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by

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INTRODUCTION

The cycling of energy/matter and minerals and the general subject of nutrient limitation, enrichment and eutrophication of aquatic ecosystems has been the subject of intense ecological research for the past several years. The limits to productivity in many natural systems are generally ascribed to the scarcity of one or more nutrient species. For aquatic-marine ecosystems, nitrogen is often cited in this regard (Pomeroy, 1975) and marsh ecosystems in particular indicate some degree of nitrogen limitation (Pigott, 1969; Valiela and Teal, 1974). Under natural conditions of nutrient availability, the successional nature of most aquatic communities tends to optimize resource utilization (both structurally and energetically) and establish rather characteristic cycles for nutrient flux. However, aquatic ecosystems perturbed by the addition of nutrients, especially to saturation levels, respond in ways not completely predictable based on the study of similar natural ecosystems.

The disposal of domestic sewage as a point source of nutrient enrichment to aquatic ecosystems has historically been a major source of nutrient addition and has led to eutrophication in many instances (see Likens,G.E.(ed),1972). However, no conclusive or quantitative statements, in a predictable sense, can be made regarding exact cause and effect relationships given the level of enrichment, existing environmental conditions and/or the previous history of an impacted area.

The continued input of limiting nutrients to levels above saturation for the natural system creates what might be termed a "nutrient block" and results in excess nutrients which cannot be metabolically utilized and/or physically stored. The system becomes "saturated" and establishes new conditions which may result in new and different nutrient exchange pathways predominating. This often results in species replacement or redistribution and may lead to fundamental changes in community structure. In effect, this forces the system into a new and different state that may or may not retain the functional attributes or behaviors of the original system.

An ecosystem normally operating at or near limiting nutrient conditions and then exposed to high or saturated conditions will change state. It will not remain functionally the same system. Therefore, studies of natural, unperturbed systems serve only as a reference for describing the impact and not as controls in the experimental sense. They offer statistical correlation but do not quantitate cause and effect relations. The studies are descriptively useful but predictably imprecise. Processess controlling nutrient dynamics are largely obscured in such analyses and can only be inferred. Studies oriented toward analysis of functional (rate) processes and adopting an ecosystem level approach should certainly aid in the solution to the present problems.

Since most questions (both basic and applied) are phrased in terms of the total systems behavior regarding the enrichment impact or systems recovery following relaxation, it seems that studies of impacted systems be holistically oriented. This view can be adopted by incorporating experimental measures of functional components with total systems' measures.

It is along these lines that we have initiated studies of a nutrient enriched marsh-creek ecosystem. Our studies are oriented toward quantifying the processes of recovery, identifying those system's components (both physical and biological) controlling nutrient dynamics in the marsh system, and relating total system's behavior (mass balance) to adjoining ecosystems.

In this report, we summarize our initial findings of a nutrient enriched aquatic ecosystem prior to relaxation of sewage input. The studies reported were directed toward characterizing the system prior to relaxation and to begin experimental studies to accomplish our general objectives. The specific areas of study were decided *a priori* and were chosen for the determination of what processes and/or system's components on which to concentrate our research effort as the project continued.

STUDY AREA: YORKTOWN CREEK

Yorktown Creek and the associated marsh is located in the southeast section of the state of Virginia at Yorktown. The creek-marsh ecosystem is an oligohaline marsh with mixed vegetation. The system is branched off the western side of the York River approximately six miles west-northwest of the lower Chesapeake Bay. Total marsh acreage is approximately 35 acres and is part of the Colonial National Historical Park. The dominant vascular plants of the marsh are _Spartina alterniflora, Spartina patens, Distichlis spicata_ and _Typha angustifolia._ Figure 1 illustrates the geographical location of the area and the general topographic features of the study area.

Yorktown Creek has been historically impacted (ca.19 years) at the head of the southern creek branch by the addition of secondarily treated domestic sewage from the city of Yorktown. Plant operation records indicate erratic daily discharges interspersed with periods of no operation. For the period June, 1975 to March, 1976 average daily discharge was 71,000 gals. (269 m^3) (Figure 2). The discharge travels approximately 1280 m to the York River and traverses nearly 25 acres of marsh (_Typha angustifolia, Spartina patens_ and _Distichlis spicata_). However, the principal impact of the sewage disposal is more than likely

FIGURE 1; YORKTOWN CREEK WATERSHED.

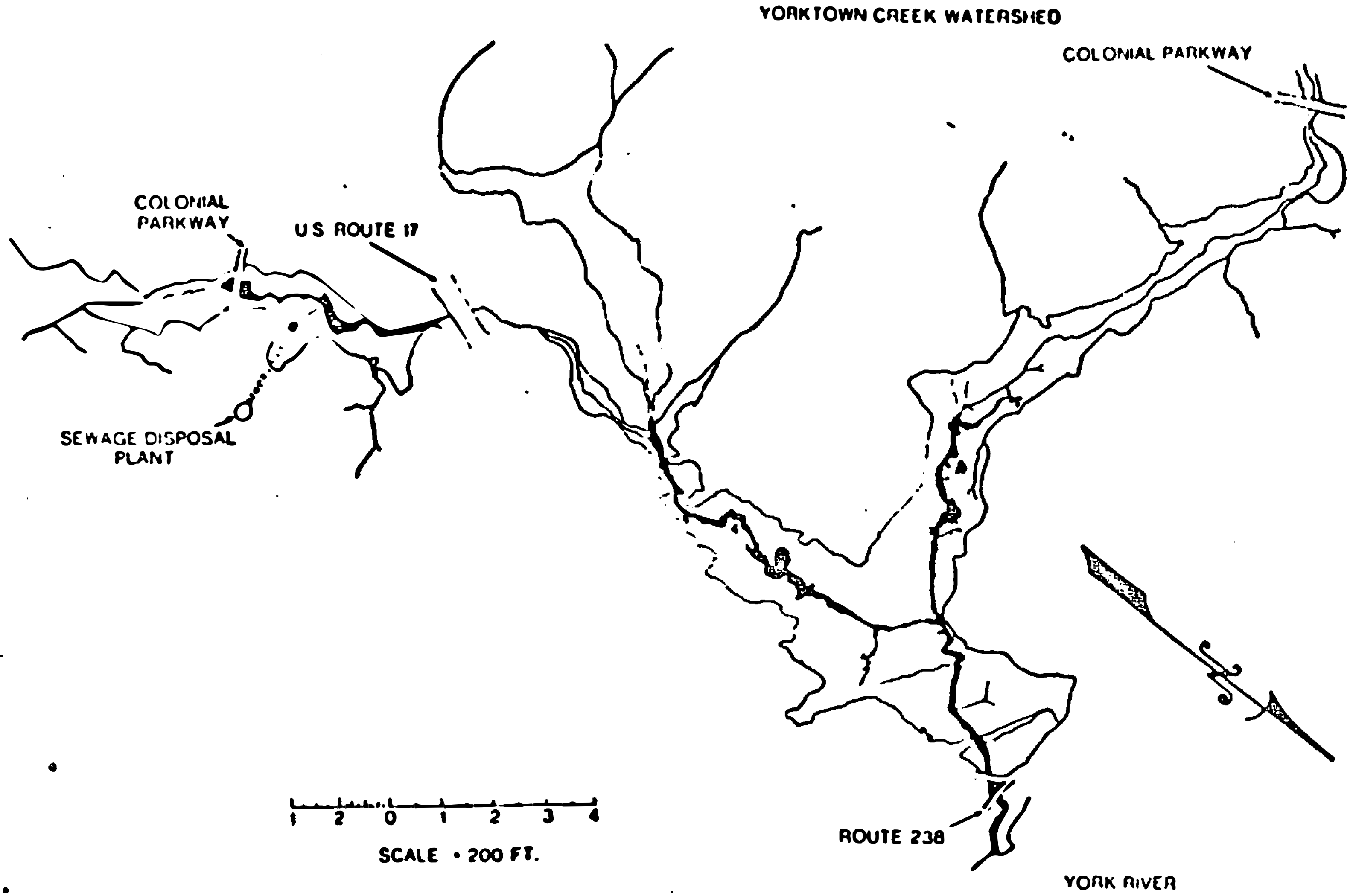
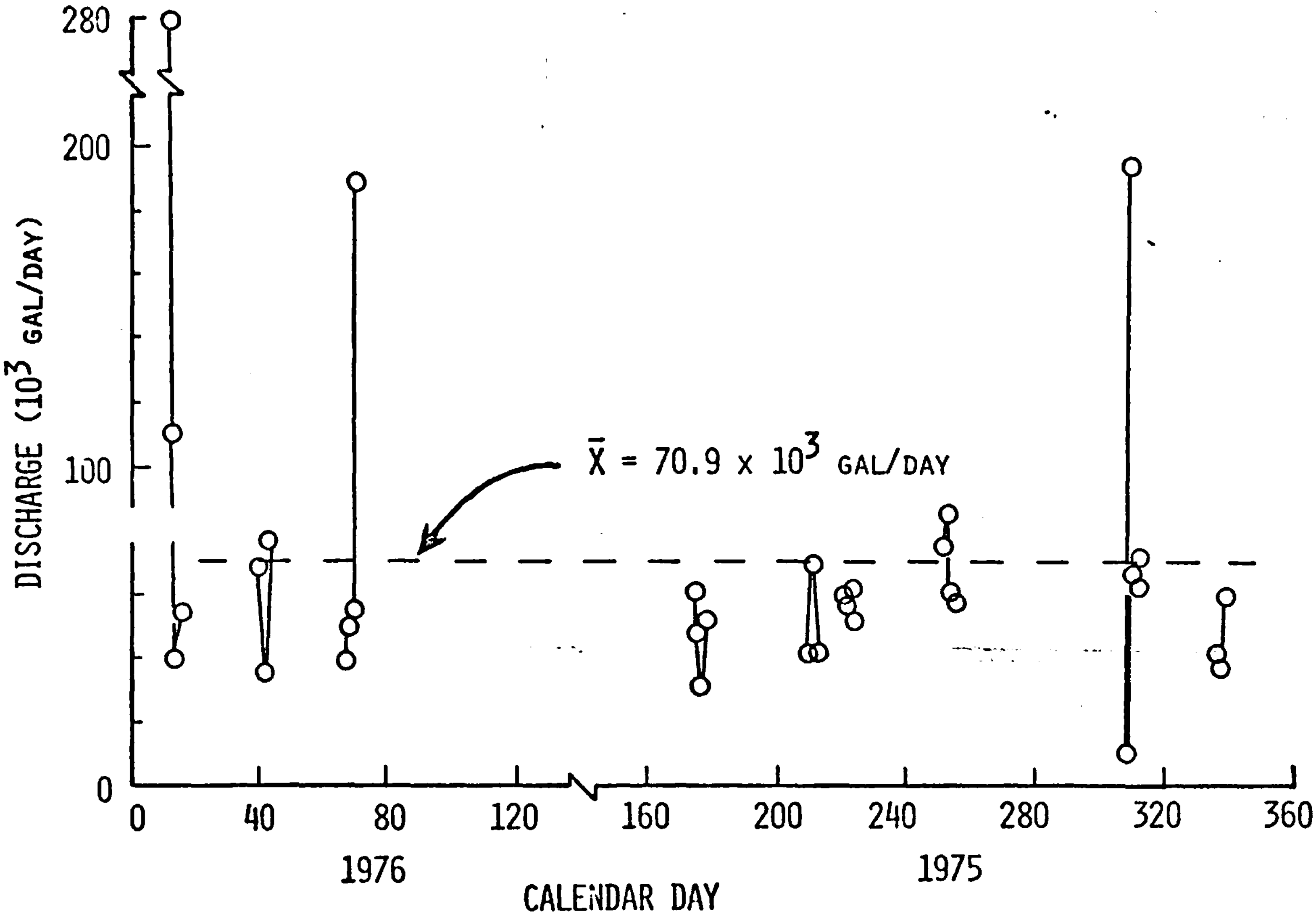


FIGURE 2; SEWAGE DISPOSAL DISCHARGE, YORKTOWN PLANT. (DATA COURTSEY NATL. PARK SERVICE, COLONIAL NATIONAL HISTORIC PARK, YORKTOWN, VA.)



limited to the creek and those marsh areas directly adjacent to the creek. Highway and road construction activities dating to Colonial times have grossly altered the hydrology of the area and influenced tidal flushing and exchange. This combined with marsh elevational changes has removed much of the Spartina-Distichlis zones from daily tidal influence and exchange with the creek system. Because of our limited hydrological data, separation of the impacts at the system's level are not yet possible. It appears, at least in gross characteristics,i.e. vascular plant distribution, the upper reaches of both creek branches have been more influenced by hydrological changes than nutrient enrichment.

