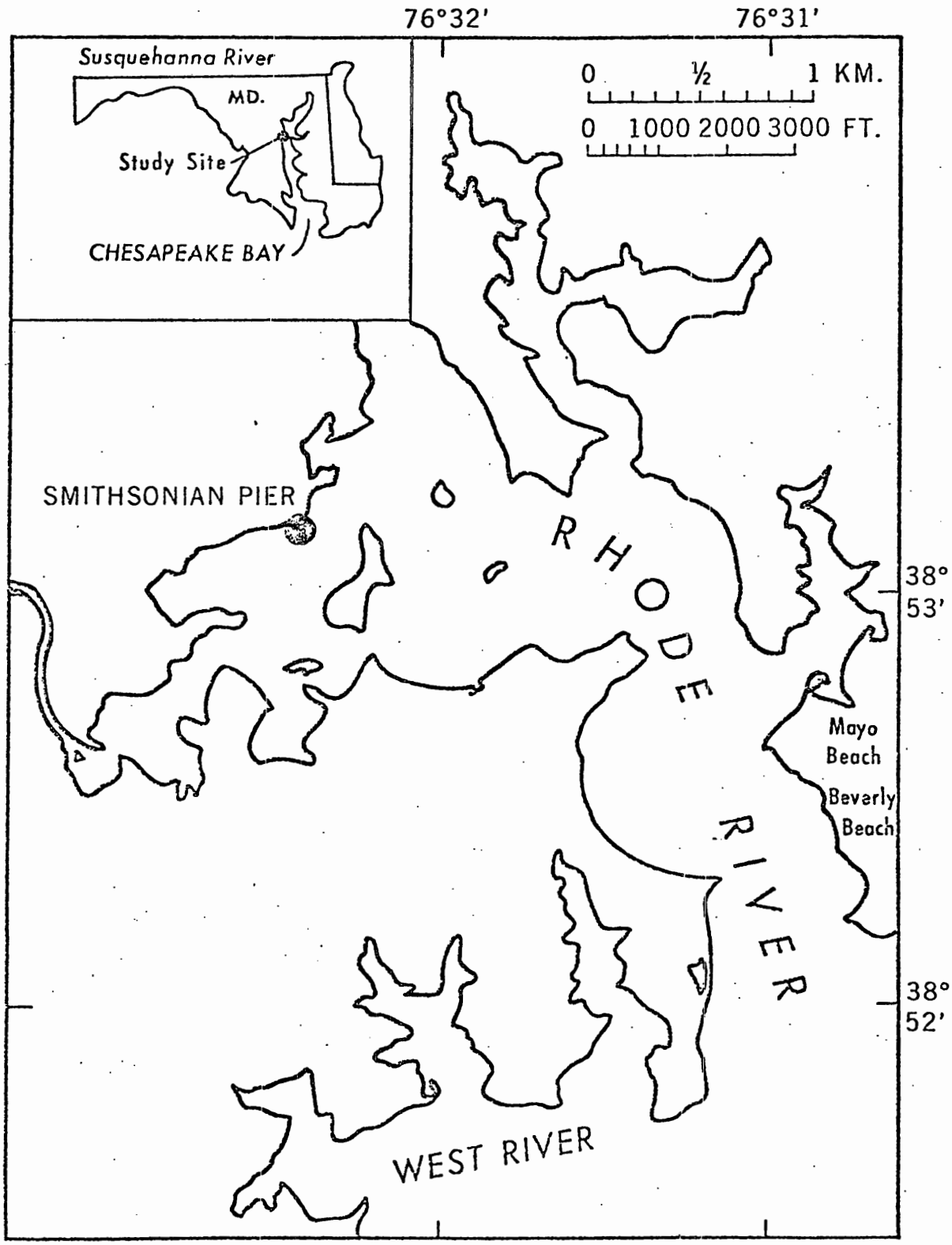


Figure 1. Map showing location of study area

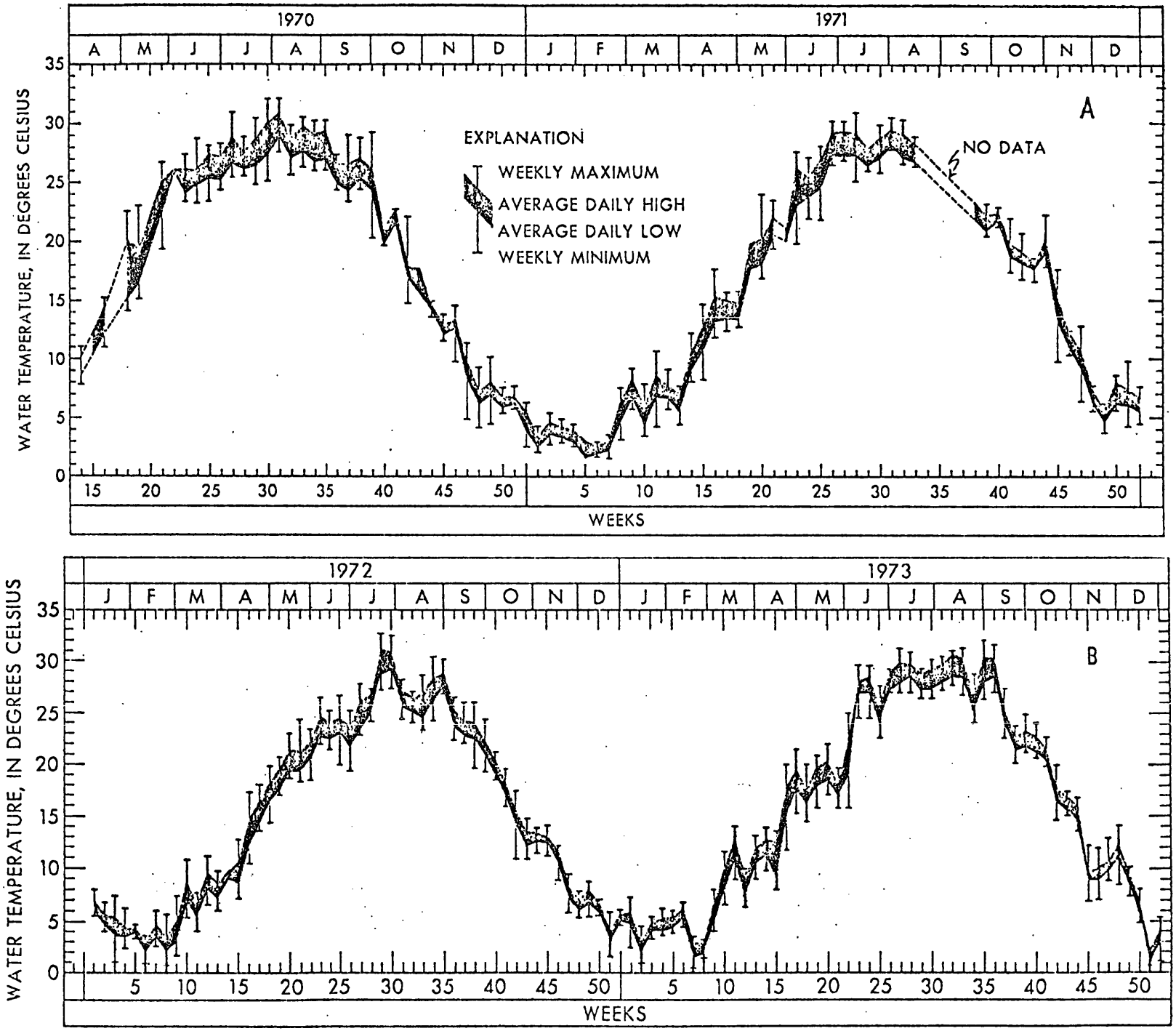


Figure 2. Hydrograph of water temperature

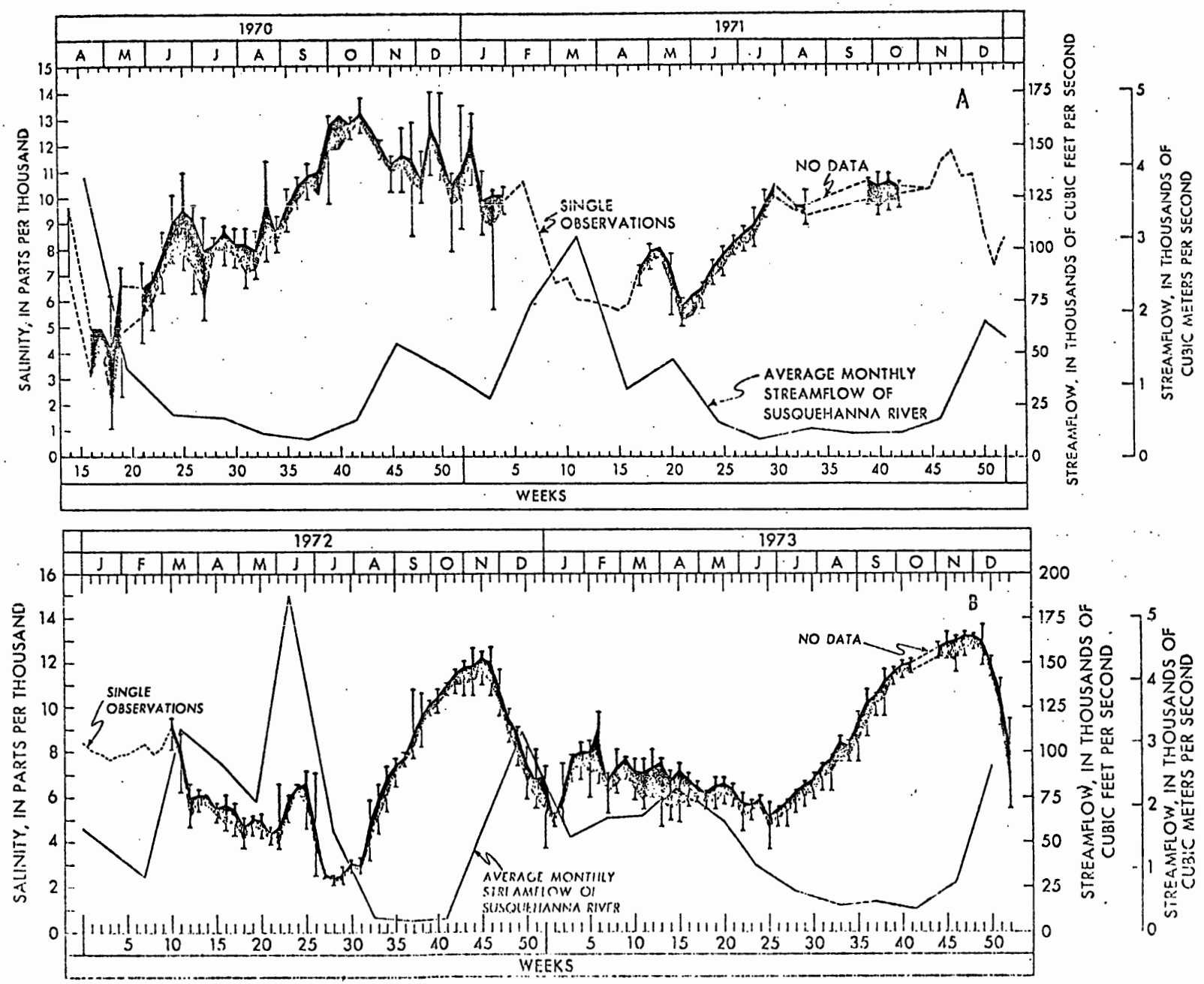


Figure 3. Hydrograph of water salinity

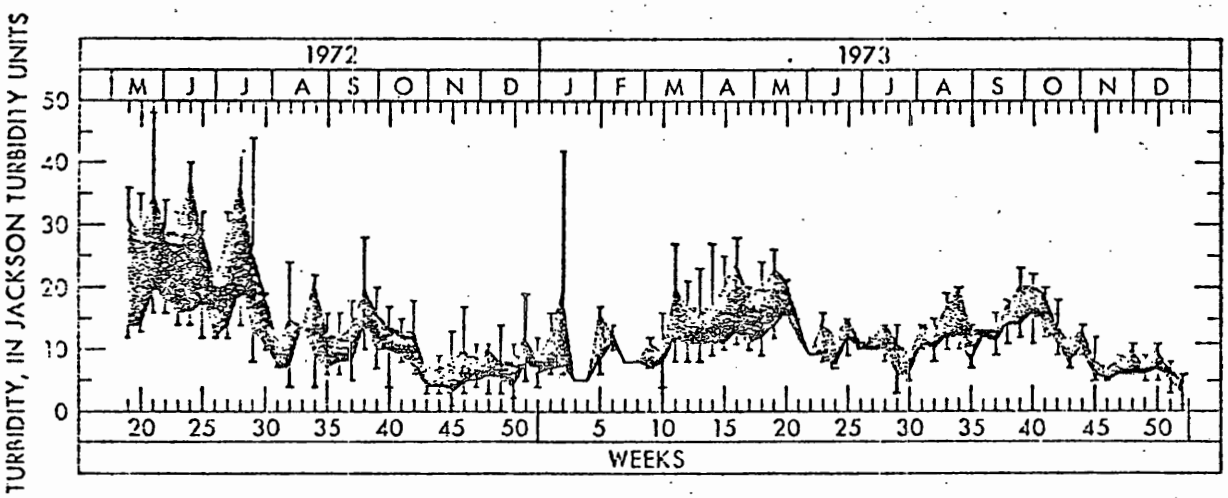


Figure 4. Hydrograph of water turbidity

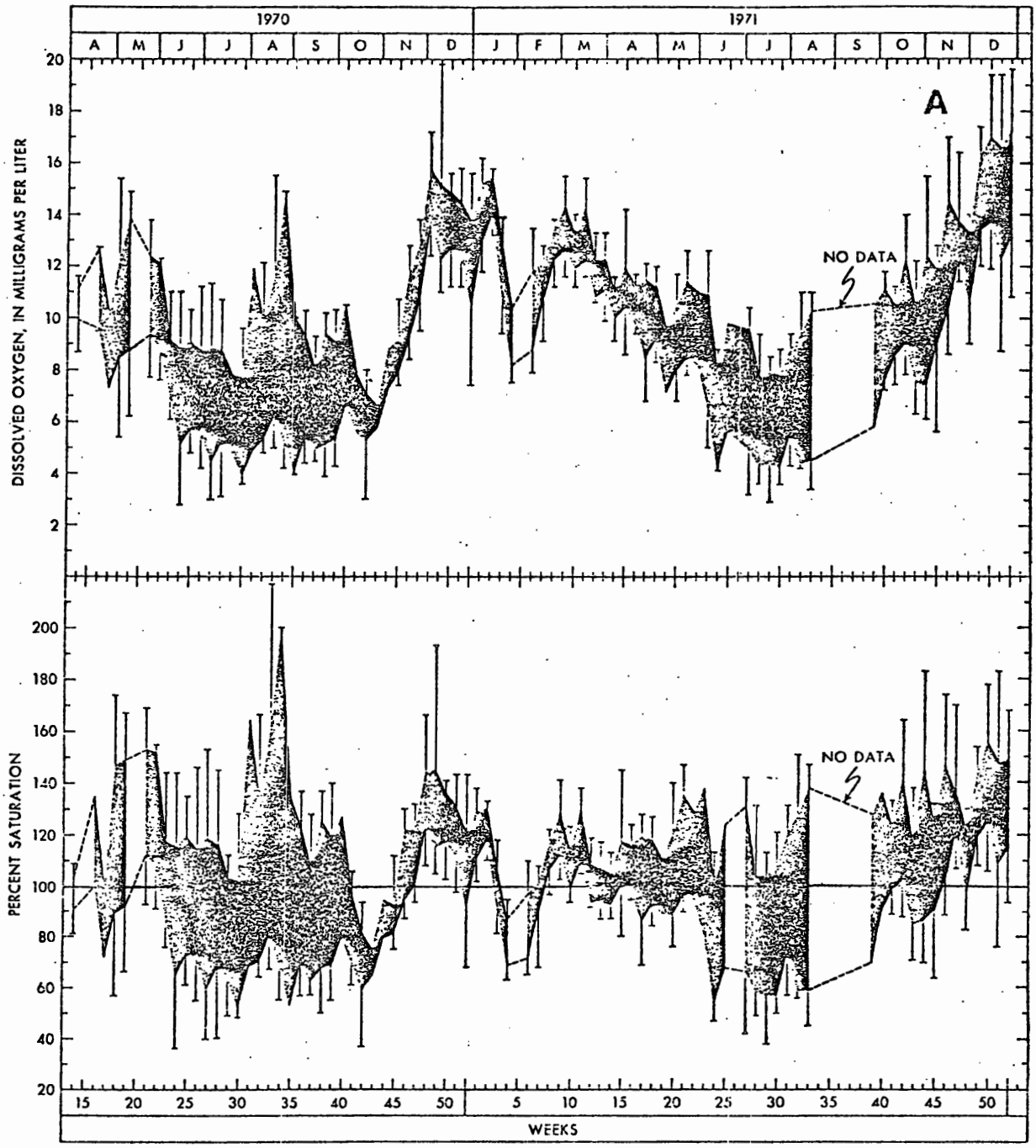


Figure 5. Hydrograph of dissolved oxygen and percentage saturation

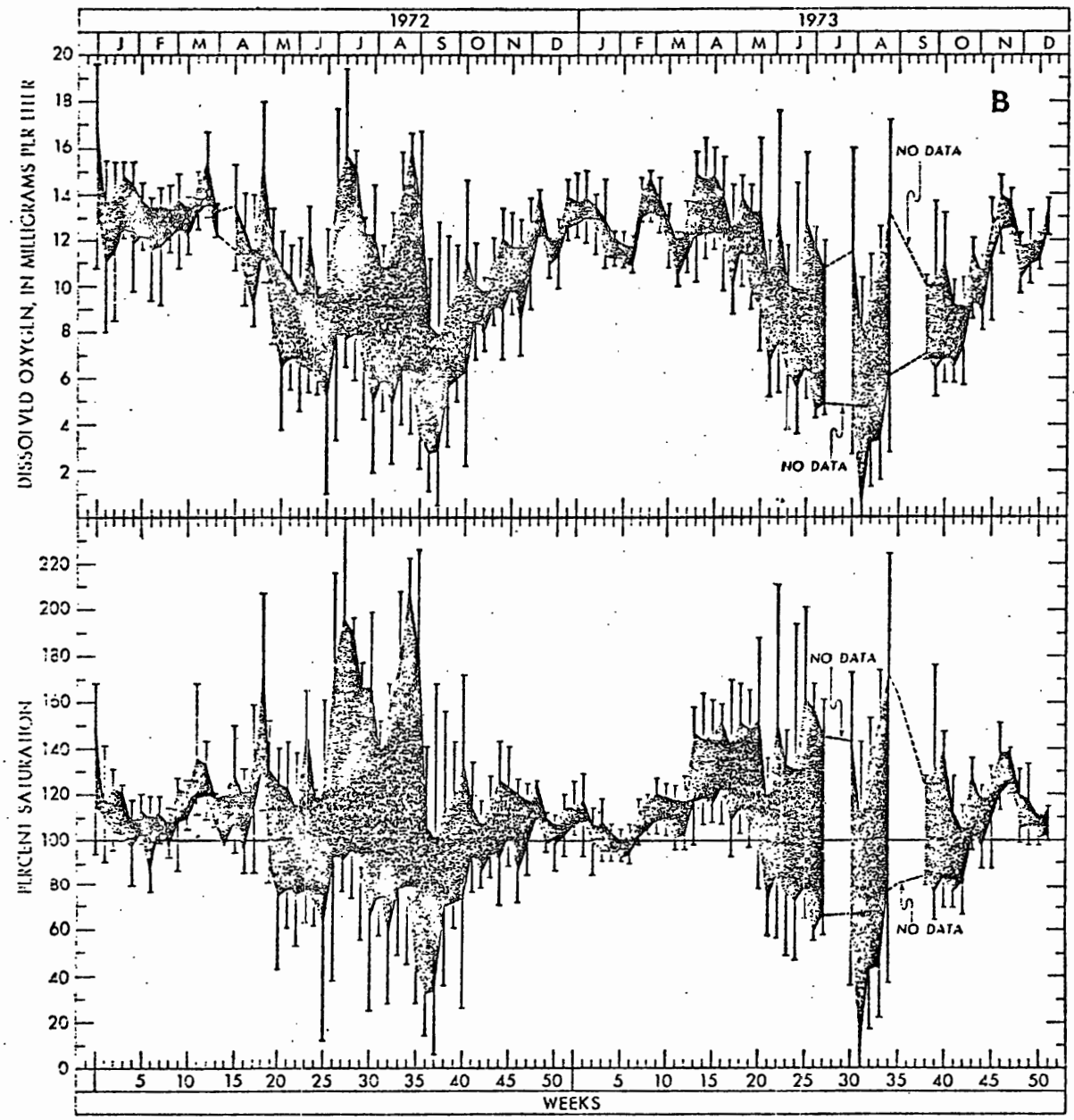


Figure 5. Hydrograph of dissolved oxygen and percentage saturation

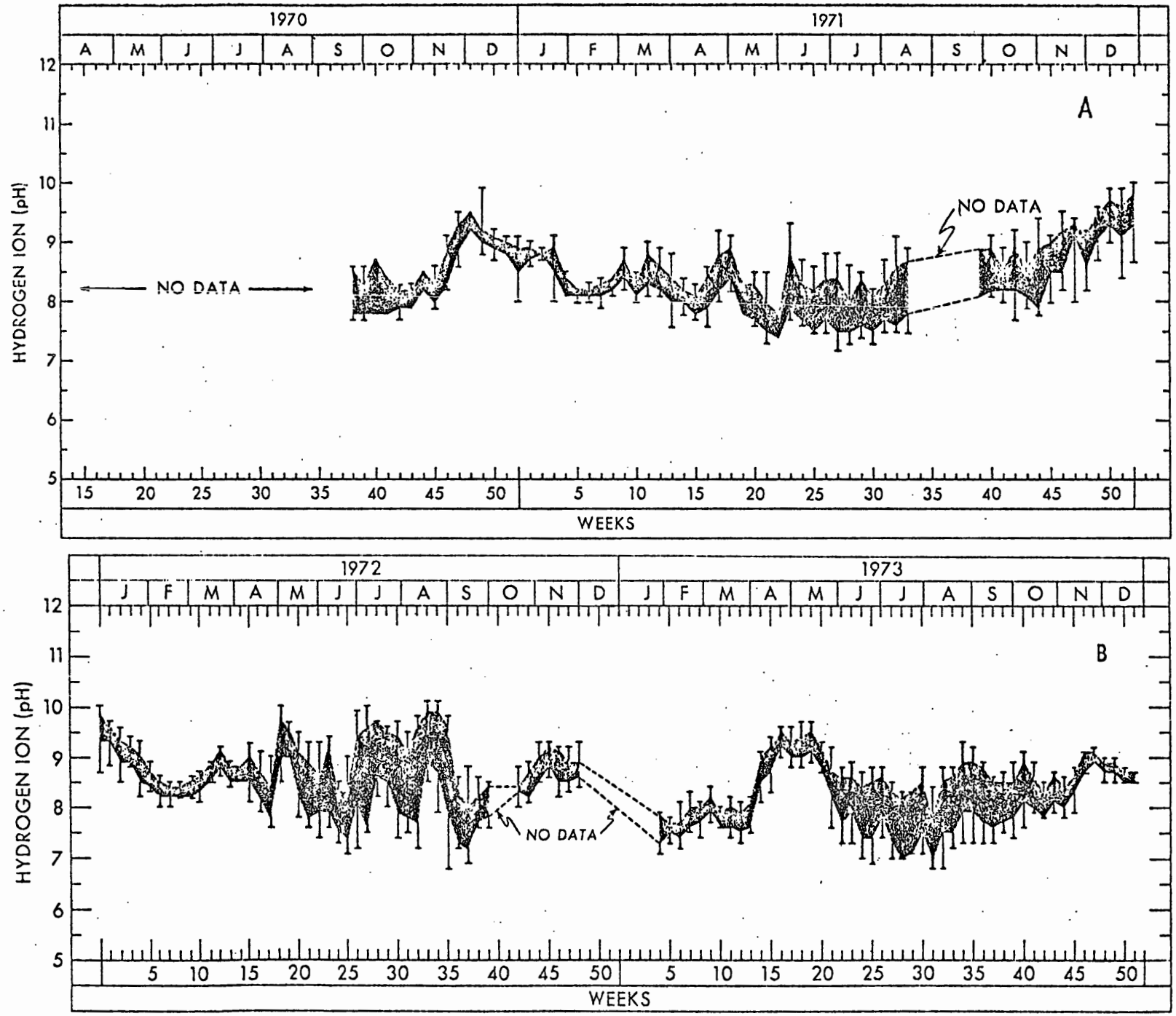


Figure 6. Hydrograph of hydrogen ion activity

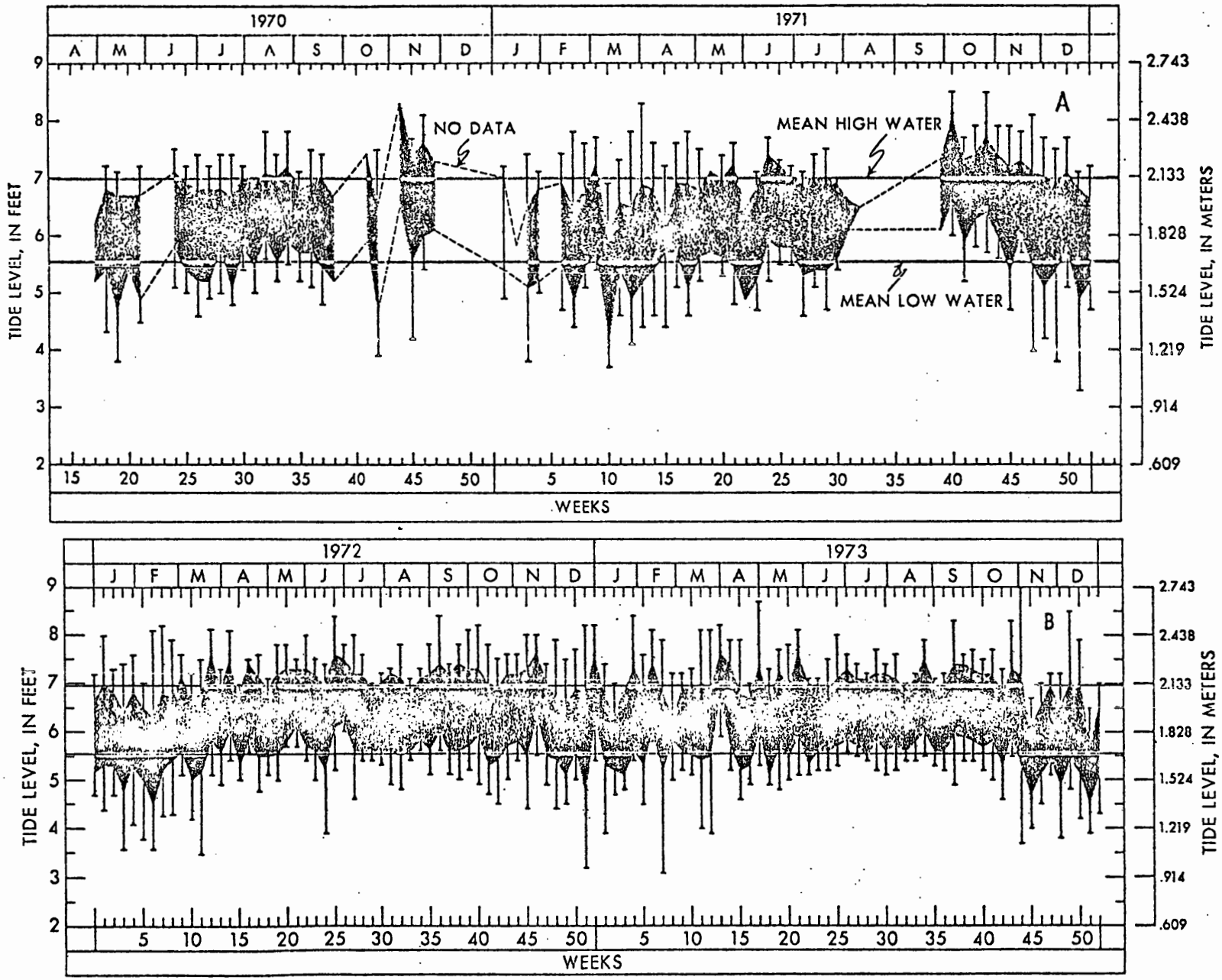


Figure 7. Hydrograph of tides and water stage

Table 1. Summary of water quality data (sample)

WEEK	DATE MO	DA	YR	TEMPERATURE DEG C MAX	TEMPERATURE DEG C MIN	pH MAX	pH MIN	DISSOLVED OXYGEN PPM MAX	DISSOLVED OXYGEN PPM SAT	DISSOLVED OXYGEN PPM MIN	DISSOLVED OXYGEN PPM SAT	TURBIDITY JCU MAX	TURBIDITY JCU MIN	SALINITY PPT MAX	SALINITY PPT MIN	TIDE HEIGHT FT MAX	TIDE HEIGHT FT MIN
13	3	25	72	7.6	6.3	8.8	8.6	13.3	118.	12.2	106.	***	***	5.77	5.52	7.1	4.9
13	3	26	72	7.6	6.0	8.7	8.5	13.6	120.	12.2	106.	***	***	6.25	5.64	7.0	5.7
13	3	27	72	7.6	7.1	8.7	8.5	13.5	120.	12.3	108.	***	***	6.38	6.13	6.8	5.9
13	3	28	72	8.6	6.7	8.7	8.5	****	****	12.6	111.	17	14	6.38	5.95	7.0	5.6
13	3	29	72	9.6	7.8	8.9	8.4	****	****	****	****	16	13	6.38	5.64	7.3	6.1
13	3	30	72	9.5	8.9	8.8	8.5	****	****	****	****	22	13	6.13	5.64	7.9	6.4
13	3	31	72	****	****	****	****	****	****	****	****	***	***	*****	*****	7.4	6.3
EXTREME				9.6	6.0	8.9	8.4	13.6	120.	12.2	106.	22.	13.	6.38	5.52	7.9	4.9
AVERAGE				8.4	7.1	8.8	8.5	13.5	119.	12.3	107.	18.	13.	6.21	5.76	7.2	5.8
									1.2								
14	4	1	72	****	****	****	****	****	****	****	****	***	***	*****	*****	8.1	6.4
14	4	2	72	****	****	****	****	****	****	****	****	***	***	*****	*****	7.3	5.7
14	4	3	72	****	****	****	****	****	****	****	****	***	***	*****	*****	6.6	5.6
14	4	4	72	****	****	****	****	****	****	****	****	***	***	*****	*****	7.2	5.4
14	4	5	72	****	****	****	****	****	****	****	****	***	***	*****	*****	****	****
14	4	6	72	12.5	10.7	****	****	****	****	****	****	***	***	*****	*****	****	****
14	4	7	72	11.5	8.4	9.0	8.1	12.6	121.	10.9	98.	24	14	5.40	5.16	6.8	5.6
EXTREME				12.5	8.4	9.0	8.1	12.6	121.	10.9	98.	24.	14.	5.40	5.16	8.1	5.4
AVERAGE				12.0	9.5	9.0	8.1	12.6	121.	10.9	98.	24.	14.	5.40	5.16	7.2	5.7
15	4	8	72	8.5	7.5	8.7	8.1	12.2	109.	10.7	95.	28	18	5.52	5.04	6.6	5.4
15	4	9	72	9.0	7.0	8.8	8.4	13.2	119.	11.4	100.	24	16	5.52	5.40	6.8	5.0
15	4	10	72	10.0	7.4	9.0	8.5	13.8	126.	11.9	105.	28	14	5.64	4.92	7.0	5.6
15	4	11	72	10.8	8.9	9.1	8.8	14.0	133.	12.2	112.	18	14	5.77	5.40	6.5	5.3
15	4	12	72	12.7	9.9	9.3	8.9	15.3	150.	12.8	119.	36	14	5.64	4.92	6.8	5.5
15	4	13	72	12.3	10.6	9.1	8.5	14.1	137.	11.4	108.	22	14	5.64	4.56	7.3	6.0
15	4	14	72	13.2	10.4	9.0	8.5	13.2	130.	11.7	111.	20	14	5.89	4.56	7.5	5.7
EXTREME				13.2	7.0	9.3	8.1	15.3	150.	10.7	95.	36.	14.	5.89	4.56	7.5	5.0
AVERAGE				10.9	8.8	9.0	8.5	13.7	129.	11.7	107.	25.	15.	5.66	4.97	6.9	5.5
16	4	15	72	13.4	12.2	8.7	8.4	12.8	127.	10.8	105.	24	16	5.52	4.68	7.4	5.6
16	4	16	72	15.0	12.2	8.8	8.1	13.0	131.	10.0	97.	24	16	5.52	4.56	7.4	5.7
16	4	17	72	15.1	12.3	8.6	8.2	11.9	121.	10.3	101.	28	16	6.13	5.16	7.3	5.8
16	4	18	72	15.9	13.0	8.5	8.0	11.5	120.	10.1	101.	34	16	5.64	5.04	7.5	6.0
16	4	19	72	17.3	14.3	8.4	7.9	11.0	118.	9.2	95.	26	18	5.77	5.28	7.2	5.8
16	4	20	72	16.4	15.7	8.1	7.8	9.9	104.	8.9	94.	60	20	5.77	5.52	6.5	5.3
16	4	21	72	18.0	14.7	8.3	7.7	10.9	119.	8.3	86.	30	18	5.64	5.40	7.5	4.7
EXTREME				18.0	12.2	8.8	7.7	13.0	131.	8.3	86.	60.	16.	6.13	4.56	7.5	4.7
AVERAGE				15.9	13.5	8.5	8.0	11.6	120.	9.7	97.	32.	17.	5.71	5.09	7.3	5.6

Rhode River and Tropical Storm Agnes

R. L. Cory - U.S. Geological Survey
M. Redding - Smithsonian Institution

Abstract

Effects of tropical storm Agnes in the Rhode River, a subestuary of the northwest shore of the Chesapeake, were assayed by use of data from a continuous recording water quality monitor.

The monitor's tide record first indicated the storm's influence. June 21 and 22, two higher than usual water levels bracketed a 20 hour period of low water. During the prolonged period of ebbing tide 2 hr. oscillations (seiches) were evident. The seiches showed diminishing amplitudes with time.

Salinity increased along with the high tides but evidence of the Susquehanna River's influence was not observed until June 23, two days post-Agnes, when salinity began to gradually decrease from 7.0 ppt to a low of 2.1 ppt by July 15. During the period of salt reduction the Rhode estuary exhibited a reverse salinity gradient in respect to the Chesapeake Bay which became nearly fresh in the waters outside the Rhode River. The Rhode River's residual salt may have been an important factor in survival of some of the benthic fauna.

Dissolved oxygen content had been decreasing prior to the storm's passage but increased significantly the week following. Seven days prior to the storm the Rhode and West Rivers contained 227 metric tons of oxygen; six days after they contained 332 metric tons. Daily minimum average oxygen at the monitor site was 5.2 mg/l and 8.0 mg/l for the same periods.

At the monitor site diel (24 hour) changes in dissolved oxygen indicate the magnitude of biological metabolism, i.e. daytime primary production and night respiration. The week before and week after showed average diel fluctuations of oxygen of 4.4 and 6.2 mg/l respectively. Comparison of daily oxygen changes in July and August with prior years showed average daily variations of about 4.0 mg/l in 1970-71 vs. 6.0 mg/l in 1972. The late summer increase in the 1972 metabolism resulted in an overall loss of oxygen from the system. Weekly average of daily minimums was about 4 mg/l in 1970-71 vs. 2 mg/l in 1972. Lowest recorded oxygen was 2.9 mg/l in 1970-71 vs. 0.5 mg/l in 1972. This trend has extended into 1973 with increases in daily changes and minimum recorded values of 0.0 mg/l being observed. Daily variations in hydrogen ion activity (pH) confirm the oxygen data.

Water Quality, S.I. Pier, June 1972

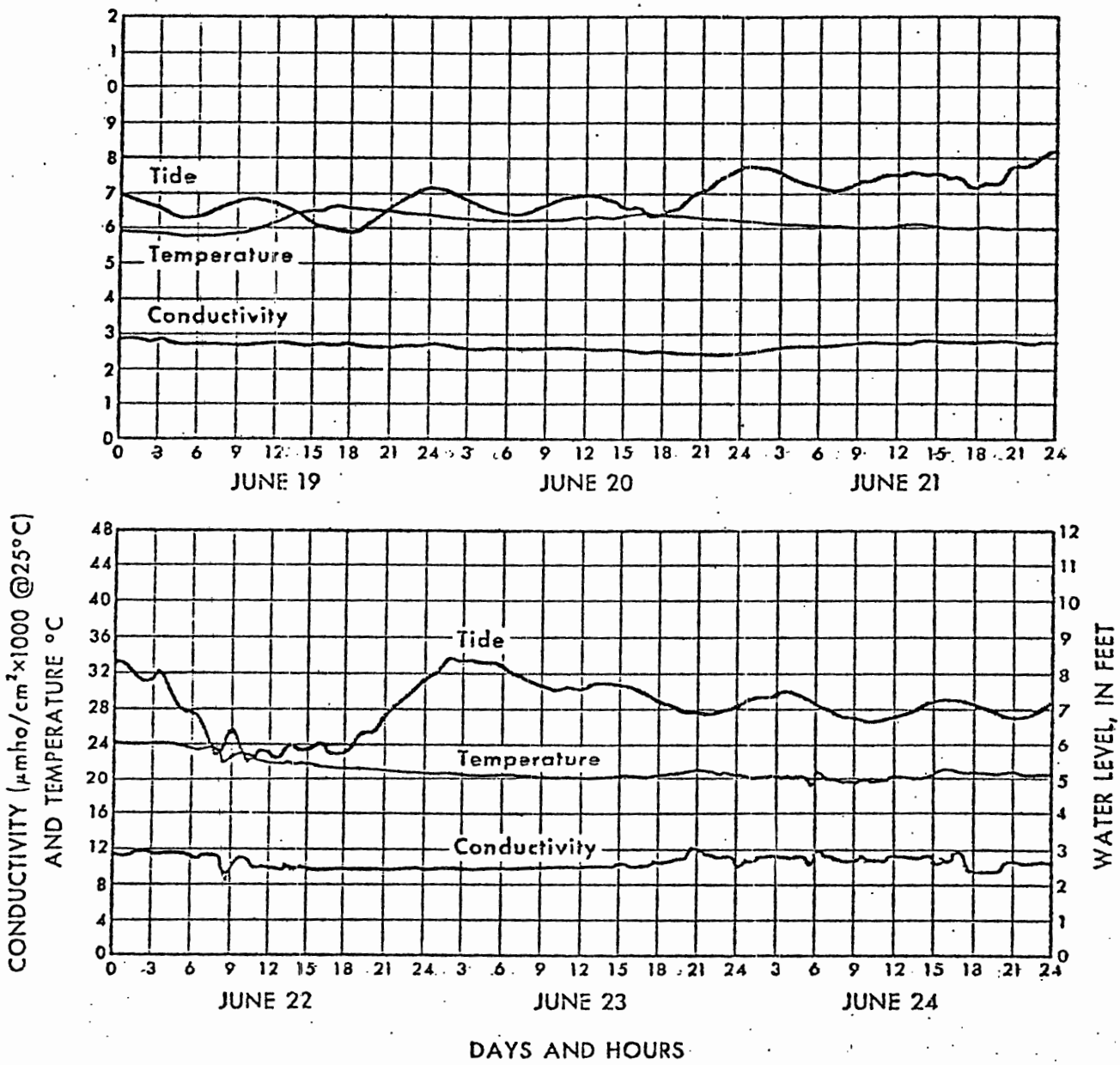


FIGURE I

HYDROCLIMAGRAPH
S.I. PIER, RHODE RIVER, MD.

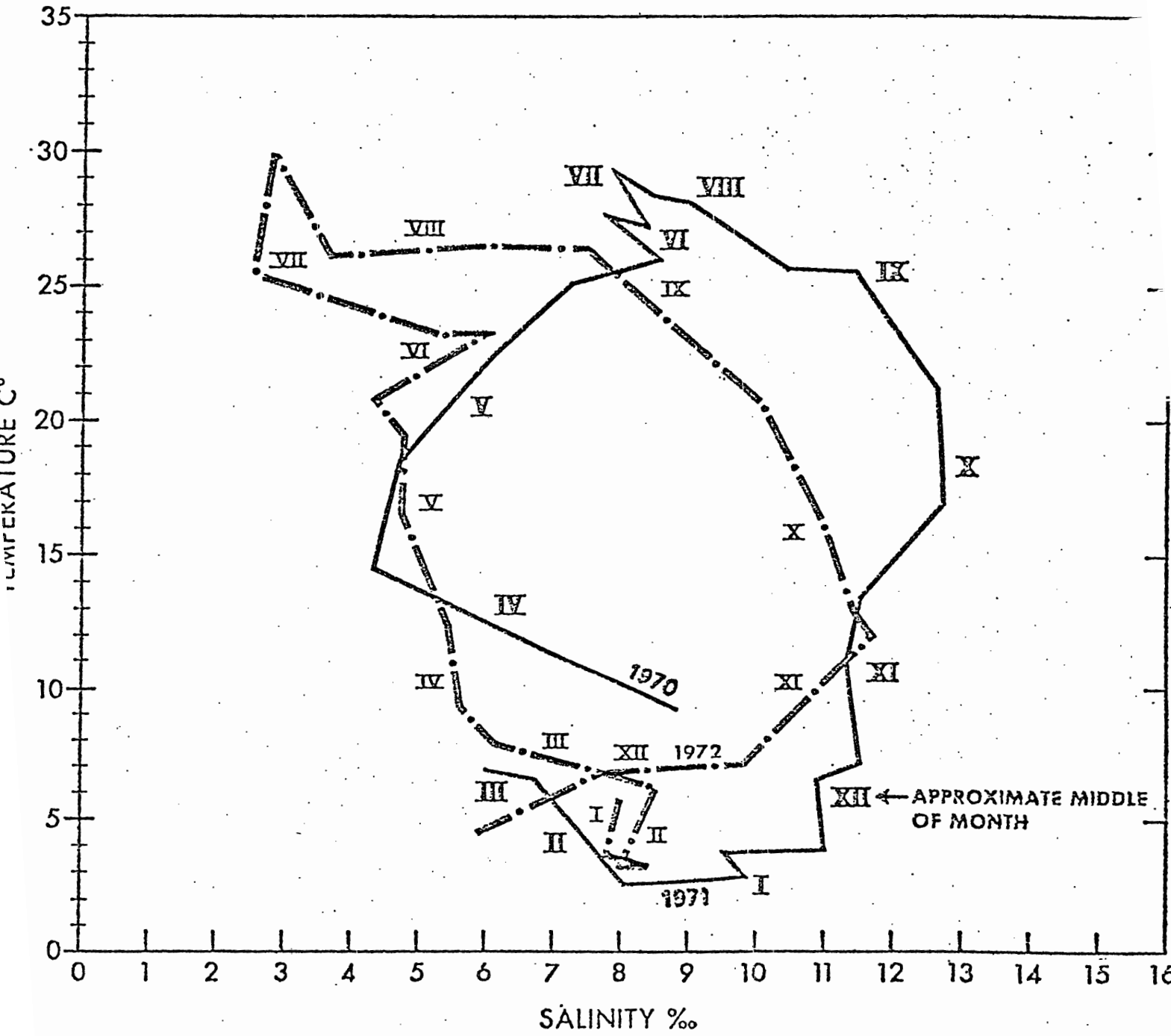


FIGURE 2

Water Quality, S.I. Pier, June 1972

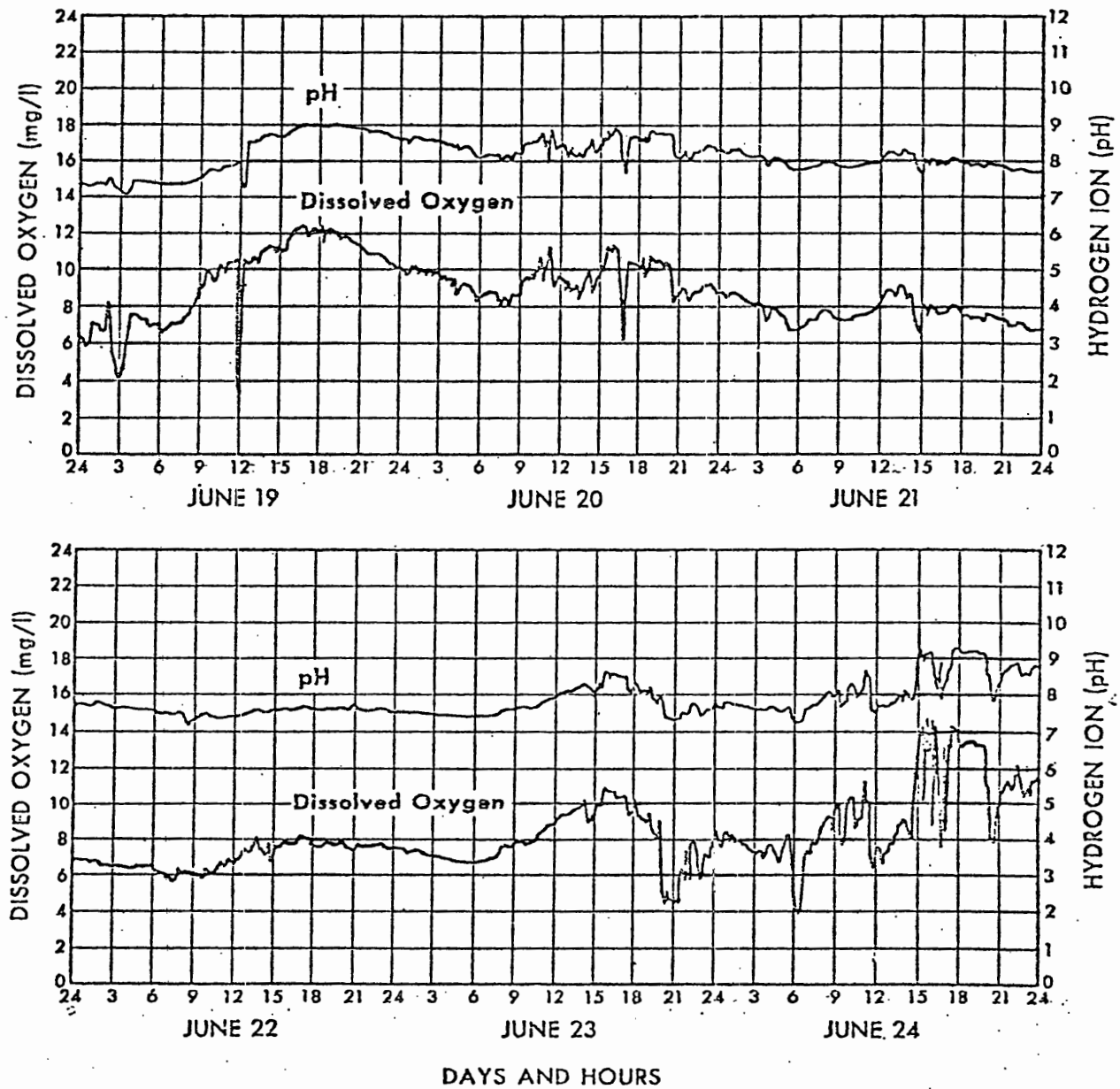


FIGURE 3

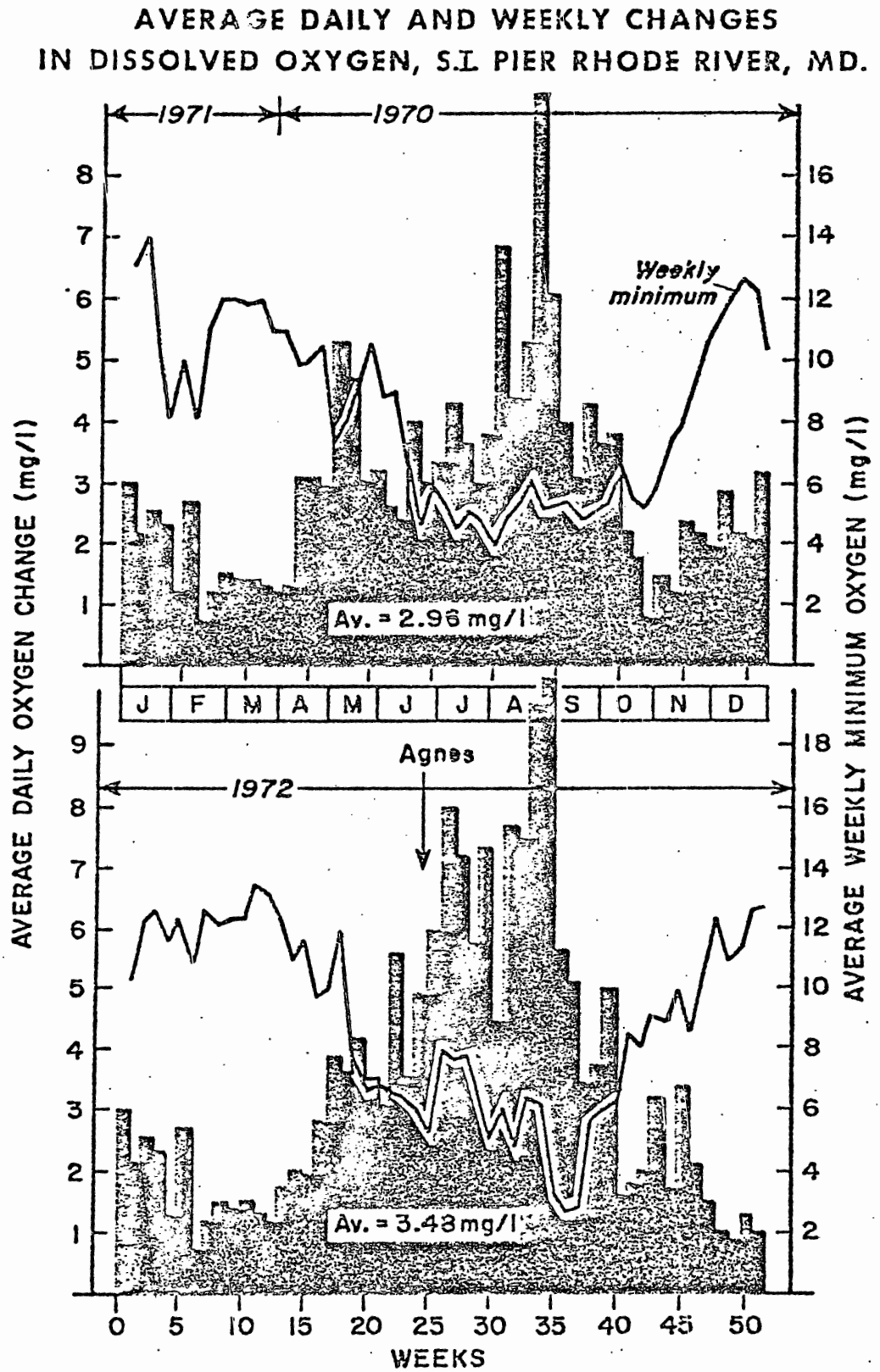

AVERAGE DAILY AND WEEKLY CHANGES
IN DISSOLVED OXYGEN, S.I. PIER RHODE RIVER, MD.
1971
1970
1972
Weekly minimum
Av. = 2.96 mg/l
Av. = 3.48 mg/l
Agnes
J F M A M J J A S O N D
AVERAGE DAILY OXYGEN CHANGE (mg/l)
AVERAGE WEEKLY MINIMUM OXYGEN (mg/l)
WEEKS
0 5 10 15 20 25 30 35 40 45 50

FIGURE 4

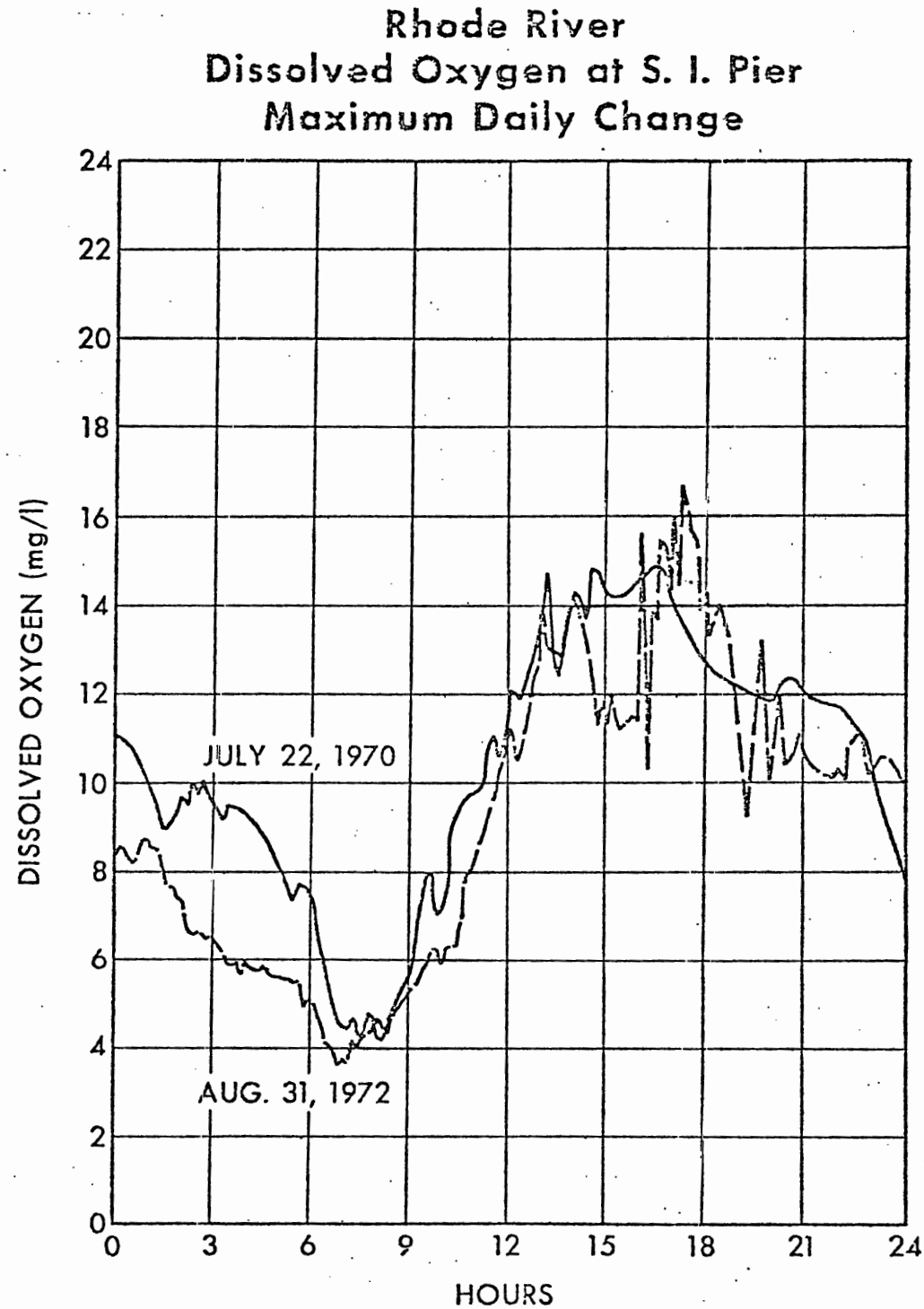

Rhode River
Dissolved Oxygen at S. I. Pier
Maximum Daily Change
DISSOLVED OXYGEN (mg/l)
HOURS
JULY 22, 1970
AUG. 31, 1972
0
2
4
6
8
10
12
14
16
18
20
22
24
0
3
6
9
12
15
18
21
24

FIGURE 5

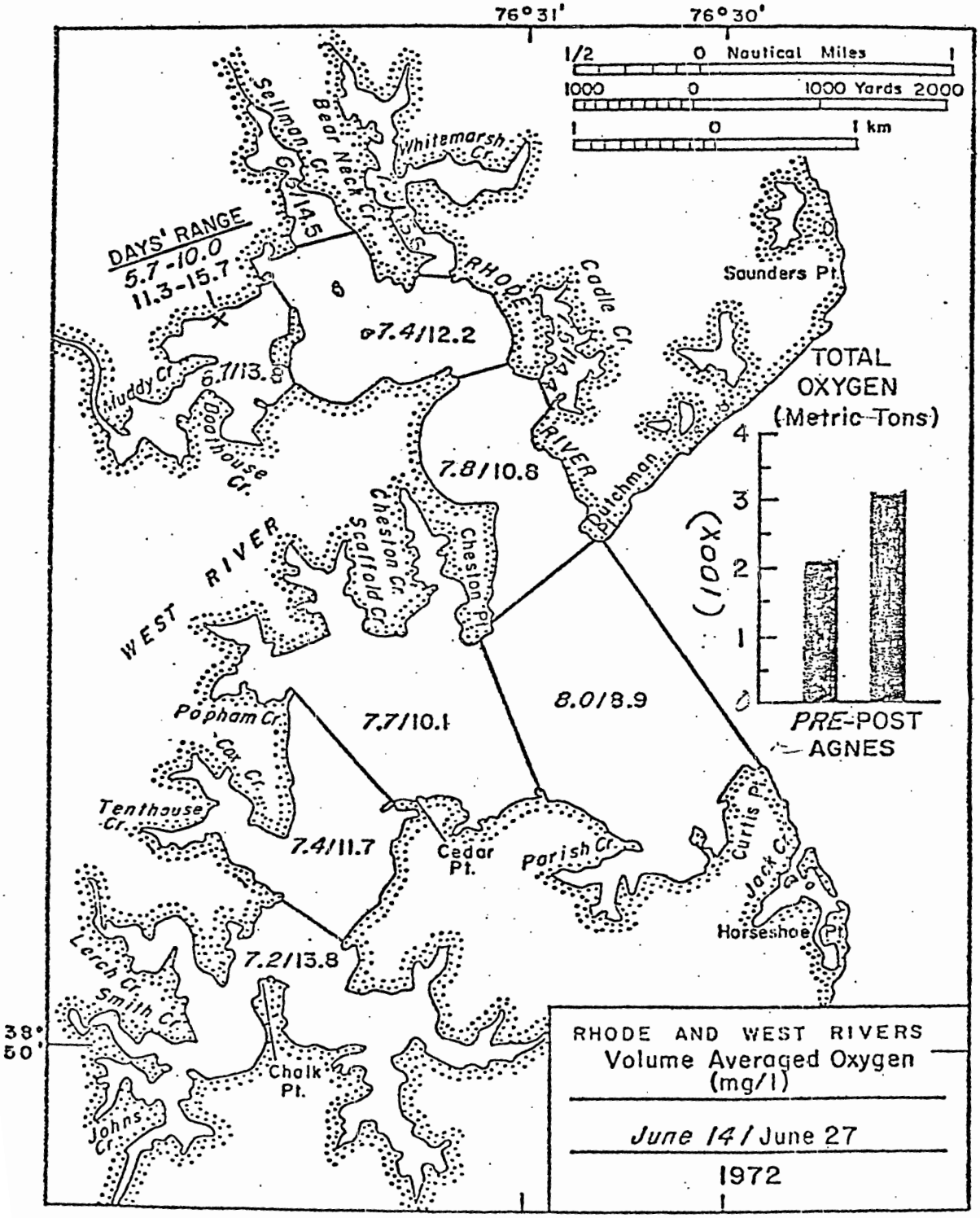

76°31'
76°30'
1/2 0 Nautical Miles 1
1000 0 1000 Yards 2000
1 0 1 km
DAYS' RANGE
5.7-10.0
11.3-15.7
Selman Cr.
Bear Neck Cr.
Whitemarsh Cr.
RHODE RIVER
Cadle Cr.
Saunders Pt.
Muddy Cr.
Goodhouse Cr.
7.4/12.2
7.1/13.9
7.8/10.8
Scaffold Cr.
Cheston Cr.
Cheston Pt.
Butchman
WEST RIVER
Popham Cr.
Cox Cr.
7.7/10.1
8.0/8.9
Tenthouse Cr.
7.4/11.7
Cedar Pt.
Parish Cr.
Curtis Pt.
Jack Cr.
Horseshoe Pt.
Lerch Cr.
Smith Cr.
7.2/13.8
Chalk Pt.
Johns Cr.
38° 50'
TOTAL OXYGEN (Metric Tons)
(100)
4
3
2
1
0
PRE-POST AGNES
RHODE AND WEST RIVERS
Volume Averaged Oxygen (mg/l)
June 14 / June 27
1972

FIGURE 6

II. Open water metabolism.

Estimates of open water metabolism, i.e. daytime net production (P) and nighttime respiration (R) have been made utilizing a modification of a method originally described by H. T. Odum and C. M. Hoskin. Basic data requirements are 24 hour measures of dissolved oxygen, temperature and, in estuarine waters, salinity. The method is dependent upon rate of change of dissolved oxygen over a day's time and will give estimates of net P and night R in grams of oxygen or carbon per cubic meter, or if depth is considered, per square meter per day. The data are analyzed by a computer program furnished by Dr. Robert Kelley, a former student of Odum's.

Data were gathered from 2 sources (1) from the water quality monitor and (2) from profiles in the Rhode and West Rivers. With the assistance of a part-time worker all useable data from the monitor record are being analyzed for the years 1973 and 1974. Approximately 500 analyses have been processed to date. These together with the profile data are being incorporated in a final paper on the Annual Metabolism and Oxygen Budget of the Rhode and West Rivers estuaries. This paper is about 3/4 completed.

The profile data are interesting because they are using the same stations and box or segment approach devised by Mr. Greg Han, PhD candidate, of the Chesapeake Bay Institute, Johns Hopkins University. Preliminary volume averaging of the profile data are accomplished using the Smithsonian's Hewlett Packard desk calculator with a program furnished by Mr. Han. To date 112 estimates were made from all 7 segments in the Rhode and West Rivers during 1973 and about 50 during 1974 in the Rhode River.

Open water metabolism estimates in suitable areas seem to ba a promising way of assessing the degree and limits of organic loading a system can tolerate.

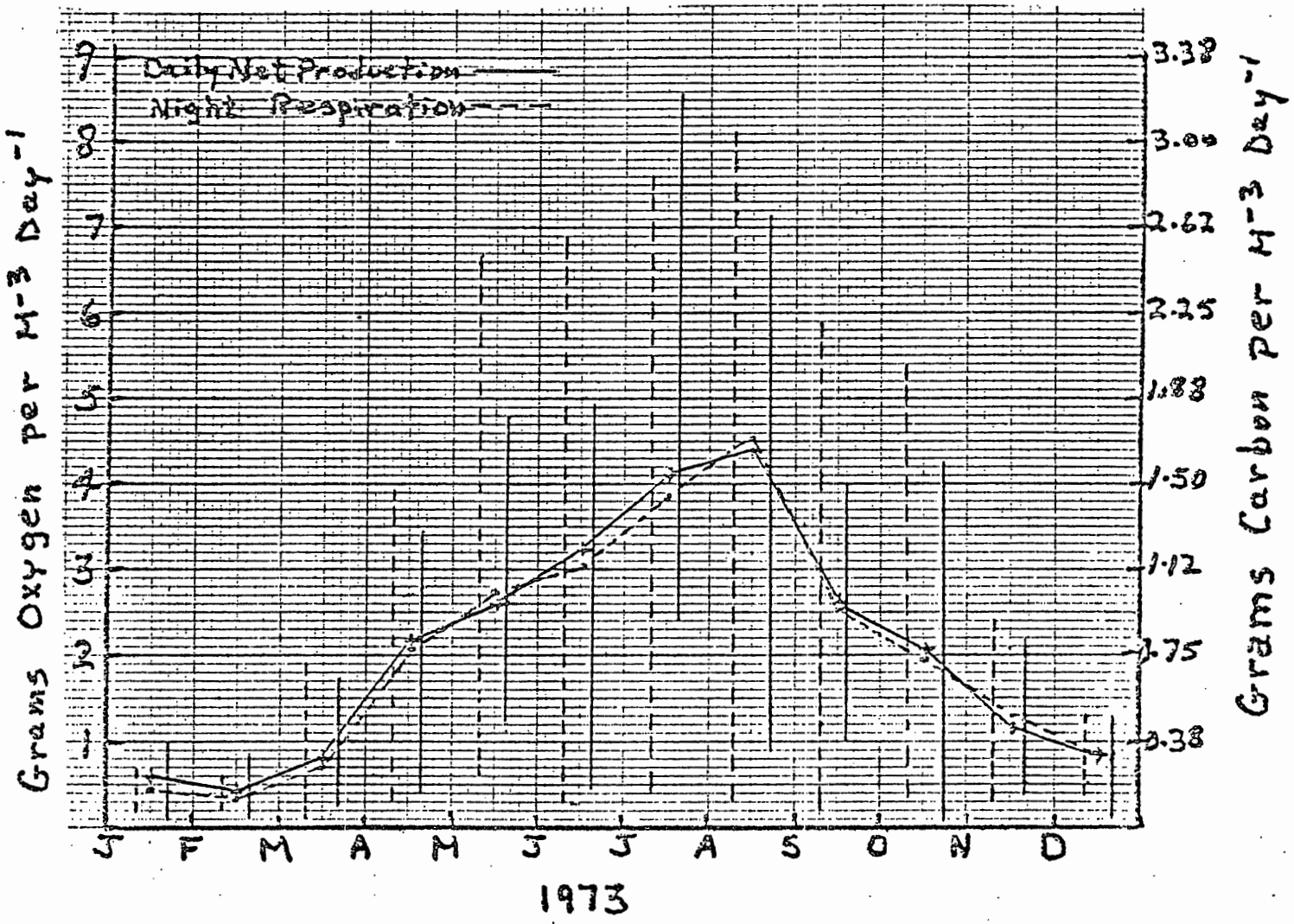
In comparison with similar studies of the Patuxent Estuary, Md. this system, particularly in the upper portion, has a high rate of metabolism. After the influx of water from tropical storm Agnes, metabolism, became excessive and the system became briefly anerobic in late summer 1972, and again in 1973. The 1974 results indicate a return to Pre-Agnes conditions.

Hydrogen Ion or pH measurements have been taken during all the metabolism data collections. Variations in pH converted to carbon dioxide equivalents can be used to estimate metabolic activity in the same manner as dissolved oxygen. To date, pH variations have only served to verify dissolved oxygen variations. As a result of this work I am of the strong opinion that a measure of pH alone if alkalinity is known could give the same answers as obtained with dissolved oxygen. The principal advantages would be superior probe dependability, stability, ease of calibration, independence of salt content of water and superior performance under conditions of biological fouling. To illustrate this point a small table of the average daily pH change during the month of August for the past four years is shown. The high metabolism observed in 1972 is evident in the large daily changes of pH.

Average 24-hr. change in pH during August:

Year	1971	1972	1973	1974
Average pH change	.90	1.42	1.00	.98

Results from 246 analyses of the monitor data for the year of 1973 (see next figure) indicate a well balanced system with production slightly exceeding respiration for 7 months, one month equal and a slight respiration excess for 4 months. As expected, day to day variationswere large, particularly during the most productive season April through October. The September data only represent the last 10 days of the month which accounts



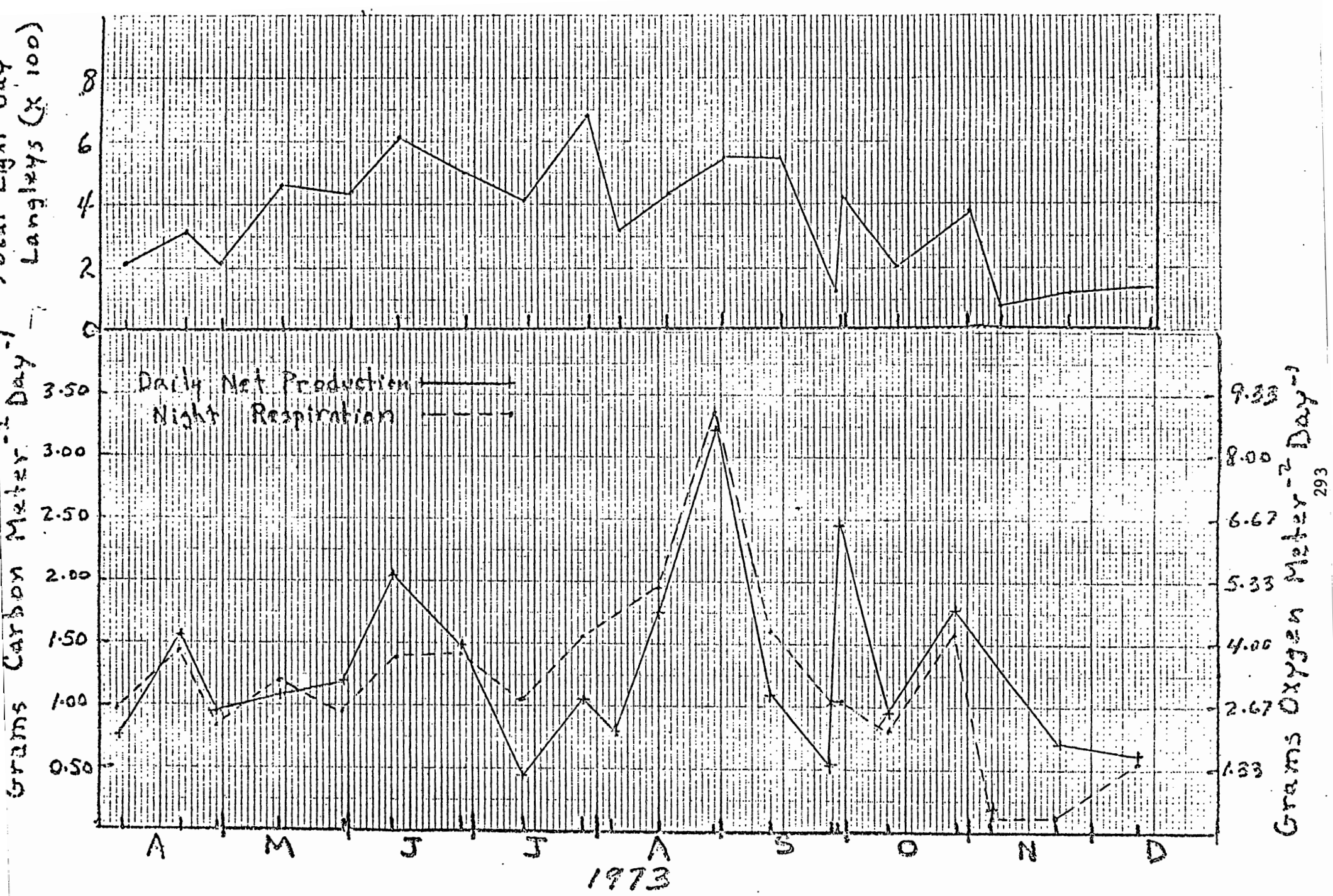
Rhode River Estuary Metabolism at Smithsonian Pier. Connected points indicate monthly average daily P and R. Vertical lines indicate daily ranges of P and R.

for the rapid drop shown on the graph. Early September metabolsim usually equals that of late August.

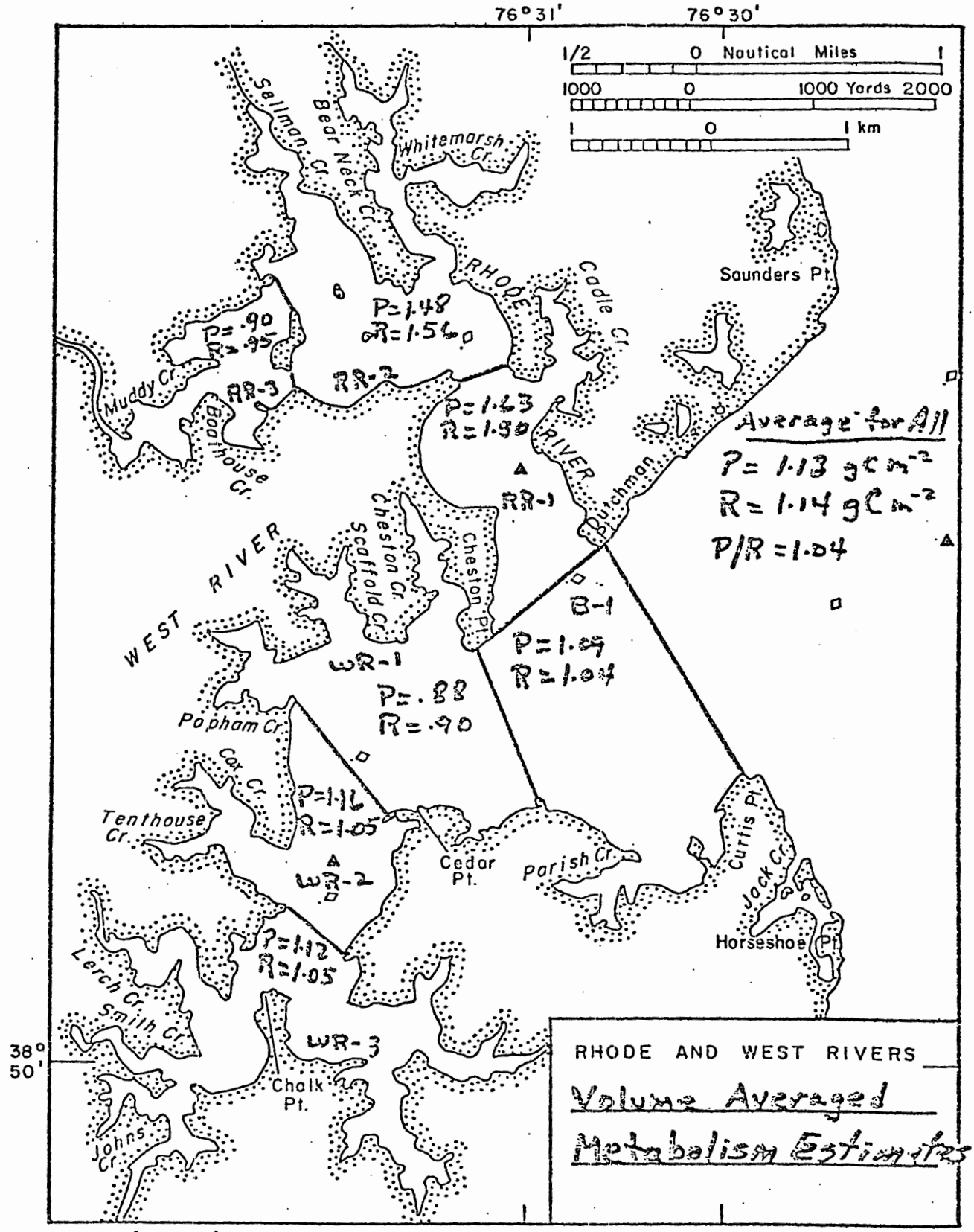
Estimates of volume averaged metabolism from the Rhode River and West River, a summary of 140 bi-weekly measures from the entire system, is shown in the figure following. The large differences between respiration and production are typical of single day analyses and roughly follow the seasonal trend shown in the previous figure. Also shown are the variatiors in light levels of days when measurements were made. Correlations with light are obvious but highest levels of metabolism occurring in July and August do not coincide with highest seasonal light levels. In September light effects are obvious when two successive days of measurements were made. The first a cloudy overcast day of about 130 langleys showed a net production estimate of 0.55 g C M^{-2} day^{-1} while the following day with 420 langleys of light had a net production of 2.45 g C M^{-2} day^{-1}, about a 4 fold increase in both.

To obtain a gross picture of the Rhode and West Rivers' metabolism the biweekly, volume averaged, data were lumped together for the entire period April through December (see figure). The data in this figure show estimates of P and R for each of the 7 segments of the two systems as well as an average for the entire system. This summary of all the data indicates a well balanced system with a P/R ratio of 1.04 indicating an exporting system. RR-3, RR-2 and WR-1 all show excesses of R over P. The greatest respiration excess occurred in RR-2 an area that briefly became anerobic after hurricane Agnes and again the following year. (See above).

Projections of these values based on mean low water depths and surface areas indicate the following for the entire system.



Volume averaged metabolism of Rhode and West Rivers. Points indicate average values for both systems combined. Measurements were taken every other week. Top figure shows light levels on days metabolism measurements were made.



P = Avg. daily net production
R = Avg. nighttime respiration
} gC meter^{-2} day^{-1}

Rhode & West Rivers	Average Yearly daytime Net P	= 6,771	Met. Tons C.
	Average Yearly nighttime R	= 6,588	" " "
	Yearly P-R	= +183	" " "

Gross production and total Respiration would be almost double these figures.

In this report only 1973 data are shown. Results from 1974 analyses are not yet complete, however, for the completed months the monitor metabolism estimates indicate a 1974 further decrease in metabolism from the 1972 high. The volume averaged data from 1974 are still being analyzed. The work from the 1974 data from Rhode River will be compared with the 1973 data. Though West River stations were omitted additional transect and segment stations were added in Rhode River as well as an additional set of profiles, i.e. 4 profiles (dawn-afternoon-sunset-dawn) at each station over a 24 hr. period as compared to 3 (dawn-sunset-dawn). These analyses will be completed when seasonal help is available in January 1975.

III. Biological Studies.

Studies of the biota include three groups of organisms (1) the epifauna, (2) the benthic fauna, and (3) the fishes. Because of the manpower limitations these studies (each of which could be a major project) are limited in scope.

(1) The epifauna have been collected at the Smithsonian pier site by means of artificial substrates. Collections began in 1970 and are continuing. Monthly and quarterly panels are analyzed for species, numbers, and biomass.

The following table lists the phyla species and their relative abundance.

Table. Species and relative abundance of epifauna collected from test panels submerged at the Smithsonian Institution Pier.

Phyla	Species	(Abundance)
Protozoa	Various species	(C)*
Coelenterata	Cordylophora lacustris	(C)
	Bimeria sp.	(S)
	Sagartia leucolena	(P)
Platyhelminthas	Stylochus ellipticus	(C)
Nemertea	Unidentified species	(C)
Bryozoa	Victorella pavida	(A)
	Bowerbankia gracilis	(P)
	Membranipora crustulenta	(P)
Mollusca	Branchiodontes recurvus	(P)
Anelida	Nereis succinea	(C)
	Polydora ligni	(A)
Arthropoda	Balanus improvisus	(A)
	Corophium lacustre	(A)
	Gammarus tigrinus	(C)
	Gammarus mucrunatus	(C)
	Parapleuestes sp.	(P)
	Orchestria platensis	(P)
	Rithropanopeus harrisi	(P)
	Leptochelia sp.	(P)
	Chironomidea (sp.)	(P)

*A = abundant; C = common; P = present

The following table shows the month of attachment and abundance of the 3 most common species which make up a significant part of the total biomass found on panels exposed for 1 month.

Time of attachment and numbers per square decimeter per 30 days of principal epifauna at the Smithsonian Pier site.

Species *Polydora ligni* (Oyster worm)

Month/Yr.	1970	1971	1972	1973	1974
J	--	0	0	0	0
F	--	0	0	0	0
M	--	0	0	0	1
A	--	0	0	1	0
M	20	16	0	22	0
J	6	100	0	747	78
J	0	119	0	985	71
A	200	119	63	228	Incomplete
S	50	321	235	13	"
O	0	37	32	41	"
N	0	98	1	0	"
D	0	0	0	0	"

Species		*Corophium lacustre* (Tube amphipod)			
Month/Yr.	1970	1971	1972	1973	1974
J	--	0	0	0	1
F	--	0	0	4	8
M	--	0	0	4	6
A	--	33	4	18	3
M	222	168	33	182	564
J	913	150	505	875	636
J	880	20	22	42	94
A	40	46	9	0	Incomplete
S	210	6	6	22	"
O	675	0	12	73	"
N	360	9	14	27	"
D		1	8	1	"

Species		*Balanus improvisus* (barnacle)			
J	--	0	0	0	0
F	--	0	0	0	0
M	--	0	0	0	0
A	--	0	0	0	0
M	2,280	11	0	11	15
J	1,635	1,000	1,515	331	37
J	0	9	325	603	0
A	0	0	18	69	Incomplete
S	1	0	5	0	"
O	2	0	0	0	"
N	90	0	0	26	"
D		9	0	0	"

The following table shows the monthly accumulation of epifaunal organic material (ash free dry weight) which was measured during the period of study. Ash free dry weights average about 25 percent of total dry weight.

Ash free dry weight of epifaunal accumulations on test panels exposed for 30 days at Smithsonian Institution Pier. Weights are grams per square meter per 30 days.

Month/Yr.	1970	1971	1972	1973	1974
J	--	--	2.5	--	1.9
F	--	--	2.5	3.7	--
M	--	4.0	1.9	3.7	7.8
A	--	3.4	4.5	13.0	9.1
M	5.2	18.3	6.0	9.3	29.5
J	249.1	251.7	591.5	305.4	62.8
J	250.5	82.2	211.0	--	24.8
A	103.3	34.0	59.8	112.4	
S	335.7	43.6	79.2	62.1	
O	83.4	37.5	37.5	13.6	
N	38.5	9.0	2.8	0.4	
D	--	1.0	1.0	1.5	

(2) Benthic Faunal Studies:

Three subsystems of the Rhode River estuary were sampled with a 6 x 6 x 9" Tall, Handheld, Eckman dredge. The pricipal purpose was to inventory the macrobenthos by species and biomass. A second objective was to compare the faunal characteristics of the 3 creeks to identify obvious differences in the fauna. A third objective was to gain a knowledge of the fauna in one of the creeks prior to receiving effluent from a proposed sewage plant.

The creeks sampled were selected because of principal differences in land use of their immediate watershed. Cadle Creek, located near the mouth of Rhode River is urbanized with houses, bulkheading of shores, several marinas and a small boat factory in the head of the creek. Sellman Creek near the head of the estuary receives the runoff from a cattle farm but is not urbanized. Muddy Creek at the head of the estuary is not urbanized but receives runoff from several small farms; it has the greatest freshwater flow of the 3 creeks and is considered to be the most pristine of the 3 creeks.

Two reports of these studies have been completed and a third is in preparation. The data are not suitable for journal publication but will be issued as open file reports making them available for comparison with any future work.

The reports include surface sediment charts, total organism abundance charts, biomass charts and charts showing distribution and abundance per square meter of each species. The most serious deficiency in these reports was the time of collection. Muddy Creek was sampled, September, 1972, following tropical storm Agnes. Cadle Creek was sampled May 1973 and Sellman Creek August 1973.

Data summarizing results from benthic collections from the 3 creeks are shown in the two tables following.

Table Comparison of Community Dominance for 3 Tributary Creeks of the Rhode River.

	Sellman Creek		Cadle Creek		Muddy Creek	
	% #	% Biomass	% #	% Biomass	% #	% Biomass
Micrura leidyi	.9	3.2	1.1	1.7	7.2	6.3
Macoma balthica	1.2	32.2*	3.5	50.0*	--	--
Macoma mitchilli (phenax)	1.7	17.7	4.9	15.1	1.5	7.5
Notomastus latericeus (Heteromastus)	8.6	14.5	8.0	5.2	4.9	7.2
Scolecolepides viridis	13.2	15.1	18.3	8.3	6.6	19.2
Laeonereis culveri	41.6*	15.0	2.5	2.2	18.8	8.7
Nereis succinea	.2	.2	1.4	5.4	--	--
Hypaniola grayi	4.5	.3	6.7	1.0	.4	.03
Cyathura politta	3.3	1.4	4.3	4.2	18.6	47.8*
Edotea triloba	.4	0	.5	.1	--	--
Corophium lacustre	.1	0	18.8	2.8	1.1	.4
Gammarus tigrinus	.4	0	7.6	1.3	.4	.05
Gammarus mucronatus	.1	0	.8	.1	--	--
Chironomidea	23.6	1.1	21.8*	2.3	40.4*	2.5
Rithropanopeus harrisi	--	--	.1	.5	--	--

* Dominant

Table Comparative Data from 3 Tributary Creeks of the Rhode River.

	Sellman	Cadle	Muddy
Date of Collection	August 1973	May 1973	September 1972
Total # organisms collected	1315	5710	920
Mean # organisms per collection	34.6	78.2	13.5
Mean # organisms/m^2	1491	3370	583
Total biomass collected	2.6193	16.4139	1.5865
Mean biomass per collection (g.)	.0689	.2248	.0233
Mean biomass/m^2	2.9696	9.6888	1.0042
Total # species	14	15	11
Diversity index $D = \frac{s - 1}{\log N}$ *	1.02	1.80	1.10

* s = total number of species

N = total number of individuals

In addition to the above benthic studies two surveys of the commercial clam and oyster beds were carried out to assess the damage caused by influxes of fresh water from tropical storm Agnes to the Rhode, West and South Rivers and adjacent Bay areas. The first survey was conducted with emergency funds supplied by the U.S. Army Engineers Corps which paid for hire of a commercial clam dredger and commercial oyster boat. The second survey was in cooperation with the University of Maryland, Chesapeake Biological Lab. They furnished the services of their clam dredge, one biologist and a boat captain. Their financial support came from the RANN grant. My RANN funded assistant aided in collections and analyses of data. A report on this study has been submitted for publication in Chesapeake Science. See following abstract and figures.

Tropical Storm Agnes and Mass Mortality of the Commercial Clam, Mya arenaria

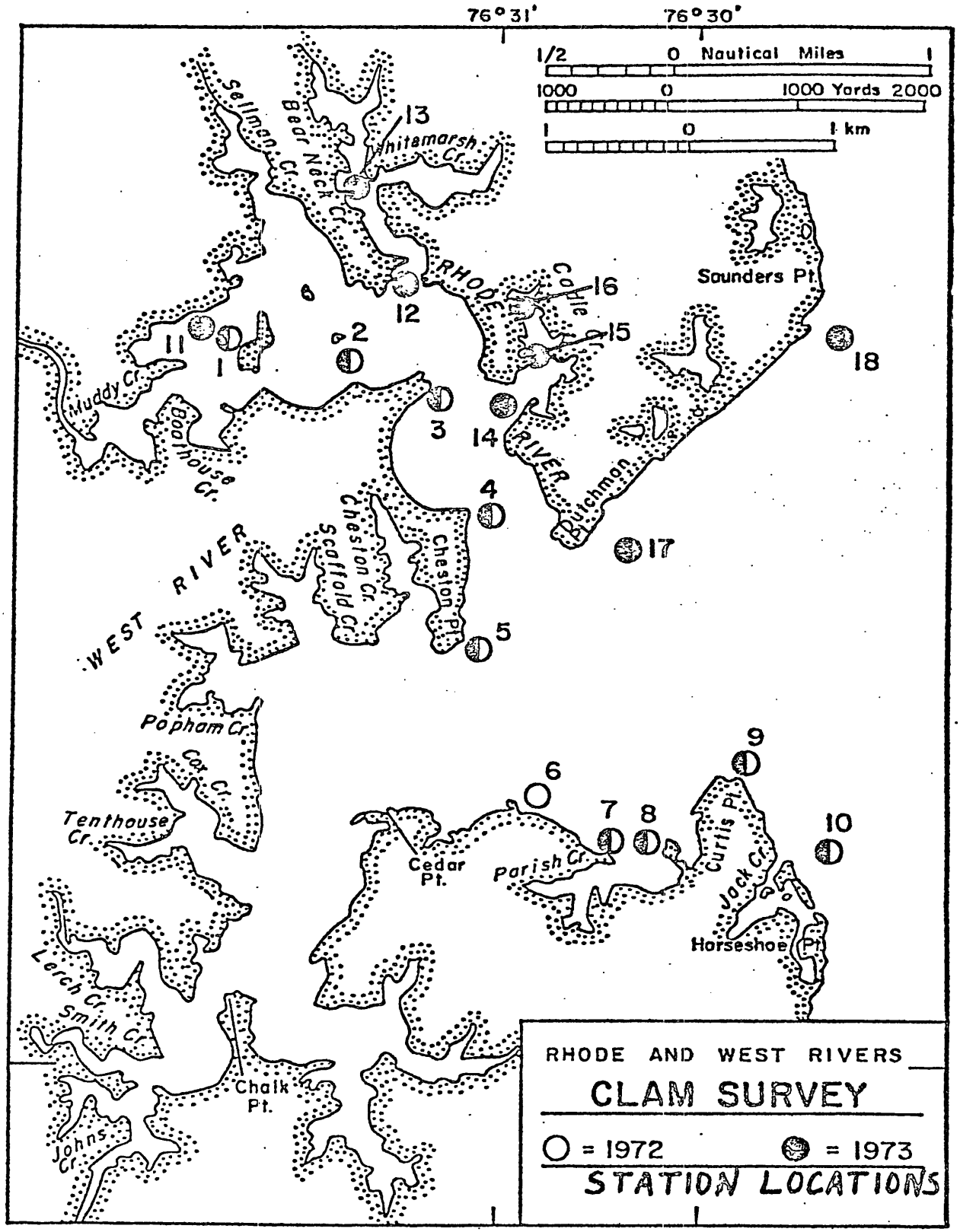
Robert L. Cory
J. Michael Reddding
Chesapeake Bay Center for Environmental Studies
Smithsonian Institution
Rt. 4, Box 622
Edgewater, Md. 21037

Abstract

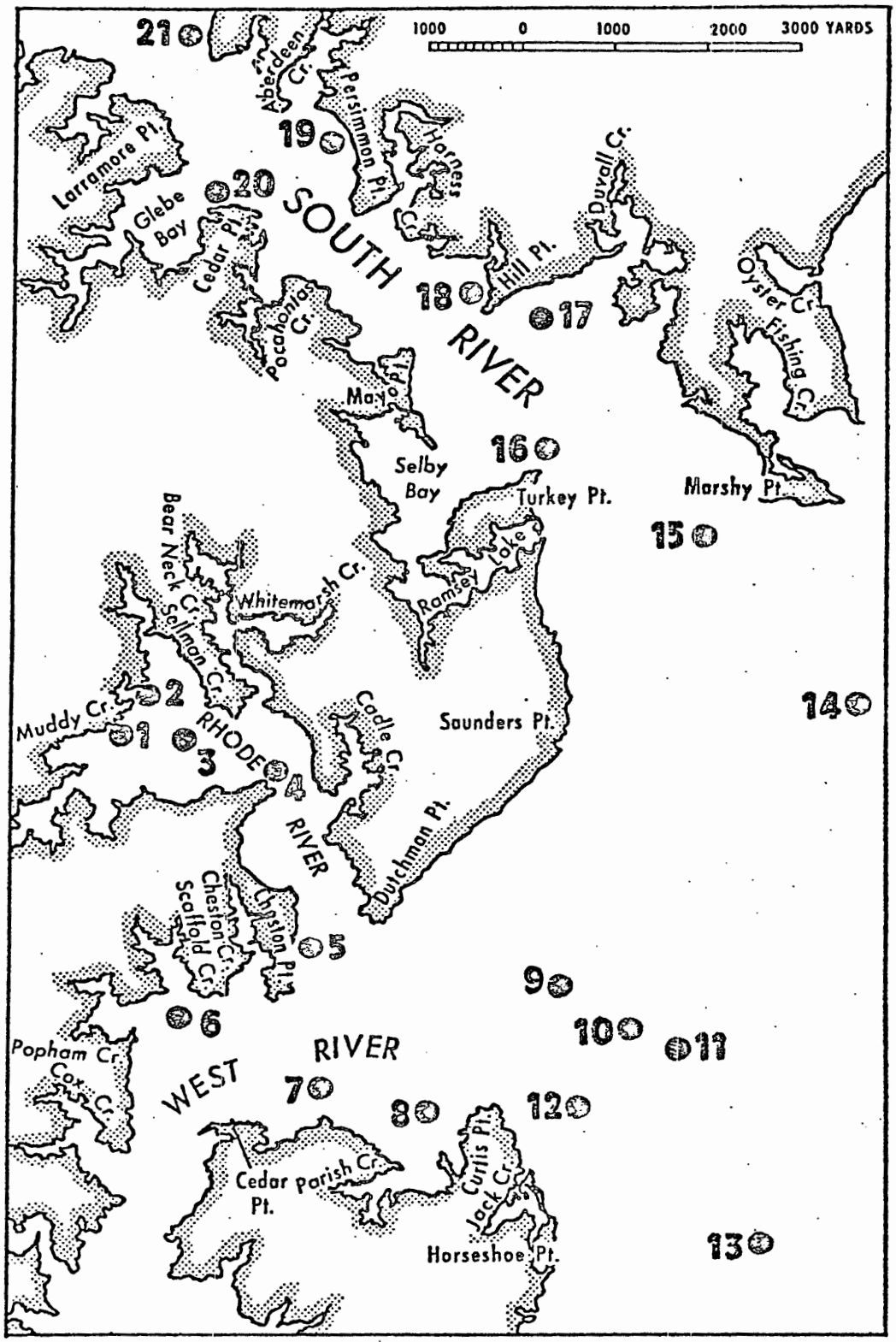
Post Agnes reconnaissance surveys of the clams in Rhode River and adjacent bay and the oysters in Rhode, West and South Rivers and adjoining bay were made in July and August 1972 by means of a Maryland hydraulic clam dredge. No live specimens of the commercial clam Mya arenaria were found. Viable specimens of 4 other molluscs commonly found in these waters were also collected and data indicated they too suffered varying degrees of mortality.