For the purposes of our nutrient enrichment studies, the creek-marsh system at Yorktown was segregated into three regions. Each branch of the creek system and that portion of the creek system resulting from the confluence of the two branches, constitute natural subdivisions in the total system, having distinct nutrient regimes and approximately equal areas. Each area, designated Control, Impacted and Mixed branches, are treated separately in the following sections of the report. To avoid boundary effects, the three zones were separated by a buffer area in which no sampling was done. Effective July 1, 1976, disposal of sewage into the Yorktown Creek Watershed was stopped. Except for the road and highway construction, the system has been left relatively unperturbed.

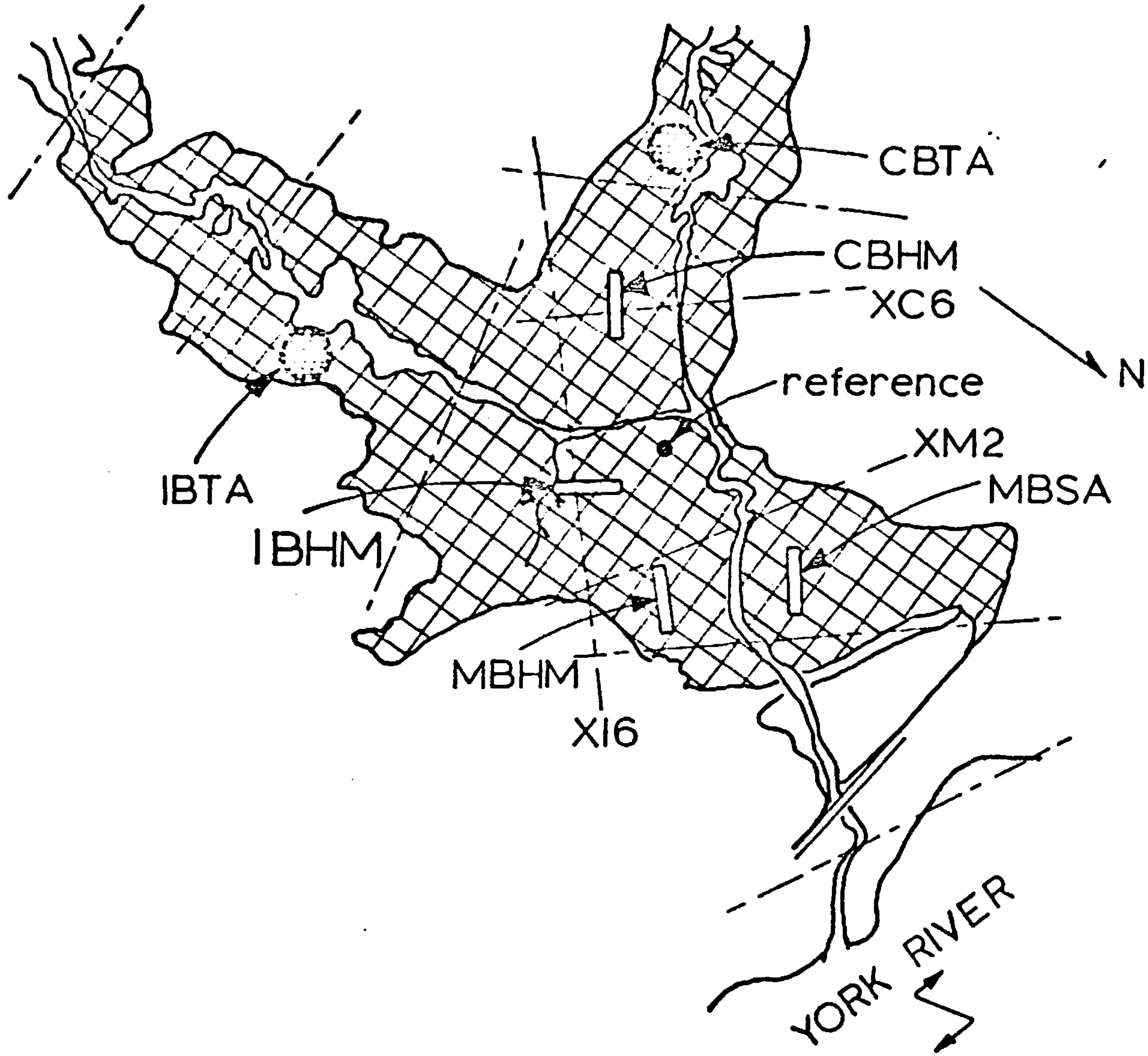
METHODS AND MATERIALS

General Methods

A ground survey of the marsh to establish transects for sampling locations during the proposed research program was completed prior to the growing season (March 1976). A photographic overflight of the area was done to 1) establish marsh zonation patterns, 2) determine total and zonal marsh acreages, and 3) check positioning of the surveyed sampling points and transects. A grid-coordinate system drafted from a ground reference point and the overflight information was used to randomly locate sampling points within a given zone. The grid coordinate system and major sampling locations are shown in Figure 3.

For sampling purposes, the creek-marsh system was stratified into three main blocks: 1) nutrient impacted, 2) control, and 3) mixed branches. Between these main blocks, 30 m boundaries measured from the convergence of the two creek branches were established in which no samples were taken other than creek water samples for nutrient analyses. The transects marked X16, XC6, and XM2 delimit the boundaries of the buffer zone (Figure 3). Preliminary dye studies using Rhodamine B were attempted to assess the degree of mixing between the impacted and control arms of the creek in an attempt to reduce the buffer zone as much as

FIGURE 3; GIRD COORDINATE AND VASCULAR PLANT SAMPLING LOCATIONS.



possible, and to determine water residence time; but they were unsuccessful. Laboratory studies conducted with Rhodamine B indicated extreme decay due to sorption of the dye when added to water and suspended sediments from Yorktown Creek. This occurred even when dye concentrations orders of magnitude in excess of the dye detection limit were added to the suspensions (see Appendix A). This result has recently been confirmed by the studies of Smart and Laidlaw (1977). However, water chemistry data indicated that mixing between the two arms was minimal and a 30 m boundary was more than adequate.

This report includes the results of our studies from November, 1975 to August, 1976 on: 1) vascular plants, 2) benthic microflora, 3) creek and interstitial water chemistry, 4) benthic infauna, 5) nitrogen fixation, and 6) 24 hour input-output (mass balance) investigations.

Vascular Plants

The basis for establishing a detailed plant surveying and sampling program were: 1) plant distribution, zonation, and productivity studies are, at present, the best comparative indices of total systems function, 2) vascular plant production and the subsequent dominant input of detrital-plant matter to the salt marsh-estuarine ecosystem forms the basis of the longer term cycles of biologically derived carbon, nitrogen and phosphorous, and 3) vascular plant tissue represents the single largest, biological storage compartment for nutrients.

Figure 3 shows the vascular plant sampling areas. Each rectangular area is 5 x 20 m. Quadrats from these permanently staked areas were randomly selected from an internal coordinate system. Permanently staking the Typha sites was found to be unfeasible and a random toss method for quadrat selection was used. Three high marsh sites (CBHM, IBHM, and MBHM), two Typha sp. sites (IBTA & CBTA), and one S. alterniflora site (MBSA) were harvested monthly using 0.1 m^2 circular, 0.25 m^2 rectangular, and 0.1 m^2 circular quadrats, respectively. In each area monthly samples were replicated 6-10 times.

Net primary production (NPP) was estimated by the method of Smalley(1958). This method is not as accurate an estimate of NPP as the originally proposed paired plots method (Wiegert and Evans, 1964). The method used is less demanding in terms of time requirements,allowing more sample replication,and is comparable to the proposed method when plant mortality and detrital disappearance between sampling intervals is small. The effects of grazing are not known for this method.

Turn-around time was greatly reduced in July after application of the technique for altering optimum quadrat size (Wiegert, 1962; Van Dyne et al., 1963). In obtaining useful results from the technique, its application had to be delayed until significant amounts of live biomass were present; hence, the pre-August results are occasionally incomplete and some sampling intervals are longer than one month.

After sampling, the clipped quadrats were returned to the laboratory and separated into live species and dead fractions, and subsampled for live plant lengths. The samples were dryed for a minimum of 48 hours at 105-110 °C and weighed to the nearest 0.1 gms. Representative subsamples were taken from the samples and ground, using a Wiley mill, for analyses of tissue carbon, nitrogen, phosphorus, and caloric content. Only the caloric content data for Distichlis spicata is included in the report. Samples were run using the Wiegert-Gentry microbomb calorimeter.

Ground truth surveys were conducted on two occassions, pre and post-peak growing seasons, for mapping and checking vegetative zones. Interstitial water, benthic microflora and benthic infauna sampling was co-ordinated with the vascular plant sampling areas.

Benthic Microflora

Analysis of the role of micro-autotrophs in Yorktown Creek was attempted using both productivity (gas concentration changes) and biomass methods (chlorophyll a). Unfortunately both approaches are hampered by a lack of methodology.

Measurements of primary production were attempted by monitoring changes in atmospheric gas concentrations in specially constructed plexiglass chambers. The chambers were incubated in the field and atmospheric gas samples taken by syringe. The samples were analysed using gas chromatography. This method shows promise, and if perfected, would eliminate the problem of microbial uptake and heterogenous isotope distribution associated with ^{14}C incubations. However, because of the short time frame of this study the studies were abandoned until an adequate methodology using GC can be worked out.

Chlorophyll a is widely used as a measure of micro-autotrophic biomass in aquatic ecosystems (Moul and Mason, 1957; Pomeroy, 1959; Estrada, et al, 1974; Gallagher and Daiber, 1974; Sullivan and Daiber, 1975). However, its measurement, especially in marsh soils presents methodological problems. The standard extraction procedure, which we followed, calls for a 24 hour dark extraction of the sample in basic 90% acetone. Sediment samples were taken by hand with a 3.17 cm (1.25 in.) PVC coring tube and frozen. The top centimeter of each core was cut off and ground with a mortar and pestle before extraction. Chlorophyll a was determined spectrphotometrically, within one month of sampling.

Since most samples for chlorophyll anaylsis contain the degradation product phaeophytin, it is desirable to correct for this pigment by acidification. Experiments in which acidifications were carried out with 5 and 10% (v/v) HCL showed discrimination between the two pigments after a minimum of 5 minutes incubation, but the differences were slight and variable for the majority of cases. It is noteworthy that only the unacidified (i.e. uncorrected) estimates of chlorophyll a approximated published values. Because of the unreliability of the acidification, the chlorophyll a values in this report are uncorrected for phaeophytin, and thus are relative and not absolute estimates of microfloral biomass.