A second survey of the clam stations in October 1973 demonstrated their successful repopulation at the bay stations and the outer Rhode River area. Length data indicated market clams would be available in 1974.

In August 1972, oysters in Rhode River had an average mortality of 24% while the bay stations and South River averaged about a 7% loss. A repeat survey of oysters was not conducted.


76°31'
76°30'
1/2 0 Nautical Miles 1
1000 0 1000 Yards 2000
1 0 1 km
Selman Cr.
Bear Neck Cr.
Whitemarsh Cr.
RHODE
Cable
Sounders Pt.
Muddy Cr.
Boathouse Cr.
RIVER
Bulchmon
WEST RIVER
Scaffold Cr.
Cheston Cr.
Cheston Pt.
Popham Cr.
Cox Cr.
Tenthouse Cr.
Cedar Pt.
Parish Cr.
Curtis Pt.
Jack Cr.
Horseshoe Pt.
Lerch Cr.
Smith Cr.
Chalk Pt.
Johns Cr.
RHODE AND WEST RIVERS
CLAM SURVEY
= 1972
= 1973
STATION LOCATIONS

RHODE AND WEST RIVERS
1972 OYSTER SURVEY



STATION LOCATIONS

Table 1. Results from July 1972 clam survey.

Species	STATIONS[1] 1	2	3	4	5	6	7	8	9	10	MORTALITY PERCENT Alive:Dead
Macoma balthica[2]	3:84	8:10	2:0	0:0	0:0	0:0	0:0	0:0	1:20	0:0	11:89
Mya arenaria	0:0	0:0	0:162	0:21	0:87	0:13	0:88	0:47	0:105	0:26	0:100
Tagelus plebius	0:0	0:1	7:1	0:0	10:20	0:0	3:10	1:0	2:6	0:0	62:38
Brachiodontes recurvus	0:0	0:0	0:0	0:0	0:0	0:0	0:0	36:16	0:0	18:1	77:23
Crassostrea virginica	0:0	0:00	5:0	6:5	0:0	18:2	0:0	11:3	0:0	9:6	75:25

1 See Fig. 1

2 Live animals:recent dead

Table 2. Results of October 1973 Clam Survey.

Molluscs	STATIONS 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
Macoma balthica	-	4	32	149	1		15	35	7	123	-	1	1	1	-	1	67	5	439
Macoma phenax	1	1	3	10	-		11	85	36	37	-	1	-	1	1	1	77	10	274
Mya arenaria	-	-	1	2	-		10	1	15	65	-	-	-	-	-	-	38	43	175
Rangia cuneata	-	-	-	1	1		1	5	7	51	-	-	-	-	-	-	6	1	73
Mulinia lateralis	-	-	-	-	-		-	-	-	1	-	-	-	-	-	-	1	1	3
Tagelus plebius	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	1	-	1
TOTAL	1	5	36	162	2		37	126	65	277		2	1	2	1	2	190	60	965

Not Sampled

Table 3. Results of August 14 and 15, 1972 Oyster Survey.

Location	Station No.	Live	Recent Dead	Total	Percent Alive:Dead
Rhode & West R.	1	0	0	0	0:0
	2	0	0	0	0:0
	3	21	11	32	66:34
	4	75	24	99	76:24
	5	2	1	3	67:33
	6	49	12	61	80:20
	7	16	17	33	49:51
	8	61	6	67	90:10
Total		224	71	295	Avg. 76:24
Chesapeake Bay	9	0	0	0	0:0
	10	24	6	30	80:20
	11	88	6	94	94:6
	12	41	3	44	93:7
	13	10	0	10	100:0
	14	59	1	60	98:2
Total		222	16	238	Avg. 93:7
South River	15	7	1	8	88:12
	16	37	3	40	92:8
	17	73	2	75	97:3
	18	30	1	31	97:3
	19	29	0	29	100:0
	20	116	1	117	99:1
	21	38	12	50	76:24
Total		330	20	350	Avg. 94:6

(3) Fish

To augment previous summer collections of fish from Muddy Creek, 3 fish traps were set weekly near the mouth of the Creek. The period of study was from March 16, 1973, through June 14, 1974. The purpose of the collections was to add to the data base on creek biological conditions prior to placement of a proposed sewage outfall. Species composition, numbers, biomass and length frequencies were determined from the trapped fish. The traps were 5 foot long, 2 ft. high, 2 ft. wide, double funneled and nylon net covered (1/2 x 3/4" mesh). The traps were efficient collectors of most of the larger fish present but small fish could easily pass through the net.

Histograms of length frequencies (not shown) and a table (see following) summarize these collections. A check list of the previous fish collections taken by haul seine is included for comparison of spring and summer populations.

From the trap data it can be seen that the catfish were the most abundant followed by the Pumpkin Seed and White Perch. These three species had size ranges from juveniles to spawning adults. The Catfish showed an interesting pattern of a predominance of juveniles in March and April with a shift to all adults in May and June. Many of the adult white perch, pumpkin seed and catfish were observed to have ripe gonads indicating their use of the creek waters as a place to spawn. The carp have been observed spawning throughout the entire length of the tidal portion of the creek.

MUDDY CREEK FISHES

collections taken with haul seine summer 1971

location	no. collections	date
creek mouth	2	8 July
mid-creek	3	1 July, 2 August
fork	2	28 June, 2 August

Rank	Species	Number	per cent
1.	Menidia menidia	1633	36.1
2.	Fundulus heteroclitus	1448 +	32
3.	Ictalurus nebulosus	522	12.2
4.	Brevoortia tyrannus	418	9.2
5.	Lepomis gibbosus	161	3.6
6.	Lepomis sp.	135	3.0
7.	Morone americanus	130	2.9
8.	Strongylura marina	21	0.5
9.	Notemigonus crysoleucas	8	0.2
10.	Leiostomus xanthurus	5	0.1
11.	Cyprinodon variegatus	4	00.08
11.	Anguilla rostrata	4	0.08
12.	Cyprinus carpio	3	0.07
13.	Anchoa mitchilli	2	0.04
13.	Fundulus majalis	2	0.04
14.	Morone saxatilis	1	0.02
14	Ictalurus catus	1	0.02

BIOMASS

Rank	Species	Number	Total weight	per cent
1.	Cyprinus carpio	3	10,800 g.	43.2
2.	Morone americanus	130	4858.6 g.	19.4
3.	Fundulus heteroclitus	1448 +	3437.4 g.	13.7
4.	Lepomis gibbosus	161	2299.1 g.	9.2
5.	Ictalurus nebulosus	522	1328.5 g.	5.3
6.	Menidia menidia	1633	1018.6 g.	4.1
7.	Brevoortia tyrannus	418	469.8 g.	1.9
8.	Ictalurus catus	1	408.7 g.	1.6
9.	Leiostomus xanthurus	5	113.9 g.	0.5
10.	Lepomis sp.	135	91.1 g.	0.4
11.	Notemigonus crysoleucas	8	73.4 g.	0.3
12.	Fundulus majalis	2	39.2 g.	0.2
12.	Morone saxatilis	1	37.7 g.	0.2
13.	Strongylura marina	21	28.5 g.	0.1
14.	Anguilla rostrata	4	3.3 g.	0.01
15.	Cyprinodon variegatus	4	0.9 g.	0.004
16	Anchoa mitchilli	2	0.5 g.	0.002

Numbers and biomass in grams of fish collected from 3 traps located in the lower section of Muddy Creek.

1973	16 March	20 March	22 March	26 March	28 March	5 April	13 April	19 April	26 April	3 May	10 May	17 May	25 May	1 June	7 June	14 June	Totals
Lepomis gibosus (Pumpkin seed)																(3 alive)	
Total number	11	749	43	4	14	19	110	9	5	10	3	11	10	40	24	13	1,075
Total biomass grams	-	10368	513	35	1028	515+	1963	334	172	535	50	650	513	2665	907	461	20,709
Lepomis sp. (sunfish)																	
Total number	-	3	3	-	-	1	7	6	-	1	-	-	4	1	2	-	28
Total biomass g.	-	31	104	-	-	4	21	550	-	81	-	-	506	172	100	-	1,569
Fundulus heteroclites (Killifish)																	
Total number	-	42	5	2	-	-	5	1	-	-	-	-	-	2	-	9	66
Total biomass g.	-	99	24	13	-	-	21	3	-	-	-	-	-	9	-	-	169
Notemigonus crysoleucas (shiner)																	
Total number	-	3	-	-	-	-	-	2	-	-	-	-	1	-	-	11	17
Total biomass g.	-	32	-	-	-	-	-	100	-	-	-	-	5	-	-	50	187
Ictalurus nebulosus (catfish)																	
Total number	30	500	9	145	424	2076	18	12	26	40	11	10	3	6	1	4	3,315
Total biomass g.	-	5756	71	7463	16340	33489	721	909	2262	15761	4008	2022	800	1665	338	1130	92,735
Morone amer. (white perch)																	
Total number	7	4	7	19	30	13	2	59	1	16	24	29	41	20	8	8	288
Total biomass g.	-	532	704	2216	3206	840+	128	4127	82	1638	1915	2910	3300	1959	597	500	24,654
Perca flavescens (yellow perch)																	
Total number	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
Total biomass g.	-	-	-	-	24	-	-	-	-	-	-	-	-	-	-	-	24
Anguilla rostrata (eel)																	
Total number	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	1	3
Total biomass g.	-	-	-	-	-	-	-	71	168	-	-	-	-	-	-	900	1,139
Cyprinus carpio (carp)																	
Total number	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1	-	3
Total biomass g.	-	-	-	-	-	-	-	100	-	-	-	-	-	-	2000	-	2,100
Calinectes sapidas (blue crab)																	
Total number	-	-	-	-	-	-	-	1	1	1	1	4	-	1	8	-	17
Total biomass g.	-	-	-	-	-	-	-	44	21	-	30	53	-	36	515	-	699

Concluding Statement

The above work was accomplished only through a cooperative effort of the U.S. Geological Survey, National Science Foundation, Smithsonian Institution and the Universities of Maryland and Johns Hopkins. This cross exchange of ideas, facilities use, computer programs and sometimes exchange of personnel seems to be a most valid approach to the needed study of these complex systems.

The Chesapeake Bay the largest estuary in the world is in a state of high biological production of a variety of seafood. The waters are extensively used for both sport and commercial activities. Despite the seemingly healthy conditions that prevail in the Bay, trouble signs are evident. Several of the industrialized tributaries are no longer in useful biological production. Organic loading from domestic sewage is becoming excessive. Tributaries such as the Potomac and Patuxent are in trouble with late summer algal blooms and low dissolved oxygen. Annual spring fish kills are now predictable in the upper Bay and intermittent kills of fish are not uncommon during the late summer.

It is my belief and hope that a variety of talent pooled in cooperative laboratories such as the Chesapeake Bay Center will furnish the data base and research necessary to manage this vast and complex water system.

In a 1966 Department of Interior Task Force Report on Estuarine Pollution they repeatedly stressed the importance of cooperative studies in understanding and managing estuaries. With the tightened fiscal policies of 1974-75 this is now more important than ever.

A.

Microbial Studies of the Carbon Cycle in the Rhode River of the Chesapeake Bay

RANN Grant Number GI 38973

Final report for the period June 1, 1973 - September 30, 1974

(Total Budget = $38,099)

Submitted by

Rita R. Colwell
Principal Investigator

and

Jayne F. Carney
Research Associate

Department of Microbiology
University of Maryland
College Park, Maryland 20742

R. R. Colwell
Professor of Microbiology

December 4, 1974
date

B. Abstract

Four different areas in the Rhode River sub-estuary of the Chesapeake Bay were examined on a monthly basis from September, 1973 through September, 1974. Water and sediment were sampled to enumerate the total, viable, aerobic, heterotrophic bacterial populations and to estimate, by means of the most probable number technique, standard indicator organisms, namely, total and fecal coliforms and fecal streptococci. No seasonal fluctuation in the numbers of heterotrophic bacteria was apparent, although there was a detectable increase in total viable bacteria in the water, January through March. The numbers of coliforms indicated a sporadic and unpredictable occurrence. However, the methods for coliform determination are not precise. More than 95% of the positive streptococci reactions were confirmed, demonstrating an acceptable reliability for the fecal streptococci index procedure employed in the Rhode River study. No Salmonella were found at concentrations of, or greater than, one organism per gram of sediment. The presence of Clostridium botulinum in sediment was confirmed, and Vibrio parahaemolyticus-like organisms (VPLO) were isolated except in the winter months, when VPLO's could be isolated only from sediment samples.

The ability of the heterotrophic bacteria to utilize chitin, casein, cellulose and urea was determined on a quarterly basis. Seasonal fluctuations in generic types were determined by isolating over fifteen hundred bacteria and identifying and classifying the pure cultures to the genus level. Water samples from the four Rhode River stations were compared with two other Chesapeake Bay stations for the ability to take up labelled substrates. Samples of water collected in

Colgate Creek, which is an oil-polluted site, and in a populated site in Cadle Creek demonstrated greatest uptake. Seasonal comparisons indicated that the lowest rates of uptake occurred in December and the highest in May.

C. Objectives

1. To determine the total numbers of aerobic, heterotrophic bacteria in the water and sediment on a monthly basis.

2. To enumerate different types of indicator organisms and to compare the various methods used for determining the presence of these bacteria.

3. To identify the frequency of occurrence of pathogens, viz. Salmonella and Clostridium botulinum.

4. To establish the capability of bacteria to utilize natural substrates, such as chitin, cellulose and urea.

5. To estimate the heterotrophic potential by measuring the amount of labelled material taken up at different areas and at different times during the year.

6. To compare the composition of the bacterial populations at each site by identifying isolates to the generic level.

D. Introduction

The construction of a large facility, such as a power plant, sewage treatment plant, or housing project, inevitably results in an increased demand on the water system into which the waste effluents run. Assessment of the ability of the given ecosystem to adjust should be a significant component of a siting study for the facility. It should be possible to construct models so that effects of system perturbations can be evaluated and such evaluations applied to related situations without repetitions and costly studies for every new facility siting. One of the objectives of this project was to collect data concerning the bacterial populations as a portion of an over-all system study. This research was directed toward the enumeration and identification of the aerobic heterotrophic bacteria present in the Rhode River sub-estuary of Chesapeake Bay, correlation of numbers of indicator organisms with presence of specific pathogenic bacteria, and evaluation of the heterotrophic potential of the bacterial populations at selected sites in the Rhode River.

Since the four sampling sites, or stations, that were carefully selected to be representative of the Rhode River estuary have different nutrient inputs, effects on the microbial populations were compared at each of the stations. The stations were sampled at monthly intervals during the period, September, 1973 through September, 1974.

E. Methods

Location of Sampling Sites

The station numbers 0.0, 3.38, 5.4 indicate distance, in miles, from the mouth of the Rhode River sub-estuary. Station number CC 0.6 refers to the distance from the entrance of Cadle Creek into the Rhode River proper (Fig. 1). Station 0.0 is located at the junction of the Rhode and West River sub-estuaries. Data collected at Station 0.0 were useful in that they provided information concerning bacteria and nutrients entering and leaving the Rhode River system. Station 3.38, located in one of the two relatively deep areas on the east side of Big Island, was selected to provide information concerning the main body of water in the Rhode River. Station 5.4 is a site in the marsh area of Muddy Creek. Cadle Creek is a populated area with some industrial effluent, i.e., from several gas stations and marinas. All four stations were sampled on a regularly scheduled basis, not only by us but also by other investigators working on the Waste Water Program in Rhode River.

Frequency of Sampling

Frequency of sampling depended upon the information sought. Aerobic, heterotrophic bacteria were enumerated monthly so that periodicities, if any, could be detected. Although indicator organisms were enumerated monthly, specific pathogens were tested for on a seasonal basis, except in the warmer months. The generic distribution of representative bacteria in water and sediment were analyzed quarterly, i.e. four times a year, so that seasonal population variations could be assessed.

Heterotrophic uptake rates are such that the incubation time required for uptake in the winter months exceeds the experimental time limits. Hence,

a majority of the experiments were conducted during the warmer months, although one extensive experiment was carried out in December (temperature ca. 9°C) and another in April (temperature ca. 12°C). Work by other investigators carried out in the Georgia marshes indicates that degradation of marsh grasses requires a time period in excess of six months. Hence, the decomposition experiments carried out as part of this program were designed for sampling every forty days for six months.

Sampling Procedures

Water samples were collected with a sterile Niskin bag sampler at a depth of one meter. The water samples were transferred aseptically to a sterile jar. In the winter months, the water samples in the sterile jars were iced until return to the laboratory for work-up. Sediment samples were collected using a Petit Ponar grab. The grab, after the sediment had been collected, was opened onto a plastic bag. The sediment samples were taken from the upper central portion of the collected sediment and were added aseptically to sterile beakers. All of the sterile beakers containing sediment were stored in ice when samples were collected in the winter months, and in an insulated cooler chest in the summer. Heterotrophic uptake experiments were performed in situ, i.e., on station, whereas samples for enumeration of total, viable, aerobic, heterotrophic bacteria, were taken to the Smithsonian field laboratory for work-up. Total elapsed time between sampling and plating was in the range of 1/2 hr to 3 hrs. Conductivity, salinity and temperature of the water and sediment samples were measured with an Electrodeless Induction Salino-

meter (Oceanography Unlimited, Hoboken, N. J.) and dissolved oxygen with a Model 51 A Oxygen Meter (YSI Instruments, Inc., Yellow Springs, Ohio). A Secchi disk was used for turbidity measurements.

Enumeration of total, viable, aerobic, heterotrophic bacteria

Enumeration of the bacterial populations was accomplished by plating, in triplicate, 0.1 ml of the samples from the dilution series (10^{-1}, 10^{-2}, 10^{-3}-water; 10^{-3}, 10^{-4}, 10^{-5}-sediment) on Estuarine Salt Water Yeast Extract (ESWYE) agar plates. Colonies were counted after the plates had been incubated for two weeks at 15C. ESWYE medium consists of 0.1% yeast extract, 0.1% proteose peptone and 2% agar, with a three salts solution employed as the diluent. The final pH of the medium was adjusted to 7.2-7.4. The three salts solution contains 1.0% sodium chloride, 0.23% magnesium sulfate heptahydrate, and 0.025% potassium chloride. Water samples were diluted in 9 ml of the three salts medium. Sediment dilutions were prepared using volume displacement in 180 ml three salts solution.

Enumeration of Indicator Organisms

All of the techniques for standard indicator organisms followed the procedures set forth by the A.P.H.A. (Standard Methods for the Examination of Water and Waste water, 1971). A five tube, three ten-fold dilution series of lactose broth was used for determination of the most probable number (MPN) of coliforms. The MPN tubes were incubated at 35C and were examined at 24 and 48 hours. Positive tubes, indicated by growth and gas production, were confirmed in Brilliant Green Lactose Bile (BGLB) broth or on Eosin Methylene Blue (EMB) plates, also incubated at 35C for 48 hours. A five tube, three

ten-fold dilution MPN series in EC broth was incubated at 44.5C (± 0.5C) for 24 hours to determine the number of fecal coliforms. The EC MPN was inoculated from positive lactose broth tubes. The positive EC tubes, indicated by growth and gas production, were confirmed on EMB medium incubated for 24 - 48 hours at 35C. All of the various media were obtained from Difco Laboratories, Detroit, Michigan, or Bioquest Laboratories, Cockeysville, Maryland.

A five tube, three ten-fold dilution series of Sodium Azide Dextrose Broth was used to determine the MPN of fecal streptococci. The Sodium Azide Dextrose Broth medium was incubated at 35C for 48 hours. Turbidity was used to indicate a positive result. Samples from positive tubes were transferred to Ethyl Violet Azide Broth and incubated at 35C for 48 hours. Although the instructions for use of Ethyl Violet Azide Broth indicate that only tubes showing a purple button are to be interpreted as positive, our biochemical studies showed that all the precipitates contained fecal streptococci. Hence, any sedimented turbidity in the tubes was regarded as positive. In several cases, all of the tubes showing turbidity were examined for the presence of fecal streptococci. The organisms were first streaked on M-enterococcus medium and the pink colonies were transferred to Nutrient Agar plates for isolation of pure cultures. The pure cultures were tested for catalase, oxidase, growth in 6.5% sodium chloride, and ability to hydrolyze starch and sodium hippurate. The method of Facklam et al. (1974) was used for the determination of hippurate hydrolysis. This series of tests also provided a measure of the efficacy of the test results.

Presence of Salmonella Organisms

Several methods were used to isolate Salmonella. The steps involved in the procedures used were as follows. First, four hundred ml quantities of water or diluted sediment were filtered and incubated in Selenite broth overlaid with mineral oil (to inhibit growth of Pseudomonas organisms) at 35C for 4 hours. Tubes showing growth were plated onto Bismuth Sulfite medium for isolation of pure cultures. A comparison of Bismuth Sulfite, Salmonella, Shigella and Deoxycholate Citrate (DC) agar media showed that positive colonies were more easily distinguished on DC medium. In the procedure finally adopted for isolation and characterization of Salmonella organisms, one liter of sample was filtered through successive 0.45 µm Millipore filters, which were folded and placed in a tube of Selenite Cystine broth, the broth overlain with mineral oil, and the tubes incubated at an elevated temperature (43C). After 48 hours, the cultures were streaked on DC medium and the plates incubated at 35C for 48 hours. Several positive cultures were isolated and tested for catalase, oxidase, urease and hydrogen sulfide production. Suspected Salmonella organisms were tested further, using the A.P.I. system. Typical Salmonella organisms were confirmed by serological methods, employing H antigen.

Presence of Clostridium botulinum

Sediment samples (ca. 1 gm) were added to sterile Cooked Meat medium which had been boiled for ten minutes and cooled immediately prior to inoculation. Samples were either overlaid with mineral oil or not, depending on the experiment. After five days incubation at 25C, samples were frozen at -70C until further testing was done. The frozen cultures were warmed to room

temperature. The culture liquid overlying the cooked meat was transferred individually to centrifuge tubes and centrifuged for 10 minutes at 10,000 rpm, using an SS 34 rotor in a Sorvall RC2-B centrifuge set at 5°C. The procedure was repeated if the supernate was cloudy; if clear, the supernate was decanted. After 4.5 ml supernate and 0.5 ml trypsin (1% aqueous) were mixed, the solution was incubated at 37C for 45 min. Two mice, one protected against Clostridium botulinum toxin and one unprotected, were injected for each station sample. Two mice, again one protected and one unprotected, were injected (to serve as controls for the group) with Cooked Meat medium processed by the procedure detailed above. One mouse was injected with 0.4 ml of the treated filtrate, while a second was injected with a combination of polyvalent antitoxin (0.1 ml) and the given treated filtrate. Injection of the antitoxin preceded injection of the filtrate. The mice were observed for 48 hours. Results given, as follows, illustrate the procedures employed:

Unprotected	Protected	Interpretation
no death	no death	1
no death	death	2
death	no death	3
death	death	4

The first situation, the most frequent, was interpreted as indication that Clostridium botulinum was not present at concentrations greater than one organism per gram of sediment. The second situation was interpreted as providing evidence that perforation of an internal organ occurred or death was

caused by shock. The third situation was interpreted as indicating that the mouse died from botulism toxin, since the protected mouse survived. In this instance, a further series of injections were performed using three mice. The entire procedure for preparation of the trypsinized filtrate was repeated, since trypsinized toxin cannot be stored. For purposes of duplication, one mouse was injected with the treated filtrate, and the other two mice with anti-B antitoxin or anti-E-antitoxin. If insufficient material was available, the B and E antitoxins were mixed. These antitoxins were employed because type B is the most prevalent botulinum toxin occurring in the East, and type E botulinum toxin is found in aquatic environments. The fourth situation indicated death from another cause, usually Clostridium tetani, or an overdose of the toxin in the filtrate. In this instance, the above procedure was modified such that each of the three mice was protected with a pre-injection of 0.1 ml anti-tetanus antitoxin approximately thirty minutes before injection with the filtrate. In the event of all three of the mice dying (which did not occur in this study), the procedure was repeated with a diluted filtrate. These procedures are a modification of those described by Dowell and Hawkin (1974).

Presence of Vibrio parahaemolyticus-like Organisms (VPLO)

Two procedures were used for detecting VPLO's. In the first, a five tube, three ten-fold dilution MPN series containing Salt Colistin broth was inoculated and incubated at 35C for 12 to 24 hours. All of the tubes showing turbidity were streaked on TCBS agar plates, which were then incubated at 35C for 24 hours. The second method was employed in this study in the warmer

months, when greater numbers of VPLO's were anticipated. It was intended that the results of the two methods be compared but a shortage of Colistin depleted our supplies of Salt Colistin medium. Consequently, samples were directly plated onto TCBS plates, using the procedures detailed under "Enumeration of total, viable, aerobic, heterotrophic bacteria." The inoculated plates were incubated at 35°C for 24 to 48 hours.

Distribution of Generic Types

Fifty-five colonies from water and fifty-five from sediment samples were selected from the ESWYE plates in October, 1973; January, 1974; April, 1974 and July, 1974. All of the colonies from one or two quadrants on a plate were picked to avoid bias in selection of the colonies. Fifty-five colonies were picked from the count plates, because it was anticipated that some of the colonies would not survive repeated transfer. As soon as pure cultures were obtained, the pure cultures were subjected to the following procedures, using a scheme developed in our laboratory (Johnson and Colwell, 1974). Cultures were streaked for isolation on ESWYE until pure, generally three times. The pure cultures were maintained on ESWYE slants at 15C until the tests were completed and then were stored under oil at 15C. All of the cultures were Gram stained (at 20-22 hours, except for very slow-growing organisms) and examined for shape, approximate size and presence of spores, and pigmentation of the colony. The cultures were tested for motility, and positive strains were flagella stained. The biochemical tests which were done included catalase, oxidase and the marine oxidative-fermentative glucose test, wherein gas production was noted using a Durham tube.

Estimation of Heterotrophic Potential

The portion of the total, viable, aerobic, heterotrophic bacterial populations capable of metabolizing casein, chitin, and cellulose was estimated using a spreader bar technique on double layer plates. The plates were prepared using an underlay of ESWYE and an overlay consisting of 1% substrate and three salts agar. A zone of clearing around a colony indicated utilization or hydrolysis, and the number of colonies was calculated as a percent of the total population on the plates. Casein plates were examined at 48 hours, since longer incubation resulted in the zone from a rapidly growing colony spreading over the plate. It was not possible to estimate these physiological types, when $\geq$ 50% of the population was capable of utilizing the given substrate because of the resulting confluent zones. Chitin and cellulose plates were examined after a month incubation. The number of urea utilizing bacteria was also estimated using a Urea broth, five tube, three ten-fold dilution MPN series, which was incubated at 25 C for 2 weeks. The highest dilutions that were positive were streaked on Urea agar plates and incubated at 25C for confirmation. Positive results were obtained when the indicator changed from orange to purple-pink.

Heterotrophic uptake rates were estimated, using the method of Williams (1970). Several water samples collected aseptically with a Niskin sampler were pooled in a large sterile carboy. For each substrate tested, a 400 ml sample was placed in a sterile stoppered bottle covered with a piece of tire tubing. A 360 ml duplicate sample was treated by addition of 40 ml of

of 36% formaldehyde solution. The latter control indicated label lost through attachment to surfaces. Radioactively labeled substrates were obtained from New England Nuclear Company. The following specific activities were employed: L-glutamic-^{14}C acid (U), 234 mc/mM; D-glucose-^{14}C (U), 196 mc/mM; palmitic-1-^{14}C acid, 12.5 mc/mM; glycerol-^{14}C (U), 7.4 mc/mM; and urea-^{14}C, 4.5 mc/mM. In each case, labeled substrate was added (0.4 µl of glucose or glutamate, or the amount of palmitate, urea or glycerol required to give 5.5×10^3 dpm/ml) to appropriate flasks at five-minute intervals. Experiments employing urea, glycerol and palmitate were sampled at 40-minute intervals over a six-hour period, whereas in the experiments employing glucose and glutamate, samples were withdrawn at ten or fifteen-minute intervals over a period of 1-1/2 - 2 hrs. A Swinney filter cascade with 10, 5, 1.2 and 0.45 µm Millipore or Nucleopore filters was prewashed with unlabeled substrate and employed in the preliminary experiments, after which only the 0.45 µm filters were used. At t=1, 10 ml sample was removed and gently filtered into a rubber capped scintillation vial. Immediately thereafter, 0.2 ml of 1N H_2SO_4 was injected to terminate uptake. The Swinney filter cascade was washed with 5 ml Rhode River water and 5 ml air. The filters were separated and placed in individual scintillation vials containing a standard omnifluor/dioxane mixture. Samples were returned to the laboratory and put through the counting procedure in which the CO_2 was pumped through a closed system into Woehlers'Solution A,diluted with an equal amount of Solution B for counting purposes. The Intertechnique Counter, Model No. SL40, was used in all of the experiments employing radioisotopes.

Decomposition of Radioactively Labeled Natural Materials

Dr. David Correll very kindly provided ^{14}C-labeled samples of redhead grass, milfoil grass, an alga, and some periphyton. The labeled samples were mixed individually with sediment collected at each of the four stations. The inoculated samples were divided into twelve parts, each of which was placed into containers prepared from rubber tubing. The tubes were positioned in racks at each of the stations in September, 1973. The samples were protected from fish and large-scale disruptions by a fine mesh screen, which also permitted passage of water and microorganisms. Experiments by Odum and de la Cruz (1967) indicated that time periods in excess of 180 days were required for most of the grasses studied to be decomposed. Therefore, duplicate samples were collected at 40 day intervals. The samples were dried at 80°C for 24 hours, and stored. However, to analyze the samples, a carbon asher is required. Hence, these data are unavailable until such time that access to a carbon ashing instrument is obtained.

F. Results

Enumeration of Aerobic, Heterotrophic Bacteria

Temperature and time of incubation influence the number of organisms growing on a given medium. The data obtained to date indicate a mixed population of bacteria occurring in Chesapeake Bay. One group of bacteria forms visible colonies rapidly within 24 to 48 hours at 25C. A second group requires three to five days incubation at 25C. Examination of plates, incubated for a two month period, revealed many small pin-point colonies, evidence that there is yet a third group of bacteria which grows very slowly. In one sample

tested, 35 replicates of each dilution were plated, ten in the cold, using chilled sterile hockey sticks. In addition, five replicates of each dilution were incubated at 2, 5, 15, 25, 35, 41 and 55C for two weeks (Fig. 2). The largest number of organisms was obtained when the plates were incubated for two weeks at 15C. The water sample was collected when the in situ water temperature was 9.8C. Most of the bacteria growing at 25C will be capable of growth at 15C, if the temperature is maintained (Sieburth, 1967). For this study, two weeks incubation at 15C was decided upon for all samples.

The total, viable population of aerobic, heterotrophic bacteria in the water column was found to vary between 1.9×10^2 bacteria/ml in December and 2.2×10^5 bacteria/ml in February (Fig. 3). The counts at Stations 0.0, 3.38, and CC 0.6 followed the same general pattern, with a few anomalous measurements, peaking during the period January - March and then leveling off. Although the total, viable bacterial populations in the water at Station 5.5 paralleled that of the other stations, January through April, sporadic increases were observed. Counts $< 1.7 \times 10^3$ bacteria/ml were never observed at Muddy Creek. The numbers of total viable bacteria in the water at Stations 0.0 and 3.38 were closely parallel. The data for Cadle Creek, while following the same pattern, never showed counts below 1.3×10^3 bacteria/ml.

There was no readily discernable pattern for numbers of total, viable aerobic, heterotrophic bacteria in the sediments (Fig. 4). Counts varied from 1.4×10^4 to 9.4×10^6 bacteria/gm. Both the extremely low and high counts were recorded at Station 5.4, in October, 1973 and September, 1974, respectively. Oscillations in the total counts were observed at all stations, without any correspondence in the numbers or time of initiation.

Most Probable Number of Indicator Organisms

Total coliforms were found in low numbers in the water at Stations 0.0 and 3.38, with both stations showing elevated coliform counts in June (Table 1). Coliform counts in the water at Station 5.4 were relatively high, falling below 100/100 ml only in November, 1973. Total coliforms at Cadle Creek were higher than at either Station 0.0 or 3.38, but generally did not reach levels obtained at Station 5.4. Total coliform counts in the sediments did not parallel counts for the water column, nor were sediment counts higher after a peak was observed for the water column the previous month. The greatest numbers of total coliforms for all four stations, in both water and sediment, were observed in June.

As expected, the numbers of fecal coliforms were much lower than the total coliform count (Table 2). Very high numbers of fecal coliforms were found in December, 1973 and June, 1974, but only at Station 5.4. The sediment counts were not notably high, with the exception in May at Cadle Creek, and in June at Stations 0.0 and 5.4. Frequently no fecal coliforms were found to be present. When the percent of coliforms of fecal origin was determined (Table 3), it was obvious that most of the total coliform population probably was not derived from fecal wastes. Fecal contamination was more often responsible for the coliforms in the water column than in the sediment. In May, almost all of the coliforms were probably derived from fecal contamination of the water.

When the variation in bacterial levels at the four stations was normalized, by determining the number of fecal coliforms per million aerobic, heterotrophic bacteria, it was apparent that the coliforms formed a relatively stable portion

of the sediment population (Table 4). Only twice, in December, at Station 5.4, and in June, at Station 0.0, did the coliforms increase significantly. In the water column, the coliforms varied in number from fewer than one fecal coliform organism per million to as many as $6,067/10^6$ at Muddy Creek. The fecal coliforms comprised less than one percent of the total population.

Table 6 lists the MPN of fecal streptococci, as obtained using Sodium Azide Dextrose broth. The latter medium provides an enrichment for the more selective Ethyl Violet Azide broth, and, as is evident from a comparison of data given in Tables 6 and 7, permits growth of organisms other than fecal streptococci. The results of direct inoculation into EVA broth showed greatly reduced numbers, demonstrating the value of an enrichment procedure. The confirmed fecal streptococci counts are given in Table 6. Very low numbers of fecal streptococci were found in the water column, except in May at Muddy Creek. With the single exception of a sampling accomplished in June, no more than two fecal streptococci were found in the water at Station 0.0 or 3.38. Low numbers of fecal streptococci were found in the sediment at the two stations, with higher numbers obtained at Station 5.4 and Muddy Creek. Samples were taken from all tubes showing a precipitate, whether white or purple. These were streaked on M-enterococcus agar and $\geq$ 95% of these cultures yielded pink colonies. At least one colony from each tube was purified and over 95% were identified by means of the tests which had been selected for the study (Table 8). Microscopic examination revealed the presence of streptococci in all the tubes. Bacillus organisms were also found in mixed culture in several tubes.

In Table 9 is presented a list of the ratios of fecal coliforms to fecal streptococci. High ratios were found much more often in the water column than in sediment, and at least three times at Stations 0.0, 5.4 and Cadle Creek. The ratios of fecal coliforms in the water to fecal coliforms in the sediments (Table 5) were observed to be between 0 and 1 for Stations 0.0 and 3.38, with one exception. At Cadle Creek, ratios varied from 0.25 to 4, while at Station 5.4 they were always greater than 1.

In Table 10 is presented a list of the physical parameters which were monitored in the study, including temperature, turbidity, condictivity, dissolved oxygen and salinity. Water was monitored constantly for these parameters at the dock site of the Smithsonian Field Station by other investigators, and these data will be incorporated at a later date, when the data have been tabulated and made available.

A strict correlation between turbidity and fecal coliforms was not observed. However, greatest turbidity was observed at Muddy Creek, as were the highest numbers of fecal coliforms. Temperature and dissolved oxygen measurements were similar at all four sites, and only one measurement has been recorded.

In Table 11 is shown that organisms capable of producing botulinum toxin were present at three different sampling times, viz., in April, June and July. In June, type E was identified, but insufficient material was available for more definitive tests to have been run in April and July. Presumptive tests for Streptococcus salivarius, a suggested indicator organism, were positive at Cadle Creek, but no cultures survived confirmation. This was tried only once, using Levan Methylene Blue agar.

An inverse correlation between the presence of VPLO organisms in the water and the water temperature was noted, insofar as no organisms were found in the water column at 2C. However, the inherent differences in the two methods employed for enumeration requires a careful interpretation of the results (Jannasch, 1965). While the numbers observed were indicative of the presence of VPLO, a zero value for plate counts did not preclude its presence. TCBS medium is strongly inhibitory, and none may have survived inoculation onto this medium.

No confirmed Salmonella organisms were detected at any of the four stations. While some organisms were always found on Bismuth Sulfite or DC agar, they were generally Proteus or H_2S negative Gram-negative organisms.

Estimation of Heterotrophic Potential

A very low percentage of the population was able to digest chitin at any time, except the sediment population sampled in April (Table 13). No chitin-digesting organisms were found in July in either the water or the sediment. No cellulose-digesting bacteria were observed to be present, except in July, when several cultures were obtained (Table 14). Much of the population was found to be proteolytic, i.e., greater than 50% at all stations in the warmer months. The lowest percent of proteolytic bacteria was found in the water column in January at Stations 0.0 and 3.38 (Table 15). Very few urease-producing organisms were found in the water column, and most of these were at Muddy Creek. All of the sediments showed the presence of populations capable of hydrolyzing urea (Table 16).

Distribution of Bacterial Genera

Tables 18, 19, 20 and 21 list numbers and relative abundance of bacterial genera at each station for October, 1973; January, 1974; April, 1974 and July, 1974 samples. During October (Table 18), strains of the genus Vibrio were predominant at three stations, 0.0, 3.38 and Cadle Creek. Other groups isolated were pseudomads and Bacillus spp. At Station 3.38 vibrios comprised 80% of the bacterial population, with the remaining isolates, Bacillus and pseudomonads, each comprising 8% of the population. The population in the sediment, however, was quite different, with 50% of the organisms identified as Bacillus spp. At Cadle Creek, Bacillus spp. were 25% of the isolates from sediment. Coryneforms were a relatively constant proportion of the populations examined, consistently in the range of 13 to 18%. Pseudomonads comprised 7 to 16% of the species examined, whereas Vibrio spp. were $\geq$ 8% only at Station 0.0 (17%).

In January (Table 19), the bacterial populations found in water were more uniform in distribution among the stations. Pseudomonads were detected in greatest numbers (55-60%), while vibrios, except at 5.4, were $\leq$ 3%. Bacillus spp. were present at ca. 3-6% of the population. Representatives of the genus Spirillum were isolated from water samples at three stations and in sediment samples in January, although none had been isolated from samples collected in October. The distribution of bacterial genera in the sediment population was not similar for samples collected at the four stations in January. Bacillus spp. were at the highest level of incidence at Station 3.38 and at Cadle Creek (57%), and dropped to 10% at Stations 0.0 and 5.4. At Stations 0.0 and 5.4, the frequency of occurrence of Vibrio spp. was high,

whereas only low numbers of Vibrio spp. were isolated at Stations 3.38 and 5.4. The incidence of coryneforms varied from 2 to 30%.

The generic distribution of populations in the water column was varied in April (Table 20). Vibrio spp. comprised 37% of the population, with pseudomonads, Cytophaga/Flavobacterium spp. and an unidentified cluster of strains also represented in samples collected at Station 0.0. At Station 3.38, a group of unidentified organisms was dominant, with pseudomonads also in abundance; no vibrios were isolated. At Station 5.4 a rather uniform distribution of bacterial genera was noted for Bacillus spp., pseudomonads, vibrios and Cytophaga/Flavobacterium spp. At Cadle Creek, the Cytophaga/Flavobacterium group predominated, followed by pseudomonads and an unidentified group of organisms. Acinetobacter spp. were isolated at significant levels at Stations 0.0, 3.38 and 5.4. The unidentified group of organisms was predominant in the sediment samples, except at Station 3.38, where Bacillus spp. comprised 54% of the population.

In July (see Table 21), Vibrio spp. and pseudomonads each comprised 30-40% of the population, except at Station 0.0, where Vibrio spp. were clearly dominant. Similarly, at Station 0.0, Vibrio spp.were dominant in the sediment population. At Station 3.38, Bacillus spp. formed 40% of the population, whereas at Station 5.4, more pseudomonads were isolated. At Cadle Creek, vibrios comprised 43% of the population, followed in numbers by pseudomonads and bacilli.

In Table 22 is listed the combined results by season. The data demonstrate that pseudomonads and vibrios are the predominant heterotrophic bacteria

in the water column, with vibrios more numerous in the fall and pseudomonads in the winter months. Enteric bacteria were isolated but only at a very low incidence, i.e., < 1%. Bacillus spp. and coryneforms were found in the water column, notably in the fall months and comprised the major portion of the sediment bacterial flora, along with Vibrio and Pseudomonas spp.

Results of glucose utilization studies are summarized in Table 23. More than half the bacterial population in October at each station consisted of organisms capable of utilizing glucose, either oxidatively or fermentatively. By January, less than 5% of the bacteria were glucose utilizers, except at Muddy Creek. An increase in glucose-utilizing bacteria was noted in April at Station 0.0. In July, glucose-utilizing bacteria comprised 40% of the bacterial strains isolated at all stations. There was no detectible correspondence, either seasonally or by station, for glucose-utilizing bacteria in sediment.

Uptake of Radioactively Labeled Substrates

Uptake of glutamate, glycerol, urea and palmitate was examined in situ in September, 1973 and again in December, 1973 at Station 3.38. Unfortunately, insufficient isotope was used in the first experiment, resulting in all of the available glutamate label being taken into the cells within the first hour. In the case of palmitate, no uptake occurred. Over 90% of the cells containing labeled glutamate and 70% of the cells containing labeled glycerol passed through a 5 μm filter. Label was evenly distributed on the 1.2 or 0.45 μm filters. Over 95% of the cells which had taken up radioactively labeled urea passed

through a 5 μm filter also, but only 4% of the label was retained on the 0.45 μm filter, most of the labeled having been collected on the 1.2 μm filter. In the experiment carried out in December, very little uptake was observed for the substrates examined.

In general, the rate of uptake was non-linear. In several experiments (Table 24) samples taken at 20, 40 and 60 minutes demonstrated a linear rate of uptake, but subsequent samples over the next 60 minutes revealed a slowly decreasing rate of uptake. Consequently, the comparisons were based on the uptake per liter which occurred in the first hour. Station 3.38 and Muddy Creek were included in the study, so that an open area and a highly productive marsh could be compared. Similar rates of uptake of labeled substrate were observed at both stations in late June, but a much greater rate of uptake was observed in July at Muddy Creek, compared with Station 3.38. Again, most of the labeled substrates employed was retained on the 1.2 μm filter, in all experiments.

A comparison of the rate of glutamate uptake at Station 3.38, September through June, revealed a very low level of uptake in December (Table 25). By April, the amount of label substrate taken up per unit of time increased by a factor of 2.5, with the increase in temperature of 11°. In May, a twelve-fold increase in uptake was noted, with a drop in rate of uptake by one-third in June and September.

The rate of heterotrophic uptake of radioactively labeled substrate at all four of the stations in the Rhode River was compared with two stations in Chesapeake Bay over a three-day period in July, 1974. Colgate Creek,

located in Baltimore Harbor, receives significant industrial pollution, especially oil from tanker traffic. Eastern Bay is a relatively unpolluted area in the upper mid-Bay area. Similar, low amounts of uptake were noted for Eastern Bay and Station 3.38, and approximately twice the rate at Station 0.0 and four times the rate at Muddy Creek. The highest amount of uptake was measured at Colgate Creek. The amounts of labeled substrate taken up by samples tested in Colgate Creek and Cadle Creek were ten times that of the Eastern Bay station.

G. Discussion and relevance of findings to the Waste Water Program

An increase in aerobic, heterotrophic bacteria in the water column was noted at all stations examined in the Rhode River during the three month period, January through March. Similar results have been recorded in studies of mercury metabolizing and hydrocarbon-utilizing bacteria examined at several stations in Chesapeake Bay (Colwell et al., 1974). Possible explanations include a slower grazing rate due to decreased metabolic rates of plankton populations or an increase in psychrophilic bacteria, which grow well at low temperatures. Since Muddy Creek is shallow and turbid (Table 10), much of the sediment is resuspended. It is also a narrow creek with a marked input from run-off. Either, or both, of these conditions could account for the greater number of bacteria present and for the sporadic increases in bacterial counts which have been observed (Fig. 2). No seasonal distribution was detected in the sediment counts. It should be pointed out that there is a vertical distribution of heterotrophs in sediment, with the greatest number of

bacteria commonly found in the upper centimeter (Bell and Dutka, 1972). It is very difficult to sample the upper centimeter of sediment in most of the grab samplers because of the sediment/water mixing that occurs at the interface and the disturbance created by closure of the grab. This is further distorted in grab samplers where the entire contents must be removed to reach the surface portion. Consequently, although attempts are always made to achieve uniform sampling, a mixture of the first few centimeters can account for differences observed for given samples.

Fecal coliforms appear to be a better indicator of sewage pollution than total coliforms, as shown in Tables 1 and 2. Serious errors arise in using these methods, since many false positives are obtained in the total coliform test, while non-lactose fermenting E. coli are not identified at all. The more rigorous conditions employed in the fecal coliform test reduce spurious results but still require that the organism possess the ability to metabolize lactose. The majority of the fecal coliforms were found in Muddy Creek and a higher fecal coliform count was noted at Cadle Creek than at Stations 0.0 or 3.38. The data do not provide information concerning the cause of the lower numbers found at Stations 0.0 and 3.38. Coliform die-off or dilution of coliforms in run-off could occur at Stations 0.0 and 3.38. It is known that enteric organisms not only survive but also reproduce in nature (Hendricks, 1972). The effect of dilution from run-off can be calculated when the data are available.

Since fecal coliforms are not necessarily from human sources, the number of fecal coliforms at Stations 0.0 and 3.38 may also indicate sources, such as domestic animals, i.e., horses observed on the southwest shore of the Rhode River. Indeed, the data given in Table 3 support the likelihood of local sources of fecal

pollution. The possibility of resuspension from sediments exists, although the volume of water at Stations 0.0 or 3.38 would require a very large number of microorganisms in sediment before resuspension would cause an increase in observed counts. In shallower areas, such as Muddy Creek or Cadle Creek, resuspension is a strong possibility. It should be noted that fecal coliforms were found in sediments more than 75% of the time. Presenting the fecal coliforms as a normalized part of the aerobic, heterotrophic population provides a means of distinguishing between an increase in the entire population versus an increase in just the indicator organisms. Using this approach (see Table 5), counts at Station 3.38 in December and at Station 5.4 in August are more significant.

Very few fecal streptococci were isolated from water samples collected at Stations 0.0 and 3.38, or in sediment at Station 3.38. In general, the number of fecal streptococci was much lower than the fecal coliforms. However, some fecal streptococci could be found in sediment at Stations 0.0, 5.4 and Cadle Creek, in $>$ 90% of the samples (Table 7). In 1969, Geldreich and Kenner suggested that the ratio of fecal coliforms to fecal streptococci be used to determine the source of pollution, with ratios greater than 4.0 indicating sewage effluents and those lower than 0.7 suggesting non-human warm-blooded animal sources. This work was supported by Feachem's separation of human and pig sources of pollution (1974). Ratios obtained at Station 0.0, Muddy Creek, and Cadle Creek indicated frequent human sources of pollution, particularly in the spring at Muddy Creek and Cadle Creek. However, this increase may be due to more rapid and widespread run-off from spring rains.

Several methods for detection of indicator organisms have been tried in these studies. In all cases, the MPN technique was chosen over the filtration technique because of difficulty in enumerating sediment populations using a filter. While no difficulty was encountered in using the coliform or fecal coliform methods, the tests themselves are no longer considered to be accurate. Not all E. coli organisms are able to ferment lactose with consequent gas production. In this study, tubes showing turbidity without gas production were observed. These cultures may have been coliforms. This is a particularly strong possibility, since, in effluents from sewage treatment plants, as shown by Braswell and Hoadley (1974), E. coli organisms injured during chlorination may not grow or may grow without producing gas in lactose broth. Survival studies should be conducted with Salmonella and other pathogens subjected to chlorination and other sewage treatment procedures. Methods employed for the enumeration of fecal streptococci were found to be more reliable than the coliform methods. Results of biochemical tests (Table 8) revealed that > 95% of positive tubes contained organisms which were subsequently identified as enterococci. However, the reliability of the fecal streptococci procedures must be established for each site under study, since a given site may contain more organisms giving false positive reactions.

Donsel and Geldreich (1971) observed a correspondence between Salmonella and fecal coliforms in the sediments. According to their study, in any given sediment sample, there would have been only a 20% possibility of finding Salmonella, even with high coliform counts. Cohen and Shuval (1973) discovered that fecal streptococci are more resistant to die-off in the natural water

environment. They suggested that better correlation for measurement was survival of fecal streptococci and presence of enteric viruses. Hoadley and Chen (1974) have shown that Streptococcus faecalis organisms are much more resistant to stress in the form of selective media than E. coli. Considering the erratic coliform counts observed in the Rhode River and the problems with the coliform procedures cited above, greater reliance might better be placed on fecal streptococci as an indicator of fecal pollution and/or the presence of potentially pathogenic organisms. Improvement of techniques for direct isolation and identification of Salmonella, as, for example, the fluorescent antibody technique (Cherry et al., 1972) may provide a better indicator system. Counts of Clostridium perfringens in the sediments (Donsel and Geldreich (1971) or enumeration of coliforms by determination of coliphage counts (Kenard and Valentine, 1974) are other approaches which might be considered. The problem of human viruses and their transmission via water has only recently been recognized. Clearly, there is a need for a more reliable and more widely applicable indicator system than the coliform procedures currently in use.

In this study, water and sediment samples were examined directly for pathogenic bacteria, including Salmonella, Clostridium botulinum, and Vibrio parahaemolyticus. None of the positive cultures on DC medium were confirmed as Salmonella, using the A.P.I. system. While occurrences of large numbers of Salmonella have been reported in water systems, in general, very large volumes of water are required for direct isolation and identification of Salmonella. An indirect method, i.e., toxin production, was used successfully

for the detection of Clostridium botulinum. The technique is simple and appears to be reliable. Unfortunately, a rather long time period is required for growth and testing, viz., a minimum of six days.

Vibrio parahaemolyticus organisms were not detected in the water during the winter months, but V. parahaemolyticus was present in sediment, as reported by Kaneko and Colwell (1973). The TCBS medium presently used for isolation and presumptive identification of V. parahaemolyticus is too inhibitory for enumeration studies. The MPN technique, employing Salt Colistin or a growth medium such as ESWYE for enrichment, followed by a confirmation on TCBS, rather than direct plating on TCBS agar, would probably allow detection of V. parahaemolyticus and related organisms, even when they occur in low numbers.

The ability of given bacterial populations to decompose such naturally occurring substrates as cellulose and chitin was examined in the course of this study. In general, only a small portion of the population was observed to be capable of utilizing chitin, although large increases in chitin digesters were noted in sediments and water at Station 3.38 during April. Very few cellulose utilizers were isolated. Several isolates were obtained in July in water at Muddy Creek. From the number of different approaches used, including anaerobic incubation of the samples, it is concluded that bacterial decomposition of cellulose is not accomplished by a single species. In contrast, a significant portion of the bacterial populations possessed proteolytic enzymes. Such isolates were notable in the spring and summer months, with numbers of proteolytic bacteria showing a decrease in the fall, with a minimum observed in January.

The low numbers of urea utilizing bacteria found in the water column was surprising, since bacteria are generally considered an important agent in the mineralization and cycling of dissolved organic material. Remsen et al. (1972) estimated that bacteria in estuaries and coastal waters play a very small role in urea decomposition. In the open ocean it has been shown that bacteria are active utilizers of urea (Taga, 1970). Whether a preferential utilization of nitrogen in the form of nitrate occurs in estuarine and coastal regions requires further study. An alternate method of detecting urea utilizers, other than the MPN method, may yield different results.

Studies of the incidence of individual bacterial groups showed a seasonal fluctuation for some genera. Acinetobacter spp. formed a low but constant portion of the sediment population, i.e., 2 - 9%. Low numbers of Acinetobacter spp. also were found in the water column, with greatest occurrence in April at three of the stations. Acinetobacter was isolated in Cadle Creek only during January. Bacillus spp. were maintained in the population sediment at a constant level in Cadle Creek, but the numbers of Bacillus spp. varied widely at the other stations, with the greatest number found in October. The greatest number of Bacillus spp. in the water also occurred in the fall. However, it should be pointed out that Bacillus spp. are low in numbers, except at Muddy Creek where their higher numbers reflect resuspension of the sediment.

The incidence of coryneforms varied widely, with the greatest occurrence in Cadle Creek sediments in January (30%). However, no pattern of seasonal incidence or distribution of coryneforms was evident. Organisms such as

Achromobacter, Alcaligenes, Aeromonas, enterics, Lactobacillus, Micrococcus, Spirillum, Staphylococcus and some of atypical pseudomonads were found in low numbers throughout the year. Micrococcus spp. comprised 25% of the sediment population in January at Cadle Creek. Although these organisms were generally present in very low numbers, a burst of growth in the Vibrio or Pseudomonas spp. would result in out-selection of the micrococci, i.e., suppress the incidence of the latter. It is difficult to assess the contribution of the Moraxella/Cytophaga/Flavobacterium groups; these organisms characteristically produce yellow pigmented colonies and commonly do not survive serial transfer in the laboratory. Nevertheless, they were isolated at all the stations with greatest frequency in the water in April. Vibrio and Pseudomonas spp. were, however, always the most prevalent isolates at every station throughout the year. All of the stations showed decreasing counts of Vibrio in the winter, except Station 5.4, where Vibrio spp. comprised up to 25% of the population, even in January. Maximum numbers of Vibrio spp. were found either in July or October. Wide variation was noted in the sediment populations. Although pseudomonads were always present, highest numbers were found in January and April.

Heterotrophic uptake of radioactively labelled substrates were directed toward elucidation of a seasonal cycle and comparison of the Rhode River stations. Glutamate was found to be more readily taken up than either glycerol or urea and little, if any, uptake of palmitate was observed. Muddy Creek and Big Island were selected as appropriate sites for comparison, since Big Island was thought to be lower in nutrient levels and an open mixing area, whereas the Muddy Creek station is in a highly productive marsh site. Studies by other workers in the Rhode River have shown that there is a relatively high nutrient concentration at Big Island. In June the rates at both areas were similar. In July the uptake rate at Muddy Creek was four times that of the Big Island station.

The results of seasonal uptake studies using glutamate were interesting. Very low rates were measured in December, and maximum uptake was found to occur in May. April was very cold, although the water temperature was higher than in December and the uptake rate was found to be only two and a half times that measured in December. Studies have shown that low temperatures greatly reduce rates of nutrient uptake (Paul and Morita, 1971), corroborating the observations made during this study in the Rhode River. It should be pointed out, however, that cultures can be adapted to the cold, such as in the Antarctic. Morita et al. (1972) found that indigenous Antarctic microflora demonstrated metabolic activities comparable to those found in temperate zones.

Comparison of results obtained for Rhode River stations with two other Chesapeake Bay stations showed that an increased pollution load can result in populations with different heterotrophic potentials. Sites such as Cadle Creek

and Colgate Creek, which can be considered similar or reasonably comparable in terms of relative nutrient inputs, demonstrated high rates of uptake. The load on the system in the form of seepage from septic tanks, marinas and gas stations in Cadle Creek, and from shipping and oil spills from tankers in Colgate Creek, have resulted in a selection for those bacteria capable of rapid mobilization of nutrients. At the present time, the methods employed in uptake experiments are being examined very carefully. Unfortunately, some serious errors in the techniques have been pointed out. For example, effect of the fixative on release of labelled compounds (Griffiths et al., 1974) is one potential source of error. In order for absolute rates to be calculated, the naturally occurring amount of substrate studied must be known. All of the values given in our study are relative rates for the purposes of seasonal and spatial comparisons. In a study where fluxes in a body of water are to be compared, it is essential that the organic components of the system be known, both qualitatively and quantitatively. All of the data in this report would have greater import, if the actual levels of nutrients at the different areas were known. Comparison of techniques used in the Rhode River (Williams, 1970) with the method of Wright and Hobbie (1965) have been carried out in the Chesapeake Bay. The technique of Wright and Hobbie is simpler to use, although more coordination is required. The latter appears to be more suited to open water work, since samples need to be taken only at one interval, whereas longer incubation times are required to gain sufficient data with the first method. Although there are criticisms of the method used for trapping carbon dioxide, the fact that it can be collected immediately makes it an

attractive method in view of the losses that can occur from loose caps or in transport with the Williams' method.

Nedwell and Floodgate (1971) have shown that, although similar numbers of organisms can be isolated in the winter and summer months, different types of bacteria can be selected by the great temperature difference between the two seasonal extremes. It has been shown by Colwell (1972) that two areas in Chesapeake Bay, having approximately the same generic composition in the total bacterial populations, can differ considerably at the species level. The present studies of generic distribution show that areas in the same sub-estuaries which are different in terms of nutrient input can demonstrate quite different generic distributions. The fact that the same relative total numbers were found suggests an external control on the population, such as grazing, or a maximum population potential. In an area such as Cadle Creek, those species with a high uptake rate can predominate, but as total numbers increase, it would be expected that increased grazing rates would act to prevent huge increases in the population, except for periods at the time of, and immediately following, large nutrient inputs. A comparison of the data accumulated for Cadle Creek and the Big Island Station 3.38 for July demonstrates clearly that it is the type of bacteria present and not merely the total number of bacteria that is important. Although the same total, viable number of heterotrophic bacteria were found, the rate of uptake was ten times greater at Cadle Creek. Examination of the generic distribution revealed approximately the same groups were represented in the same proportion, demonstrating the importance of distinct bacterial species. In conclusion, this work has pointed out two major problems

in environmental microbiology. 1) Improper reliance is being placed on the coliform indicator test. In the Rhode River, fecal streptococci methods appear to be more reliable. 2) Neither total bacterial numbers nor crude approximations of bacterial groups are sufficient to characterize the bacterial populations in situ. The plate count and coliform MPN should not be the sole measure of the environmental impact on the microbial ecology of a given geographical site. The relationships within a microbial population and between populations are very complex, and the microbial activities in nutrient cycling and mobilization are too fundamental to the health of an estuary to be incorrectly estimated.

Acknowledgments

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Figure 1. A map of the Rhode River sub-estuary showing the four sampling stations: 0.0, 3.38, 5.4 and CC 0.6.

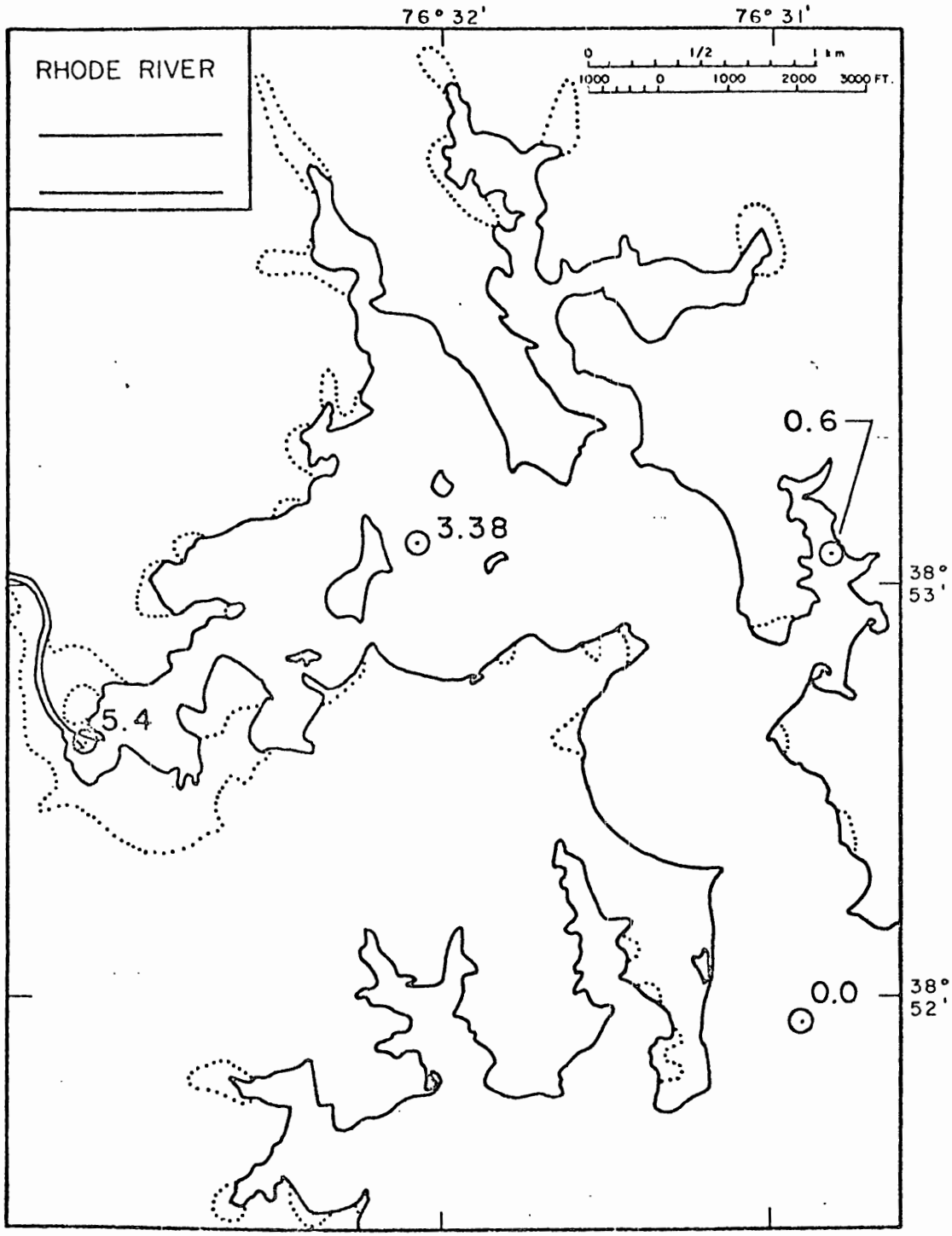
76° 32'
76° 31'
RHODE RIVER
0 1/2 1 km
1000 0 1000 2000 3000 FT.
0.6
3.38
5.4
0.0
38° 53'
38° 52'

Figure 2. The effect of varying length and temperature of incubation on the total numbers of bacteria.

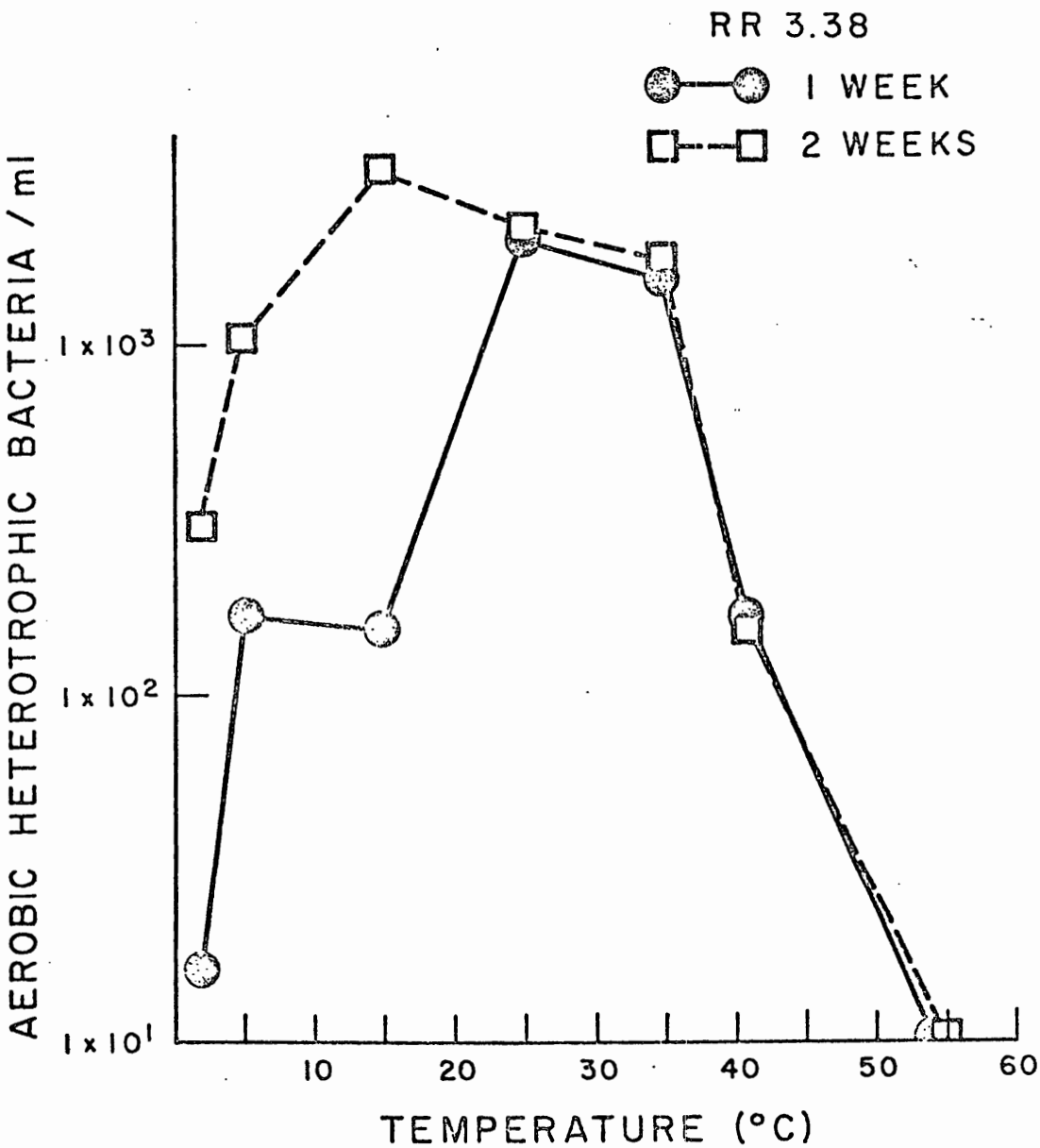
RR 3.38
1 WEEK
2 WEEKS
AEROBIC HETEROTROPHIC BACTERIA / ml
1 x 10³
1 x 10²
1 x 10¹
10
20
30
40
50
60
TEMPERATURE (°C)

Figure 3. The total number of aerobic, heterotrophic bacteria per ml water from September, 1973 through September, 1974.

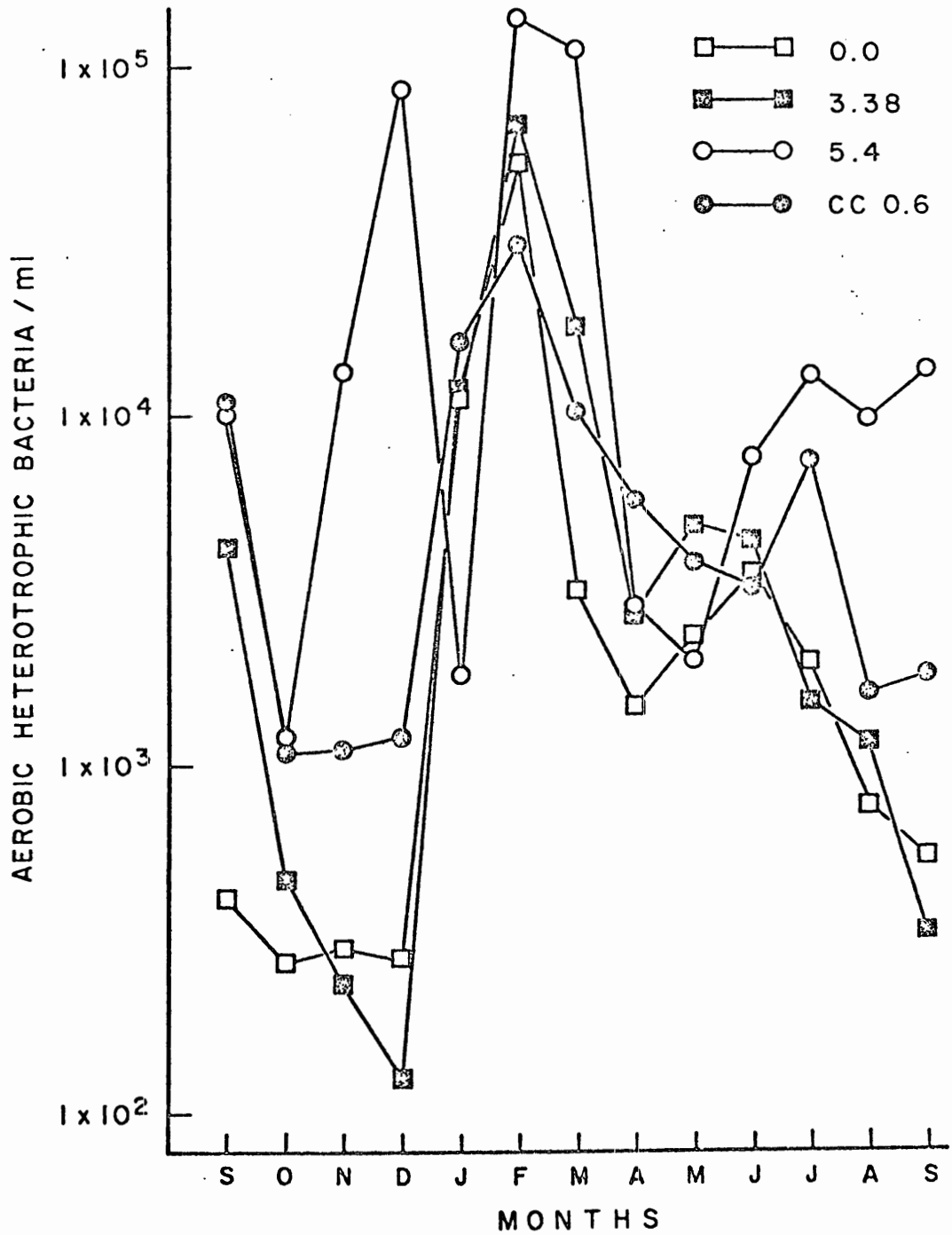
AEROBIC HETEROTROPHIC BACTERIA / ml
1 x 10^5
1 x 10^4
1 x 10^3
1 x 10^2
0.0
3.38
5.4
CC 0.6
S O N D J F M A M J J A S
MONTHS

Figure 4. The total number of aerobic, heterotrophic bacteria per gram sediment from September, 1973 through September, 1974.

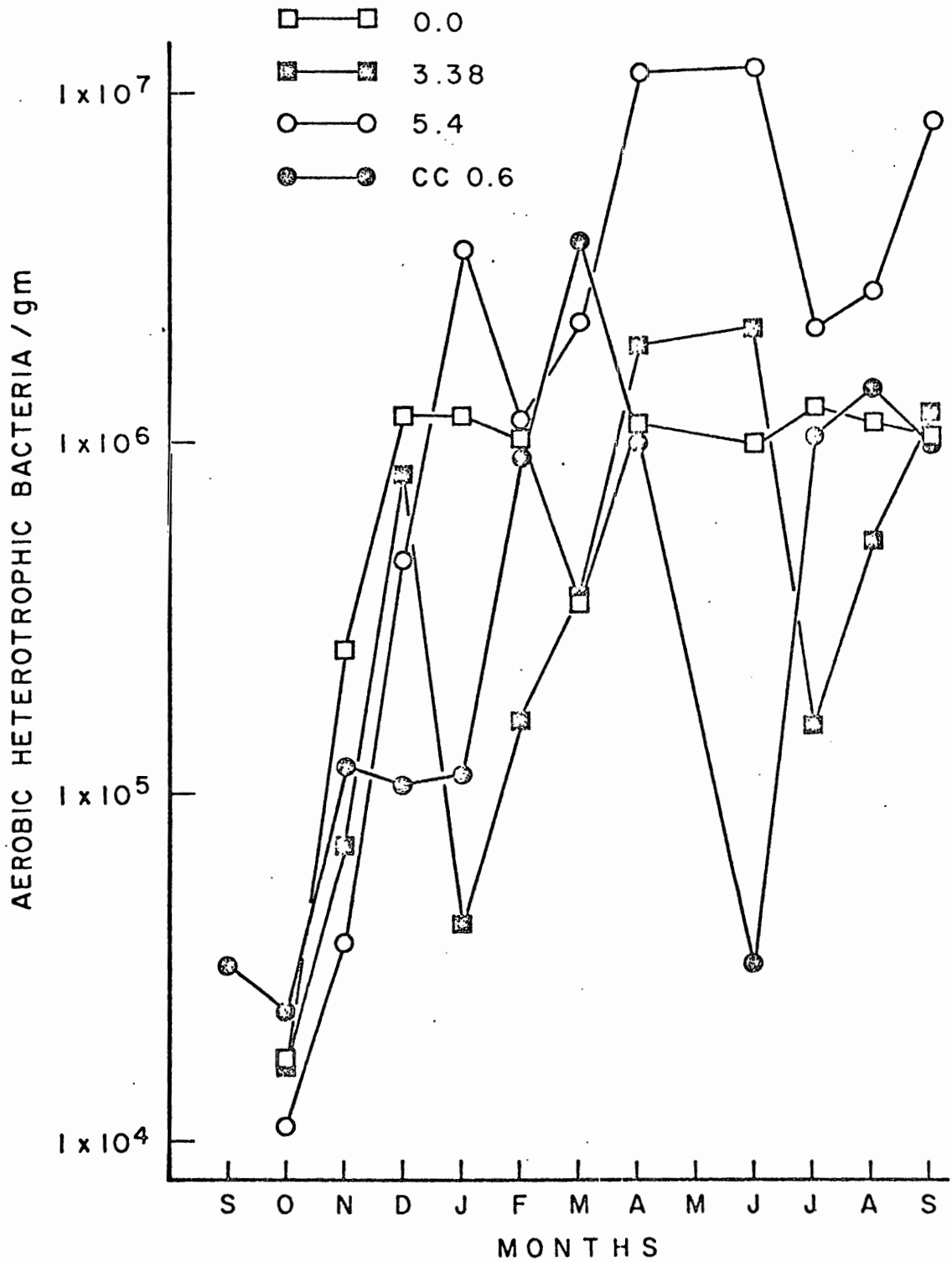
0.0
3.38
5.4
CC 0.6
1 x 10^7
1 x 10^6
1 x 10^5
1 x 10^4
AEROBIC HETEROTROPHIC BACTERIA / gm
S O N D J F M A M J J A S
MONTHS

Table 1. Most probable number of total coliforms per 100 ml water or 10 gm sediment sample.

Sample	Water				Sediment			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Month								
September	33	33	≧2400	920				
October	17	11	240	22				
November	0	70	11	240				
December	17	79	≧2400	540	140	140	260	170
January	130	33	130	240	-	350	1600	350
February	30	50	540	90	170	240	920	90
March	10	20	350	90	280	920	≧2400	350
April	60	220	1600	180	≧2400	920	≧2400	350
May	49	79	540	79	140	350	1600	1600
June	3500	790	5400	24000	≧2400	5400	3500	24000
July	0	40	490	490	460	1300	1300	1400
August	20	80	28000	2400	60	1100	1700	3300
September	7	33	540	23	26	79	180	350

Table 2. Most probable number of fecal coliforms per 100 ml water or 10 gm sediment sample.

Sample	Water				Sediment			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Month								
September	2	2	46	17				
October	17	5	33	17				
November	-	-	-	-				
December	0	22	≥2400	130	0	70	260	0
January	5	0	49	70	-	-	-	-
February	20	0	20	10	0	0	20	10
March	10	10	220	90	20	10	40	40
April	20	90	60	40	10	10	60	10
May	49	79	220	79	140	240	17	1600
June	170	0	5400	110	330	0	490	0
July	0	0	80	17	50	20	20	50
August	0	20	130	20	0	50	70	80
September	0	17	49	5	0	0	8	2

Table 3. Percentage of coliforms of fecal origin in the water and in the sediment.

Sample	Water				Sediment			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Month								
September	6	6	2	2				
October	100	45	14	77				
November	-	-	-	-				
December	0	28	100	24	0	50	100	0
January	4	0	38	29	-	-	-	-
February	66	0	4	11	0	0	2.1	11
March	100	50	63	100	7.1	1.0	1.7	2.9
April	33	41	4	22	0.4	1.0	2.5	2.9
May	100	100	41	100	100	68.5	1.0	100
June	5	0	100	0	13.7	0	14	0
July	0	0	16	3	10.8	1.5	1.5	3.5
August	0	25	0	0	0	4.5	4.1	2.4
September	0	51	9	22	0	0	4.4	0

Table 4. Number of fecal coliforms/10^6 aerobic heterotrophic bacteria.

Sample	Water				Sediment			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Month								
September	30	3.0	460	13				
October	35	71	194	130				
November	-	-	-	-				
December	0	1157	255	76.0	0	7.6	37	0
January	3.5	0	14.8	24	-	-	-	-
February	2.6	0	0.9	1.8	0	0	1.3	1.0
March	17.9	3.0	15.7	82	0.33	1.6	0.97	0.6
April	80	183	117	50	0.66	2.9	0.35	1.0
May	13.9	109	594	127	-	-	-	-
June	1.6	0	6067	200	330	0	2.7	0
July	0	0	38	20	2.5	7.1	0.5	3.8
August	0	125	1300	68	0	6.6	1.4	3.2
September	0	298	21.0	14	0	0	0.08	0.2

Table 5. Ratio of fecal coliform bacteria in the water column to coliform bacteria in the sediments.

Station	0.0	3.38	5.4	cc0.6
Month				
December	0	0.3	9.2	∝
January	-	-	-	-
February	∝	0	1	1
March	0.5	1	5.5	2.25
April	0.5	9	1	4
May	0.35	0.33	12.9	0.05
June	0.51	0	11.0	∝
July	0	0	4	0.34
August	0	0.4	1.9	0.25
September	0	∝	5.1	2.5

∝ = nofecal coliforms in sediment

0 = no fecal coliforms in water or sediment

Table 6. Most probable number of fecal streptocci per 100 ml or 10 gram sediment sample.

Sample	Water				Sediment			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Month								
January	540	540	≧2400	1600	≧2400	≧2400	≧2400	≧2400
February	5	0	0	0	≧2400	14	17	≧2400
March	11	8	≧2400	240	≧2400	≧2400	540	540
April	49	240	≧2400	49	≧2400	≧2400	≧2400	≧2400
May	140	33	≧2400	23	≧2400	≧2400	≧2400	≧2400
June	790	0	170	3500	≧2400	700	5400	5400
July	130	0	330	170	460	2400	490	16000
August	130	210	700	790	≧2400	≧24000	16000	≧24000

Table 7. Most probable number of confirmed fecal streptococci per 100 ml water or 10 gram sediment sample.

Sample	Water				Sediment			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Month								
January	2	0	23	5	23	0	≧2400	6
February	2	0	0	0	7	0	10	14
March	2	0	17	17	9	17	17	33
April	0	0	13	7	33	0	26	5
May	0	0	≧2400	8	17	0	≧2400	≧2400
June	13	0	0	70	33	0	79	160
July	0	0	17	10	0	0	0	220

Table 8. Biochemical confirmation of fecal streptococci from Ethyl Violet Azide broth medium

Month	Number Tested	Hippurate	6.5% NaCl	Starch	Catalase
May	18	3	18	18	18
June	20	0	20	20	20
July	18	1	18	18	18
Enterococci	a	0[b]	+	+	+
Other fecal Streptococci	a	+	0	0	+

a Results from Facklam et al. (1974).

b Variable, less than 20% positive.

Table 9. Ratio of fecal coliform bacteria to fecal streptococci bacteria in water and the sediment.

Sample	Water				Sediment			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Month								
January	2.5	∝	2.1	14				
February	10.0	0	∝	∝	0	0	0.2	0.7
March	5.0	∝	12.9	5.3	2.2	2.5	2.3	1.2
April	∝	∝	4.6	5.7	0.3	∝	2.3	2.0
May	∝	∝	0.1	9.9	8.2	∝	0.	0.5
June	13	0	∝	1.6	10	0	6.2	0
July	0	0	4.7	1.7	∝	∝	∝	0.2

∝ Fecal Coliforms not detected

Table 10. Physical data: Measurements of temperature, salinity, conductivity, dissolved oxygen and turbidity.

Month	Turbidity[a]				Conductivity[b]			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
September	0.75	0.5	0.25	0.25	17.9	16.9	16.0	17.3
October	0.75	0.75	0.5	0.5	12.1	12.0	17.1	12.1
November	1.0	1.0	0.5	0.75	15.8	15.1	14.2	15.1
December	-	-	0.25	-	-	-	-	-
January	1.75	2.0	-	2.0	8.2	5.5	-	-
February	1.0	-	-	1.5	-	-	-	-
March	1.25	1.5	0.75	-	9.3	10.3	7.3	10.3
April	0.33	0.33	< .25	0.25	-	-	-	-
May	-	-	-	-	-	-	-	-
June	1.0	0.75	0.25	0.75	10.5	10.1	0.0	10.2
July	0.5	0.5	0.25	0.25	16.6	17.1	15.7	17.1
August	0.75	0.5	0.20	0.50	19.5	19.4	18.1	19.8

(continued. . . . .)

Table 10. (con't)

	Dissolved Oxygen[c]	Temperature[d]	Salinity[e]			
Month						
Station			0.0	3.38	5.4	cc0.6
September	7.8	28.9	10.3	9.5	9.1	9.7
October	8.7	20.4	18.0	18.0	11.4	18.0
November	10.4	9.4	13.4	12.9	12.1	12.9
December	-	3.8	-	-	-	-
January	13.5	2.0	8.3	5.5	-	5.2
Feburary	-	3.0	-	-	-	-
March	11.4	8.4	8.0	8.5	6	8.7
April	-	12.0	-	-	-	-
May	-	16.2	-	-	-	-
June	8.6	20.0	6.6	6.5	0.0	6.6
July	7.4	26.8	9.8	10.0	9.4	10.2
August	8.5	25.3	11.8	11.8	11.1	12.0

a Turbidity - meters

b Conductivity - millimhos/cm

c Dissolved Oxygen - ppm

d Temperature - degrees Centrigrade

e Salinity - 0/00

Table 11. Determination of Clostriduim botulinum

Month	Location	Sample	Presence	Type
January	0.0	w+s	0	-
January	3.38	w+s	0	-
January	5.4	w+s	0	-
January	cc0.6	w+s	0	-
April	0.0	w+s	0	-
April	3.38	w	0	-
April	3.38	s	+	B or E
April	5.4	w+s	0	-
April	cc0.6	w+s	0	-
May	0.0	s	0	-
May	3.38	s	0	-
May	5.4	s	0	-
May	cc0.6	s	0	-
June	0.0	s	0	-
June	3.38	s	0	-
June	5.4	s	+	E
June	cc0.6	s	0	-
July	0.0	s	0	-
July	3.38	s	0	-
July	5.4	s	+	B or E
July	cc0.6	s	0	-
August	0.0	s	0	-
August	3.38	s	0	-

(Continued . . . .)

Table 11. (Con't)

Month	Location	Sample	Presence	Type
August	5.4	s	0	-
August	CCO.6	s	0	-

Table 12. Enumeration of Vibrio parahaemolyticus like organisms in water and sediment.

Water					
Station		0.0	3.38	5.4	cc0.6
Month	Method		(VPLO /ml)		
January	a	0	0	0	0
April	a	17	42	49	131
June	b	100	100	0	700
July	b	100	120	280	50

Sediment					
Station		0.0	3.38	5.4	cc0.6
Month	Method		(VPLO/gm)		
January	a	111	0	0	15
April	a	100	100	100	100
June	b	6000	0	100	0
July	b		5500	3700	44000

a= MPN Salt Colistin broth, confirmed on TCBS medium.

b= plate counts on TCBS medium.

Table 13. Number of chitin-digesting bacteria in water and sediments reported as the percentage of the total aerobic, heterotrophic bacterial populations.

Water	0.0	3.38	5.4	cc0.6
October	0	0	3.8	0.3
January	1	0.5	2.0	1.2
April	0	21	1.4	1.0
July	0	0	0	0

Sediment	0.0	3.38	5.4	cc0.6
October	-	-	-	-
January	7	0	0.5	0
April	21	10	7.0	33
July	0	0	0	0

0 = $\leq$1 colony /100 capable of utilizing chitin.

Table 14. Number of cellulose digesting bacteria in water and sediment reported as the percentage of the total aerobic, heterotrophic bacterial population.

Water	0.0	3.38	5.4	cc0.6
October	0	0	0	0
January	0	0	0	0
April	0	0	0	0
July	0	0	<1	0
Sediment				
October	0	0	0	0
January	0	0	0	0
April	0	0	0	0
July	0	0	0	0

0 = $\leq$1 colony/100 capable of utilizing cellulose

Table 15. Number of proteolytic bacteria in water and sediment reported as the percentage of the total aerobic heterotrophic bacterial population.

Water	0.0	3.38	5.4	cc0.6
October	13.8	15.5	24.1	9.8
January	3.0	4.0	48	9.0
April	>50	>50	>50	>50
July	>50	>50	>50	>50

Sediment	0.0	3.38	5.4	cc0.6
October	-	-	-	-
January	0	26	19	29
April	>50	>50	>50	>50
July	>50	>50	>50	>50

Table 16. Most probable number of urea utilizing bacteria per 100 ml water or 10 gm sediment.

Sample	Water				Sediment			
Station	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Month								
February	0	0	0	0	33	70	350	79
March	0	6	240	0	350	≧2400	140	280
April	2	0	23	0	540	920	920	180
May	2	0	33	0	220	1600	33	180
June	0	0	0	0	790	790	1300	940
July	0	0	0	0	70	1300	330	330

Table 17. South River data (April)

Data	Water	Sediment
TVC	1×10^3/ml	1.8×10^6/gms.
Total coliforms	330/100 ml	$\geq$2400/10 gm
fecal coliforms	33/100 ml	2/10 gm
urea utilizers	0	140/10gm
chitin utilizers(%)	0	0
cellulose utilizers(%)	0	0
proteolytic(%)	>50	>50

Table 18. Generic comparison of bacteria isolated from samples collected at stations during October 1973.

Genus	0.0		3.38		5.4		cc0.6		0.0		3.38		5.4		cc0.6	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Acinetobacter	2	3.9	1	2	2	4.9			2	6.9	3	5	2	3.6	2	6
Achromobacter					1	2.4					1	1.7				
Alcaligenes													1	1.8	1	3
Aeromonas	1	1.9					1	2.5							1	3
Bacillus	8	15.4	4	8	8	19.5	7	17.5	15	51.7	30	50	30	53.6	8	25
Coryneform	3	5.8	1	2	2	4.9	5	12.5	5	17.2	11	18.3	10	17.9	4	12
Entero	1	1.9			1	2.4										
Lactobacillus					1	2.4									1	3
Micrococcus																
Moraxella Cytophaga/Flavo	1	1.9			3	7.3	4	10			1	1.7	5	8.9	8	25
Pseudomonas	3	5.8			6	14.6	1	2.5	2	6.9	4	6.7	2	3.6		
Pseudomonas Gp.3	6	11.5	3	6	4	9.8	3	7.5			3	5	4	7.1	5	16
Atypical Gp. 3			1	2							2	3.3				
Atypical Gp. 2	1	1.9														
Atypical Gp. 1					2	4.9										
Spirillum																
Staphylococcus																
Vibrio	26	50	40	80	11	26.83	19	45	5	17.2	5	8.3	2	3.6	1	3
Total No. strains examined	52		50		41		40		29		60		56		31	

Table 19. Generic comparison of bacteria isolated from samples collected at the stations during January, 1974.