The chlorophyll a values reported are not corrected for interference of chlorophylls b and c. Trichromatic techniques which correct for the presence of chlorophylls b and c were initially done, but were found to alter the final calculation of chlorophyll a concentration only slightly, while tripling the analysis time. For this reason, the trichromatic technique was discontinued and the concentrations of chlorophyll a were calculated from optical densities read at 665 nm using a Spectronic 20. Chlorophyll a was calculated using the equations given by Vollenweider (1974).

Samples for chlorophyll a analysis were taken from each arm of Yorktown Creek in the high marsh (CBHM & IBHM), high marsh-*Typha sp*. mixed vegetation zones (CBMV & IBMV) and *Typha angustifolia* (IBTA & CBTA) zones, as well as at the creek mouth (MBSA), below the confluence of the two branches (Figure 4). In March it was not possible to enter the CBTA zone. Chlorophyll a concentrations in the sediments were determined from four streamside marsh replicates in each of the vegetational zones at low tide in March and May. In June, two high and low tide samples were taken from the CBHM & IBHM zones above the confluence of the two branches to determine possible differences between impacted and control arms and between high and low tide samples.

Chlorophyll a concentrations in the creek system were measured during the 24 hour study (June 14-15,1976) at the mouth of Yorktown Creek to determine the flux of chlorophyll in the marsh-creek system. Duplicate 10 ml water samples were taken and filtered every half hour, and the chlorophyll-containing filters were frozen on dry ice until analysis. For analysis, the filter was homogenized in 10 ml. of 90% acetone and stored cold, in the dark, for an extraction period of 24 hours. Chlorophyll a was then determined by flourescence using a Turner 111 flourometer.

Creek and Interstitial Water Nutrients

Water samples were taken at monthly intervals beginning November, 1975. The sampling locations are shown in Figure 4, and Table 1 gives a verbal description of each site by station number.

At each station, water samples were taken in duplicate , by hand with 125 ml, acid-washed, polypropylene containers. The samples were returned to the laboratory and immediately filtered using Type A glass fibre filters and the filtrates preserved by the addition of several drops of 20% HgCL. The samples were stored at -20°C until analysis. Each sample was analysed for NH_3 , NO_2^-, NO_3^-, urea, PO_4^{-3} and chlorinity.

After reduction to nitrite, nitrate and nitrite were determined colorimetrically by a diazotization reaction on a cadmium-copper column. Sample flow through the cadmium

FIGURE 4; WATER NUTRIENT AND CHLOROPHYLL SAMPLING LOCATIONS.

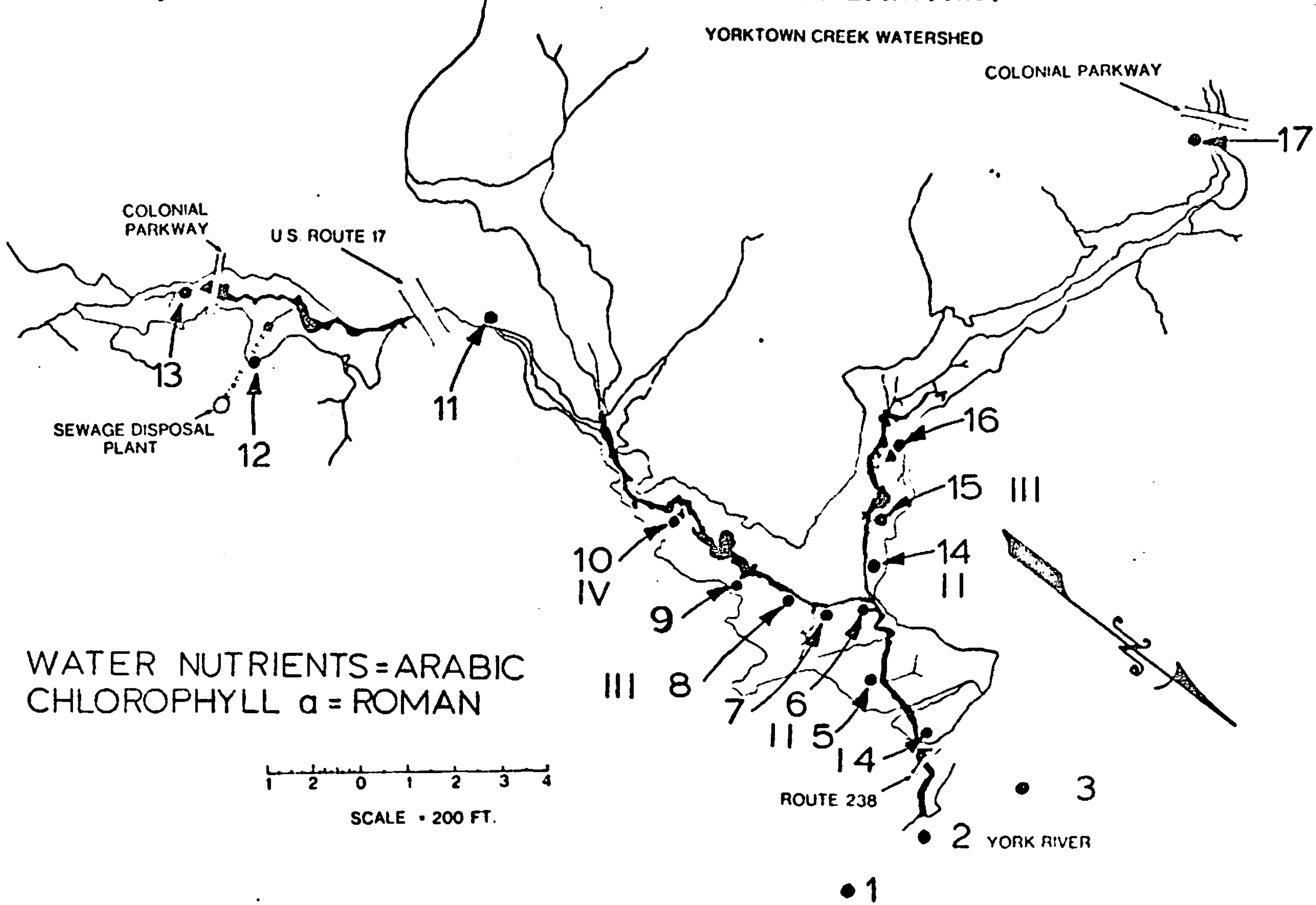


Table 1: Description of Water Sampling Sites in Yorktown Creek

Site	Nutrient Regime	Description
1	---	York River, 60 m downstream from the mouth of Yorktown Creek. 40 m from shore.
2	---	Sand Spit at the mouth of Yorktown Creek.
3	---	York River, 60 m upstream from the mouth of Yorktown Creek. 40 m from shore.
4	Mixed	*Spartina alterniflora* dominates vegetation. Zone MBSA.
5	Mixed	*Spartina alterniflora* dominates vegetation. Zone MBSA.
6	Mixed	*Spartina alterniflora* dominates vegetation. Zone MBSA.
7	Impacted	High marsh (*S. alterniflora*, *Spartina patens* & *Distichlis spicata*) dominates vegetation. Zone IBHM.
8	Impacted	High marsh dominates vegetation, transition to *Typha angustifolia*. Zone IBMV.
9	Impacted	*Typha angustifolia* dominates vegetation. Zone IBTA.
10	Impacted	*Typha angustifolia* dominates vegetation. Zone IBTA
11	Impacted	Below outfall. Creek drains woodland at this point.
12	Impacted	Sewage Outfall.
13	Unimpacted	Above sewage outfall. Mixed vegetation of *Typha* sp., *Sagittaria* sp., *Peltandra* sp.
14	Control	High marsh dominates vegetation. Zone CBHM.
15	Control	High marsh dominates vegetation, transition *T. angustifolia*. Zone CBMV.
16	Control	*T. angustifolia* dominates vegetation. Zone CBTA.
17	Control	Creek drains woodland at this point.

column and addition of reagents were controlled by a peristaltic pump after the automated method described by Stainton (1974). Samples were read manually on a Beckman DU-2 spectrophotometer. Ammonia was determined by the method of Solorzano (1969) and urea by the method of Newell, et al.(1967). Both of these methods were carried out manually on 5 ml subsamples of the filtrates. Reactive phosphorous was determined by the method of Murphy and Riley (1962). Chlorinity was determined by the standard titrametric technique (Strickland and Parsons, 1968).

Interstitial, or pore water, nutrient concentrations were determined on squeezed core samples taken in July, 1976. The cores were taken by hand from marsh zones MBHM, IBHM, CBHM, MBSA, IBTA and CBTA. After sampling, the cores were returned to the laboratory and the interstitial water extracted by compressing the core sample (top 5 cm) using high pressure O_2. The core sample was separated from the gas supply by a latex rubber sheet. The same sample preservation, storage, and analysis procedures used for the water nutrient samples were followed for the interstitial water samples.

Benthic Infauna

Core samples were taken on one occasion for the present study (July 7, 1976) as a gross characterization of the benthic infauna of the marsh soils. Sampling sites for the coring stations were located in marsh zones IBTA, CBHM, CBMV and IBMV.

Cores measuring 9.6 x 16.7 cm were taken by hand, returned to the laboratory, and preserved in 10% buffered formalin. Each core was sectioned into top and bottom 5 cm portions and analysed separately. The infauna of each core were hand picked under magnification, and divided into major taxonomic groups. Counts of each group were recorded for the total number of organisms in each group present in the core sample.

Because of the short time frame of this preliminary investigation and the time required to sort the core samples, only one set of samples could be processed. We have planned routine sampling of benthic infauna using a coring technique for the future work.

Nitrogen Fixation

Nitrogen fixation (N_2 to amino-nitrogen or ammonia) was determined by the acetylene (C_2H_2) reduction method. Our adaptation of the Stewart *et al.* (1967) method utilized light and dark incubations in an atmosphere of 15% C_2H_2 in air and helium. Samples are taken from the incubation at intervals of 15-30 minutes and analyzed by gas chromatography for CH_3, C_2H_2, and C_2H_4. Rates are calculated from linear regressions of C_2H_4 increase, ignoring any lag period (Webb *et al.*, 1975; Wiebe *et al.*, 1975).

Samples were taken on one occassion during our present studies: June 2, 1976. Samples were taken along the creek marshes in both control and impacted areas from:1) *Spartina alterniflora* and *Spartina patens* intertidal zones, 2) floating algal mats associated with tidal pools, and 3) the blue-green algal mats predominately found in the control area. Vegetation samples were also taken in the areas where sediment cores were taken. The samples were placed in small ambient temperature water baths and returned to the laboratory for preparation.

All sediment and blue-green algal mat samples were taken as cores, using 5 cc syringes and transferred immediately to 13.5 cc serum bottles. The cores were approximately 3 cc in volume and 2.0 to 3.0 cm in length. Whole S. alterniflora and S. patens shoots were removed (as vegetation samples) and 2.0 to 3.0 cc volume of rhizomes and mud were analyzed. The floating algal mats located in tidal pools were scooped into sampling dishes and 2.0 to 3.0 cc volumes of sample were placed in the serum bottles. The serum bottles, fitted with rubber serum stoppers, were filled with environmental air following the methods of Wiebe, Johannes, and Webb (1975) Englund and Meyerson (1974), and Jones (1974). Acetylene (1.0 cc) was injected, establishing an internal pressure of 15 atm or 15% gas phase. The samples were immediately shaken for 10 seconds to compensate for the effects of acetylene dissolution in water (Flett et al., 1975). After a short equilibration period at ambient temperature, normal atmospheric pressure was restored.