Month: January	Water								Sediment							
Genus	0.0		3.38		5.4		cc0.6		0.0		3.38		5.4		cc0.6	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Acinetobacter	1	1.9			1	1.8	4	10.5	1	1.9	2	3.9	2	5.6		
Achromobacter					1	1.8					1	1.9	1	2.9		
Alcaligenes																
Aeromonas																
Bacillus	3	5.7	2	4.3	2	3.6	2	5.3	5	9.8	30	57.7	4	11.1	30	55.
Coryneform	3	5.7							1	1.9	10	19.2	3	8.3	16	29.
Entero			1	2.1	1	1.8							1	2.9		
Lactobacillus																
Micrococcus																
Moraxella Cytophaga/Flavo.	5	9.4	2	4.3	5	9.1	6	15.8			2	3.9	8	22.2		
Pseudomonas	12	22.6	12	25.5	16	29.1	4	10.5	9	17.7	3	5.8			1	1.9
Pseudomonas Gp. 3	26	49.1	19	40.4	10	18.2	18	47.4	3	5.9			5	13.9	1	1.9
Atypical Gp. 3					3	5.5										
Atypical Gp 2					1	1.8										
Atypical Gp. 1					2	3.6			1	1.9						
Spirillum	2	3.8	10	21.3			3	7.9	2	3.9	3	5.8	3	8.3	2	3.7
Staphylococcus																
Vibrio	1	1.9	1	2.1	13	23.6	1	2.6	29	56.9	1	1.9	9	25	4	7.4
Total No. strains examined	53		47		55		38		51		52		36		54	

Table 20. Generic comparison of bacteria isolated from samples collected at the stations during April, 1974.

Month: April	Water								Sediment							
Genus	0.0		3.38		5.4		cc0.6		0.0		3.38		5.4		cc0.6	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Acinetobacter	3	7.5	5	11.9	6	13.0			4	8.7					1	2.0
Achromobacter																
Alcaligenes											2	3.5	1	1.9		
Aeromonas					1	2.2										
Bacillus	1	2.5			6	13.0			1	2.2	31	54.4	3	5.8		
Coryneform					2	4.4					4	7.0			2	4.1
Entero																
Lactobacillus																
Micrococcus	1	2.5							6	13						
Moraxella Cytophaga/Flavo.	6	15	4	9.5	8	17.4	13	38.2			2	3.5	3	5.8	10	20.4
Pseudomonas			2	4.8	2	4.4	7	20.6	4	8.7	2	3.5				
Pseudomonas Gp. 3	5	12.5	9	21.4	9	19.6	4	11.8	5	10.9			8	15.4	3	6.1
Atypical Gp. 3			1	2.4	1	2.2										
Atypical Gp. 2																
Atypical Gp. 1					2	4.4										
Unknown	8	20	23	54.8	1	2.2	9	26.5	14	30.4	12	21.1	28	53.9	32	65.3
Staphylococcus	1	2.5														
Vibrio	15	37.5			9	19.6	1	2.9	12	26.1	4	7.0	9	17.3	1	2.0
Total No. strains examined	40		42		46		34		46		57		52		49	

Table 21. Generic comparison of bacteria isolated from samples collected at the four stations during July, 1974.

Month: July	Water								Sediment							
Genus	0.0		3.38		5.4		cc0.6		0.0		3.38		5.4		cc0.6	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Acinetobacter											2	4.2			1	20
Achromobacter			1	1.9	2	3.9					1	2.1				
Alcaligenes			5	9.6												
Aeromonas							1	2.2					2	4.1		
Bacillus	5	10			8	15.7			4	7.1	20	41.7	10	20.4	5	10
Coryneform			1	1.9					2	3.6	4	8.3	2	4.1		
Entero							1	2.2							2	4
Lactobacillus																
Micrococcus													1	2.1		
Moraxella Cytophaga/Flavo.	2	4	4	7.7	1	1.9	2	4.4	2	3.6	5	10.4	2	4.1	4	
Pseudomonas	3	6	1	1.9	6	11.8	7	15.2			7	14.6	5	10.2	7	1
Pseudomonas Gp. 3	9	18	22	42.3	16	31.4	12	26.1			1	2.1	10	20.4	3	
Atypical Gp 3																
Atypical Gp 2																
Atypical Gp 1																
Spirillum	1	2	1	1.9			5	10.9			5	10.4	12	24.5	6	1
Staphylococcus																
Vibrio	30	60	17	32.7	18	35.3	18	39.1	48	85.7	3	6.3	5	10.2	21	
Total No. strains examined	50		52		51		46		56		48		49		49	

Table 22. Seasonal generic distribution of all bacteria strains isolated during October, 1973 through July 6, 1974

Totals	Water								Sediment							
Genus	October		January		April		July		October		January		April		July	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Acinetobacter	5	2.7	6	3.1	14	8.6			9	5.1	5	2.6	5	2.4	3	1
Achromobacter	1	0.6	1	0.5			3	1.5	1	0.6	2	1.0			1	0
Alcaligenes							5	2.5	2	1.1			3	1.5		
Aeromonas	2	1.1			1	0.6	1	0.5	1	0.6					2	0
Bacillus	27	14.8	9	4.7	6	3.7	13	6.5	83	47.2	69	35.8	35	17.2	39	
Coryneform	11	6.0	3	1.6	2	1.2	1	0.5	30	17.1	30	15.5	6	2.9	8	
Entero	2	1.1	2	1.0			1	0.5			1	0.5			2	
Lactobacillus	1	.6							1	0.6						
Micrococcus	1	.6			1	0.6									1	
Moraxella Cytophaga/Flavo.	8	4.4	18	9.3	31	19.0	9	4.5	14	7.9	10	5.2	21	10.3	13	
Pseudomonas	10	5.5	43	22.3	11	6.8	17	8.5	8	4.6	13	6.7	6	2.9	19	
Pseudomonas Gp. 3	16	8.7	64	33.2	27	16.6	59	29.7	12	6.8	9	4.7	16	7.8	14	
Atypical Gp. 3	1	0.6	3	1.6	2	1.2			2	1.1						
Atypical Gp. 2			1	0.5												
Atypical Gp. 1	3	1.6	2	1.0	1	0.6					1	0.6				
Spirillum			9	4.7	14	8.6	6	3.0			4	2.1	25	12.3	1	
Staphylococcus					1	0.6										
Vibrio	95	51.9	16	8.3	25	15.3	83	41.7	13	7.4	43	22.2	26	12.8	77	
Unknown			16	8.3	27	16.6	1	0.5			6	3.1	61	29.9	22	
Total No. Strains examined	183		193		163		199		176		193		204		20	

Table 23. Type of glucose utilization, reported as the percentage of the Gram-negative bacterial strains tested.

	Water				Sediment			
October Sample	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Alkalizers	12.2	10.6	26.7	47.7	0.0	30	72.2	83.3
Fermenters	68.3	82.2	36.7	47.6	60	25	11.1	11.1
Oxidizers	7.3	0.0	30	4.8	10	0.0	11.1	0.0
Non-utilizers	12.2	2.1	6.7	0.0	30	45	5.6	5.6
January Sample	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Alkalizers	55.3	36.4	37.7	28.9	6.7	33.3	43.3	0.0
Fermenters	2.1	4.6	26.4	2.6	64.4	8.3	33.3	50.0
Oxidizers	0.0	0.0	33.9	0.0	6.7	33.3	3.3	0.0
Non-utilizers	42.6	59.1	1.9	68.4	22.2	25.0	20.0	50.0
April Sample	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Alkalizers	30.8	30.2	34.2	17.7	13.3	68.2	81.6	63.8
Fermenters	41.0	0.0	26.3	2.9	26.7	18.2	18.4	2.1
Oxidizers	0.0	6.9	7.9	0.0	2.2	4.6	0.0	0.0
Non-utilizers	28.2	62.8	31.6	79.4	57.8	9.1	0.0	34.0
July Sample	0.0	3.38	5.4	cc0.6	0.0	3.38	5.4	cc0.6
Alkalizers	27.7	56.9	42.1	31.3	2.0	0.0	45.9	29.6
Fermenters	68.1	37.3	42.1	39.6	96.0	16.0	18.9	52.3
Oxidizers	4.3	3.9	15.8	10.4	0.0	0.0	10.8	0.0
Non-utilizers	0.0	1.9	0.0	18.8	2.0	84.0	24.3	18.2

Table 24. Comparison of Glucose Uptake at Big Island and Muddy Creek.

Temperature	Date	Location	%	%	Uptake	ug/ l/ hr.
23C	6/25	3.38	94.3	5.7	15	.13
28C	6/25	5.4	69.5	30.5	19	.17
29C	7/9	3.38	87.9	12.1	6.2	.057
31C	7/9	5.4	94.3	5.7	22.3	.2

Table 25. Seasonal Comparison of Glutamate Uptake at 3.38

Month	Temperature	Percent Upatke	ug/ l/ hr.
September	29C	15	0.2
December	4C	2	0.019
April	15C	3.5	0.049
May	23C	43.5	0.6
June	25C	13.8	0.19

Table 26. Glucose Uptake measured at stations in the Rhode River subestuary and in Chesapeake Bay (July, 1974).

Location	Description	Temperature	% Uptake	Rate (ug/l/hr)
0.0	open water	25C	11.0	0.1
3.38	open water	29C	6.2	0.057
5.4	marsh	31C	22.3	0.2
Cadle Creek	populated	27C	45.7	0.42
Eastern Bay	unpolluted	27C	4.3	0.04
Colgate Creek	oil polluted	28C	53.6	0.49

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Title: Characterization of Environmental Impact of Various Nutrient Loading Levels on Phytoplankton and Bacteria. Appearance and Survival of Coliform Bacteria in the Rhode River Estuary

Principal Investigator: Maria A. Faust, Smithsonian Institution

Collaborators: David L. Correll, Smithsonian Institution
Jack W. Pierce, Smithsonian Institution

Research Assistant: Michael T. Hargadon

Total Budget: $32,159.00

Project Summary: A complete survey on the source of entry and survival of fecal coliforms (FC) in the Rhode River estuary was studied. It was determined that FC bacteria enter from the watershed indeed and the influx of FC is greater during the summer months than during the other seasons. Only about 1% of the FC bacteria produced by the livestock in 5 separate watersheds totaling 849 hectares entered into the estuary through water runoff. The number of FC entering the Rhode River estuary from runoff water was not correlated with any of the following factors: rate of water runoff, nitrate, total phosphate, organic nitrogen and sediment load.

Fecal coliforms that entered the Rhode River estuary from the watershed were diluted as the Muddy Creek and Rhode River enlarged. High correlation existed between FC numbers and water volume of the Rhode River segments. In contrast, correlation between FC numbers and nitrate, organic nitrogen, chloride concentrations, pH and temperature existed at some but not all Rhode River stations during this study. Thus, dilution of FC that enter the estuary from the watershed was probably the overriding factor in assessing FC numbers in this estuary.

Fecal coliform survival experiements indicated that FC cell numbers increased during *in situ* incubation the first 24 hours, but died off thereafter. The rate of survival of FC was dependent on temperature, montmorillonite content and dissolved oxygen concentration of the water. The optimum values for survival were: 17 C, 50 ug/ml montmorillonite, 6.5 mg/L dissolved oxygen and 6‰ salinity respectively. The survival of FC cell numbers and chemical parameters measured *in situ* indicated that the best conditions existed within the Muddy Creek area of the Rhode River estuary where the nutrient concentrations and montmorillonite concentrations were high and considerable amount of fresh water diluted the saline water environment.

Concentration gradient of nutrients existed in the Rhode River. We examined bacterial and algal utilization of orthophosphate in an unpolluted estuarine environment by segregating the population using filter stacks of 5.0 um - 1.2 um - 0.45 um pore size. The role of bacteria and algae in P-uptake was elucidated. Bacteria appear to play a more important role in P-uptake than shown before. Bacteria assimilated P at a higher rate throughout the year. P-uptake by algae was considerable only from May to July. The summer P-uptake by algae was large, because cell numbers and biomass of dinoflagellate species were large and not because P-uptake rates of algae were higher. P-uptake of unit biomass is independent of phosphorus concentrations, but dependent on the given algal and bacterial flora. Dissolved orthophosphate concentrations in the water did not influence the rate of P-uptake per algal or bacterial biomass. P-uptake rates by algae and bacteria varied seasonally. It was concluded that rate of P-uptake is governed by the diversity of microorganisms rather than by phosphorus levels which were present at relatively high concentrations in the estuary throughout the year.

I. Subprogram:

A. Title: Contamination of Rhode River estuary by coliform bacteria from non-point sources.

B. Abstract: Fecal (FC) and total (TC) coliform bacteria in the runoff water of 849 hectare watershed was determined from February to September and along the length of Muddy Creek and Rhode River from June to September of the following year. The FC in the runoff water changed seasonally reaching the highest values in May and June. Number of FC in the runoff water was not correlated with water flow, nitrate, organic nitrogen, total phosphorus and sediment concentrations. During two sampling times May and June FC bacterial counts at the Rhode River stations exceeded safe water standards indicating that pollution from non-point source can become serious with a density of 0.65 animal/ha watershed area. Fecal coliform numbers decreased from the Muddy Creek entry toward the mouth of the Rhode River. The decrease in FC was correlated with increase in water volume of the Rhode River. Number of FC in the estuary was not correlated with nitrate, organic nitrogen, sediment, chloride and hydrogen ion concentrations and temperature. Calculations revealed that FC concentrations in the estuary can be largely accounted for by pollution from non-point sources.

C. Project Objectives: To measure the degree of contamination of total coliforms and fecal coliforms to the environment from rural areas as affected by storm water runoff; to establish the abundance of coliform bacteria on a seasonal basis in the Rhode River estuary; to correlate the numbers of coliform bacteria to total load and concentrations of nutrients, organic and mineral particulates delivered to the estuary on a seasonal basis.

D. Introduction: Stormwater runoff can carry coliform bacteria from fecal sources, deposited in the watershed by animals into the estuary. Municipal sewage treatment facilities will have limited effect upon water quality of an estuary unless the above non-point sources of fecal pollutants are dealt with effectively.

Morrison and Fair (7) studied a stream having no known pollution from domestic sewage and concluded that runoff from the surrounding watershed was the most important source of bacterial contamination. In another study of stormwater runoff from 27 acres residential area free of sanitary and industrial waste discharge the fecal coliform and fecal streptococci averaged 11,000 to 21,000 cells per ml of water (9). Spraying pig excrement over a pasture resulted in 20-900 fold increase in fecal bacterial numbers in discharge waters (3). In Scotland surface drainage system of 0.7 acre was studied and the coliform contamination from this area was proportional to the flow rate of drain discharge, the number of bacteria in or on the soil and vegetation (3). A review of water-born diseases arising from fecal contamination occurring from 1946-60 lists many cases when rainfall washing of animal feces into streams, water reservoirs caused diseases (10).

The estuarine environment is a complex intermix of fresh and saline water systems that circulate through ebbs and flows in a basin before dispersion to the open sea. Therefore, it is important that the degree of contamination of estuarine waters with coliform bacteria from non-point sources is established and the dissemination of bacteria in the estuary is measured. Information gathered so far indicate that coliforms found in the Muddy Creek-Rhode River system originate from the pasture areas of the watershed. This study was extended to 849 hectare of five watersheds with individual runoff collecting points and 6.7 km long waterway of Muddy Creek-Rhode River estuary for establishing the sources and appearance of coliforms in the water.

The survey of the five separate watersheds were initiated in February 1974 and was conducted for eight months with brief periods at some watersheds without runoff. The above limitations, relatively short duration of survey and occasional absence of runoff should be considered in using the presented

information. However, this survey was conducted as an integrated study of watershed-estuary interaction for coliform contamination on the largest watershed-estuary area so far examined.

E. Materials and Methods:

Sampling sites: Map of the watersheds of the Rhode River estuary whose drainage enter the Rhode River is shown in Fig. 1. The water collecting stations are designated as North Branch (Weir #1), Blue Jay Branch (Weir #2), Williamson Branch (Weir #3), Steinlein Branch (SL) and Fox Creek (F). These stations are instrumented with stream gauging weirs and with automated volume averaging water samplers. On sampling days the water flow data and samples for sediment and organic content determinations were taken from the integrated water samples. On the same days, surface water samples were taken for bacterial examination and for nutrients determinations. The study was conducted at monthly intervals from February to September 1974.

Sampling stations along the Rhode River axis are designated in Fig. 2. Station 0.0 km indicates the mouth of the Rhode River and 6.7 km the last station at the Muddy Creek. One station designated as -1.2 km is located between the Rhode and West Rivers. Surface water samples were collected from numerous stations for bacteria, nutrients and sediment content. The study was conducted at monthly intervals from June 1973 to September 1974.

The Rhode River watershed has been surveyed for total drainage area, for the area of various watersheds and for land use types (natural areas, pasture lands, cultivated cropland, wet area, marshes and swamps, grassland and residential non-sewered areas and summarized in Table 1.

Enumeration of coliform bacteria: Surface water samples were taken in sterile bottles from the surface of the water running through the weirs and at the Rhode River stations. Samples were returned to the laboratory within two hrs. of collection. The Multiple-Tube Fermentation Technique

Figure 1

Fig. 1. Drainage areas within the Rhode River watershed are illustrated.

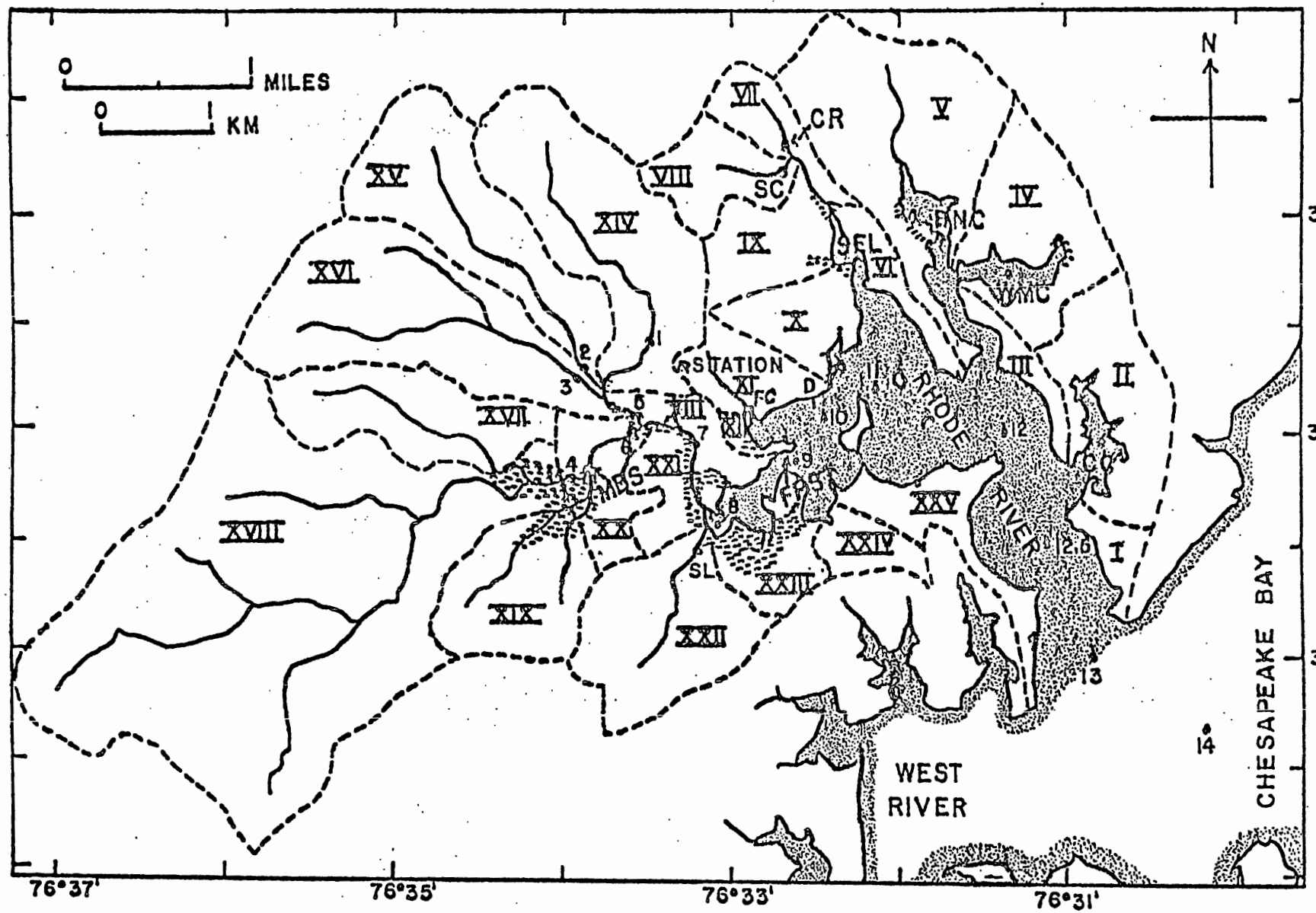

Figure 2

Fig. 2. Sampling stations are designated along the Rhode River axis.

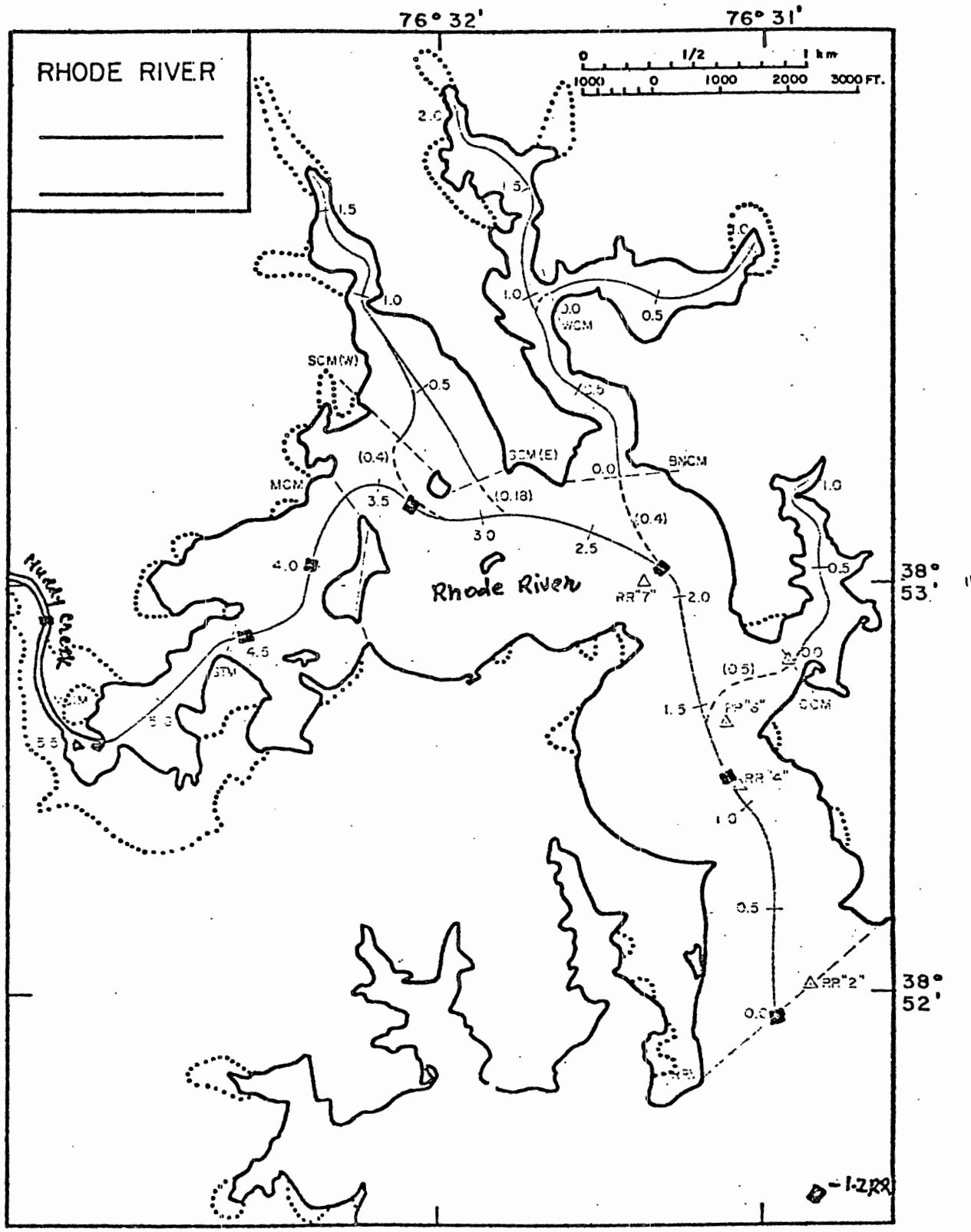

Table 1. Land use types and total drainage area of various subwatersheds at Rhode River estuary.

Watershed	hectares in each land use category in 1972						
	Cultivated crops	Wet area (open water + marshes + swamps)	'Natural' areas (forest, and old fields)	Grass-lands (pasture + other)	Residential + others (bare, paved, roads, dumps)	Total area	Livestock number of animals
All of Rhode River	615	18	1,882	379	366	3,260	-
North Branch (Weir #1)	69.7	1.9	107.7	46.9	13.2	239.4	326
Blue Jay Branch (Weir #2)	47.2	2.0	92.6	27.3	11.0	180.1	39
Williamson Branch (Weir #3)	18.2	0.45	188.5	31.8	15.5	254.4	84
Steinlein Branch (Weir SL)	62.0	0.36	72.9	7.4	4.1	146.8	77
Fox Creek (Weir FC)	2.35	0	24.39	2.21	0.43	29.38	30

was used for the identification and enumeration of coliforms recommended and described in Standard Methods for Enumeration of Water and Waste Water (1). Total coliform (TC) numbers were determined by inoculating into each 5 tubes of Lactose Broth, 10, 1 and 0.1 ml of water samples. These tubes were incubated for 24-48 hrs. at 35.5 C. Positive tubes, that produced acid with gas were estimated and TC bacterial numbers by the Most Probable Numbers (MPN) per 100 ml water determined. All of the above positive cultures were further tested for the presence of fecal coliforms (FC). An aliquot of the positive cultures were transferred to EC Broth containing tubes, and incubated in a water bath for 24 hrs. at 44.5 C. Positive cultures producing acid and gas at above elevated temperature indicated the presence of fecal bacteria. Bacterial numbers were estimated as MPN/100 ml of water.

Identification of bacteria other than coliforms: Water samples were diluted in sterile 0.01 M phosphate buffer pH 7.5 and plated on Nutrient agar collected from the weir stations. Samples collected from the Rhode River stations were plated on Estuarine agar (0.3% yeast extract, 1% peptone, 0.5% sodium chloride and 2% agar at pH 7.2-7.4). Each sample was plated in duplicate on the above media at each of two dilutions. Dilutions ranged from 10^1 - 10^3, depending upon the numbers of the population expected to be present. Plates were incubated at room temperature (18-20 C) for seven days. Total counts, identification of isolates were obtained from Nutrient and Estuarine agar plates. Single colonies were selected from the plates and were purified and tested for the following characters: cell shape and size, flagellation and motility, colony appearance, pigmentation, oxidative and fermentative ability of carbohydrate utilization, catalase and oxidase production (2, 5, 8).

Nutrients and sediment determinations: Total phosphorus, nitrate, chlor organic nitrogen, and sediment concentrations of the water samples (Table 2) were determined in Drs. D. L. Correll and J. W. Pierce laboratories. Physical parameters pH and temperature were also recorded at the same time. Methods are given in the corresponding parts of the report.

F. Results:

Coliform and other bacteria in runoff water: The number of coliform bacteria in the runoff water of the watersheds changed during the season. Coliform numbers were relatively low from February to April, considerably increased during May and June and decreased again in July and August (Table 3). During the late two months some weirs were dry and water samples could not be collected. The proportion of FC and total coliforms (TC) numbers varied throughout the season. The ratio of FC/TC changed from 0.04 - 1.0 with the highest values being in May and July. This pattern was uniform between watersheds, when coliform numbers were high at one weir it was generally high at others.

There was no correlation between flow rate of runoff water and coliform numbers of the water (Table 4). The water runoff ranged from low of 0.1×10^3 L/ha/day to 16.8×10^3 L/ha/day of watershed. The coliform bacteria, however, did not incredase proportionally to runoff. When one examines individual watersheds such as Weir #1 the absence of correlation is clear. In April 13.4×10^3 L/ha/day carried 353×10^6 FC/day, in May 12.8×10^3 L/ha/day carried $73{,}958 \times 10^6$ FC/day and in June a decreased flow rate of 4.4×10^3 L/ha/day carried high bacterial numbers $117{,}216 \times 10^6$ FC/day. Other values are also similar.

The coliform numbers in the runoff water were also not correlated neither with the animals located at the watersheds, nor with the pasture area of the given watershed. Coliform numbers estimated in runoff FC/day/animal

Table 2. Nutrient concentration of surface water samples collected at designated stations.

Stations	Constituents	Days of 1974 52	77	105	133	168	259	273
Weir #1	NO_3 - N ug/L a)	378.0	314.0	491.0	213.0	330	Streams dry	
	Total PO_4-P ug/l	50.0	73.0	191.0	116.0	119		
	Organic N - N ug/l	291.0	451.0	567.0	698.0	640		
	Total solids mg/L b)	14.8	26.8	74.9	26.4	-		
	Organic matter mg/L	9.2	4.6	10.5	43.8	-		
Weir #2	NO_3 - N ug/L	249.0	179.0	347.0	10.0	175.0		
	Total PO_4 - P ug/L	55.0	151.0	106.0	60.0	111.0		
	Organic N - N ug/L	73.0	334.0	320.0	523.0	495.0		
	Total solids mg/l	-	24.2	111.2	11.0	9.4		
	Organic matter mg/L	-	15.1	11.5	20.8	31.2		
Weir #3	NO_3 - N ug/L	170.0	146.0	121.0	41.0	286.0		
	Total PO_4 - P ug/l	39.0	77.0	300.0	167.0	137.0		
	Organic N - N ug/L	58.0	356.0	691.0	553.0	313.0		
	Total solids mg/L	20.0	21.7	118.2	78.4	36.6		
	Organic matter mg/L	12.9	21.2	8.7	11.8	10.5		
Steinlein Br.	NO_3 - N ug/L	326.0	236.0	410.0	82.0	250.0		
	Total PO_4 - P ug/L	21.0	77.0	231.0	118.0	158.0		
	Organic N - N ug/L	124.0	182.0	451.0	538.0	545.0		
	Total solids mg/L	21.2	34.2	54.0	26.4	43.5		
	Organic matter mg/L	4.5	15.2	12.1	14.3	16.2		
Fox Cr.	NO_3 - N ug/L	229.0	216.0	384.0	73.0	398.0		
	Total PO_4 - P ug/L	30.0	63.0	171.0	59.0	58.0		
	Organic N - N ug/L	145.0	153.0	407.0	451.0	284.0		
	Total solids mg/L	71.3	27.9	47.4	57.2	7.6		
	Organic matter mg/L	11.3	25.1	10.3	12.4	21.7		

a) Nitrate, total phosphate and kjeldahl N concentrations were determined in Dr. D.L. Correll's laboratory.

b) Total solids and oxidizable organic concentrations were determined in Dr. J.W. Pierce's laboratory.

Table 3. Estimated total and fecal coliforms in surface water samples collected at designated stations.

Days of 1974	Sites	TC	FC	Ratio FC/TC
		MPN/100 ml		
52	Weir #1	170	7	0.04
	#2	130	27	0.20
	#3	280	180	0.64
	SL	94	33	0.35
	F	17	4	0.23
77	Weir #1	130	130	1.00
	#2	540	170	0.31
	#3	110	46	0.41
	SL	17	13	0.76
	F	22	22	1.00
105	Weir #1	79	11	0.13
	#2	130	17	0.13
	#3	79	33	0.41
	SL	33	11	0.33
	F	49	33	0.67
133	Weir #1	2,400	2,400	1.00
	#2	2,400	2,400	1.00
	#3	920	920	1.00
	SL	350	220	0.62
	F	920	280	0.30
168	Weir #1	24,000	11,000	0.45
	#2	1,500	1,100	0.73
	#3	1,100	1,100	1.00
	SL	4,600	750	0.16
	F	4,600	1,100	0.23
259	Weir #1	93	93	1.00
	#2	-	-	-
	#3	-	-	-
	SL	-	-	-
	F	2,400	2,400	1.00
273	Weir #1	4,600	1,100	0.24
	#2	11,000	460	0.04
	#3	-	-	-
	SL	-	-	-
	F	2,400	2,400	1.00

Table 4. Fecal coliforms in surface water samples affected by water runoff and livestock density at various watersheds.

Days of 1974	Sites	Flow L x 10^6/day	Flow L x 10^3/day/ha	FC x 10^6 discharged/day	FC x 10^6 discharged/day/animal
52	Weir #1	1.13	4.7	80	0.2
	#2	1.45	5.2	253	6.5
	#3	1.28	5.0	2,306	27.4
	SL	0.76	5.2	251	3.2
	F	0.12	4.2	5	0.2
77	Weir #1	2.37	9.9	3,088	9.5
	#2	1.72	9.6	2,937	75.3
	#3	2.59	10.2	1,192	14.2
	SL	1.29	8.8	165	2.1
	F	0.24	8.4	53	1.8
105	Weir #1	3.21	13.4	353	1.1
	#2	2.44	13.6	416	10.7
	#3	-	-	-	-
	SL	0.74	5.1	82	1.0
	F	0.27	9.4	1	0.03
133	Weir #1	3.06	12.8	73,958	226.8
	#2	2.30	12.8	51,840	1,329.2
	#3	2.95	11.6	27,158	323.3
	SL	1.85	12.7	4,118	53.5
	F	0.14	4.9	403	13.4
168	Weir #1	1.06	4.4	117,216	359.5
	#2	3.02	16.8	3,326	85.2
	#3	3.31	13.0	3,643	43.4
	SL	0.47	3.2	3,564	46.3
	F	0.04	1.4	475	15.8
259	Weir #1	0.01	0.4	8	0.02
	#2	-	-	-	-
	#3	-	-	-	-
	SL	-	-	-	-
	F	0.001	0.5	34	1.1
273	Weir #1	0.32	1.4	3,643	11.2
	#2	0.09	0.5	423	10.8
	#3	-	-	-	-
	SL	-	-	-	-
	F	0.002	0.1	69	2.3

ranged from a low 0.2 x 10^6 to 1329 x 10^6, the high values generally occurring during the months of May and June (Table 4). If we accept the data of Geldreich (4), that a cow produces 5428 x 10^6 FC/day than we can calculate that in average 1% of FC produced by the animals were washed off and ended up in the runoff water and ranged from trace to 6.6% with one exception of 24.4% occurring at the watershed of Weir #2 in May.

Fecal coliform numbers estimated in the runoff did not correlate with the chemical and physical parameters of the samples (Table 5). The measured parameters included nitrate and organic nitrogen, total phosphorus, particulate levels, pH and temperature. Occasionally when one parameter correlated with FC numbers of the same weir throughout the year a significant figure was obtained. However, when the data from all weirs were considered the occasional significance cannot be considered important. Temperature and FC numbers correlated significantly at three weir samples out of 5 throughout the sampling period (Table 5).

Bacterial genera other than coliforms were also identified in weir samples between June and September 1974 (Table 6). Bacillus sp. were the most numerous organisms isolated from 4 weir samples. Aeromonas sp. Streptococcus sp. and Flavobacterium sp. were the next numerous. Proteus sp. and Pseudomonas sp. were found only in one weir sample each. Bacteria originated from natural sources were Bacillus sp. Aeromonas sp. and Flavobacterium sp. and organisms associated with pollution were Streptococcus sp., Pseudomonas sp. and Proteus sp.

Coliforms and other bacteria in the estuary: The distance on the Rhode River axis from km 4.5 to 6.7 is considered as part of the Muddy Creek and the distance between km 0 to 4.0, the Rhode River (Fig. 2). There was a considerable change in coliform numbers between the Muddy Creek and Rhode River stations. The TC and FC cell numbers were higher at stations in the

Table 5. Relationship between fecal coliforms and nutrients, total solids, organic content, pH and temperature of surface water samples at designated stations. a)

Constituents	Weir #1		Weir #2		Weir #3		Steinlein Br.		Fox Cr.	
	n b)	r c)	n	r	n	r	n	r	n	r
NO_3-N ug/L	7	0.52N.S.	5	0.95*	6	0.15N.S.	6	0.12N.S.	7	0.67N.S.
Total PO_4-P ug/L	7	0.06N.S.	5	0.51N.S.	6	0.38N.S.	6	0.54N.S.	7	0.48N.S.
Kjeldahl-N ug/L	7	0.89*	5	0.91*	6	0.37N.S.	6	0.72N.S.	7	0.49N.S.
Total solids mg/L	5	0.49N.S.	4	0.49N.S.	5	0.56N.S.	5	0.55N.S.	5	0.88N.S.
organic matter mg/L	5	0.98*	4	0.91N.S.	5	0.31N.S.	5	0.61N.S.	5	0.67N.S.
pH	5	0.95*	3	0.77N.S.	4	0.94N.S.	4	0.82N.S.	5	0.84N.S.
Temperature °C	8	0.74*	6	0.53N.S.	6	0.83*	6	0.72N.S.	9	0.90*

a) correlation calculated for all sampling sites from Feb.-Sept. 1974.
b) n sample size
c) r correlation coefficient *statistical significance $P < 0.05$
N.S. not significant

Table 6. Bacterial general other than coliforms identified from water samples collected at all weir sites from June to September, 1974.[a]

	Stations				
genera	Weir #1	Weir #2	Weir #3	Steinlein br.	Fox Cr.
Bacillus	+[b]	+	+	+	-
Aeromonas	+	+	-	+	-
Pseudomonas	+	-	-	-	-
Chromobacter	+	+	-	-	-
Proteus	-[c]	-	+	-	-
Streptococcus	+	+	-	-	+
Flavorbacterium	-	-	-	+	+
Chromobacterium	-	-	-	-	-

a) Colonies were identified from nutrient agar plates.

b) genera present

c) genera absent

Muddy Creek, than at the Rhode River (Tables 7 & 8). Since the obvious source of TC and FC was from the watershed of the Muddy Creek the possibility of dilution of coliforms was examined. The 5.4 km point at the Rhode River axis was designated as one and by using the volume of Rhode River segments corresponding to sampling stations, the relative dilution figures were calculated for each segment (Table 9). The volume of each segment and FC numbers correlated at 1% and 5% level from April to July sampling dates, indicating that FC numbers are diluted along the Rhode River (Table 10). During the months of August and September the FC numbers were very low and because of this, significant dilution did not occur. In contrast, no correlation existed between TC and volume of Rhode River. It is likely that TC cell numbers are affected by different factors than FC and therefore their numbers declined along the Rhode River axis differently from FC. The TC numbers decreased rapidly after 5.4 km Muddy Creek stations and their numbers remained about the same approaching the 0.0 km of Rhode River station. This perhaps indicates that certain types of TC were eliminated but others were able to multiply so their numbers were not diluted.

Bacteria other than coliforms were identified from various stations at the Rhode River (Table 11) from water samples collected from June to September 1974. Aeromonas sp. Streptococcus sp., Salmonella sp. and Proteus sp. that were found in the weir (Table 6) samples were eliminated after 4.5 km stations. In contrast, Bacillus sp. Flavobacterium sp. and Pseudomonas were recovered from the outermost sampling sites of the Rhode River. Bacteria belonging to Serratia sp. and Chromobacterium sp. were only found in samples collected from the Muddy Creek stations.

Nutrient levels and physical parameters of collected samples of Muddy Creek and Rhode River stations from km 6.7 to 0.0 are presented in Tables 12 and 13. The temperature of the water changed from a low of 3 C to a high of

Table 7. Seasonal distribution of total coliforms of surface water samples at the Muddy Creek and Rhode River Stations.

Day of 1973-74	Stations on Rhode River axis (Distance in Km.). 6.7	6.8	6.1	5.4	4.5	4.0	3.4	2.2	0.0
				MPN/100 ml					
178	2,400	2,400	-	2,400	-	33	221	-	-
208	2,400	2,400	-	2,400	-	5	240	-	-
232	1,600	1,600	-	1,600	-	170	22	-	-
262	540	540	-	920	-	350	130	-	-
288	1,600	920	-	350	-	280	350	49	-
323	130	79	-	33	-	13	2	8	-
347	2,400	130	-	350	110	33	17	11	-
23	79	79	170	49	17	8	17	8	-
52	350	350	220	920	110	17	9	7	6
77	1,600	350	350	350	140	79	33	46	14
105	540	110	540	1,600	94	23	33	13	5
133	2,400	2,400	2,400	2,400	1,600	220	920	110	22
168	11,000	11,000	4,600	1,100	1,600	460	21	1,100	93
203	2,400	460	4,600	4,600	2,400	93	93	75	9
232	2,400	2,400	4,600	150	23	240	210	15	20
260	2,400	1,100	1,100	1,100	93	150	93	14	93

Table 8. Seasonal distribution of fecal coliforms of surface water samples at the Muddy Creek and Rhode River Stations.

Day of 1973-74	Stations on Rhode River axis (Distance in Km.) 6.7	6.8	6.1	5.4	4.5	4.0	3.4	2.2	0.0
				MPN/100 ml					
178	2,400	2,400	-	1,600	-	17	17	-	-
208	2,400	2,400	-	1,600	-	0	140	-	-
232	1,600	1,600	-	1,600	-	70	12	-	-
262	540	540	-	540	-	33	33	13	-
288	540	350	-	240	-	13	22	0	-
323	17	27	-	0	-	0	0	0	-
347	79	22	-	70	49	13	7	4	-
23	49	79	130	17	7	0	0	0	-
52	220	4	17	23	2	0	0	0	0
77	1,600	70	130	79	79	17	0	17	11
105	170	17	540	1,600	94	13	2	2	0
133	2,400	2,400	2,400	2,400	1,600	220	920	110	7
168	11,000	11,000	4,600	540	350	150	7	43	9
203	2,400	240	1,100	2,400	240	9	15	4	4
232	1,100	240	460	93	9	9	9	4	4
260	2,400	460	240	93	9	23	15	4	7

Table 9. Water Mass for Segments of Rhode River.

Segment km point R.R. axis	Volume at mean low water x $10^4 m^3$	Surface area at mean low water ha	Average depth mean low water m	Cumulative volume of water x $10^4 m^3$	Relative portion c water volu
5.4					1.0000
5.4 - 4.5	14	26	0.55	14	0.0714
4.5 - 3.4	83	56	1.50	97	0.0103
3.4 - 2.2	375	152	2.47	472	0.0021
2.2 - 0.0	349	145	2.41	821	0.0012
0.0 - (-1.17)	620	326	1.95	1441	0.0007

Table 10. Relationship between total and fecal coliforms and volume of Rhode River segments.

Days of 1974	Stations Km on Rhode River axis	Relative portion of water volume	TC MPN/100ml	FC MPN/100ml	Relationship TC river volume r	Relationship FC river volume r[a)]
105	5.4	1.0000	1,600	1,600	0.57N.S.	1.00**
	5.4 - 4.5	0.0714	94	94		
	4.5 - 3.4	0.0103	22	13		
	3.4 - 2.2	0.0021	23	2		
	2.2 - 0.00	0.0012	5	0		
	0.0 - (-1.2)	0.0007	23	2		
133	5.4	1.0000	2,400	2,400	0.59N.S.	0.99**
	5.4 - 4.5	0.0714	1,600	1,600		
	4.5 - 3.4	0.0103	220	70		
	3.4 - 2.2	0.0021	515	182		
	2.2 - 0.0	0.0012	22	7		
	0.0 - (-1.2)	0.0007	8	0		
168	5.4	1.0000	1,100	540	0.59N.S.	0.95*
	5.4 - 4.5	0.0714	1,600	350		
	4.5 - 3.4	0.0103	460	150		
	3.4 - 2.2	0.0021	560	25		
	2.2 - 0.0	0.0012	122	9		
	0.0 - (-1.2)	0.0007	93	23		
203	5.4	1.000	4,600	2,400	0.54N.S.	0.99**
	5.4 - 4.5	0.0714	240	240		
	4.5 - 3.4	0.0103	93	9		
	3.4 - 2.2	0.0021	84	10		
	2.2 - 0.0	0.0012	15	4		
	0.0 - (-1.2)	0.0007	4	0		
232	5.4	1.0000	150	93	0.52N.S.	0.74N.S.
	5.4 - 4.5	0.0714	23	9		
	4.5 - 3.4	0.0103	240	9		
	3.4 - 2.2	0.0021	113	7		
	2.2 - 0.0	0.0012	15	7		
	0.0 - (-1.2)	0.0007	9	0		
260	5.4	1.0000	1,100	93	0.60N.S.	0.63N.S.
	5.4 - 4.5	0.0714	93	9		
	4.5 - 3.4	0.0103	150	23		
	3.4 - 2.2	0.0021	54	10		
	2.2 - 0.0	0.0012	57	6		
	0.0 - (-1.2)	0.0007	9	4		

a) correlation coefficient
** statistical significance at $P < 0.01$
* " at $P < 0.05$
N.S. Not significant.