Following the above treatments, the samples were incubated at ambient temperature and light conditions. Gas samples were taken approximately every two hours and analyzed immediately. Ethylene production was determined by gas chromatography using a Varian Aerograph 600-D chromatograph (H-flame ionization detection) fitted with a Poropak N column (5 mm x 18 m) maintained at 60 to 70 C. Nitrogen was used as the carrier and maintained at a flow rate of 25-27 ml/min. Control and blank peak heights were subtracted from sample peak heights for calculation of sample gas concentrations. Ethylene peaks were calibrated using peak heights of known concentration. Methane was used as an internal standard by computing a ratio to correct the ethylene values. The rate of acetylene reduction was converted to nitrogen fixation by assuming a molar ratio of 3:1 for ethylene formed to ammonia formed (Schollhorn and Burris, 1967; Hardy _et al_., 1968). The calculated rates were expressed in terms of dry weights for sediments and surface areas for the algal mats.

Mass Balance Studies

Diel studies were conducted on two occasions, June 15-16 and August 4-5, 1976, in an attempt to characterize the nutrient mass balance properties of the Yorktown Creek-Marsh ecosystem and its interaction with the lower York River system. Dissolved inorganic nutrients (NH_3, NO_2^-, NO_3^-, urea, PO_4^{-3}) chlorophyll _a_, and adenosine triphosphate (ATP) were measured at 30 minutes and one hour intervals, respectively, during each of the studies. At each sampling period, a bucket sample was taken midstream at the mouth of Yorktown Creek between the two road culverts (See Figure 3). Input to the creek was monitored at the disposal outfall using an automatic sampler set at the same sampling frequency. Each 24 hour study started in early afternoon during low tide. Results and analyses for the August study have not been completed and are not included in the report.

Nutrient and chlorophyll a analyses were done as previously discussed. ATP was assayed using the methods of Bancroft, Yetka, and Wiebe (1974).

Current velocities and relative tide heights were recorded at each sampling interval. Relative tide heights were used to calculate the cross sectional area of the creek, and water volume either ebbing or flooding, was calculated as the product of current velocity (cm/sec) times the cross sectional area (cm^2).

Total exchange (x_t) for a given parameter was calculated as;

$$X_T = \sum_{n=1}^{t} (x_t) \cdot \pm dL_{30t},\ n=1,2,3...t(t=48).$$

where: X_T = daily mass balance for the 'x' parameter.

x_t = x concentration x L^{-1} at 't' sampling interval.

$\pm dL_{30t}$ = volume discharge (L) during the 't' 30 minute sampling interval.

+ = flooding tide

− = ebbing tide

Thus, a positive X_T value would indicate a net flux of the parameter into the marsh-creek system and a negative term would indicate a net export of the parameter of interest from the system. The tidal transport studies were designed after the methods of Boon (1975).

RESULTS

Vascular Plant Studies

Table 2 lists (alphabetically) the principle vascular plant flora of the Yorktown Creek-marsh system for samples collected between 18 February and October, 1976. It is not a complete list of all the flora present. A total of 18 species was identified in the samples taken from the six vegetative zones. Species richness (Margalef, 1969) was not determined for the various zones since it depends on the identification of individuals of plants species. However, the number of species occurring in the samples was consistently higher in the control branch compared to similar vegetation zones in the impacted area. The differences in species composition of the control and impacted areas were always attributable to species elimination and not replacement in the impacted branch; i.e. there were no species occurring in the impacted that did not occur in the control branch.

Figure 5 illustrates end of growing season (1976) vascular plant distribution and dominance within the six vegetation zones. The data was compiled from both the regular sampling program and an end of growing season ground survey (October, 1976). The major features of the plant distributions were persistent throughout the growing

Table 2: Vascular Plant Flora of Yorktown Creek-Marsh (February - October, 1976)

Species	IBTA	CBTA	MBSA	MBHM	IBHM	CBHM
Aster subtulatus	x	x				
Aster tenuifolius				x		
Cyperus erythrorhizos	x	x				x
Cyperus sp.		x				
Distichlis spicata			x	x	x	x
Echinochloa sp.		x				
Eleocharis sp.		x				
Galium tincturium		x				
Hibiscus sp.	x					
Hydrocotyle sp.	x	x				
Kosteletzkya virginica		x				x
Labiatae spp.	x	x				
Pluchea purpurascens						x
Polygonum punctatum	x	x				
Scirpus robustus				x		
Spartina alterniflora			x	x	x	x
Spartina patens			x	x	x	x
Typha angustifolia	x	x				x
Total Species 18	7	11	3	5	3	7

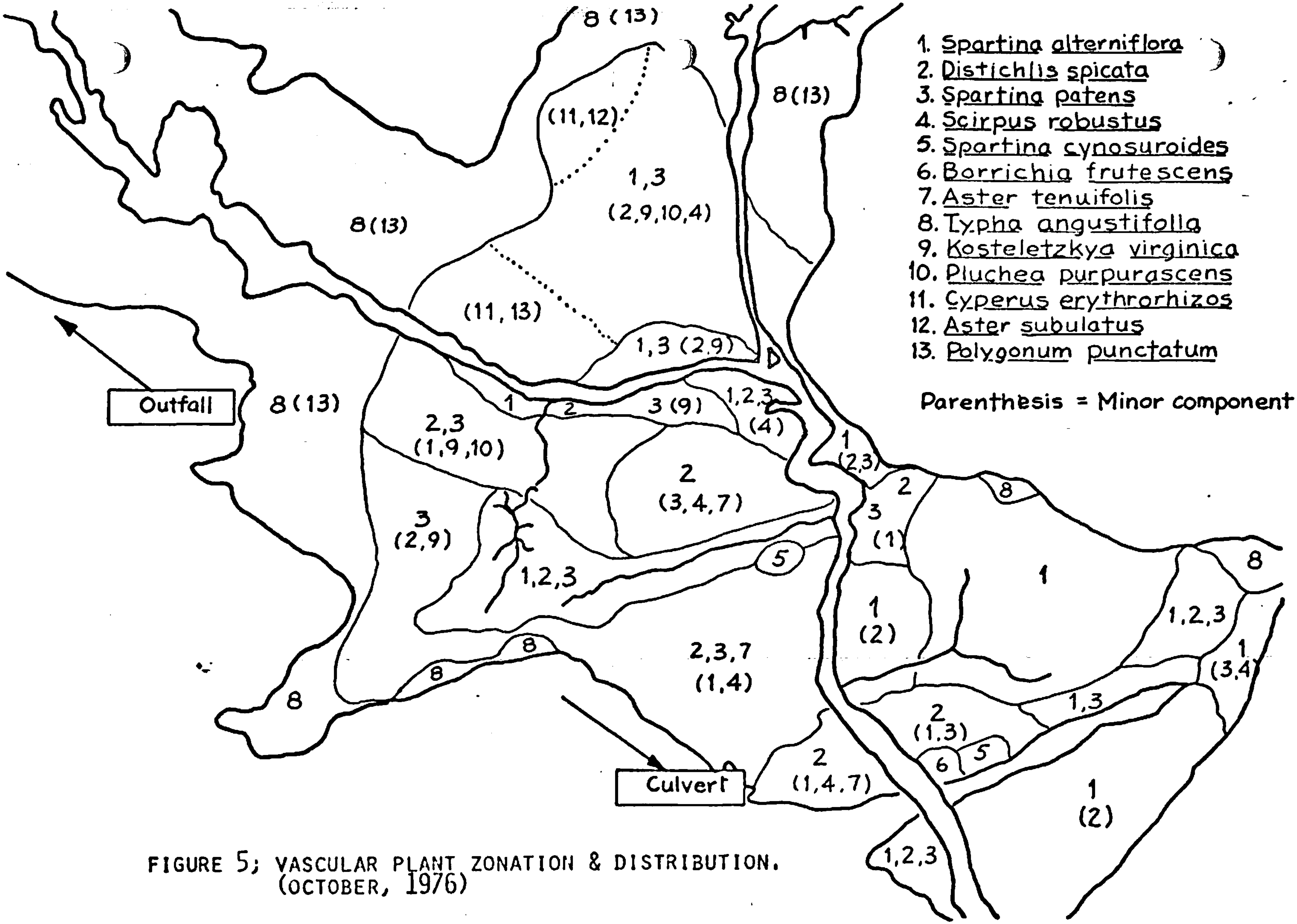


FIGURE 5; VASCULAR PLANT ZONATION & DISTRIBUTION. (OCTOBER, 1976) season and no significant changes in either dominance or zonation were noted between comparable areas in control, mixed or impacted sections. The species distribution reflects both the mixed nature of the entire system and the interactive effects of sewage discharge, reduced tidal flushing and exchange with the York River and elevational changes. The system grades in the upper reaches from freshwater flora (Typha angustifolia) through brackish water species in the middle and mixed section (Distichlis spicata) to typically higher salinity species (S. alterniflora and S. patens).

Table 3 summarizes our measures of aerial net primary production and peak live biomass for the sampling areas and dominant species. The Typha zones of both impacted and control branches dominated production for the entire creek-marsh ecosystem. The Typha communities from both areas account for 79.1% of total systems net aerial primary production while dominating 66.5% of the area. High marsh (IBHM, CBHM, MBHM) and Spartina (MBSA) zones contributed 13.1% and 7.8% of total systems production while occupying 33.5% of the area. Comparison of peak live biomass (PLB) for the dominant species within the zone and total net primary production ($g.m^{-2}.yr^{-1}$) in the Typha zones (IBTA and CBTA) and mixed branch Spartina zone (MBSA) indicates the near total dominance within the respective zones by a single species. Total peak live biomass and net primary production ($g.m^{-2}.yr^{-1}$) in the impacted Typha zone was slightly greater than in the control branch. Peak live biomass and net primary production

Table 3: Aerial Net Primary Production and Peak Live Biomass By Sampling Zone; February to October, 1976

Site	Area (hectares)	Dominant Species	PLB Species	($g.m^{-2}$) Total	NPP Site $g.m^{-2}.yr^{-1}$	Production Area (metric tons . $area^{-1}$ $.yr^{-2}$)
IBTA	3.15	<u>T. angustifolia</u>	651.6	725.4	1506.5	47.4
CBTA	4.55	<u>T. angustifolia</u>	643.0	649.0	1359.3	61.8
IBHM	.88	<u>S. patens</u> <u>D. spicata</u>	333.0 208.0	544.0	641.8	5.68
CBHM	.73	<u>S. patens</u> <u>S. alterniflora</u>	531.3 89.0	560.4	971.2	7.09
MBHM	1.04	<u>S. patens</u> <u>D. spicata</u>	233.0 250.0	507.0	507.0	5.27
MBSA	1.23	<u>S. alterniflora</u>	683.0	683.0	881.7	10.8
TOTAL	11.58 (28.6 acres)					138.0 (152.1 tons)

* PLB = Peak Live Biomass

($g.m^{-2}.yr^{-1}$) within the Spartina zone was similar to other natural areas. Production and peak live biomass within the high marsh zones (IBHM, MBHM, CBHM) was variable for individual species and differed between areas. Total vascular plant production in each of the areas was similar however. Highest annual production (estimated by PLB) was by Spartina patens (531.3 $g.m^{-2}$) growing in association with S. alterniflora. Comparison of S. patens production and peak live biomass within impacted and mixed zones and growing in association with D. spicata indicated higher production in the impacted zone relative to the mixed zone. D. spicata production was higher in the mixed area. Comparison of the two principal areas of S. alterniflora occurence, CBHM growing in association with S. patens and MBSA (a nearly monospecific stand of S. alterniflora), indicated approximately a 10 fold increase in peak biomass in the monospecific stand compared to the mixed branch S. patens - S. alterniflora stand. The only physical separation between the areas was the mixed branch of the creek system.

Figures 6 and 7 indicate the seasonal changes in live and dead vascular plant biomass for the vegetation sampling areas. End of growing season samples were not significantly different between comparable marsh areas for live plant material. However, it appears that both the initiation and duration of the growing season was different for all areas between impacted, mixed and control sites (Fig.6 and 7). The growing season seems to begin earlier and lasts

FIGURE 6; LIVE-DEAD VASCULAR PLANT PRODUCTION:

<u>TYPHA ANGUSTIFOLIA</u> ZONES.

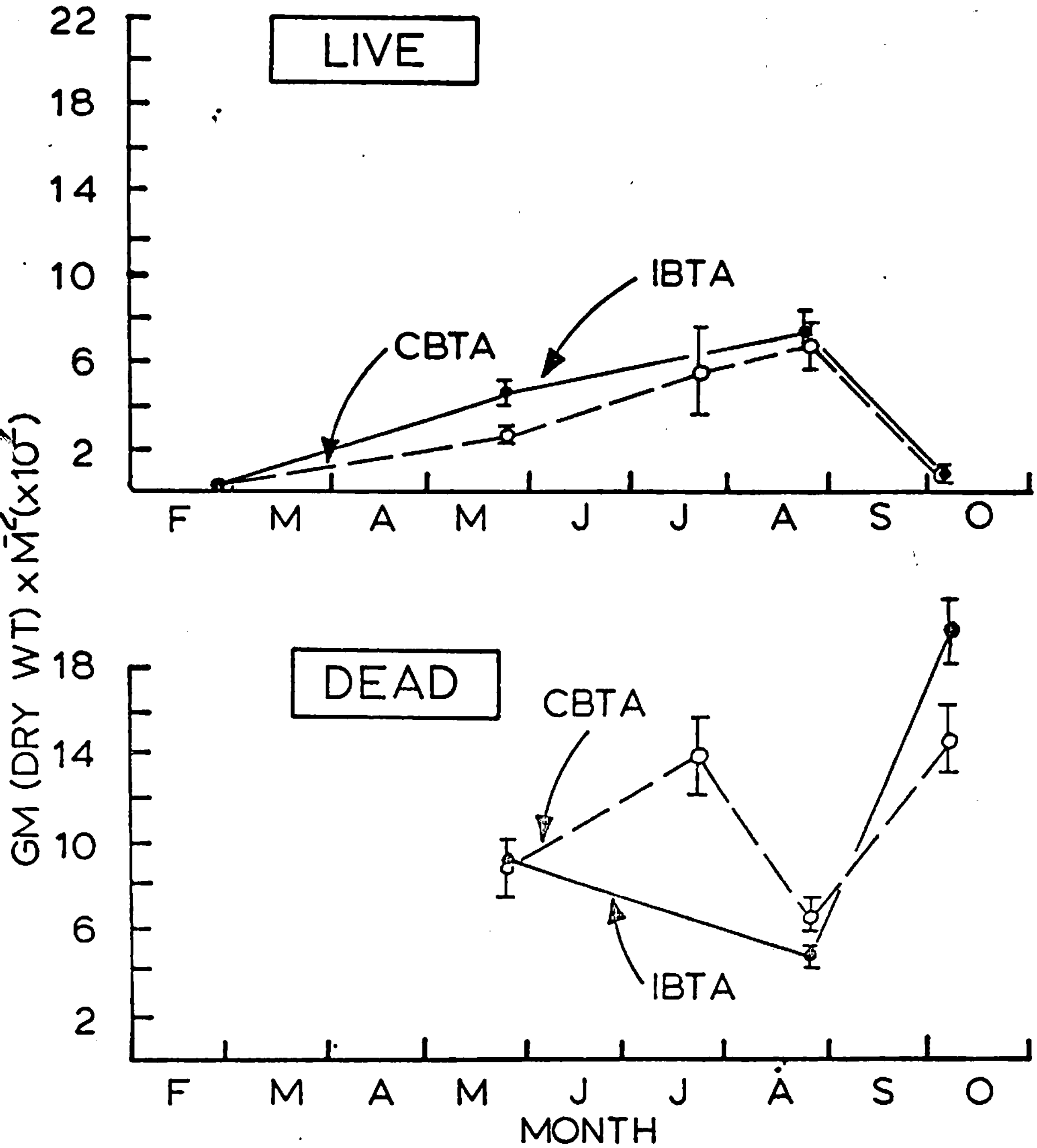
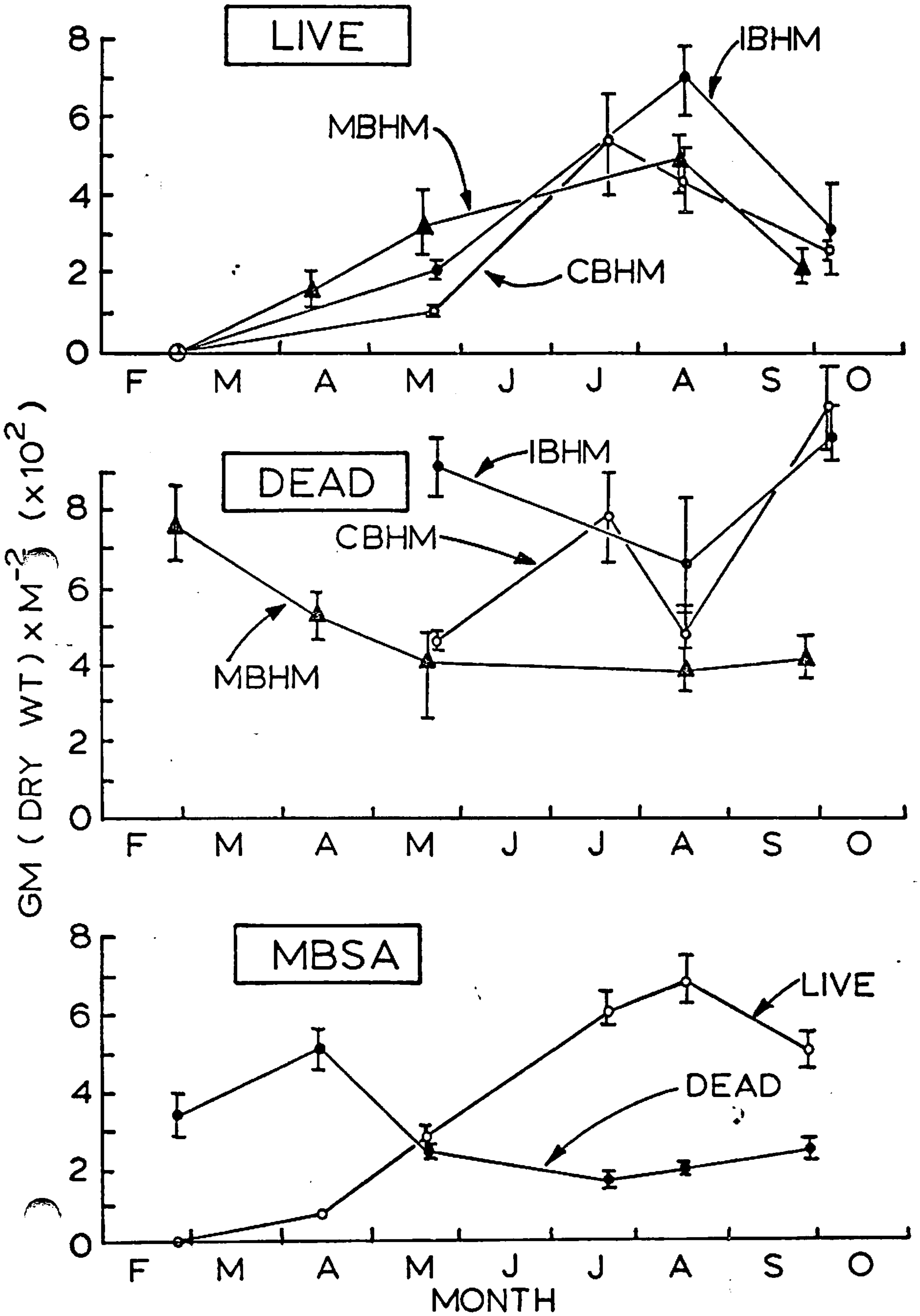


FIGURE 7; LIVE-DEAD VASCULAR PLANT PRODUCTION:
HIGH MARSH & S. ALTERNIFLORA ZONES.



longer in the nutrient impacted areas. Accumulation of dead plant material due to plant mortality begins earlier in the control than impacted sites, substantiating the notion of a somewhat shorter growing season in the un-impacted zones.

Table 4 summarizes stem length data for the predominate plants in the six zones. In most cases, interspecific comparisons of mean stem lengths by month were significantly greater for plants occuring in the nutrient enriched zones.

Benthic Microflora

Sediment chlorophyll a concentrations were used as an indicator of microautotrophic biomass. Sediment chlorophyll a concentrations were sampled in vegetation zones IBTA, IBHM, CBTA, CBHM, MBSA plus the two vegetative transitional zones between *Spartina* and *Typha* areas and designated as IBMV and CBMV for impacted and control branches respectively (see Figure 4). Table 5 summarizes the results of our sediment chlorophyll a measures.

Mean monthly chlorophyll a concentrations in vegetation zones of the impacted branch were compared with corresponding zones of the control branch using Student's - t test (p=0.05). In March, prior to vascular plant growth, chlorophyll a concentrations were not significantly different between either impacted, mixed or control areas or between sampling stations along the branches. However, the March samples were 5-10 times greater than samples taken when vascular

Table 4: A Summary of Mean Stem Lengths for Dominant Flora of Vegetative Sampling Zones.[1]

Species	Month	IBTA	CBTA	IBHM	CBHM	MBHM	MBSA
Typha angustifolia	May	122	121	..	..	..	..
	July	-	145	..	..	..	..
	Aug.	182*	142				
S. Patens	April	..	..	-	-	23	..
	May	..	..	35	-	31	..
	July	..	..	-	40	-	
	Aug.	..	..	38**	33	35	..
	Oct.	..	..	35*	34*	28	
D. Spicata	April	..	..	-	-	14	..
	May	..	..	28**	-	25	..
	Aug.	..	..	37*	-	34	..
	Oct.	..	..	33	32	33	..
S. alterniflora	April	..	..	..	-	19	30**
	May	..	..	..	-	33	47**
	July	..	..	..	44	-	59**
	Aug.	..	..	..	51	43	69**
	Oct.	..	..	..	63	-	73*

1 Significance of mean stem lengths indicated by *; p=0.05 and **; p=0.01 (Student's t-test). Underline indicates significant mean comparisons. A - indicates no data available. A .. indicates not applicable for the particular species.

plants were at or near peak standing crop in the various zones (Table 5). Highest recorded levels of sediment chlorophyll _a_ occurred in March in the lower mixed branch section, _Spartina alterniflora_ zone. A one-way analysis of variance ($p=0.05$) comparing zones in each branch with MBSA indicated it to be significantly higher than any zone during this sampling period.