Table 11. Bacterial genera identified from water samples collected at stations along the Rhode River axis from June to September, 1974.[a]

Genera	Stations on Rhode River axis (Distance in km)						
	6.7	6.8	6.1	5.4	4.5	4.0	3.4
Bacillus	+[b]	+	+	+	+	+	-
Aeromonas	+	-[c]	-	-	+	-	-
Chromobacterium	+	+	+	+	-	-	-
Streptococcus	+	+	+	-	-	-	-
Flavobacterium	+	+	+	-	+	+	+
Pseudomonas	+	+	+	+	+	+	+
Proteus	+	+	+	-	-	-	-
Serratia	+	+	-	+	-	-	-
Salmonella	+	+	+	-	-	-	-

a) single colonies from Rhode River estuarine agar were taken for identification of bacteria

b) + genera present

c) - genera absent

Table 12. Temperature, pH and chloride ion concentrations of surface water samples collected along the Rhode River axis.

Days of 1973-74	Constituents	Stations on R.R. axis (distance in Km) 6.7	6.8	6.1	5.4	4.5	4.0	3.4	2.2	0.0
347	Temp. C	3.0	3.3	-	4.3	4.5	5.0	5.0	5.5	-
	pH	6.4	6.5	-	7.3	8.2	8.2	8.2	8.1	-
	[Cl] parts/ 1000	0.5	0.04	1.0	4.7	5.6	5.7	5.7	5.9	-
23	Temp. C	7.0	6.0	6.5	7.5	7.0	5.0	5.5	5.0	-
	pH	6.4	6.5	-	7.7	7.8	8.2	8.2	8.0	-
	Cl	0.07	0.02	0.3	1.3	3.5	3.7	3.8	4.1	-
52	Temp. C	4.0	3.5	4.5	5.5	5.5	4.5	5.5	4.0	4.0
	pH	6.2	6.4	5.7	6.7	7.5	8.0	8.1	8.0	8.0
	Cl	0.04	0.01	0.2	0.5	3.3	4.5	4.5	4.3	4.7
77	Temp. C	12.0	10.5	12.0	10.0	9.0	7.0	7.0	7.3	6.5
	pH	6.2	6.4	6.5	6.5	7.4	8.4	8.5	8.3	7.9
	Cl	0.02	0.01	0.03	0.06	3.3	4.3	4.1	4.2	4.3
105	Temp. C	16.0	15.5	16.0	17.5	16.5	15.0	14.0	13.0	13.0
	pH	6.4	6.6	6.5	7.2	8.6	8.4	8.4	7.9	7.6
	Cl	0.02	0.01	0.08	0.3	2.6	2.9	3.0	3.0	3.1
133	Temp. C	18.5	15.5	16.5	18.0	18.5	17.5	18.0	18.0	16.5
	pH	6.7	6.7	6.7	6.9	8.1	8.5	8.5	8.3	8.1
	Cl	-	-	-	-	-	-	-	-	-
168	Temp. C	20.0	18.5	21.5	22.0	24.5	27.5	27.5	24.5	24.0
	pH	6.6	6.6	6.7	6.9	8.0	9.2	9.2	8.9	8.6
	Cl	0.7	0.2	1.3	1.9	3.3	3.6	3.5	3.7	4.0
203	Temp. C	22.0	21.0	22.0	21.0	27.0	25.0	24.0	24.5	24.0
	pH	8.3	7.7	6.6	7.9	8.3	8.0	7.5	8.1	8.3
	Cl	3.7	3.2	4.2	4.7	5.0	5.0	5.1	5.1	5.2
220	Temp. C	26.5	26.5	27.5	27.0	28.5	28.0	28.0	28.5	28.5
	pH	7.0	7.0	8.1	7.2	7.2	-	-	-	-
	Cl	4.2	4.1	4.9	5.2	5.4	5.5	5.5	5.4	5.5
232	Temp. C	23.5	23.5	23.5	24.5	25.5	26.1	26.1	26.0	25.7
	pH	6.2	6.5	7.1	6.6	6.6	7.6	7.6	7.8	8.2
	Cl	4.7	4.6	5.6	5.8	5.8	5.4	5.4	5.4	5.8
246	Temp. C	26.0	26.0	26.0	26.3	27.8	27.4	27.4	27.4	26.9
	pH	7.1	7.2	6.6	7.3	7.5	7.4	7.4	7.8	7.6
	Cl	4.0	3.3	5.0	5.8	5.9	6.5	6.5	6.7	4.6
260	Temp. C	21.0	21.0	21.5	22.5	23.5	23.7	23.7	23.9	24.0
	ph	7.0	7.0	7.1	7.3	7.6	7.8	7.8	7.5	8.2
	Cl	4.4	4.1	5.3	5.8	5.6	6.5	6.5	6.6	-
274	Temp. C	15.0	15.5	17.5	17.0	18.0	-	-	-	-
	pH	6.9	6.9	6.8	6.8	6.7	7.4	7.4	7.5	-
	Cl	2.7	2.2	3.8	5.3	6.1	-	-	-	-

Table 13. Nutrient concentrations of surface water samples collected at stations along the Rhode River axis.

Days of 1973-74	Constituents N ug/L	Stations on Rhode River axis (Distance Km). 6.7	6.8	6.1	5.4	4.5	4.0	3.4	2.2	0.00
347	NO_3	410	452	316	47	5	1	42	87	-
	Organic N	367	248	-	716	586	593	443	485	-
23	NO_3	400	316	338	358	390	400	460	484	-
	Organic N	222	153	135	659	156	448	434	347	-
52	NO_3	244	163	189	163	184	384	351	327	368
	Organic N	190	148	219	203	358	457	360	377	289
77	NO_3	218	157	192	194	69	95	116	159	370
	Organic N	462	291	416	547	571	509	656	744	490
105	NO_3	193	182	158	72	6	88	208	432	587
	Organic N	170	163	272	849	632	586	593	455	328
133	NO_3	115	98	87	58	3	11	6	41	213
	Organic N	481	346	173	706	1,077	1,197	1,225	1,033	517
168	NO_3	6	126	2	3	1	2	3	4	3
	Organic N	219	768	1,125	1,353	983	761	756	674	667
203	NO_3	13	9	13	9	9	12	17	12	7
	Organic N	2,883	2,130	1,759	1,647	1,227	1,048	1,043	1,198	833
220	NO_3	9	11	5	7	10	6	6	5	7
	Organic N	2,192	2,074	1,643	1,444	1,128	761	761	815	755
232	NO_3	23	23	7	9	7	3	3	6	3
	Organic N	1,917	1,497	1,028	1,285	763	648	648	1,000	996
246	NO_3	0	1	0	4	7	0	0	0	6
	Organic N	1,992	2,431	1,563	1,247	1,119	1,252	1,252	1,027	1,064
260	NO_3	10	7	5	5	5	11	11	6	10
	Organic N	1,395	1,444	1,171	1,186	1,053	1,283	1,283	1,180	971
274	NO_3	50	50	18	8	4	8	8	8	-
	Organic N	-	-	-	-	-	-	-	-	-

28 C during the year. In general the water temperature at the 2.2 and 3.4 km stations were higher than in the shallow waters of 6.7 km station with the exception of February and March, when the reverse was true. The pH of the water changed between 5.4 and 4.5 km sampling stations from near neutral to slightly alkaline affected by tidal actions. During spring the freshwater input at the Muddy Creek was high and resulted in lowered chloride content of the samples. The chloride content of samples increased during the summer months. The nitrate and organic nitrogen concentrations remained about the same at all stations along Muddy Creek and Rhode River during the winter months. However, nitrate levels decreased and organic nitrogen concentrations increased toward the mouth of the Rhode River during the rest of the year.

The relationship between FC numbers and nutrient and physical parameters (nitrate, organic nitrogen, chloride ion concentrations, pH and temperature of samples collected during this study at the Rhode River stations is listed in Table 14. Correlation between the above parameters was observed in some but not on all stations evaluated along the length of Rhode River. This can indicate that the dilution of FC that enter the Rhode River from the watershed was the overriding factor in assessing FC pollution in estuarine environment.

G. Discussion: This study allows a preliminary analysis of sources and dissemination of coliform bacteria in the Rhode River estuary. Degree of contamination of coliform bacteria in runoff water varied with the season and an appreciable proportion of coliforms were of fecal origin. The analysis of FC/TC ratios and coliform numbers revealed that during the months of May and June the FC numbers were very high and there were no other than FC type of coliforms present. This contrasted with the winter months, when the FC represented only about 4-64% of the total coliform bacteria. The seasonal

Table 14. Relationship between fecal coliforms and nutrients, pH, temperature and chloride of surface water samples at designated Rhode River stations.[a)]

Stations Distance Km	Constituents NO_3 - N ug/L		Organic N - ug N/L		pH		Temperature °C		Chloride $[Cl^-]$ parts/1000	
	n[a)]	r[b)]	n	r	n	r	n	r	n	r
6.7	13	0.91**	12	0.68*	14	0.47N.S.	14	0.68**	12	0.20N.S.
6.8	13	0.51N.S.	12	0.05N.S.	14	0.12N.S.	14	0.52N.S.	12	0.42N.S.
6.1	13	0.69**	11	0.43N.S.	12	0.31N.S.	12	0.65*	12	0.30N.S.
5.4	13	0.59*	12	0.59**	14	0.42N.S.	14	0.65*	12	0.18N.S.
4.5	13	0.51N.S.	12	0.69*	13	0.52N.S.	13	0.45N.S.	12	0.47N.S.
4.0	13	0.61*	12	0.60*	18	0.50*	18	0.50*	11	0.51N.S.
3.4	13	0.51N.S.	12	0.72**	13	0.43N.S.	13	0.34N.S.	11	0.82**
2.2	13	0.59*	12	0.59N.S.	12	0.71**	12	0.55N.S.	11	0.55N.S.
0.0	10	0.60N.S.	10	0.69*	9	0.66*	10	0.44N.S.	8	0.29N.S.

a) n sample size

b) r correlation coefficient * statistical significance $P < 0.05$
** " " $P < 0.01$
N.S. Not significant

variation of FC was uniform between watersheds, when coliform numbers were high at one weir, they were generally high at other weir stations.

Water runoff/ha/day from the watershed at the various weir stations differed little from one weir to the other. Similarities in water runoff rates coupled with the uniformity of precipitation falling of these watersheds indicates that the hydrological relationship is relatively uniform throughout the Rhode River watershed. However, the relationship between FC numbers and runoff rates did not increase porportionally with water flow. Our data in regard to lack of correlation between water flow and FC numbers is similar to those of Leininger and McCleskey (6), they also did not find correlation between rainfall and FC numbers. In a different type of study on a small area (0.7 ha), where the water first penetrated the soil and was collected through drainage pipes, the rate of runoff and FC correlated well. On this area the addition of liquid animal excrement to the surface of soil increased the FC numbers proportionally in the drainage water samples (3).

It appears that the discharge of FC from animal excrement is a major pollutant from non-point sources of the Rhode River watershed. Data calculated by using Geldreich (4) estimates for coliform bacteria discharged/animal/day and the comparison of these values with the runoff rate of FC at the weir stations revealed, that in average only about 1% of FC was washed off from the Rhode River watershed. When such small fraction of bacteria is washed off, the FC numbers in the runoff cannot be expected to correlate with the volume of water runoff. Interpreting this data one must consider that the watershed was 849 hectares with 0.65 animal/ha population. The above reasoning also can be used for the lack of correlation for FC discharge/animal/day between various watersheds. The animals moved freely on the watershed and the possibility that certain times the excrement was deposited closer

to the water cannot be overlooked.

With 0.65 animal/ha the largest daily FC discharge from the entire 849 ha watershed area was 157 x 10^9 FC/day. For this large FC number 2.2 million m^3 of receiving water is necessary to keep coliform numbers below 70/100 ml of water which is recommended by the Public Health Department as safe level for human use. The volume of water at the Muddy Creek and Rhode River (Table 9) and the estimate of water volume per segments indicate that the 2.2 million m^3 of water starting from the Muddy Creek 6.7 km point is between the 3.4 km and 2.2 km stations. Comparison of this data with FC numbers revealed that indeed the coliform numbers were high between 6.7 km and 2.2 km stations (Table 8). Although these direct comparisons are evident we have to consider other factors. Fecal coliform bacteria survive for a period of time in the Rhode River estuary and only 849 ha out of 3260 ha of the total watershed was surveyed for coliform discharge.

We have also estimated existing FC numbers in May in the total water volume at Rhode River. If one calculates the total FC per m^3 of water from the values given in Table 8 and the FC numbers are multiplied with the total volume of water for each river segments, the total numbers of FC in the total volume of water from Rhode River stations, 0.0 to 6.7 km are calculated. For example, on the 133rd day of 1974 the Rhode River estuary contained 4251 x 10^{10} FC bacteria. Evans and Owens (3) estimated that coliform bacteria survive about 57 days in the drainage water. Usingthe 57 day survival value, the daily bacterial influx of FC are determined by dividing 4251 x 10^{10} by 57, which gives an entering value of FC into the estuary from the weir stations 74.6 x 10^{10}FC/day. Since we determined only the FC influx from 849 ha out of 3260 ha total Rhode River watershed, we further must divide the FC numbers by a factor of four.

This calculation gives us a number of 18.6×10^{10} FC/day that entered from the 849 ha watershed area into the Rhode River estuary. This value is very close to the theoretical value of daily FC discharge of 15.7×10^{10} FC/day by 0.65 animal/ha from 849 ha watershed area.

In the above calculations we used the assumption that pollution from the entire watershed is similar to that of determined portion of the watershed and that FC bacteria survive in the salt water at the same rate as they do in fresh water. Because of these assumptions our calculation does not give positive proof, but points to the strong possibility that FC bacteria in the Rhode River estuary can be largely accounted for from non-point sources of pollution.

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II. Subprogram

A. Title: Survival of Escherichia coli MC-6 in the Rhode River estuary

B. Abstract:

The survival of Escherichia coli MC-6 has been studied in estuarine environment using dialyzing chambers. Viable cell numbers remained high at high dissolved oxygen levels, but their numbers declined rapidly when temperature and salinity of the water increased. Addition of montmorrilonite to the cell suspension prolonged the survival of bacteria but, illite had no effect. Viable cell counts were similar in chambers containing either millipore or nucleopore filters.

C. Project Objectives:

The survival of fecal E. coli (strain MC-6) was determined by using dialyzing chambers. The effects of physical parameters such as temperature, conductivity, dissolved oxygen, salinity and suspended particulate matter on the survival of the above bacteria were elucidated.

D. Introduction:

The presence of Escherichia coli in the Rhode River estuary has been the subject of an intensive study in the past year. The above organism is found in the intestine of humans and warm blodded animals, and their presence in the estuarine environment indicates fecal pollution. Escherichia coli enters the estuary from the watershed by means of leaking septic tanks or stormwater run-offs. Monitoring of the fecal pollution in the estuary is important, because estuaries are breeding gounds of fishes, clams, crabs and oysters. They are also used for bathing, swimming and various other recreations purposes by a great number of people. Some strains of E. coli are pathogenic and can cause disease when water and seafoods containing the bacteria are consumed.

Our studies indicate that E. coli enters the Rhode River estuary from the watershed in great numbers, and their numbers rapidly decline toward the mouth of Rhode River. The possibility exists that the decline in E. coli numbers is not caused by dilution alone. Parameters such as salinity, temperature and turbidity may affect the E. coli population also.

Survival of E. coli has been extensively studied in marine and freshwater environments. In estuarine conditions the tidal area is periodically subjected to an influx of freshwater causing certain amount of stress on these pathogenic organisms. Very little quantitative data is available on the persistence and survival of fecal coliforms in an estuary.

E. Materials and Methods:

Bacteria and Media. The bacterium used in this study was isolated from the Muddy Creek within the Rhode River estuary. It was identified as Escherichia coli by the following cultural characteristics: growth on Brilliant Green Lactose Bile broth, characteristic colonies on Eosin Methylene Blue agar, growth and gas production in 24 hours in EC broth at 44.5 C and idole, methyl red, Voges-Proskauer, citrate reactions of ++--. This bacterium was designated as E. coli MC-6.

All cultures used in these experiments were grown in lactose broth (Difco) for 24 hours at 37 C. Cells were harvested by centrifugation (3000x g) for 15 minutes and washed twice with sterile 0.01M phosphate buffer. After the final wash, cells were resuspended in sterile buffer and diluted to the desired population density using a Bechman DU Spectrophotometer at 600 nm wavelength.

Chambers. Dialyzing chambers were purchased from their designers, McFeters and Stuart (1972). It is composed of three pieces of plexiglass, held together with six nuts and bolts. Millipore filters of 0.45 um pore

size are used to enclose bacterial suspension, creating a chamber bordered by plexiglass and filters. Each sterile chamber is aspetically inoculated with 20 ml of cell suspension. Three to five chambers were used in each treatment.

First, the plexiglass pieces are washed thoroughly. The filters are cut into circles of 7.5 cm in diameter. The plexiglass pieces and filters are then placed under the ultraviolet light for at least 30 minutes. They are carefully assembled with sterilized forceps. Extra care is taken to insure the sterilization of the parts that actually is in contact with the cell suspension.

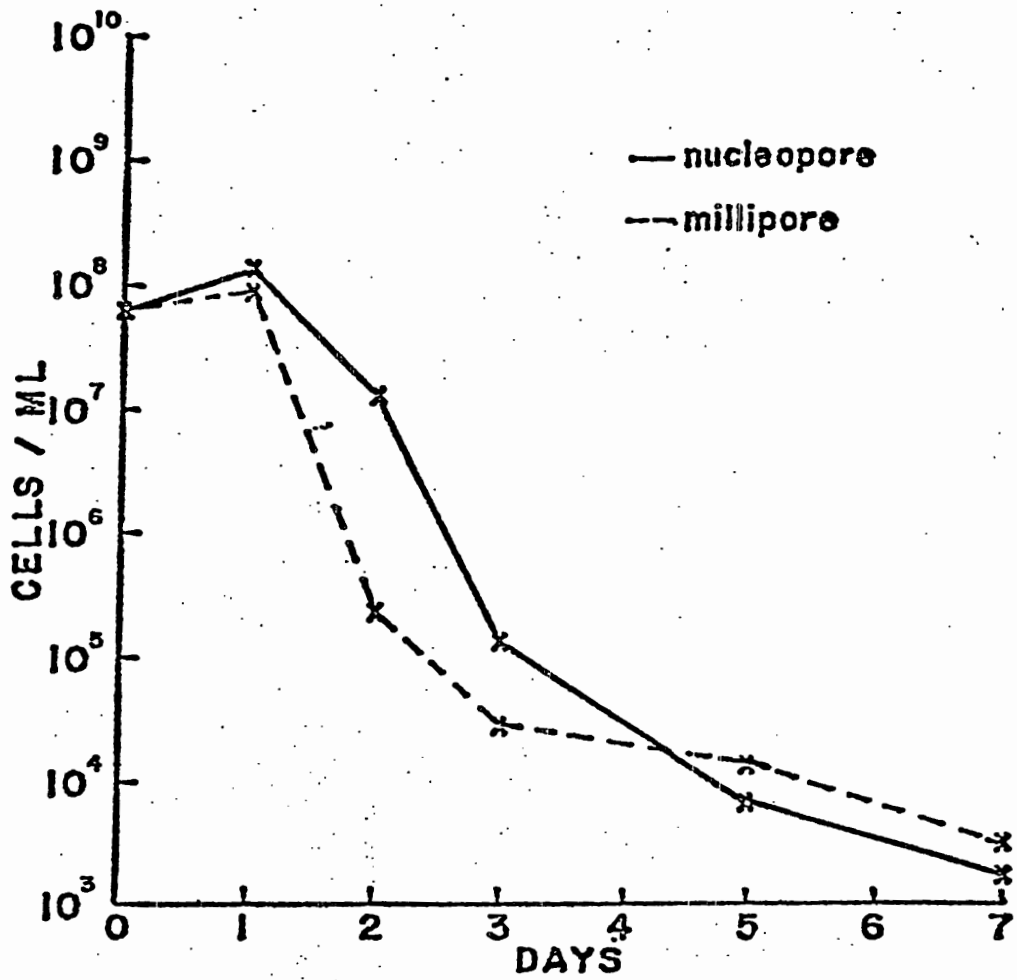
Survival experiments. The chamber inoculated with 20 ml of the washed cell suspension (10^7-10^8 cells/ml) was placed in a plastic, polyethylene container punched with numerous holes to allow free movement of the river water and to provide some protection for the chamber. Each dialysis chamber was suspended from the lid of the container with a stainless steel wire. Elastic clamps held the top and the bottom of each container together. The entire setup was lowered into the river, one meter below the surface, by a rope and left there, tied to the dock on the Smithsonian Institution property.

Samples were taken daily for the first three or four days and then on the sixth and/or seventh day. One milliliter sterile syringe was used to obtain the samples. The chamber was placed in the river water and the syringe was pumped at least twenty times before the sample was taken in order to resuspend the cells in the chambers. Samples were removed with sterile syringes under aseptic conditions for bacterial enumeration. Cells were diluted in 0.01 M sterile phosphate buffer solution.

For bacterial enumeration, petri plates containing Endo or EMB agar were completely dried by incubating them for several hours at 37°C. The complete dryness of the agar plates were needed to obtain single colonies of the organisms for counting purposes. 0.1 ml of the diluted cell suspension were added to the center of each plate and spread over the agar surface with a sterile glass spreading rod. Appropriate dilutions were obtained to contain 30-300 cells per plate. Viable cell numbers were determined by averaging the counts from three to five chambers per treatment. Nutrient agar and nutrient agar plus 5% salts were used to detect any other contaminants. Spread plates were incubated at 37°C for 18 hours.

Temperature experiment. Temperature was controlled using a Hotpack circulating water bath (Hotpack Co., Philadelphia, Pa.). Since the object of our experiments was to keep the chamber *in situ* conditions as possible, a few changes were necessary. A small Masterflex tubing pump was used to bring the river water ten feet up into a container that enclosed the dialysis chambers. Throughout the experiment the river water was pumped constantly (2 ml/min.) into the bottom of the container and overflowed through the hose at the top. The container enclosing the dialyzing chambers was placed into the cooling bath. The compressor of the water bath cooled the river water within the container and kept at the desired temperature.

Particulate experiment. Clay minerals, montmorillonite and illite, were used to test whether the particulates that are abundant in the sediments of Rhode River affected the survival of *E.coli* MC-6. These clays were in dried, powdery form. Autoclaving of these clays were avoided since such action alters the properties of the clay. Known weight of the clay was thoroughly mixed with a known volume of cell suspension (10^7-10^8 cells/ml) and the chambers were inoculated in the same fashion as described previously.



Effect of millipore and nucleopore filters on E. coli HC-6 survival in Rhode River. x—x millipore filter, x—x nucleopore filter.

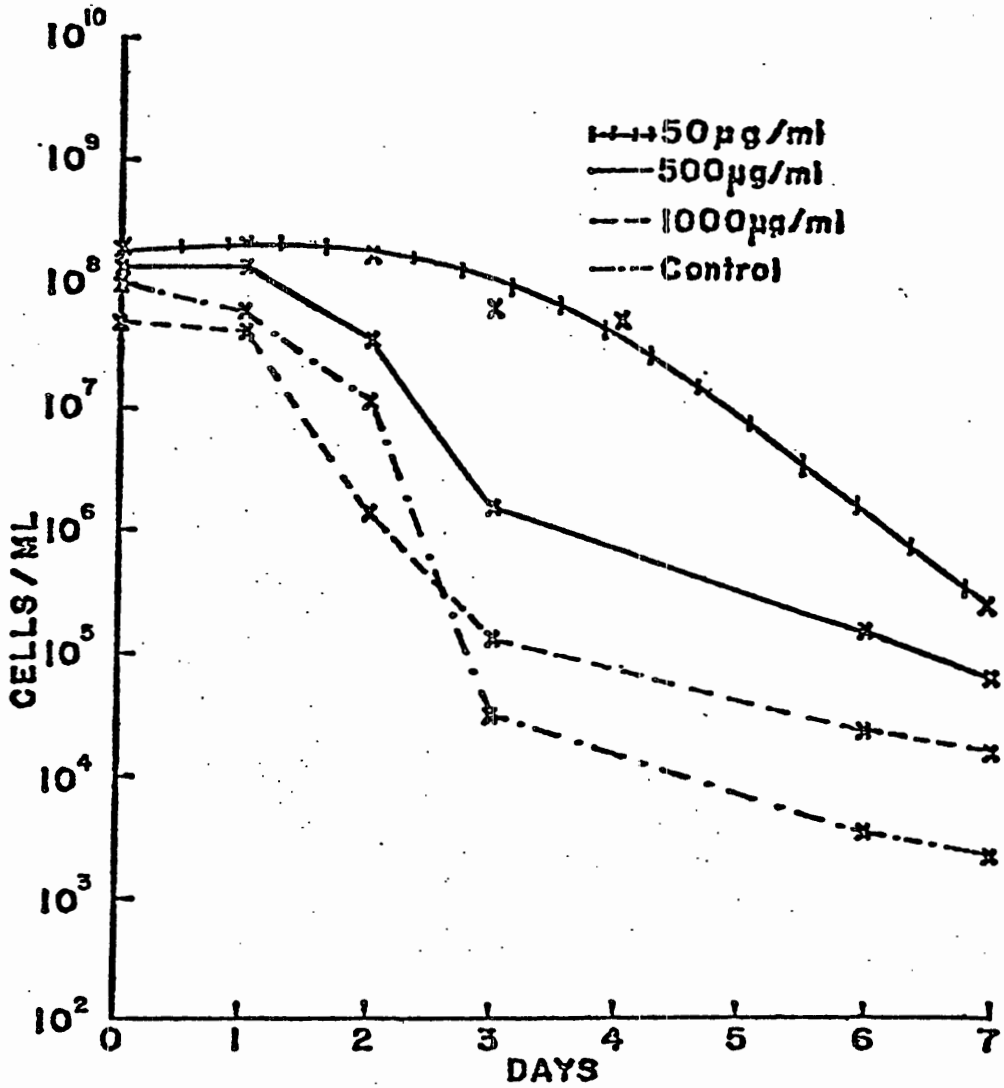
Fig. 1

Filters. Millipore membrane sheets (HAWP 304F0, 0.45 um pore size, Millipre Corporation, Bradford, Mass.) were used. The above filter has a tear-resistant microweb support. Nucleopore filters of 0.45 um pore, 9.0 cm in diameter was also tested (Nucleopore Corp., Pleasanton, Calif.). These filters have a smooth surface relative to the microweb Millipore membranes.

F. Results:

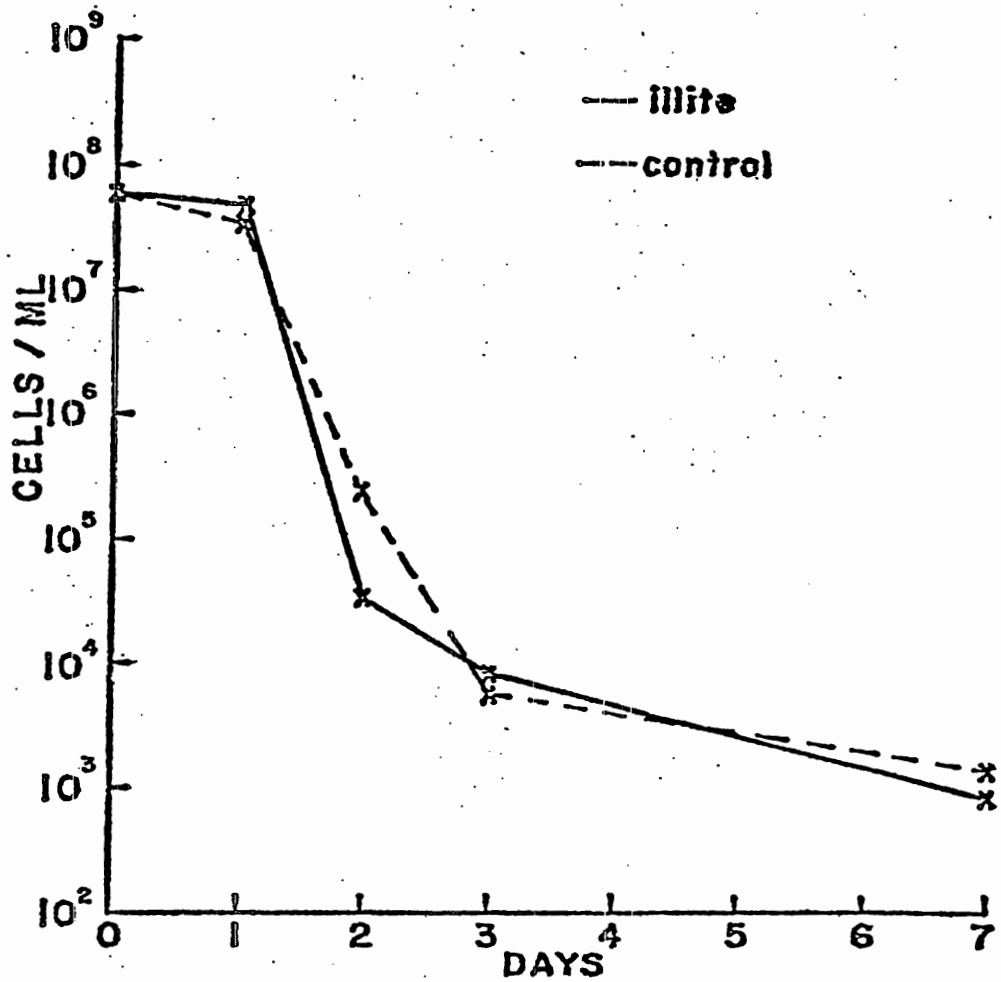
Effect of filters. It is indicated in the literature (Brown, 1973), that various filters affect the survival of bacteria differently. Since filters were an essential part of the dialyzing chambers, the effect of nucleopore and millipore filters on the survival of E.coli MC-6 was elucidated. No difference was observed in the viable cell counts remained in the chambers containing either nucleopore or millipore filters after 7 days incubation in the Rhode River (Fig.1).

Effect of clays. Sediments of the Rhode River estuary contain large amount of montmorillonite and illite clay particulates, therefore, the survival of E.coli MC-6 affected in the presence of clays has been investigated. Montmorillonite at 50, 500 and 1000 ug/ml concentrations aided E.coli MC-6 survival in the Rhode River (Fig.2). The lowest montmorillonite concentration 50 ug/ml enhanced the survival of bacteria by a magnitude of 2, 500 ug/ml by a magnitude of 1 and 1000 ug/ml about 5 times above the cells suspended without clay within the chambers after 7 days in situ. In contrast illite did not show similar protection of the bacteria at 1000 ug/ml concentration (Fig.3). Particulates can protect bacteria to certain extent in their natural habitat from phage attacks, salinity and pH changes caused by tidal movements and by making more nutrients available for cells on particulate surfaces.



Effect of montmorillonite of E. coli MC-6 Survival in Rhode River ⊹-⊹ 50 ug/ml, x——x 500 ug/ml, x---x 1000 ug/ml and x-·-·-x Control.

Fig. 2



Effect of illite on E. coli HC-6 survival in the Rhode River.
x—x 1000 ug/ml , x—x control.

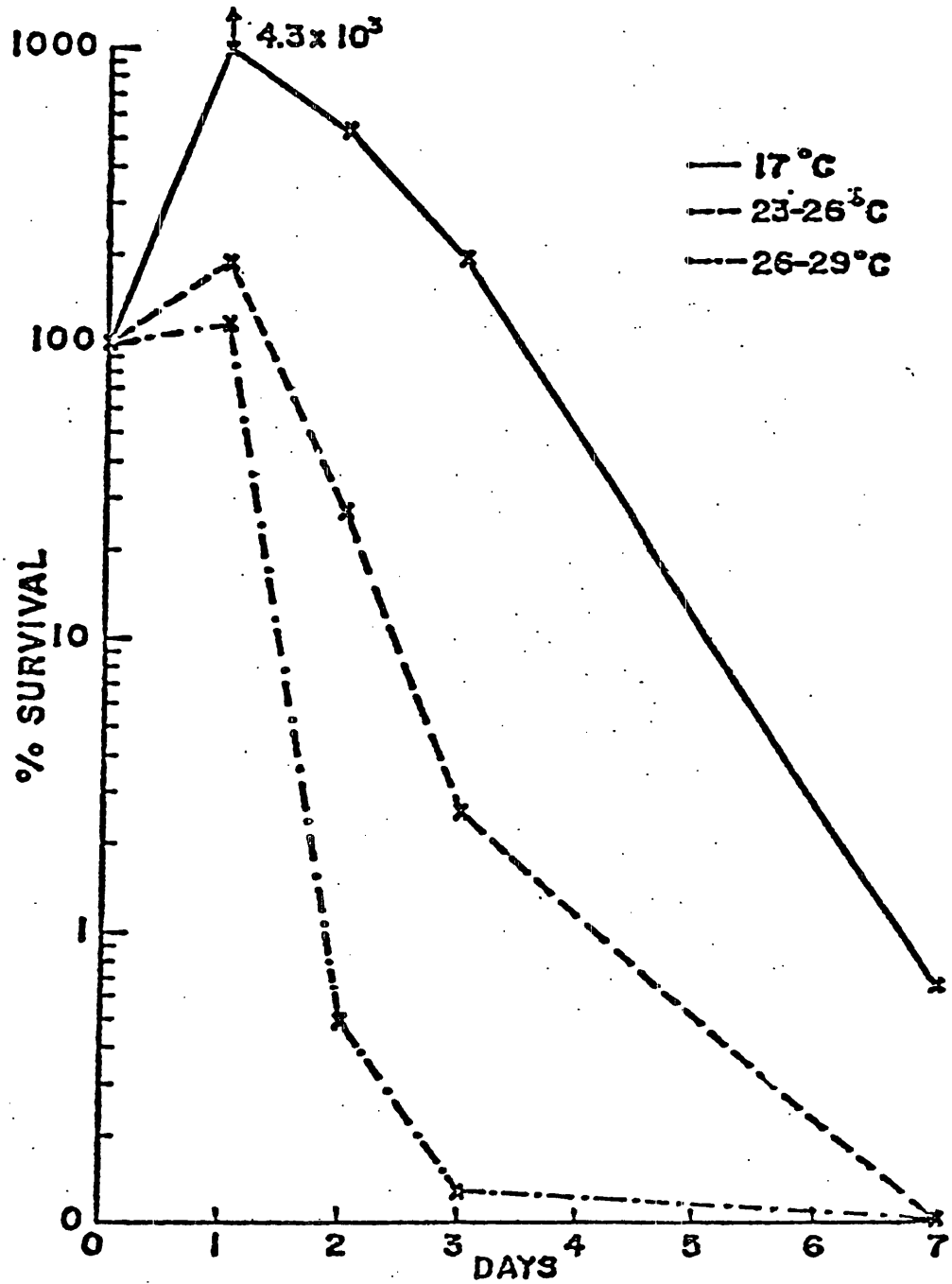
Fig. 3

Effect of temperature. The survival of E.coli MC-6 at various temperatures were also examined (Figs.4 & 5). Cell numbers increased above the initial inoculum at the first day at 17 C, in contrast, the cell numbers remained the same at 23-28 C and their numbers declined at different rates thereafter. On the third day there was no surviving bacteria at the highest temperature range, slightly over 1% at the middle range and most bacteria were alive at the lowest temperature tested (Fig.5).

Effect of other physical parameters. Conductivity, salinity and dissolved oxygen concentrations of the water all affected the survival of E.coli MC-6. Surviving cell numbers decreased as conductivity and salinity increased (Figs. 6 & 7) from 5% to 11% salinity. Dissolved oxygen concentrations were directly related with increased survival of bacteria (Fig.8). All cells remained viable after 3 days in the Rhode River estuary at 6 mg/L dissolved oxygen level of the water and only half of the cells remained viable at 4 mg/L dissolved oxygen level.

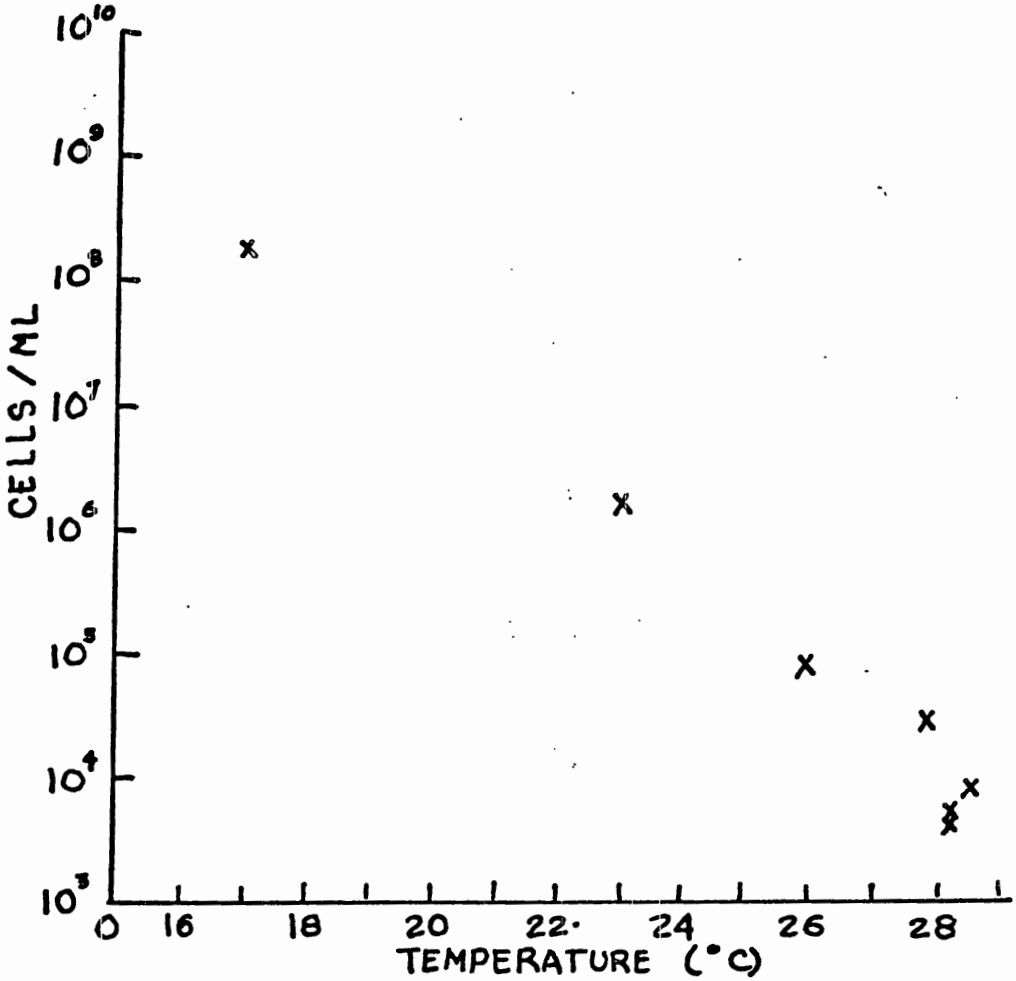
G. Discussion

Apparent success and convenience of the dialysis chambers designed by McFeters and Stuart (1972) led us to try their system in our survival experiments. These chambers allowed an in situ examination of the survival of E.coli MC-6. The bacteria remained within the chamber, yet they were also experiencing most of the natural, physiochemical properties of the river, since the membrane allowed the river water to flow through the chamber. The biological properties of the river were somewhat limited, since some other organisms could not enter the chambers, thus there was a decrease in predation and competition. For our studies, this system proved to be practical, convenient.



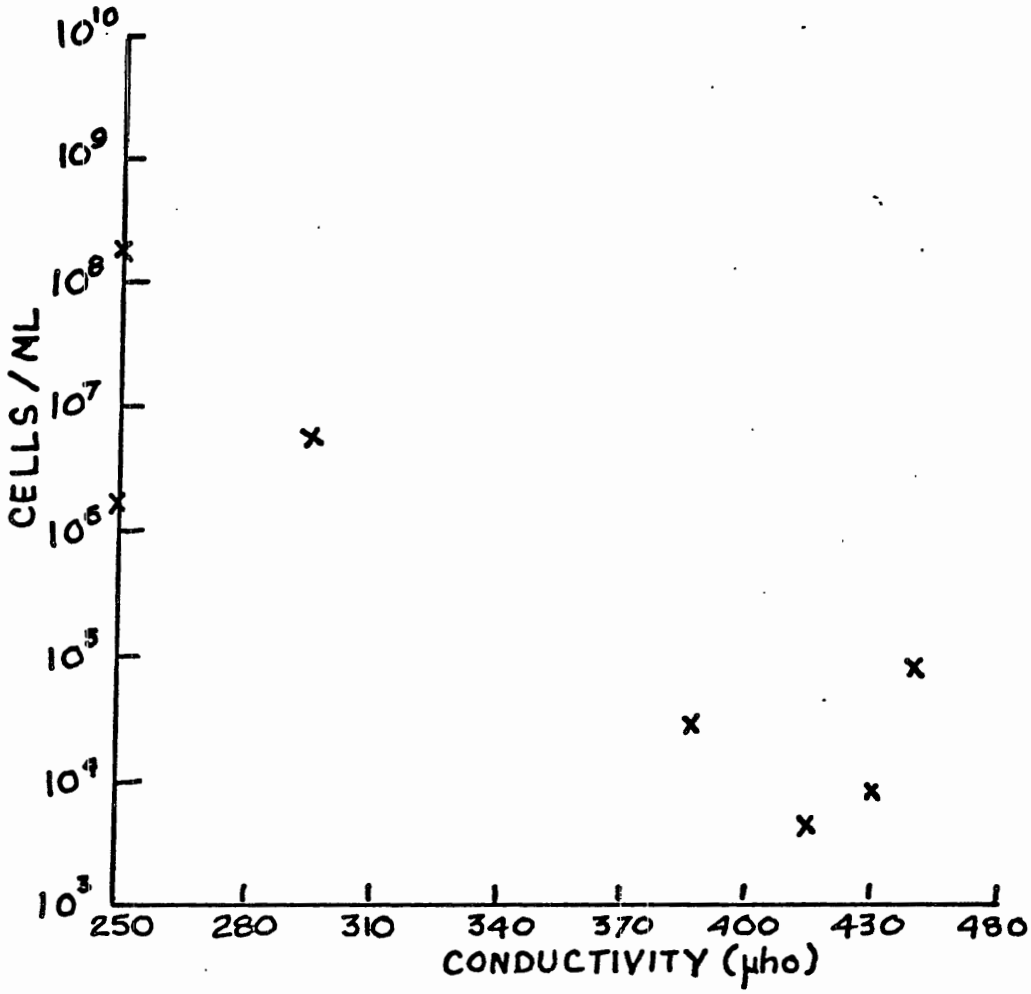
Rate of survival of E. coli HC-6 at various temperatures in situ.
x——x 17°C, x—x 23-26°C and x-----x 26-23°C.