Sampling during May and June indicated no significant differences between zones except in the high marsh areas (IBHM and CBHM). In May the control branch area of the high marsh was significantly higher than the corresponding area in the impacted branch. This may in part be due to increased shading in the impacted branch due to higher plant biomass (See Figure 7). In June the situation is reversed with significantly higher chlorophyll _a_ levels occurring in the impacted area. Also for the June samples, high and low tide comparisons were made and in all cases, low tide sediment chlorophyll _a_ concentrations were significantly higher than high tide samples ($p=0.05$). No causal explanation for this significant difference was suggested by our data.

Creek and Interstitial Water Nutrients

The results of the water sampling program conducted between October, 1975 and September, 1976 were compared in three manners;1) between stations, 2) between branches, and 3) with regard to seasonal dynamics for NO_3^-, NO_2^-, NH_3, Urea,

Table 5: Sediment Chlorophyll a in Vegetative Zones of Yorktown Creek

chl.a = mg/m^2 mean ± S.D., at low tide

Zone	March	May	June
IBTA	198 ± 20	27 ± 3	
CBTA	-	29 ± 3	
IBMV	255 ± 78	35 ± 17	
CBMV	282 ± 52	32 ± 6	
IBHM	356 ± 199	19 ± 4*	86*
CBHM	352 ± 273	65 ± 10*	43*
MBSA	573 ± 97	23 ± 3	

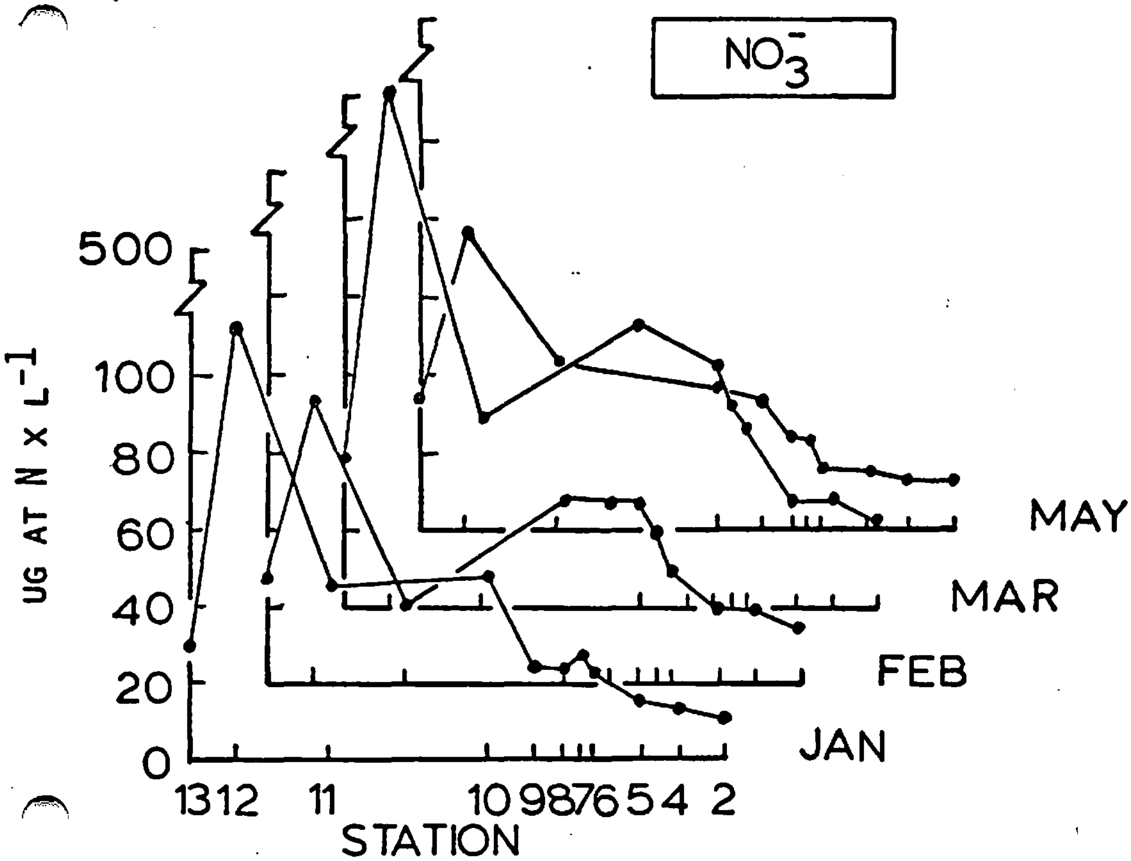
* Significantly different ($p = 0.05$)

and PO_4^{-3}. A one-way, fixed effects analysis of variance ($p=0.05$) was used for statistical comparison among stations and a paired t-test ($p=0.05$) for comparison of zonal classifications. All statistical comparisons were made by month.

Nutrient levels of NO_2^-, NH_3 and Urea among stations in the impacted branch were not significantly different. PO_4^{-3} and NO_3^- concentrations, however consistently showed significant differences among stations and were attributable to the inclusion of station 12 (outfall) in the analysis. Dropping the outfall station from the ANOVA resulted in no significant difference among stations (i.e. stations 11-7). Comparisons among station 17-14 for the control branch and stations 6-4 for the mixed branch indicated no statistically significant differences among stations. It should be pointed out, however, that for NO_2^- and NH_3 there was generally a nutrient gradient proceeding upstream (high) to downstream (low) in the impacted branch. There were always differences between stations 7 (impacted) and 6 (mixed) due to dilution by the control branch. Figures 8-10 illustrate nutrient concentration changes for the impacted and control branches proceeding left to right from the freshwater pond above the outfall (#12) to the York River.

Comparisons of nutrient concentration between the three branches of the marsh were done by lumping stations 7,8,9 and 10 in the impacted branch, stations 4,5,6 in the mixed branch, and stations 14, 15, and 16 in the control branch. For nearly all comparisons (monthly), significant differences between branches were obtained. Nutrient concentrations in

FIGURES 8-10 ; SEASONAL NUTRIENT CONCENTRATIONS; IMPACTED CREEK BRANCHES.



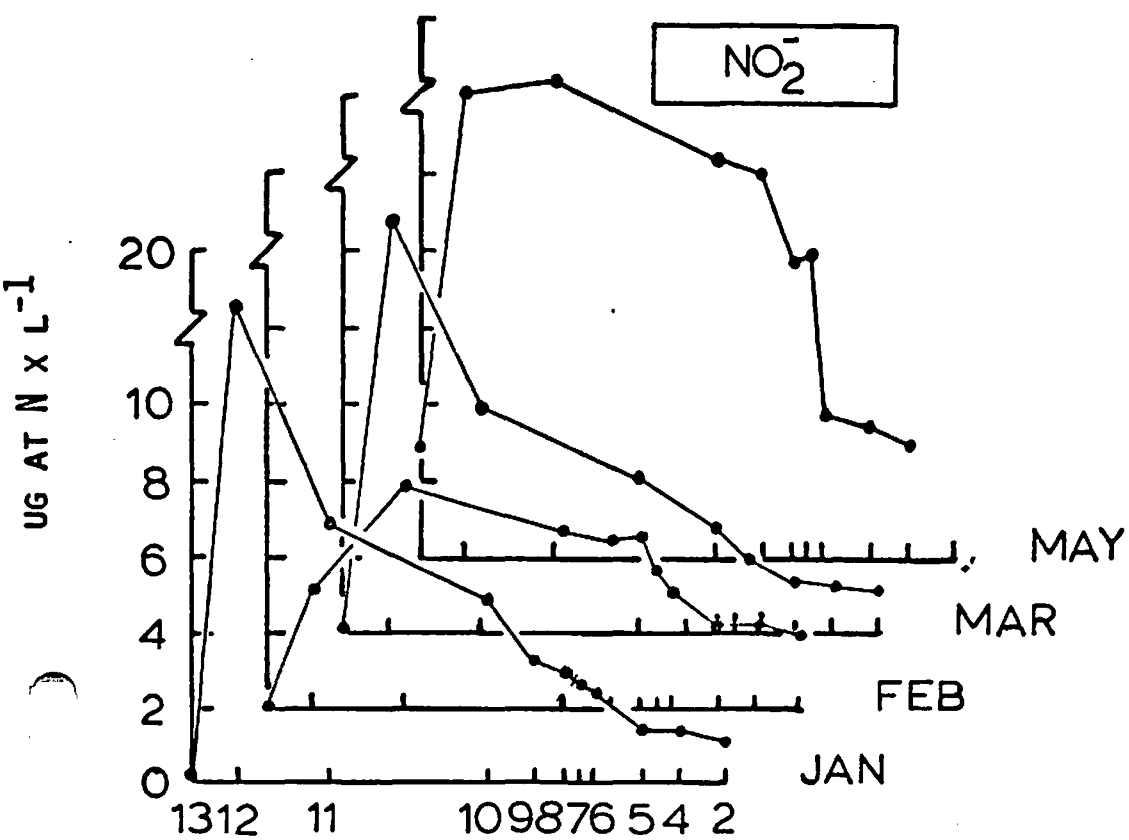
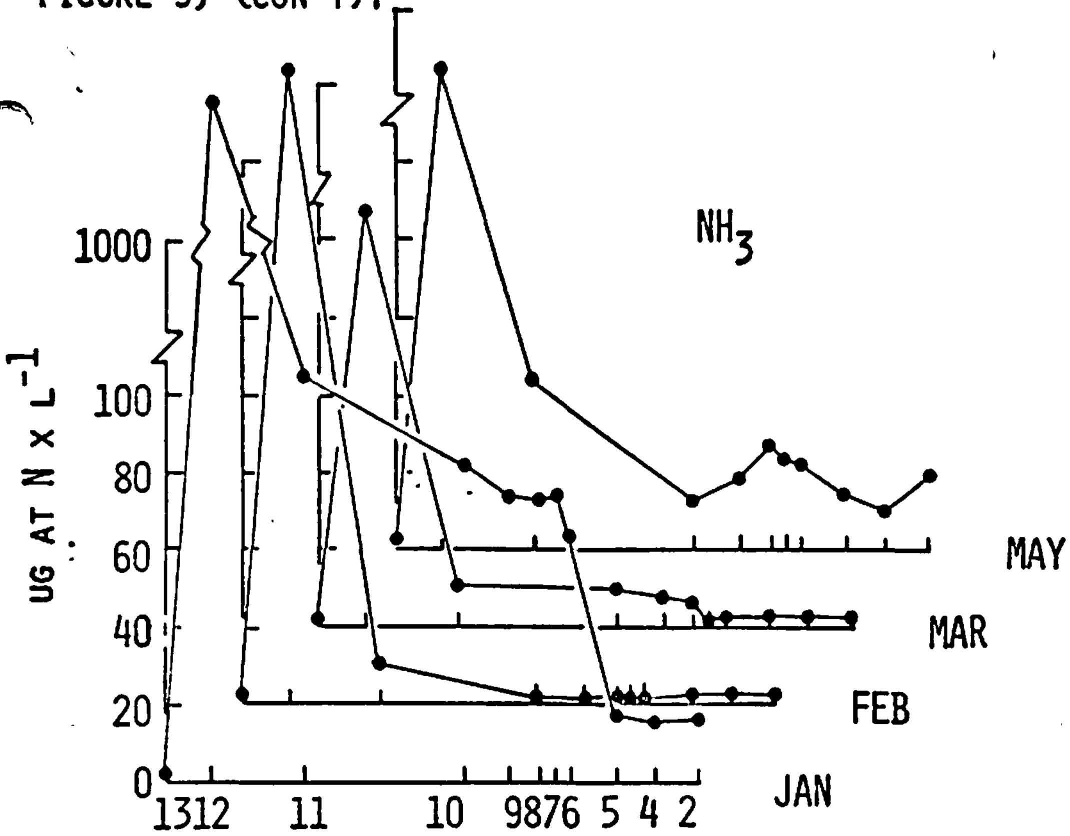


FIGURE 9; (CON'T).