Fig. 4



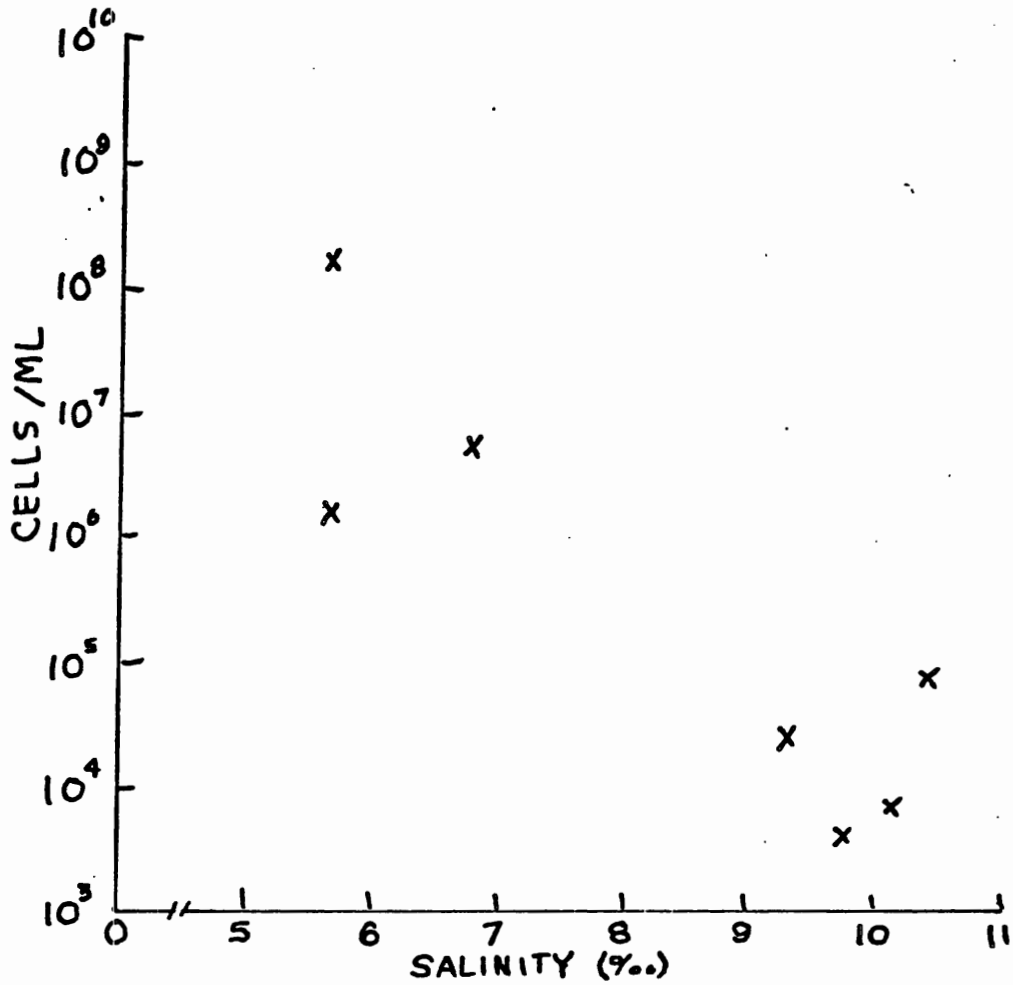
Effect of temperature on E. coli MC-5 survival after 3 days in the Rhode River

Fig. 5



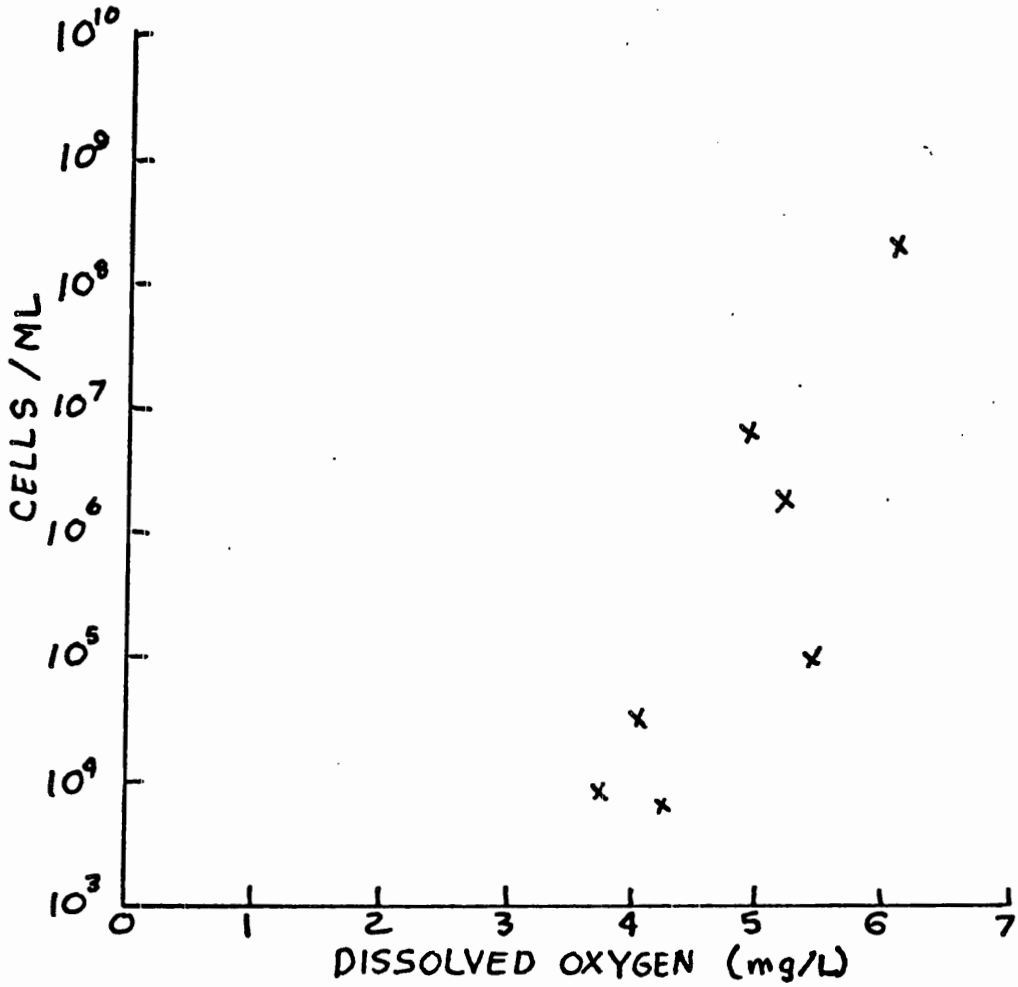
Effect of conductivity on E. coli Mc-6 survival after 3 days in the Rhode River.

Figure 6



Effect of salinity on E. coli MC-6 survival after 3 days in Rhode River water.

Fig. 7



Effect of dissolved oxygen (D. O.) on E. coli after 3 days in Rhode River water

Fig. 8

Decrease in E.coli MC-6 viable counts along the Rhode River axis towards the Chesapeake Bay has been observed. Similar pattern has been described by Pramer, et al (1963) in marine environment. Ketchum, Ayers and Vaccaro (1952) found that the decline in coliform numbers in an estuary was not due to dilution, but was due to the bactericidal effects of the saline water. Pramer, et al (1963) have attributed the decrease in E.coli numbers not only to dilution, but to adsorption, aggregation and sedimentation, whereas the death of these organisms were attributed to predation, starvation and bactericidal action of sea water. Pramer, et al (1963) have also said, that more attention should now be focused on the physiochemical aspects of the water rather than the biological factors to seek an answer to why bacteria die off in nature.

We have considered several physiochemical aspects in our survival study that would have major effects on the survival of coliform organisms. Vaccaro et al (1950) have recorded faster die off of E.coli in sea water during the summer. Higher temperature seems to increase the rate of death also in our experiments. Survival of E.coli MC-6 not only decreased at higher temperature, but also as conductivity, and salinity levels increased. Decreased dissolved oxygen values, however, resulted in more rapid decline in the viable cell population.

Biological parasitism and predation maintain a normal microbial balance in the natural environment. Adsorption and sedimentation of bacteria to clays gave some protection against parasites and predators (Roper and Marshall, 1974). Clay minerals, colloids and organic matter form a protective envelope around E.coli in the water, preventing predation by bacteriophages has been reported by the above investigators. Montmorillonite protected E.coli MC-6 survival in our experiments, probably protecting the

cells by similar mechanism described by Roper and Marshall (1974). Montmorillonite protection on the survival of coliform organisms probably indicate a physical protection of this clay.

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III. Subprogram:

A. Title: Distinction between bacterial and algal utilization of inorganic phosphate in an estuarine environment.[a)]

B. Abstract:

P-uptake by algae and bacteria varied with the season and it was governed by species composition and P-assimilating ability of the population. From August to March P was assimilated mostly by bacteria, and algal contribution to P-uptake was less than 5% of the total. P-uptake by algae increased by 47%, 22% and 44% above the total algal P-uptake in May, June and July respectively. Bacteria appear to play a more important role in P-uptake than shown before. P-assimilation by bacteria was independent of temperature, whereas algal P-assimilation was enhanced by it. P-uptake per unit biomass is independent of phosphorus concentrations, but dependent on the given algal or bacterial flora. Merely measuring the relationship between phosphorus concentrations and total productivity does not reflect the responses of algae and bacteria in this process. Sampling through a year, and segregating the microbial population based upon their size and morphology, the diversity of algae and bacteria and the changing character of the flora in an unpolluted estuarine environment has been illustrated. Bacterial and algal utilization of orthophosphate in an estuarine environment has been separated using filter-stacks of 5.0 um - 1.2 um - 0.45 um pore size.

C. Project Objectives:

To measure and distinguish between bacterial and algal utilization of inorganic phosphate in estuarine environment; to establish the diversity of the microbial population based upon size and morphology; to use radioactive tracers to measure not only total P-uptake rates, but also assess P-uptake rates based on biomass of microorganisms and seasonal variations.

a) This work was carried out with the collaboration of Dr. D. L. Correll.

D. Introduction:

One of the major difficulties in establishing the path of phosphorus cycling in estuarine environment is the lack of information about the role of various microorganisms in this process. The role of algae in phosphorus uptake was emphasized in one investigation (23). Others looked upon bacteria as the major microorganism responsible for primary absorption of this element (4, 15 & 16). Part of the controversy stems from the fact that most investigators used filters to fractionate the plankton and did not attempt to identify the exact group of microorganisms in phosphorus uptake.

Coarse sizing of the plankton in a water sample using filter paper and bolting cloth was used by Rigler (21), Johannes (15 & 16), and Harris (10), to remove larger cells. In addition, Rigler (21) and Johannes (15 & 16) both used 0.45 um pore size filters to collect the filtrate of the water after coarse filtration. Plankton net was also used in measuring phosphorus uptake of larger cells in 35 um and 10 um size fractions, but small cells, nanoplankton and bacteria, were not accounted for in these experiments (23). Taft (23) indicated that fractions of the plankton which passed the 10 um nylon mesh were responsible for most orthophosphate uptake. Those workers who used only 0.45 um filters (19 & 25) collected all particulates of the sample, without discriminating between algal and bacterial phosphorus uptake. Correll, et al (4) reported a different fractionation technique. They used filter stocks of 5.0 um - 1.2 um - 0.45 um pore size filters. Their results indicated that the bulk of inorganic phosphate was taken up into particulates which passed through the 5.0 um filter and was deposited on the 1.2 um and 0.45 um filters.

From the above experiments the conclusions emerged that direct phosphate uptake by zooplankton (10) and filter-feeding protozoans (14) is insignificant. Concommitantly the involvement of bacterial phosphorus up-

take was strongly implicated (4, 15 & 16). Some investigators thought that both algae and bacteria were important (10 & 21), and contrary to the others, Taft (23) concluded that algae were the principal consumers of phosphorus.

There are other types of evidence for the involvement of the small microorganisms in phosphorus uptake. Inorganic phosphate uptake was similar in both light and dark bottles (4 & 23), indicating that orthophosphate was taken up by heterotrophic organisms rather than by autotrophic cells. This strongly indicates bacterial involvement, but cannot be accepted as positive proof for several reasons. Although algal phosphorus uptake is expected to be photosynthetic, it may not be so. Several algae are either obligate heterotrophs (5) or facultative heterotrophs if the necessary carbon source is available (13 & 14), and inorganic phosphate uptake of algae is generally stimulated, but not dependent on light (12).

It appears that in certain conditions bacteria may be more important than algae in phosphorus uptake. Bacterial accelerated phosphate return from the sediment to lake water (11); they rapidly utilized phosphate from lake water and appeared responsible for the rapid turnover of phosphorus (21). In laboratory cultures algal growth was severely limited in the presence of bacteria, but growth of bacteria was hardly affected by algae (20). Johannes (15) warned that laboratory experiments in which bacteria competed for phosphorus were unnatural, because bacterial densities were several orders of magnitude greater than those attained in the sea. The available dissolved phosphate concentration is also important. Three strains of soil bacteria could not compete with diatom species at low 1-3 u atom/L dissolved orthophosphate levels, but did successfully at higher concentrations (8).

Fuhs and Conelli (7) used cellular autoradiography for identifying orthophosphate utilizing microorganisms in lake water. They found marked difference in phosphorus uptake by algal species. Because the species composition of microorganisms in the estuary varies with season (24), measuring total phosphate uptake into particulates are only gross estimation of this process. The differential filtration techniques used previously do not give required details of phosphate uptake by individual microorganisms. To understand phosphorus cycling in the aquatic environment it is essential to know the type of microorganisms actively participating in this process.

In this study we distinguished between bacterial and algal utilization of inorganic phosphate in the estuarine habitat. Our aim was to use radioactive tracers to measure not only total P-uptake rates, but also to assess P-uptake rates based on biomass of microorganisms and seasonal variation.

E. Materials and Methods:

Sampling

Monthly sampling was carried out in the deeper water basin at the Rhode River subestuary an arm of Chesapeake Bay from March 1973 through February 1974. The sampling site of the subestuary has a depth of 9-10 feet. The freshwater enters the study area from the Muddy Creek watershed that is covered by natural areas such as forest, brushland, marshes, nonpasture grassland, tobacco, soybeans, corn, and pasture areas. It is relatively unpopulated. The salt water enters into the system from the Chesapeake Bay through the Rhode River.

Plankton Bottle Experiments

Clear glass cylindrical bottles of 250 ml capacity were equipped with rubber stoppers through which two 16 gauge syringe needles had been placed. One needle was short and one extended well into the bottle. The bottle was

attached by a one meter line to a float. Dark bottles were made by dipping a clear bottle in black epoxy paint. The initial sample of plankton was taken from the desired depth with a peristaltic pump (designed in our laboratory) (4) and at that time water samples were also taken for chemical analysis. Labelled ^{32}P orthophosphate (carrier free) was then added to the bottle of plankton (2 uc/250 ml) and the contents were mixed thoroughly, the two syringe needles were capped and the bottle was incubated at the depth of one meter from which the sample was obtained. At appropriate time intervals (2, 4, 6, 10, 15, 20, 25, 30, 40, 50, 60 minutes) the bottle was recovered, mixed by swirling, the needle caps were removed, and a 10 ml aliquot was removed through the long needle with a 20 ml glass syringe. About 10 ml of air was pulled into the syringe and the sample was passed through a set of filters consisting of three 25 mm diameter Swinex filter holders (Millipore Corp., Bedford, Mass.) and of various filters. The first holder contained Nitex screening with a pore size of 5 um (Tobler, Ernst, and Traber, Inc., Elmsford, N.Y.) which was coated around the edges with silicone rubber to facilitate sealing. The second and third holders, respectively, contained 1.2 um and 0.45 um pore size membrane filters (Millipore Corp., Bedford, Mass.). Enough air was forced through after each sample to remove any chance of excess moisture on the filters. Each filter and a 1 ml aliquot of the final filtrate were counted. Metabolically inhibited controls were run by dissolving Iodoacetic acid (IAA) to a final concentration of 0.05 M, five minutes prior to adding the ^{32}P-orthophosphate.

Radioisotope Counting

Samples were counted in a Packard, Model 3320, liquid scintillator. The scintillation liquid was composed of 5.5 g 2.5-diphenyloxazole and 0.5 g p-bis-[2-(4-methyl-5-phenyloxazoyl)] benzene dissolved in 800 ml toluene and 200 ml Triton X-100 (Rhom and Haas, Philadelphia, Pa.).

Uptake of ^{32}P on various filters was plotted as cpm against time. An intercept was calculated from the uptake in the presence of IAA, and marked on the uptake chart of the corresponding filter. The linear portion of the uptake was estimated between the intercept of IAA uptake and the latest time interval when uptake still appeared linear. This time interval varied between 6 and 30 minutes. Uptake after this point was not linear, it was considered at least partially recycling, and was not used in the calculations. Using the data points during the linear uptake a regression was calculated and the uptake rate per hour per liter was determined from the slope. Specific activity of P, calculated for the given P concentration of water, was taken into account. The uptake was then expressed as ug P/hr/L. Cells retained on the 5 um pore size filter were considered algae and those retained on the 1.2 um and 0.45 um filters as bacteria. Uptake was also expressed as ug P/hr/mm^3 of cell biomass by using the calculated uptake rate from the 5 um filter using algal biomass and the sum of uptake from the 1.2 um and 0.45 um filters with estimated bacterial biomass.

Water chemistry

Water samples for phosphorus analysis was taken as described in plnakton bottle experiments. Samples were analyzed for orthophosphate colorimetrically by reaction with ammonium molybdate and reduction with stannous chloride and for total phosphorus by the same procedure after potassium persulfate digestion. The dissolved organic phosphate fraction was calculated from the dissolved total phosphorus minus dissolved orthophosphate values (1).

Biomass Determinations

Cell numbers of bacteria (21) and algae (3) were determined by direct count procedures on samples fixed to a final concentration of 0.4% glutaraldehyde and 0.01 M sodium phosphate buffer pH 7.0. Live material was also collected for the identification of algae. Fixed samples were then

processed in the laboratory. First they were centrifuged for 20 min. at 2000x g. The pellet resuspended and washed two more times with distilled water and resuspended in 3 ml final volume. Each sample was then sonicated for 5-10 seconds to break up any clumps of cells (Model W 140C, Heat Systems Co., at 60 watts output). Cell suspensions were applied to clean microscopic slides. Bacteria were determined on slides stained with Gram stain. Rod shaped forms and cocci were counted separately, registering the number of cells in differing size classes. Algal cells were counted similarly but without staining. The quantity of cell suspension used for bacterial and algal determinations was slightly different. For the bacterial count 0.01 ml of fixed water sample was used and this volume was spread on 400 mm^2 area. The number of bacteria were counted on 100 microscopic fields of each sample. Cell numbers of algae were estimated using several 0.05 ml aliquots from the 3 ml final solution and spread under the cover slip of known area. The number of algae were counted on 500-800 microscopic fields of each sample. From this known area and known volume, calculation of the number of cells per unit volume were made for each species; at the same time the species were examined and identified. From the mean diameter of each size group and the number of microorganisms present the biomass was calculated as described by Rodina (22). Taking the dilution into account the biomass per unit of water was then determined.

Electron Microscopy

Water samples were collected from 1 m below the surface. Suspended organisms were obtained by fixing the collected sample with 4% gluteraldehyde (in 0.1 M phosphate buffer pH 7.0) final concentration. Fixed cells were dehydrated in alcohol, and embedded in epon (9). Thin sections were examined in a Philips 300 electron microscope.

F. Results:

Association of microbial cells with particulates

Uptake calculated from disappearance of ^{32}P from the solution cannot be used when uptake into various fractions is desired, because the disappearance rate provides information on the total uptake only. The disadvantage of using the direct counts on the filters is that various nonliving particles, which may adsorb ^{32}P, are also filtered out and the absorption by the nonliving particulates has to be estimated. The adsorption of ^{32}P to particulates can be readily measured by adding IAA to the sample 5 minutes before the ^{32}P is added. This metabolic inhibitor effectively eliminates metabolic uptake and an intercept can be calculated from a usually horizontal uptake line. This intercept then can serve as the starting point for metabolic uptake plotted as cpm against time. After a certain time period which may vary between 6 and 30 minutes, the uptake levels off. This is considered due to recycling. Therefore, we calculated the P-uptake rates only from data fit to the linear uptake line.

Association of algae and bacteria to particulates as found in estuarine water samples are illustrated in transmission electron micrographs. Some cells were free, but the majority of cells were surrounded by particulates adhering to specialized structures of the microbial cell surface (Plate 1a).[a] The adhesive material on these cells varied in structure. Some consisted of fibrous strands of various length forming a thin layer (arrows) on a blue-green alga cell surface, others in a form of short dense fibers illustrated on the surface of a cyst-like organism (Plate 1b and 1c). Particulates also varied in shapes and sizes and some were associated with the cells.

a) Illustration of the electron micrographs (Plate 1) is omitted, because it cannot be copied with the xerox machine.

Changes in algal and bacterial population and biomass during the year.

The number of bacteria, estimated by direct counts, 1 m below the water surface ranged between $5x10^5$ - 10^7 cells/ml throughout the year (Fig. 1a). The highest bacterial numbers were observed during two different algal blooms, a Prorocentrum mariae-lebouriae bloom in May and a Gymnodinium nelsonii bloom in July. After the July bloom bacterial numbers declined and remained almost constant from September to February at levels slightly above 10^6 cells/ml. In general, small Gram negative rods, 1 um in size, dominated in spring and summer and larger rods, 0.5 - 2.0 um, 1.0 - 2.0 um and 1.2 x 7.5 um, were prominent during the fall and winter including gram positive organisms (Table 1). The number of algal cells (determined also by direct counts) were between 10^2 - 10^3 cells/ml with the exception of two algal blooms, which occurred in May and July, when algal count reached 10^5 and 10^4 cells/ml, respectively (Fig. 1a). There was no apparent increase in total number of either bacteria or algae in November except that Katodinium rotundatum was the dominant algal species. The total cell numbers of various algal classes and their seasonal occurrence are shown in Table 2.

The highest bacterial biomass was 16.9 mm^3/l during the P. mariae-lebouriae bloom and varied between 1.1 - 6.3 mm^3/L the rest of the year (Fig. 1b). The algal biomass followed the algal numbers and was 150 times higher in May and 10 times higher in July, during the two algal blooms, than the rest of the year (Fig. 1b). Large dinoflagellates were the dominant algal species with respect to biomass during the summer and nanoplankton of less than 10 um size, during the rest of the year.

At 3 occasions the dominant algal species were P. mariae-lebouriae, G. nelsonii and K. rotundatum. It was more difficult to determine the species of bacteria, and they were merely classified into various size classes (Table 1).

Fig. 1

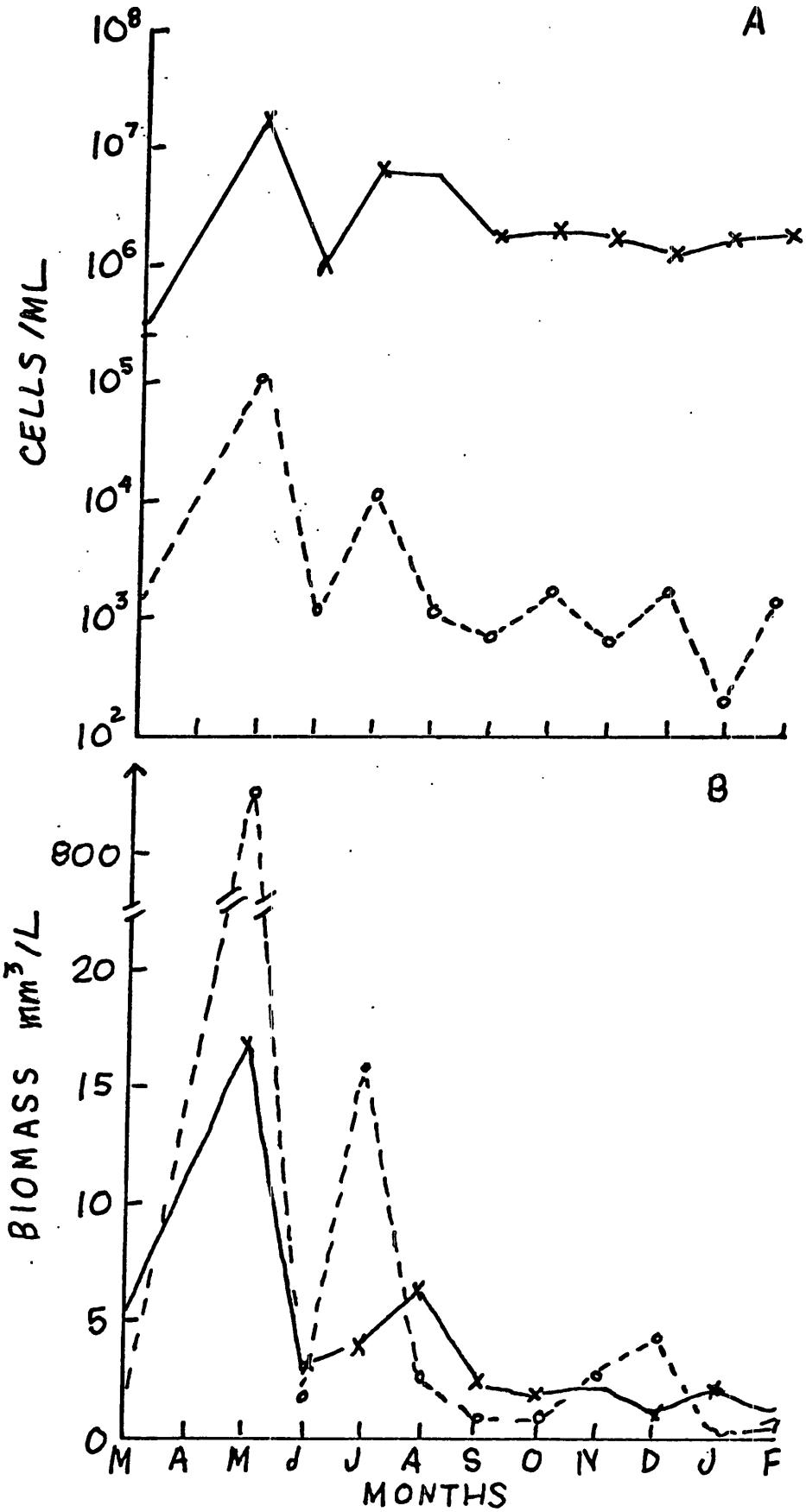

Fig. 1 Seasonal distribution of bacterial X——X and algal (o----o) cell numbers (Fig. 1A) and biomas (Fig. 1B) at the Rhode River sampling station at depth of 1 m.

Table 1. Total cell numbers of various bacterial size classes and their seasonal occurrence in Rhode River Sampling Station - 200 m south of Rhode River Km 3.20. Depth 1 m.

Bacterial Size Classes um	Day of 1973 - 74										
	87	137	165	190	219	249	304	330	352	29	59
	cells x10^4/ml										
0.6 x 0.6	9.7	0	0	100.0	0	110.0	0	0	0	5.8	8.9
1.0 x 1.0	8.1	980.0	12.8	710.0	400.0	112.0	120.0	51.3	11.3	4.4	2.2
1.7 x 1.7	0.8	0	39.4	10.0	0	0	0	0	0	4.5	18.9
0.5 x 1.5	8.5	110.0	0	0	245.0	0	150.0	98.4	49.1	191.3	215.3
0.5 x 5.0	26.7	0	0	0	0	3.6	0	103.2	80.6	16.4	11.9
0.5 x 8.5	0	670.0	0	6.0	100.0	15.3	0	47.0	19.1	2.6	2.7
1.0 x 5.0	0	0	46.0	2.0	26.0	7.8	22.0	10.0	0	8.7	2.7
1.0 x 100	0	0	0	0	0	1.4	0	0	0	0	0
2.0 x 4.0	0	0	0	0	5.0	0	0	0	0	0	0
Total cells	53.8	1,760.0	98.2	828.0	871.0	250.0	292.0	309.9	160.0	233.7	262.6

Table 2. Total cell numbers of various algal classes and their seasonal occurrence in Rhode River Sampling Station - 200 m south of Rhode River Km 3.20. Depth 1 m.

	Day of 1973 - 74										
Algal Classes	87	137	165	190	219	249	304	330	352	29	59
	cells/ml										
Dinophyceae	--	200,000	702	8,061	950	599	408	597	682	59	14
Chrysophyceae	--	--	384	--	160	36	250	14	50	80	240
Parasinophyceae	--	--	--	--	--	--	94	40	640	11	60
Chlorophyceae	250	--	74	--	80	58	434	--	174	--	59
Bacillariophyceae	100	--	292	--	82	22	1,475	66	186	25	241
Cryptophyceae	60	300	--	230	110	14	382	26	226	115	180
Euglenophyceae	110	--	--	1,200	942	18	22	--	580	--	--
Haptophyceae	--	--	--	--	92	--	252	58	778	--	98
Flagellates	140	75	--	230	41	6	4	--	--	8	130
Nanoplankton	800	50	48	404	160	56	4	24	384	16	113
Total cells	1,460	200,425	1,500	10,125	2,617	809	3,325	825	3,700	314	1,135

The morphology of bacteria varied according to the seasons. The number of cells in certain size classes were very high at one sampling and zero at several subsequent samplings. The correlation coefficients between bacterial and algal cell numbers ($r = 0.95^{**}$)[a] and biomass ($r = 0.94^{*}$) were high (Fig. 2 and biomass not shown) respectively, indicating that conditions favoring bacterial growth were also favorable to algal growth.

Availability of phosphorus in the water.

The total phosphorus rapidly increased during the month of June and July, remained high until September and rapidly decreased to low levels thereafter (Fig. 3). The various inorganic P fractions all followed the same pattern but at lower magnitudes. Because the majority of P was inorganic, the total Phosphorus pattern was similar to that of its components with the exception of the month of May when the high level of organic P broadened the shoulder of the total P peak (Fig. 3a). The organic phosphorus pattern was significantly different. It was high during the two algal blooms and slightly increased in November. Comparison of water temperature and the various fractions of inorganic P indicated that the inorganic P concentration of the water was high when its temperature was high. In contrast, there was no correlation between temperature and organic P composition of the water (Fig. 3b).

^{32}P uptake into particulates.

In all experiments only about 5% of ^{32}P was incorporated into particulates retained on the 5 um pore size filters; about 75-95% was incorporated into particulates retained on 1.2 um pore size filters; and 5-15% was incorporated into particulates retained on 0.45 um pore size filters (Fig. 4). Direct microscopic observations of the 1.2 um and 0.45 um filters indicated chlorophyll containing algal cells were retained only occasionally on 1.2 um pore size filters, and none on 0.45 um pore size filters. This has led to the

a) r correlation coefficient, * significance at 5% and ** at 1% levels.

Fig. 2.

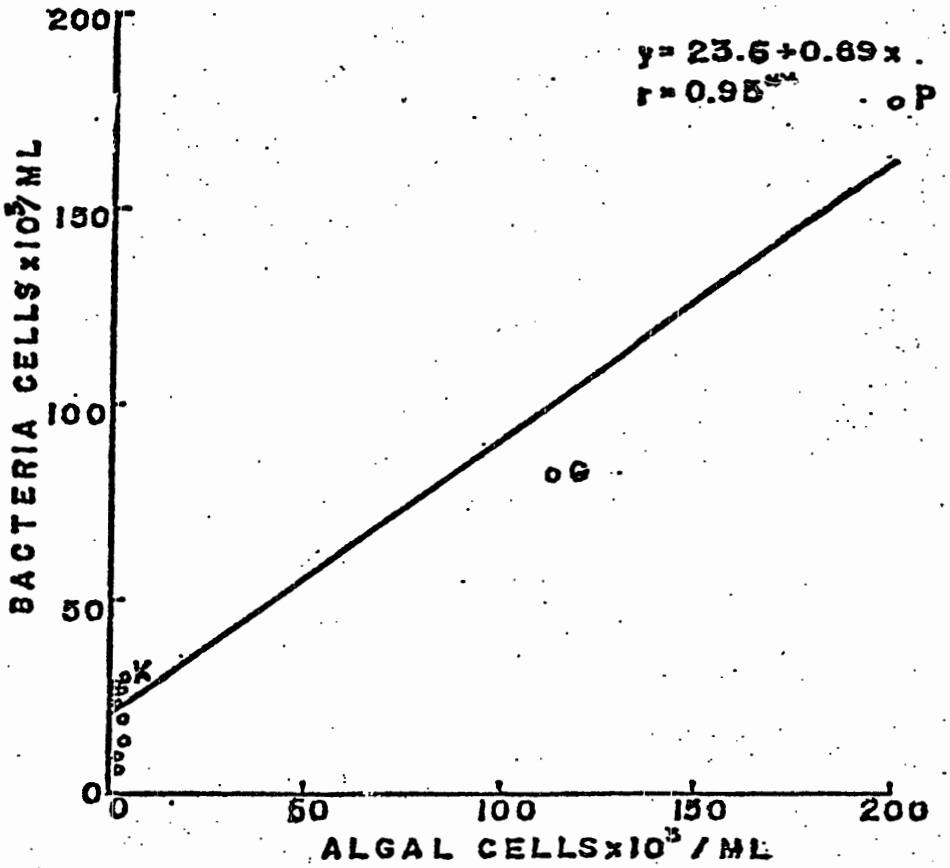

Fig. 2. The relationship between bacterial and algal cell numbers throughout the year estimated by direct count procedure. Specific point discussed in text P. mariae-lebouriae bloom in May (P), G. nelsonii bloom in July (G) and the dominant algal species K. rotundatum in Novermber (K)

Fig. 3.
Seasonal changes of phosphorus concentrations and temperature of the water at the Rhode River sampling station at depth of 1 m.

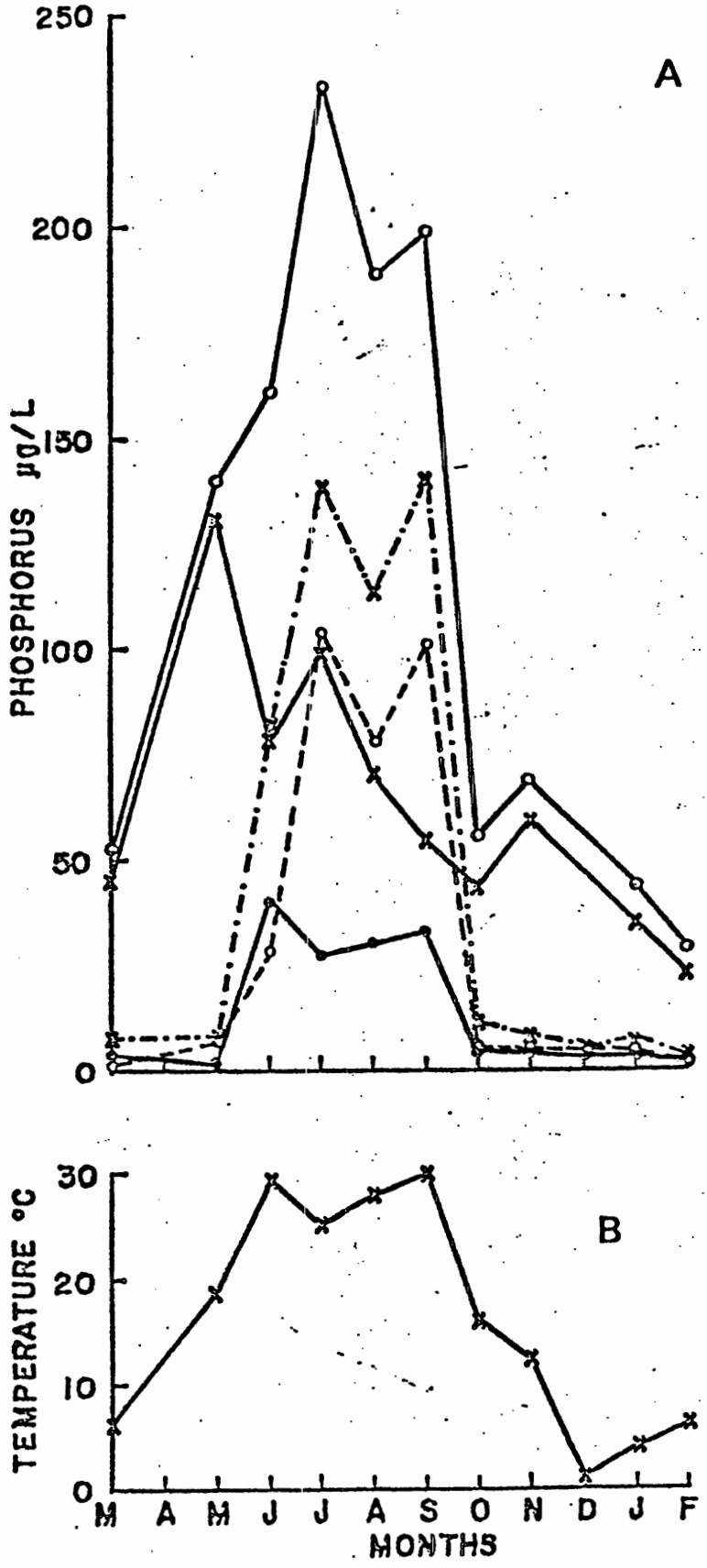

Fig. 3A. Total phosphorus O——O; dissolved organic phosphorus X——X; total orthophosphate X---X; soluble orthophosphate O--O; and particulate orthophosphate •——•.

conclusion that bacteria were the significant component retained on the above two filters. The proportions of ^{32}P retained on various filters changed little during the year indicating that when changes occurred in the biota influencing the total uptake the number of organisms increased in all size classes keeping the proportions of ^{32}P uptake constant (Fig. 4).

The total amount of P incorporated into the particulates (ug P/hr/L) varied with sampling time, but significant correlation ($r = 0.56^{**}$) existed between bacterial and algal P-uptake calculated for the season (Fig. 5). P-uptake (ug P/hr/L) was very high during the algal blooms in May, July and November. There was some difference in P-uptake between the light and dark bottles. P-uptake in the dark bottles measured on 5 um pore size filters were reduced by 2-3 fold from the light bottles from May through November during which period algae were more active in P-uptake. The reduction of P uptake due to absence of light was the highest during July. No difference in P-uptake was observed between light and dark bottles from December through March, and P uptake was very similar on all 1.2 um and 0.45 um pore size filters. Although an overall correlation existed between bacterial and algal P-uptake when P-uptake was expressed as ug P/hr/L, important differences should be pointed out. Bacterial P-uptake was high, whereas algal uptake was low in November. The opposite existed during the P. mariae-lebouriae bloom when algal uptake was high and bacterial uptake was low. During the G. nelsonii bloom uptake by both, bacteria and algae, were high.

P-uptake was also expressed per biomass of algae and bacteria as ug P/hr/mm^3 (Fig. 6). Significant correlation ($r = 0.470^{*}$) existed between P-uptake and biomass of each, algae and bacteria, but the character of their role in this process was somewhat different, than as P-uptake per volume of water (Fig. 5). P-uptake using biomass (ug P/hr/mm^3) of bacteria and algae

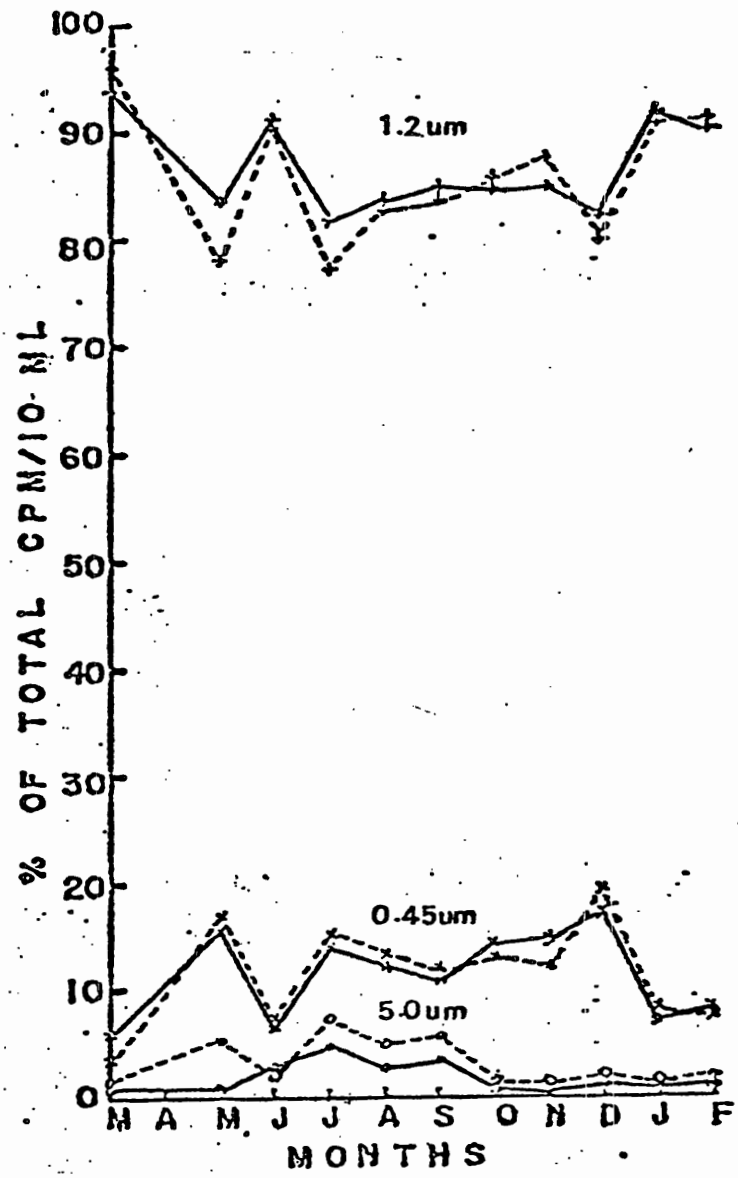

Fig. 4. ^{32}P-uptake into particulates in light and dark retained on the various filters throughout the season. Broken lines (---) P-uptake in light and solid line (——) P-uptake in dark. Particulates retained on 5.0 um pore size filters (0); on 1.2 um pore size filters (+) and on 0.45 um pore size filters (X).

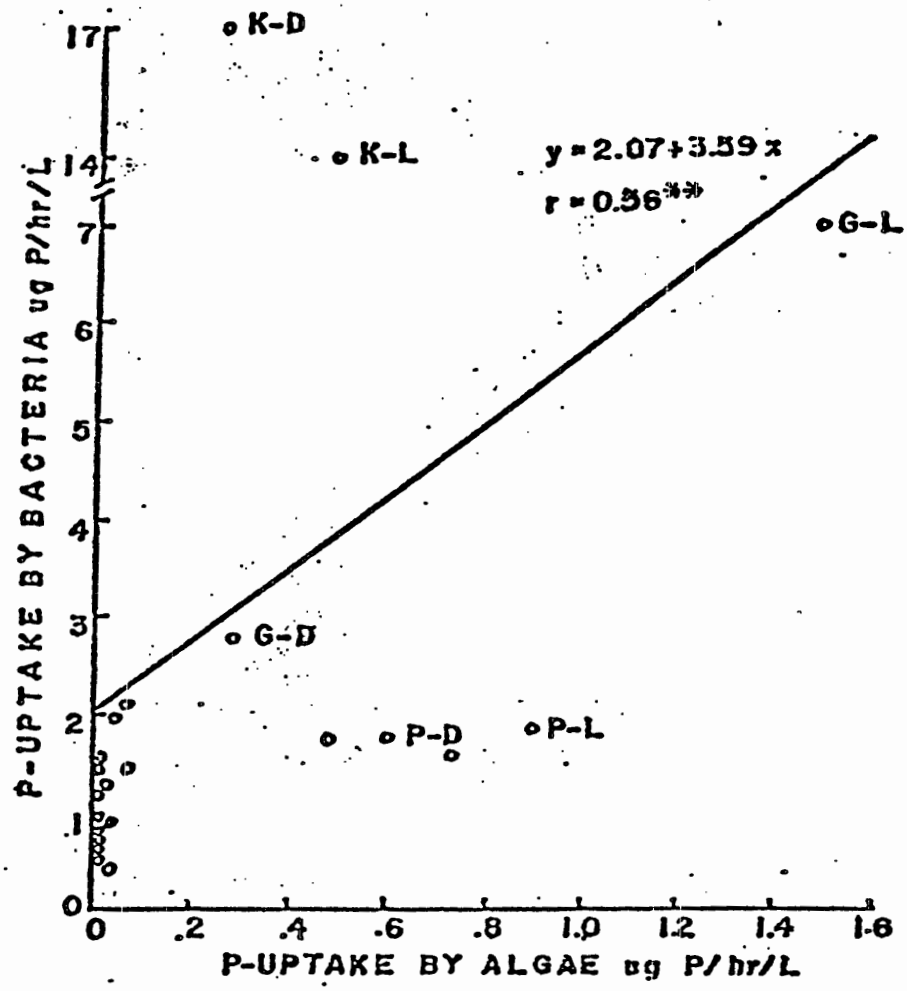

Fig. 5. The relationship between P-uptake of bacteria and algae per liter of water. P-uptake in light and dark are designated by letters L and D. P. mariae-lebouriae (P-L, P-D); G. nelsonii (G-L, G-D) and K. rotundatum (K-L, K-D) respectively.

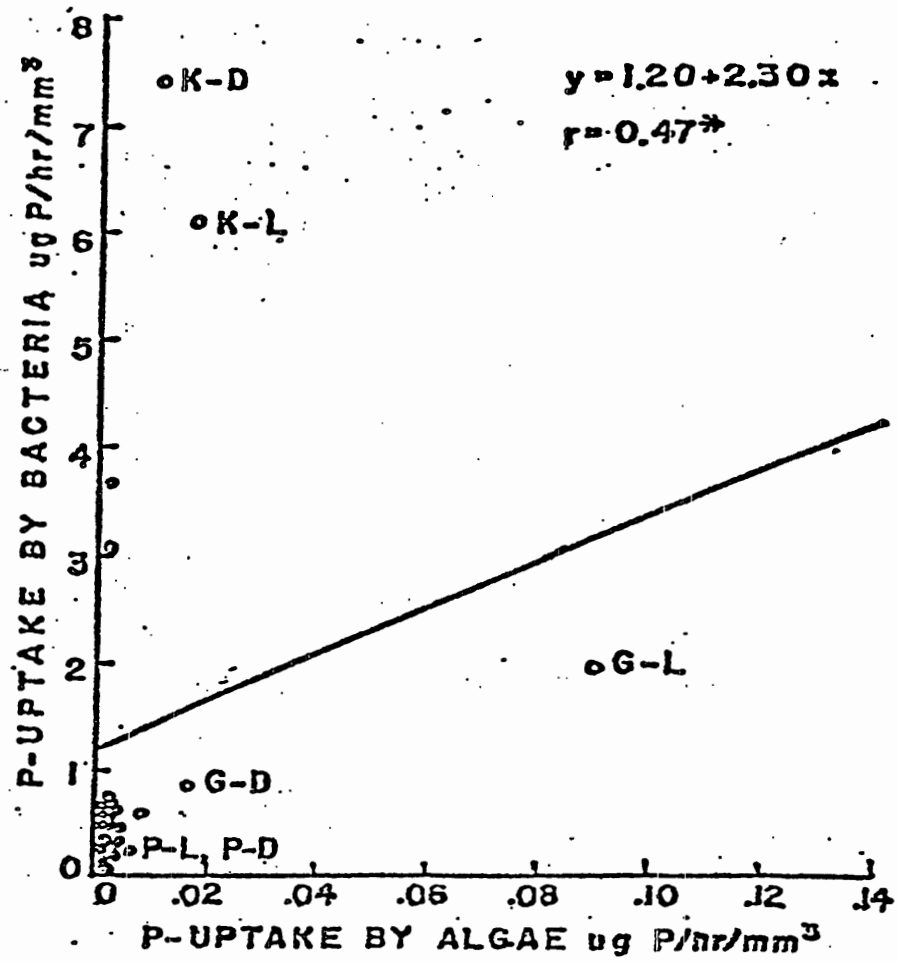

Fig. 6. The relationship between P-uptake of bacteria and algae per cell biomass. Light and dark uptake are designated by letters L and D. P. mariae-lebouriae (P-L, P-D); G. nelsonii (G-L, G-D); and K. rotundatum (K-L, K-D).

indicated (Fig. 6), that during Prorocentrum bloom both bacterial and algal activity of P-uptake were low in May in contrast with the relative high P-uptake per volume of water (Fig. 5). In comparison P-uptake per biomass of bacteria was very high when K. rotundatum was the dominant species which corresponded to the P-uptake per volume of water (Figs. 5 & 6) despite the fact that the bacterial biomass at that time was low, 2.4 mm^3/L. During the Gymnodinium bloom P-uptake calculated per biomass and by volume of water was high in the light, but it was reduced 5 folds in the dark bottle, indicating requirement of light for P-uptake by this alga (Fig. 6).