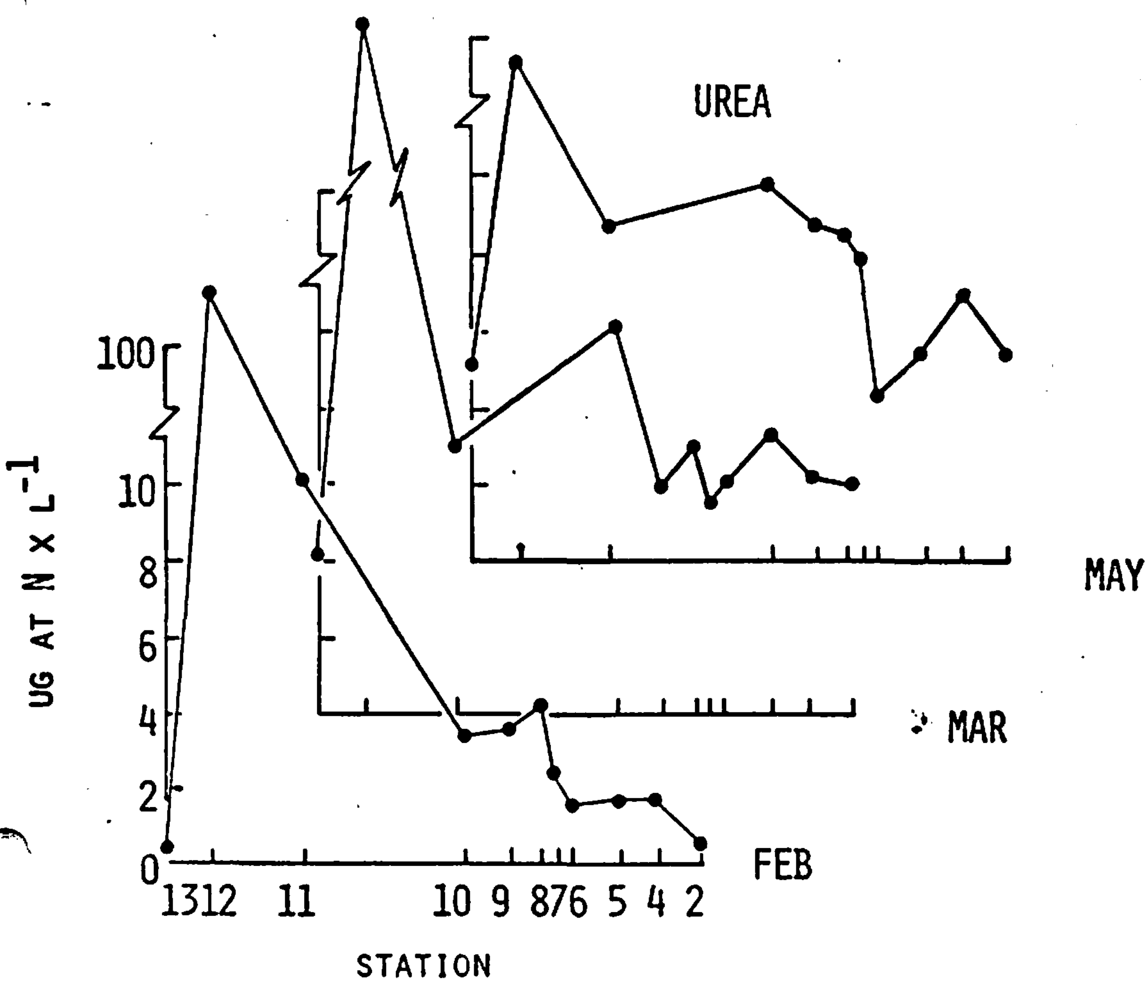
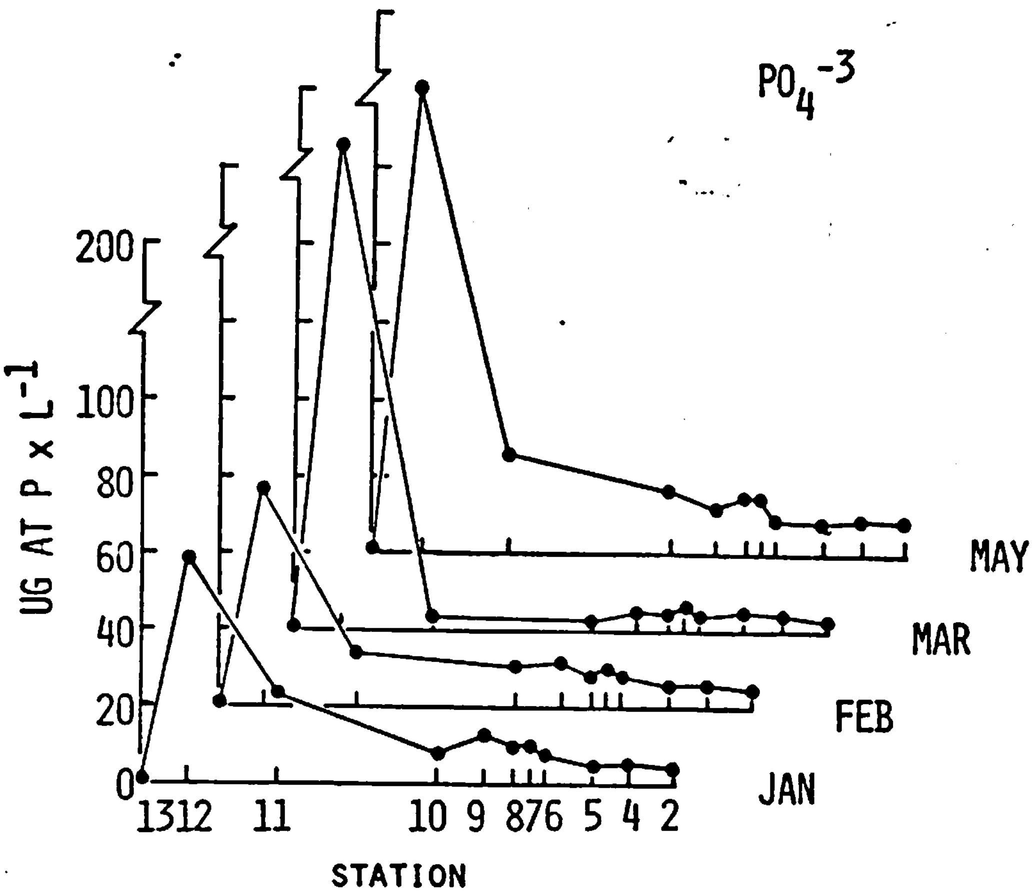


FIGURE 10; (CON'T).



the mixed branch were less than the impacted branch while concentrations in the control branch were always one to two orders of magnitude lower than either.

Water nutrient concentrations were also examined by vegetative zone and statistically compared using a paired-t test (p=0.05). Comparisons were made between similar vegetation zones for impacted and control branches and between different zones along a branch. For the listed nutrient species, all comparisons between similar vegetation zones in impacted and control branches were significantly different. Comparisons along the branches were not. Figures 11-13 illustrate the spatial, temporal and site specific changes in nutrient concentrations for the period November, 1975 through May, 1976. Table 6 summarizes between vegetation zone comparisons.

Figure 14 summarizes the seasonal trends in nutrient concentrations by creek branch. Station 12 (sewage outfall) is plotted separately and represents the predominate nutrient input to the impacted and mixed branches of the Yorktown Creek system. Nitrate and ammonia concentrations in the impacted and mixed branches reflect the pattern of sewage disposal input (station 12) and consistently show a 50 to 90% reduction proceeding downstream from station 12. Because of the similarity in patterns between input and downstream stations and the dissimilarity between impacted and control branches, this reduction in concentration is probably due to physical-hydrological processes (i.e.dilution) rather than

Table 6: Comparison of Water Nutrient Concentrations by Vegetative Zones*

Zone	$\bar{x}$ (ug at x l^{-1})			
	NO_3^-	NO_2^-	NH_3^+	PO_4^{-3}
IBHM	41.18**	5.88**	31.03**	12.07**
CBHM	0.50	0.15	2.33	0.57
N	7	7	7	6
IBMV	36.54**	6.00**	34.80**	12.30**
CBMV	0.47	0.15	2.04	0.38
N	7	7	7	7
IBTA	32.89**	6.95**	39.47**	—
CBTA	0.49	0.17	1.65	—
N	3	3	3	—

* IB__ = Impacted branch
CB__ = Control branch
__HM = High marsh
__MV = Mixed vegetation
__TA = *Typha angustifolia*

** Significantly different (p=0.05)

FIGURE 12; SEASONAL WATER NUTRIENT BEHAVIOR BY VEGETATION ZONE.

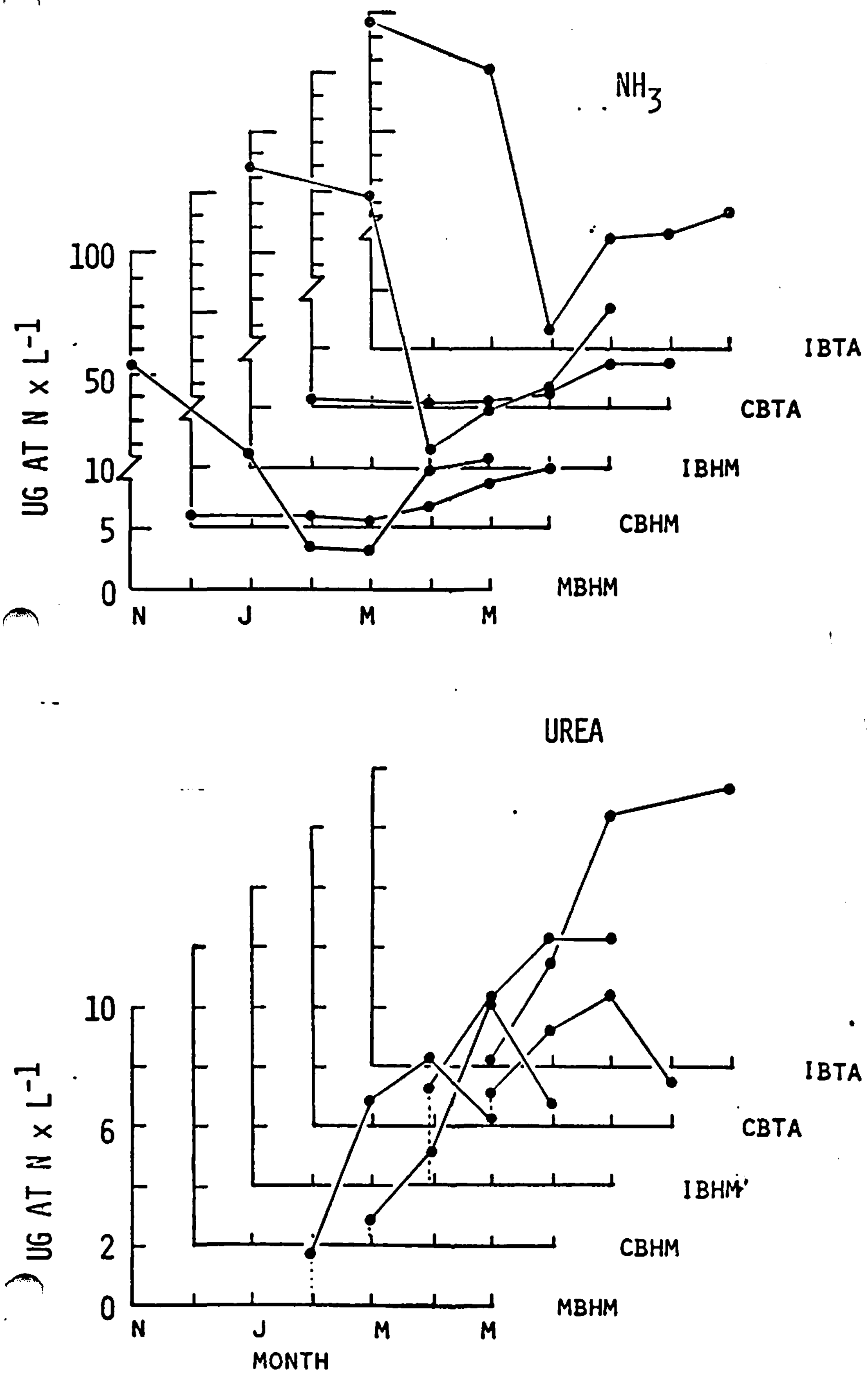


FIGURE 13; SEASONAL WATER NUTRIENT BEHAVIOR BY VEGETATION ZONE.