Comparison of algal and bacterial P-uptake and concentrations of various inorganic P fractions of the water indicated that P concentrations did not influence P-uptake by algae or by bacteria and the correlation between P-uptake and P concentrations were not significant (Figs. 7a & 7b). Significant correlation only occurred ($r = 0.660^*$) between algal P-uptake and particulate orthophosphate concentrations of the water (Fig. 7b). Bacteria in November were able to assimilate P from a very dilute solution, whereas bacteria during the Prorocentrum bloom did not assimilate P from the similarly dilute P solutions. In contrast, on several occasions, when all orthophosphate levels were high, neither bacteria nor algae were able to assimilate large amounts of P per cell biomass. The effect of temperature upon P-uptake was also determined. P-uptake by algae calculated a P-uptake per biomass was significantly ($r = 0.580^{**}$) influenced by the temperature of the water, whereas bacterial uptake was unaffected.

G. Discussion:

We have attempted to separate P-uptake by algae and bacteria in an ecosystem of an estuary by using differential filtration technique. All experiments were coupled with estimation of existing biomass throughout a season.

Fig. 7

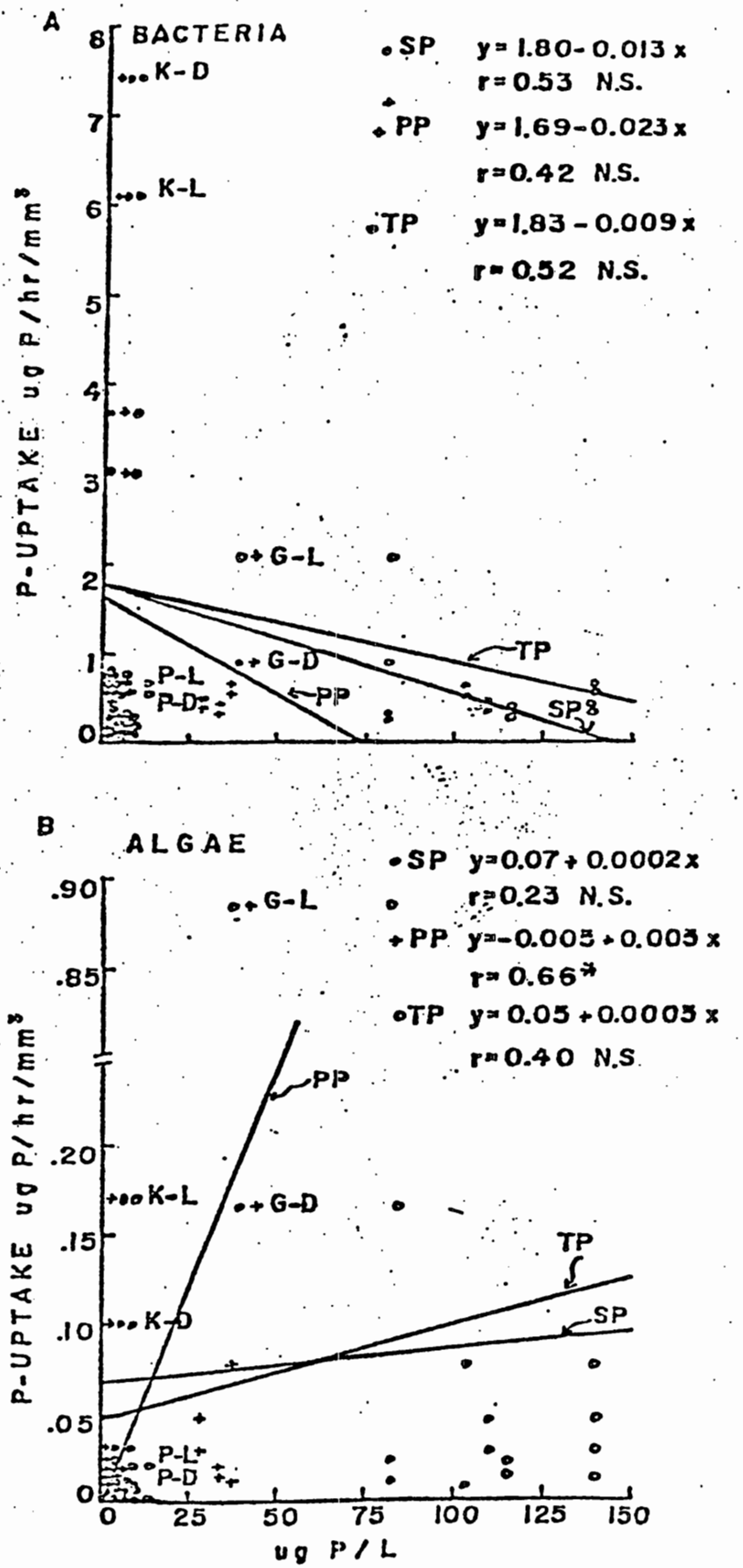

Fig. 7. The relationship between P-uptake per biomass of bacteria (Fig.7a) and algae (Fig.7b) and inorganic phosphate concentrations of the water. SP = soluble orthophosphate (•); PP = particulate phosphate (+) and TP = total phosphate (o) concentrations.

This allowed us to calculate P-uptake per biomass, and also per volume of water, which gave us not only a measure of total productivity within environmental conditons, but also a measure of metabolic activity of the organisms in regard to P-uptake.

This technique is based on the observation that the greater part of the bacterial population passes through a 5 um pore size filter whereas phytoplankton are retained on it. The detailed examination of bacterial sizes confirmed the original observation, that most of the bacteria were smaller than 5 um in size. Even though we separated algae from bacteria as well, as our conditions permitted, we recognized the difficulty in separating microorganisms into clearly defined size groups from natural waters. Some rods of bacteria were longer than 5 um, others adhered to nonliving particulates, or to various algae reported by others (2 & 17), which could prevent their passing through the 5 um pore size membrane. In contrast, some broken cells were retained on the smaller pore size filters in some samples. With all these difficulties the 5 um filter still appeared useful separating the algae from the bacteria.

We have faced another problem discriminating between ^{32}P-orthophosphate bound to nonliving particulates and ^{32}P incorporation into living particulates. By determining the ^{32}P-uptake rate into the particulate fraction of the sample in the presence of IAA, the slope and intercept should give us the adsorption of the tracer on surfaces which are largely composed of nonliving particulates. The orthophospate pool size and its specific activity as well as very frequent data points in the early phase of the experiment are also needed (4). It is also difficult to determine the time intervals when recycling of ^{32}P starts. Determining this point is important, because data for the P-uptake rate calculations can be used only until the beginning of P-cycling. The

determination of the straight portion of the P-uptake is greatly helped by knowing the intercept of the non-metabolic adsorption of this tracer. The above procedures were used to estimate P-uptake by microorganisms from the adsorbed P values in this study.

Throughout this study we wanted to express P-uptake per biomass of cells. To estimate volume of cells we needed to know their dimensions which can only be done by direct microscopic examination. Jones (17) has discussed the validity of direct microscopic counts and other methods to establish the degree of association of algae and bacteria in freshwater environment. He has found positive correlation between algal cell numbers and viable bacterial counts, but not with direct counts of bacteria. However, we obtained positive correlation between algal and bacterial cell numbers using direct counts. It appeared that bacterial numbers followed algal cell numbers. This trend gave positive correlation not only with cell numbers but also with biomass estimations in an estuarine environment.

Differentiation between P-uptake of algae and bacteria and between the total P-uptake and the P-assimilating ability of various microbial cells were accomplished by using filter stacks and by comparing P-uptake per ug P/hr/L with ug P/hr/mm^3 biomass. High P-uptake from the water sample appeared to be related to either high cell numbers, or to high P-assimilating ability of the population. In any given situation P-uptake of algae and bacteria was governed by the combination or presence of the following four factors: cell numbers of algae, cell numbers of bacteria, P-assimilation by algae and P-assimilation by bacteria. We have found several combinations: the algal and bacterial numbers were both high and the population had a very low P-assimilating ability in May (Prorocentrum bloom); both algal and bacterial numbers were relatively high and these organisms had high P-assimilating ability in July (Gymnodinium bloom); both algal and bacterial cell

numbers were low, the algal population with low P-assimilating ability and the bacterial population of high P-assimilating ability existed in November (K. rotundatum); and both algal and bacterial cell numbers were low and their P-assimilating ability were also low the rest of the year. The above observations reflect the changes in biomass and species composition as well as P-assimilating ability of a natural population which existed within a year in Rhode River estuary. Differences in nutrients and temperatures appeared to affect the seasonal change of phytoplankton in another shallow estuary (24).

Differential filter technique clearly demonstrated that the contribution of algae and bacteria to P-uptake varied with the season. During the period from August to March P was absorbed mostly by bacteria and the algal contribution to P-uptake was less than 5% of total. During the months of May, June and July P-uptake by algae increased 47%, 22% and 44% above the total algal P-uptake respectively. The fact that P-uptake by bacteria throughout the rest of the year was 5-700 times higher than that of algae, clearly indicates the importance of bacteria in P-absorption from estuarine environments, similar to that shown from marine environment (15). High P-uptake due to algae apparently is only possible in the summer, when algal numbers are high and not because their P-assimilating ability are high. An increase in population density and a decrease in primary productivity per unit of standing crop has been reported (6) and may arise through extinction of light, nutrient competition, and increased concentration of excreted algal products. These factors may have occurred in the Rhode River during times when algal population was high and when the highest supported biomass of algae was 150 times and bacteria 20 times higher than the rest of the year.

Relatively low orthophosphate concentrations which prevailed throughout the year, with the exception of the summer did not influence the P-assimilating ability of bacteria or algae. Except in November when neither algae nor bacteria assimilated P from water at low orthophosphate concentrations. Thus,

our result indicates that P-uptake per unit of biomass is independent of phosphorus concentrations, but dependent on a given microbial population. This is different from the results of those who correlated P-levels and total algal productivity (18).

Competition between algae and bacteria has been described in laboratory cultures, where algal growth was severely limited by the presence of bacteria after external P has been exhausted, but growth of bacteria was unaffected by the presence of algae (20). The algal-bacterial competition functions through growth rate, the faster growing bacteria have the advantage above the slower growing algae in a mixed population with regard to P-uptake. When conditions conductive to P-uptake by bacteria exists, the kind of bacteria present may be important. P-uptake by the bacteria was high and by the algae was low, at the time when water temperature was 12 C and the orthophosphate level was 6 ug P/L in November. It is obvious that if competition existed between K. rotundatum and associated bacteria, these bacteria competed much more effectively than other species present throughout the year.

This study demonstrated that merely measuring the relationship between P-concentrations and total productivity does not show the responses of algae and bacteria in this process. However this is possible by using differential filtration. Bacteria appear to play a more important role in P-uptake than shown before. Various bacterial species are more active in P-uptake than others. Bacterial population also changes with changing algal populations. Because of the important role of bacteria in P-uptake a detailed study to identify the existing populations of bacteria is underway.

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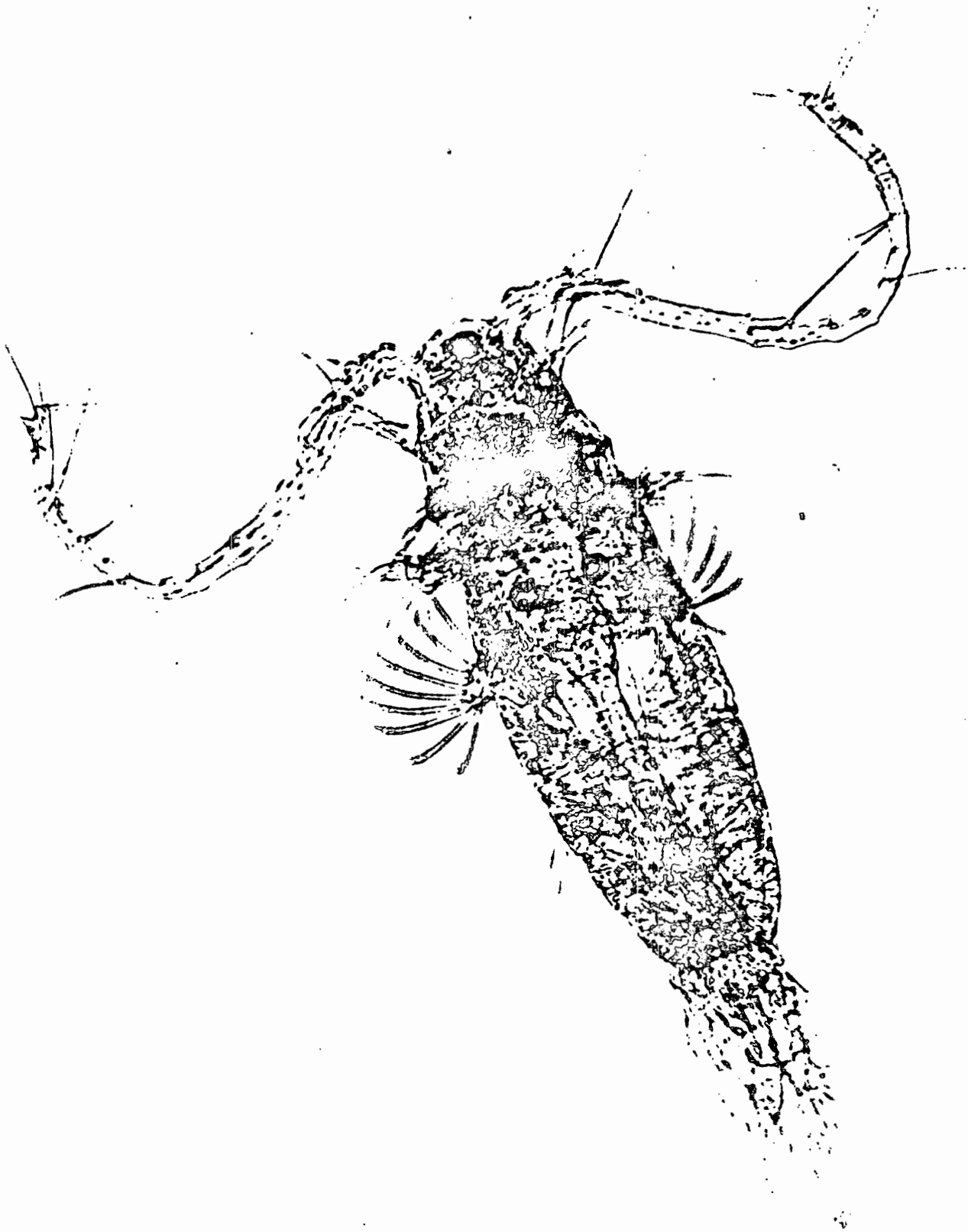
FINAL REPORT ON:

THE ROLE OF MICRO-CONSUMERS IN THE FOOD WEB OF THE RHODE RIVER ESTUARY

J. David Allan
Department of Zoology
University of Maryland
College Park 20742

Period of support: 1 June 1973 - 30 September 1974

Amount of support: $32,320



Acartia tonsa (Dana) X 120 is the predominant copepod in the Rhode River throughout the year. The extended maxillae are used as a feeding scoop in very much the same way as the cirral net of barnacles. Acartia is thought to be primarily a herbivore. See Conover (1956 Bull. Bingham oceanogr. Coll. Vol 15, pp 156-233) for description. Photo by J. Mazur.

Eurytemora affinis (Poppe) X500 is the spring dominant in the Rhode River and accounts for the largest standing crop and production observed throughout the year. Eurytemora is probably deriving most of its energy from detrtitus. Photo by J.D. Allan.

ABSTRACT

The principal objectives of this study were to investigate the population dynamics and feeding ecology of the micro-consumer food web in the Rhode River. Substantial progress was made in determining seasonal variations in numbers, diversity, biomass and production of zooplankton. However, only very preliminary results were obtained on feeding biology of the dominant species.

This report documents seasonal fluctuations in zooplankton abundance and estimates standing crops and production for two dominant copepods, Acartia tonsa and Eurytemora affinis, in the Rhode River subestuary of the Chesapeake Bay. A February-March peak which is primarily E. affinis sustains far higher biomass (218·6 mg/m^3) and production (20·5mg/m^3/day) than does an August peak of A. tonsa (biomass 16·3 mg/m^3, production 9·8 mg/m^3/day). The Rhode River has low standing crop and production relative to the much larger Patuxent estuary (Heinle, 1966). These results are discussed in the context of the theory of Williams et al. (1968) that shallow embayments have lower standing crops, and the statistical accuracy of their statement is disputed.

PROJECT OBJECTIVES

The following primary questions were asked in the original proposal:

a. What are the seasonal variations in numbers, biomass and diversity of the micro-consumers?

b. What degree of feeding selectivity is exhibited by the predominant grazers?

c. What are the feeding rates and how do feeding rates vary with the size and concentration of the particle grazed?

d. What losses to predators are sustained by the predominant micro-consumers?

e. Integrating the above information, what may we conclude about (1) the role of resource subdivision in species coexistence? and (2) species succession through the season as determined by a changing resource base?

INTRODUCTION

Substantial progress was made towards answering objective (a) and a manuscript is now ready for submission to an appropriate journal. I plan to submit it to Chesapeake Science. Questions (b) and (c) received serious and intensive investigation, but are not answered at this time. Question (d) is answerable by inference, but ought to be investigated further. Question (e) requires very detailed information from questions (a) through (d), and is therefore not answerable at this time.

The report that follows consists of the manuscript documenting the results towards answering question (a), an appendix discussing progress made on questions (b) and (c), and a second appendix discussing progress made on question (d). The methods are quite different for the various questions, and this seems to be the simplest way to report results.

This is the final report on the funded project, and unfortunately does not answer all the questions posed. However, it should be noted that the project was of only sixteen months duration and did not have previous Rhode River zooplankton studies to draw upon. Additional funding has been obtained from another source, and I hope that more complete answers to all of the questions posed will be forthcoming in the near future.

ABUNDANCES AND PRODUCTION OF COPEPODS IN THE RHODE RIVER SUBESTUARY OF CHESAPEAKE BAY

by

J. David Allan,
Thomas Kinsey, and
Melody C. James

Department of Zoology
University of Maryland
College Park 20742

ABSTRACT

We document seasonal fluctuations in zooplankton abundance and estimate standing crops and production for two dominant copepods, Acartia tonsa and Eurytemora affinis, in the Rhode River subestuary of Chesapeake Bay. A February-March peak which is primarily E. affinis sustains far higher biomass (218·6 mg/m^3) and production (20·5 mg/m^3/day) than does an August peak of A. tonsa (biomass 16·3 mg/m^3, production 9·8 mg/m^3/day). The Rhode River has low standing crop and production relative to the much larger Patuxent estuary (Heinle, 1966). These results are discussed in the context of the theory of Williams et al. (1968) that shallow embayments have lower standing crops, and the statistical accuracy of their statement is disputed.

INTRODUCTION

As part of a broad ecosystem study of one subestuary of Western Chesapeake Bay, we investigated the seasonal population dynamics and production of zooplankton. We describe the composition of the zooplankton community as it varies seasonally and spatially towards its junction with Chesapeake Bay; and estimate the standing crops and biomass production for the two dominant copepods, Acartia tonsa and Eurytemora affinis.

METHODS

Study area:

The Rhode River is a small estuary of western Chesapeake Bay less than 5 km in length and usually less than 1 km in width. Depths range typically between 1 and 3 m, and average 1.94 m (Pritchard and Han, 1972). It is fed by several small creeks, the most substantial of which is Muddy Creek. A description of the physical hydrology is presented by Pritchard and Han (1972). Four sampling stations were designated from the mouth of Muddy Creek to the opening of the Rhode River to Chesapeake Bay. Station 9 is at the mouth of the Creek, station 11 is midway between Big and High Islands, station 12 is located in mid-channel off Locust Point and station 13 is located between Cheston and Dutchman Points at the mouth of Rhode River. These stations form a transect 0, 1.1, 2.3 and 4.5 km from the mouth of Muddy Creek.

Zooplankton sampling:

Samples were collected with a #20 (64 mu) Wisconsin net from July 1973 to July 1974. At each station 10 to 20 vertical

hauls were made along a transect line 30 m long. The volume of water sampled was approximately 500 l. per sampling site. Samples were preserved in 8% formalin, after a seiving procedure was employed to remove ctenophores. Depending on zooplankton densities, a 5 or 10% subsample was later removed for analysis. All copepods were identified to species and counted in the following categories: nauplii, copepodids and adults. The first 200 individuals in each sample were measured as total body length, and eggs were counted for all gravid females.

Biomass and Production estimates:

Zooplankton biomass and production for Acartia tonsa and Eurytemora affinis were estimated by the method of Heinle (1966, 1969). The instantaneous death rate for nauplii is given as:

$$-d_n = (\ln C - \ln N)/t_n \qquad (1)$$

where d_n is the instantaneous death rate, C is the total number of copepodids and N the total number of nauplii at a point in time, and t_n is the temperature-dependent developmental time from nauplius to copepodid. Similarly

$$-d_c = (\ln A - \ln C)/t_c \qquad (2)$$

where A is the total number of adults and the subscript c in d_c and t_c refers to copepodids.

The finite death rates are given as:

$$D_n = 1 - e^{-dn}$$

$$D_c = 1 - e^{-d_c} \qquad (3)$$

and turnover times are simply the recipricols of finite death rates

$$T_n = 1/D_n$$
$$T_c = 1/D_c \qquad (4)$$

Since production may be estimated as biomass/turnover time it is necessary to estimate the standing crop biomass. Again following Heinle (1966), the numbers in each naupliar stage is estimated by assuming a constant exponential death rate (d_n) for all naupliar classes. Then

$$N_1 = N/1 + e^{-d_n t_n} + e^{-2d_n t_n} + e^{-3d_n t_n} + e^{-4t_n d_n} + e^{-5d_n t_n}$$
$$N2 = N1 \cdot e^{-d_n t_n} \qquad (5)$$

where N1 is the number of naupliar stage 1, N2 the number of naupliar stage 2, and so on. Similarly, the number in each copepodid stage is estimated by apportioning total copepods observed according to a constant exponential death rate (d_c). The weights of the twelve developmental stages N1 through adult were determined from body length data and Heinle's (1969) length-weight data for A. tonsa and E. affinis. Finally, standing crop biomass (B) for nauplii, copepodids and adults was determined from the product of numbers and average weight of each stage. Then production was estimated as:

$$P = B_n/T_n + B_c/T_c + B_A/T_A \qquad (6)$$

where P is production in $mg/m^3/day$, and we assume equal turnover times for copepodids and adults.

RESULTS

Abundances

The predominant copepods were Acartia tonsa, Eurytemora affinis and Scottolana canadensis, which together usually comprised more than 90% of the copepods. In addition, low densities were observed of Acartia clausi, Eugrasilus caeruleus, Hemicyclops americanus, Oithona brevicornis, and Pseudodiaptomus coronatus. Total numbers showed a well-defined peak in February and March of 1974, with the peak occurring first at station 9 near the main fresh-water input and last at station 13 at the mouth of the estuary (Figure 1). Maximum densities were of similar magnitude at the four stations, ranging from 10^5 to 3.6 X 10^5 per cubic meter. A second, less pronounced peak occurred in August of each year. A graph of relative abundances (Figure 2) reveals that A. tonsa predominated during most of annual cycle, with S. canadensis occasionally abundant in the fall and spring, and E. affinis most abundant during the spring peak.

Although rotifers were not identified to species, their total numbers are included (Figure 1). Brachionus (plicatilis ?) was observed to be the predominant species in our samples throughout the year. For most stations and sites rotifers were of a similar or lesser order of magnitude to copepods, and also reached peaks in spring and late summer. However, during the spring bloom at station 9 rotifers were a full order of magnitude more abundant reaching a peak density of approximately 2.5 X 10^6 per cubic meter.

Production

Instantaneous death rates and turnover times were estimated for A. tonsa and E. affinis using the developmental time estimates of Heinle (1969) and Rhode River temperature data. Results for A. tonsa were

determined from samples collected between 18 July and 9 October 1973, and for the mean population during this period (Table 1). Subsequent dates were not estimated because of the presence of A. clausi nauplii which could not be distinguished from A. tonsa. Turnover times for the mean population were 2.26 days for the nauplii, and 1.80 days for the copepodids. Individual sample estimates were all fairly close to this mean. Results for E. affinis were determined from samples collected between 20 November 1973 and 31 July 1974, and for the mean population between 8 January and 22 May 1974 (Table 2). A greater range of values was obtained, reflecting the wide range of temperatures and hence developmental times from winter to summer. Turnover times for the mean population were 14.20 days for nauplii and 11.96 days for copepodids.

The standing crop biomasses were obtained from abundances and length-weight relationships, and calculated for all sample dates. A. tonsa standing crop was greatest in mid-summer and in December-January (Table 3), and E. affinis standing crop was greatest in February (Table 4). Although A. tonsa was present in substantial numbers in the spring peak (Figure 2), most were nauplii and in terms of biomass the peak was almost entirely E. affinis. Maximum biomasses were 16.3 mg/m^3 for A. tonsa on 27 July 1973, and 218.6 mg/m^3 for E. affinis on 14 February 1974.

Production, estimated as biomass/turnover time, was 2.71 mg/m^3/day for the mean summer population of A. tonsa, and a maximum of 9.79 mg/m^3/day. Production of the mean E. affinis population was only slightly higher, 3.83 mg/m^3/day, but the maximum was much greater, at 20.47 mg/m^3/day. In the Rhode River copepod production was substantially greater during the February-March peak than during the secondary August peak.

DISCUSSION

Estimates of copepod abundances present notorious difficulties owing to their rapid avoidance ability (Fleminger and Clutter 1965). The likely result is to underestimate total numbers, particularly of older stages. In turn death rates will be overestimated and biomass underestimated. However, production estimates may not be affected substantially (Heinle, 1966). Our raw counts included copepodid stage V with adults as any individual with a well-developed fifth leg was scored in the adult category. We corrected for this by apportioning those individuals recorded as copepodids into four stages and those recorded as adults into two stages, using d_c and a modified equation 5. Then we determined a new estimate of total copepodids which included all five stages, and a new estimate of total adults. These were the data for equations (1) through (6); the correction raised death rates and lowered biomasses relative to uncorrected data but did not change production substantially, underscoring Heinle's (1966) point above.

We assumed that estimates of development times and length-weight relations based on A. tonsa and E. affinis from the Patuxent estuary (Heinle, 1969) could be applied to our populations. As the estuaries are separated by less than 100 km and subject to similar temperature and salinity regimes this seems reasonable.

Negative values for the instantaneous death rate (d_c) were obtained on two occasions (8 August and 2 October, Table 1) and reveal that one assumption of the approach is not met. That is, individual sampling dates do not represent true steady state conditions and on these occasions adults from an earlier period of high abundances survived into a subsequent

period of lower copepodid abundance. However the negative values are small (respectively -.03, -.01), indicating that this assumption does not meet with extreme exceptions.

Comparison of our values to Heinle's (1966, 1969) indicate that copepod biomass and production are substantially less in the Rhode River then in the Patuxent estuary. In the Rhode River biomass of A. tonsa is about 10%, and of E. affinis about 50% of those observed in the Patuxent. Turnover times are fairly similar, hence total production varies to the same extent as does biomass.

Williams, Murdoch and Thomas (1968) suggested that shallow embayments have low standing crops, and that zooplankton may be less important as a herbivore link in these shallow areas. Since the Patuxent is a considerably larger estuary than the Rhode River, this seems at first glance consistant with our observations.

Unfortunately, Williams et al (1968) made a critical error in their calculations. Standing crop per cubic meter was multiplied by average depth to determine total standing crop per square meter of surface area, and plotted against depth. If standing crop per cubic meter was independent of the depth of the embayment this would result in a slope of 1.00 (depth vs depth). Williams et al's (1968) finding of a slope of 1.145, not significantly different from 1.00, represents the fact that standing crop per cubic meter (their Table 3) shows a slight positive but not significant correlation with depth. Hence their conclusions have no statistical basis. (Furthermore, their Figure 4 which plots phytoplankton photosynthesis/zooplankton standing crop against depth is similarly suspect, although the data are not complete enough in their

paper for a careful re-computation).

We attempted to estimate upper and lower bounds on rotifer production in the Rhode River to determine whether they surpassed copepods in importance. As we do not have accurate counts by species, growth rates and biomass figures, these results are highly speculative. Nevertheless, using published data for Brachionus plicatilis (Theilacker and McMaster, 1971; Walker, 1973) we estimate that a Brachionus population comparable in total numbers per cubic meter to our total copepod populations would produce from 4 to 40 $mg/m^3/day$. This is up to an order of magnitude above copepod production, and probably errs toward the high side as most rotifers in Rhode River are smaller than Brachionus. If correct, this comparison would tend to support the conclusions of Williams et al (1968) regardless of their statistical mistake.

Finally, the differences between the Rhode and Patuxent Rivers was greatest for the summer Acartia bloom, least for the spring Eurytemora bloom. The Eurytemora peak may be associated with marsh detritus since it occurs early and is greatest at the mouth of Muddy Creek (February peak) and appears to proceed outwards toward the main bay (March peaks at stations 12 and 13). Heinle and Flemer (1974) calculate that the carbon demand of Patuxent E. affinis populations can only be met by substantial grazing on detrital carbon. Hence it may be that the smaller Rhode River is less productive primarily with respect to summer phytoplankton and resulting Acartia tonsa populations, and more similar in its detritus food chain.

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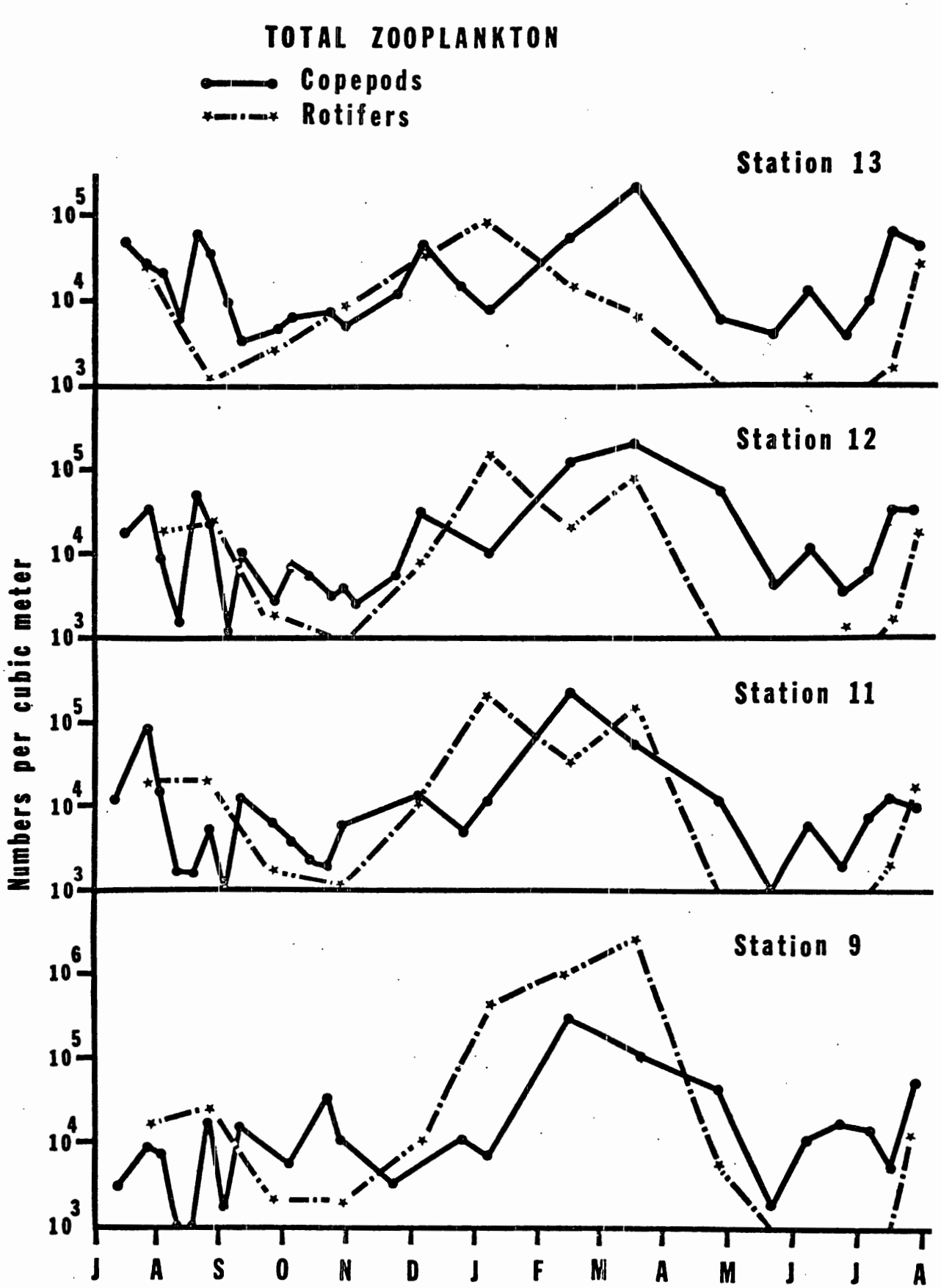
Figure 1. Total numbers of copepods and rotifers collected at each of four sampling stations in the Rhode River, 1973-74

Figure 2. The relative abundances of predominant species of copepods, averaged over all four stations, in the Rhode River, 1973-74


TOTAL ZOOPLANKTON
Copepods
Rotifers
Station 13
Station 12
Station 11
Station 9
Numbers per cubic meter
10^5
10^4
10^3
10^6
J
A
S
O
N
D
J
F
M
A
M
J
J
A

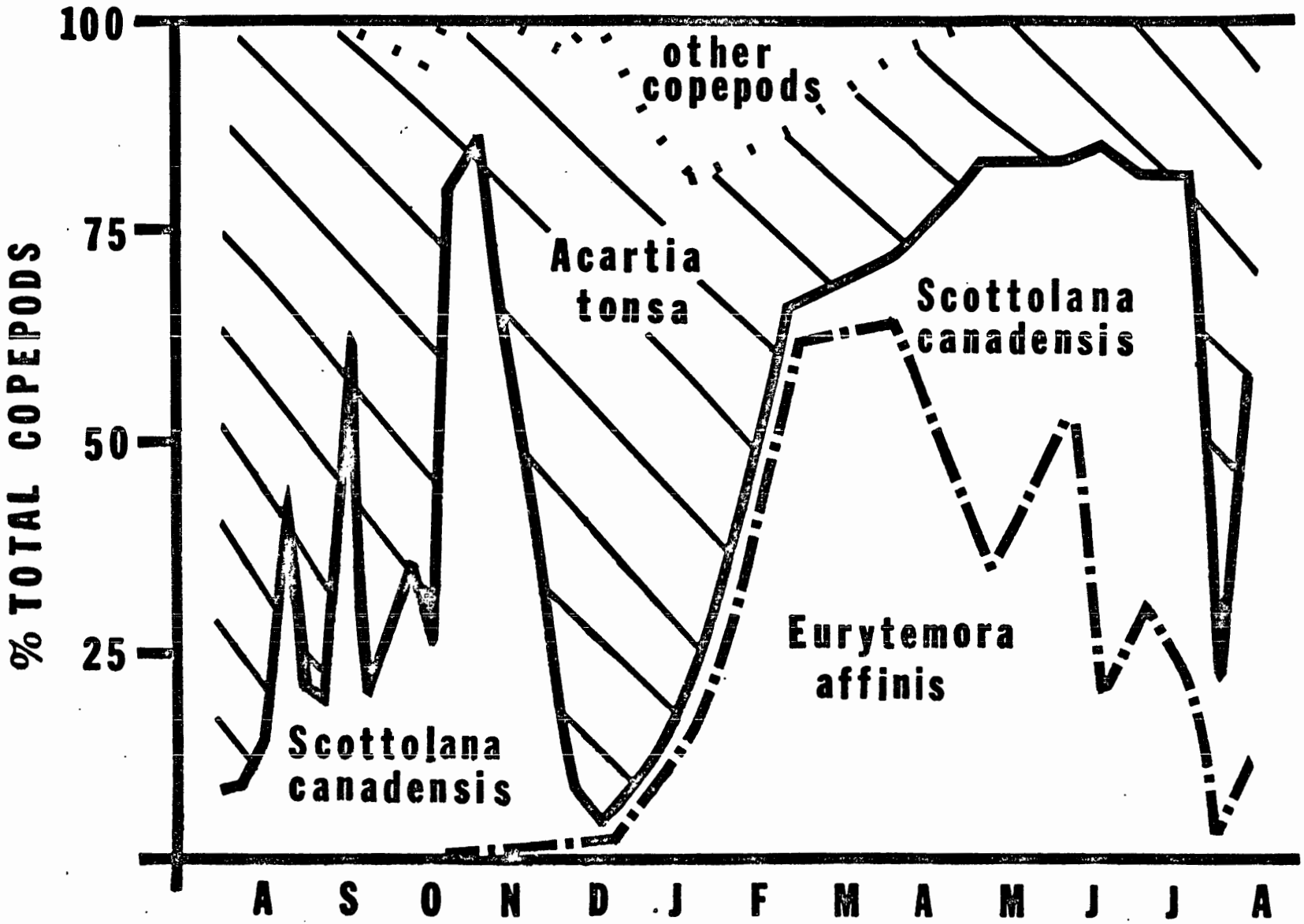

100
75
50
25
% TOTAL COPEPODS
other copepods
Acartia tonsa
Scottolana canadensis
Eurytemora affinis
Scottolana canadensis
A S O N D J F M A M J J A

Table 1. Instantaneous death rates (d) and turnover times (T) of *Acartia tonsa* nauplii and copepodids averaged over four stations in the Rhode River subestuary. Time units are days.

DATE	d_n	d_c	T_n	T_c
July 18, 1973	.50	1.1	2.54	1.5
27	.46	.66	2.71	2.06
Aug. 1, 1973	.38	1.12	3.16	1.48
8	1.17	-.03	1.45	-
16	.51	1.17	2.51	1.45
22	.97	.65	1.61	2.10
Sept. 4, 1973	.98	.01	1.80	87.65
11	.77	.93	1.87	1.65
25	1.05	1.07	1.54	1.52
Oct. 2, 1973	.55	-.01	2.38	-
9	.44	.45	2.81	2.75
mean population, 18 July 1973 - 9 Oct. 1973	.59	.81	2.26	1.80

Table 2. Instantaneous death rates (d) and turnover times (T) of Eurytemora affinis nauplii and copepodids averaged over four stations in the Rhode River subestuary. Time units are days.

DATE	d_n	d_c	T_n	T_c
Nov. 20, 1973	.38	-	3.15	-
Dec. 4, 1973	.33	-	3.59	-
26	.11	.04	9.51	27.82
Jan. 8, 1974	.20	-	5.58	-
Feb. 14, 1974	.05	.05	19.28	21.55
Mar. 22, 1974	.12	.24	8.76	4.65
April 26, 1974	.76	-	1.87	-
May 22, 1974	.28	.46	4.12	2.69
June 5, 1974	1.08	-	1.51	-
19	.66	.78	2.07	1.85
July 3, 1974	1.61	-	1.25	-
17	1.41	-	1.32	-
31	1.24	.69	1.41	2.0
mean population, 8 Jan. 1974 - 22 May 1974	.073	.087	14.20	11.96

Table 3. Biomass and productivity of Acartia tonsa averaged over four stations in the Rhode River subestuary.

Date	Standing Crop Biomass (Mg/m^3) Nauplii	Copepodids	Adults	Total	Production (mg/m^3/day)
July 18, 1973	2.33	3.77	1.00	7.10	4.11
27	3.78	8.09	4.43	16.30	9.79
Aug. 1	1.11	2.30	.59	.06	2.32
8	0.19	.07	.21	.47	.13
16	2.63	4.09	.97	7.69	4.54
22	1.80	1.32	.74	3.86	2.11
Sept. 4	.13	.09	.19	.41	0.08
11	1.00	.99	.35	2.34	1.46
25	1.11	.56	.17	1.84	1.20
Oct. 2	.46	.49	1.16	2.11	.49
9	.26	.29	.14	0.69	.25
16	.19	.16	.08	.43	- *
23	.23	.03	.16	.42	-
Nov. 20	.72	.41	1.22	2.35	-
Dec. 4	3.16	3.24	6.85	13.25	-
26	.88	3.34	10.82	15.04	-
Jan. 8	.47	1.75	11.17	13.39	-
Mar. 22	1.12	.25	1.85	3.22	-
June 19	.19	.05	0	.24	-
July 3	.16	.01	0	.17	-
17	2.41	.69	.07	3.17	-
31	1.32	.12	.06	1.50	-
Mean population, 18 July 1973 - 9 Oct. 1973	1.46	2.38	1.31	5.15	2.71

* Production not estimated after this date due to presence of A. clausi

Table 4. Biomass and productivity of Eurytemora affinis averaged over four stations in the Rhode River subestuary.

Date	Standing Crop Biomass (Mg/m^3) Nauplii	Copepodids	Adults	Total	Production (mg/m^3/day)
Nov. 20, 1973	.04	0	0	.04	.01
Dec. 4	.13	0	0	.13	.04
26	.54	.36	.31	1.21	.30
Jan. 8, 1974	.45	0	1.71	2.16	.08
Feb. 14	38.57	104.71	75.34	218.62	9.94
Mar. 22	26.45	73.1	8.59	108.1	20.47
April 26	.33	0	0	.33	.17
May 22	.38	.38	.09	.81	.27
June 5	.31	0	0	.31	.20
19	.55	.11	0	.66	.32
July 3	.35	0	0	.35	.28
17	.12	0	0	.12	.09
31	.91	.04	0	.95	.67
Mean population, 8 Jan. 1974 - 22 May 1974					
	12.33	20.66	14.63	47.62	3.83

APPENDIX I

Introduction:

Questions (b) and (c) of the project objectives concern attempts to determine the feeding rates and feeding selectivity of the predominant zooplankton species. With this information it would be possible to determine the food base for the zooplankton community, the efficiency with which it is consumed, and potential interactions among consumers.

Methods:

Grazing experiments followed the electronic particle counting approach to determine the number and sizes of particles removed. One litre bottles of filtered Rhode River water were utilized for all experiments. Using cultured algae as the food source, a number of copepods were added to one set of bottles while another set served as a control. All experiments were run at 15° in the dark on a wheel rotating at 2 revolutions per minute. After a specified time period the number and size distribution of algae in each bottle were measured and the number of surviving copepods in the control set were counted.

Results and Discussion:

Results are expressed as ml of water swept clear of algae per copepod·day assuming 100% clearing efficiency. A range of values obtained with Eurytemora affinis (Table 1) serve only to show that filtering rates are comparable to those reported by other investigators, but are too variable to allow a confident statement about mean values. Further experiments now in progress should provide more definite answers in the near future.

Appendix I, Table 1. Filtering rates of *Eurytemora affinis*.

Algal Food	Number of Copepods	Duration of Experiment (hours)	Filtering rate (ml/copepod/day)
Isochrysis	10	22	2.4
Isochrysis	12	22	24.7
Isochrysis	6	22	90.9
Isochrysis	10	6	126.4
Isochrysis + *Phaeodactylum*	6	22	6.0

APPENDIX II

Introduction:

The role of zooplankton in transferring energy to higher trophic levels may be approached from estimates of death rates of zooplankton populations and/or feeding rates of predator populations. This study utilized the first approach, and from death rates and age distribution we may infer high mortality rates.

Methods:

See Methods section of manuscript reporting findings on population dynamics and production.

Results:

Instantaneous death rates for Acartia tonsa nauplii and copepodids indicate high death rates throughout the July - October period for which estimates could be made (Table 1). Death rates tended to be higher for copepodids than for nauplii, suggesting that the older, larger copepodids suffered higher mortality. Instantaneous death rates for Eurytemora affinis (Table 2) are typically much lower, especially in the cold weather periods when E. affinis is abundant. Again copepodids show slightly higher mortalities than nauplii.

Discussion:

High death rates and resulting rapid population turnover suggest that predation is substantial. A slight trend towards size-selectivity is revealed, but not of the order observed in freshwater systems (Brooks and Dodson, 1965). Evidentally much of the predation pressure is non-selective. Jellyfish and ctenophores are likely non-selective predators and may be a major source of mortality in this system (Heinle, 1974). Fish are likely to select larger

prey items, and my student Adam Myers has demonstrated that the silverside Menidia will consume only the largest copepodids and adults of this zooplankton community. However, it is unlikely that fish are the major predators or more of a bias towards higher copepodid mortality would be expected.

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