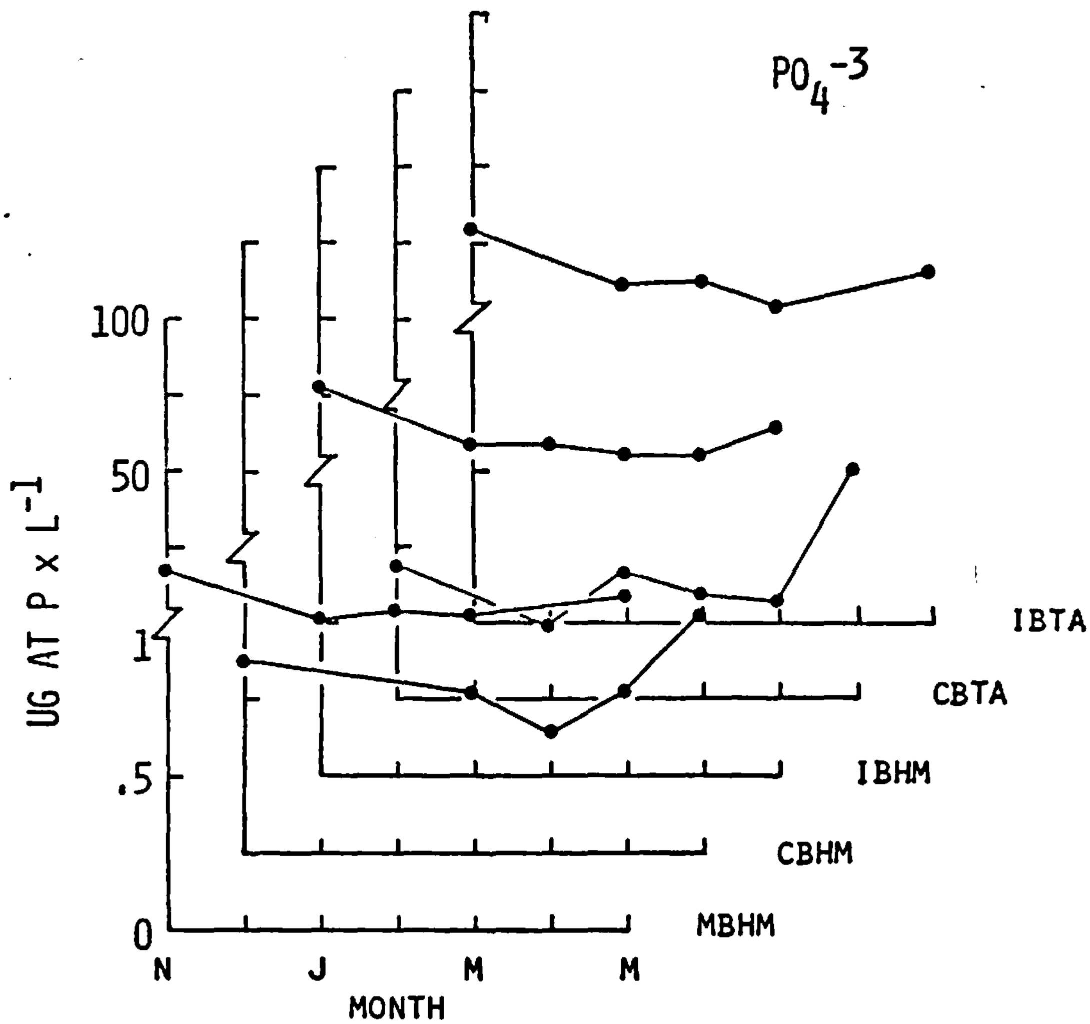
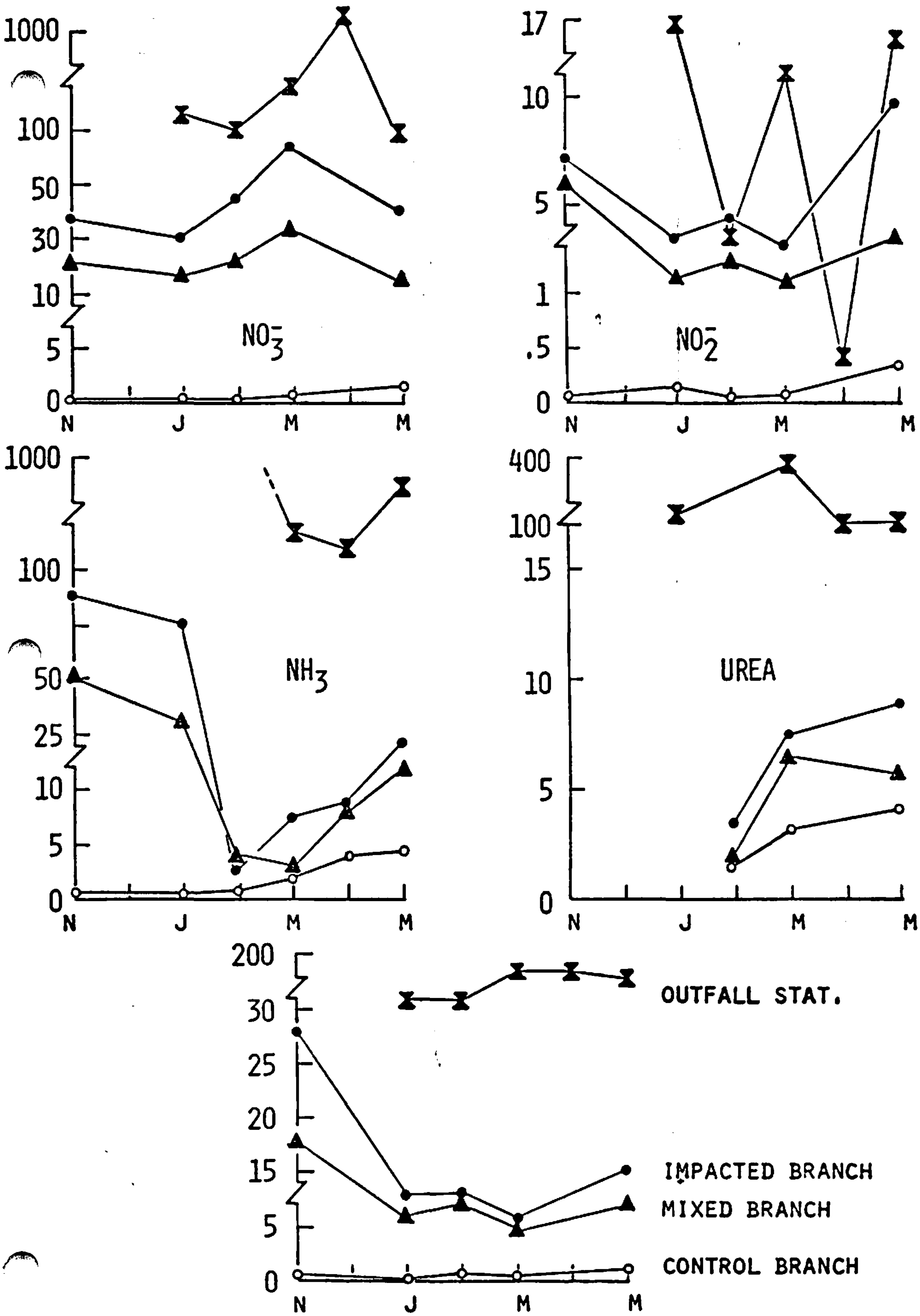


FIGURE 14; SEASONAL WATER NUTRIENT BEHAVIOR BY CREEK BRANCH.*



* ALL CONCENTRATIONS EXPRESSED AS UG AT X L^{-1}.

biologically mediated or controlled processes. The drastic reduction between stations 12 and 11 (1st downstream impacted station) supports this contention (see Figs. 8 and 9). Nitrite, urea and phosphorus do not reflect the input pattern. Nitrite on two occasions actually increased downstream while urea and phosphorous showed behavior similar to the control branch. Absolute concentrations were always higher in the impacted sections.

Table 7 summarizes our analysis of interstitial (pore water) nutrient determinations and chlorinity for the various vegetation zones. The nutrient values were lumped by branch and analyzed by a one-way, fixed effects ANOVA ($p=0.05$). No significant differences among branches resulted. For some parameters, the sample size was too small for valid statistical comparisons. In general, however, the interstitial concentrations did not reflect what would or might have been expected from the creek water analyses. For example, the lowest NO_3^- levels were found in sediments of the impacted *Typha angustifolia* zone where the highest creek water NO_3^- concentrations were found. Highest pore water NO_3^- concentrations were found in the control branch. Nitrite concentrations are similar throughout the creek-marsh system except in the mixed branch, *Spartina alterniflora* zone. Ammonia levels are similar in all areas and are higher in absolute concentration than the other nitrogen species. Chlorinity determinations within the various zones indicate the extreme spatical heterogeneity of the marsh system. As would be expected, soil chlorinity

Table 7: Interstitial Water Nutrients; Yorktown Creek*

Site	CL^{-}**	NO_3^-	NO_2^-	NH_3^+	EN	PO_4^{-3}	N/P
MBHM	218.38 (3)	1.14 (3)	0.59 (1)	6.36 (2)	9.37 (1)	2.41 (3)	24.00 (1)
CBHM	139.70 (2)	1.54 (2)	0.39 (2)	7.47 (2)	9.40 (2)	7.14 (2)	1.26 (2)
IBHM	193.70 (2)	0.58 (2)	0.37 (2)	3.33 (2)	4.28 (2)	2.84 (2)	1.63 (2)
MBSA	226.30 (3)	1.01 (3)	2.30 (1)	3.63 (3)	6.30 (1)	0.38 (2)	14.70 (1)
CBTA	55.60 (2)	1.73 (2)	0.42 (2)	2.12 (1)	5.29 (1)	1.40 (2)	15.40 (1)
IBTA	34.60 (2)	0.41 (2)	0.52 (2)	8.92 (2)	13.91 (1)	47.20 (1)	0.29 (1)

* $\bar{x}$ (µg at x 1^{-1}) Number in parenthesis = N

** Chlorinity expressed as ppm

is highest in the S. alterniflora zone (also closest to the York River) and lowest in the T. angustifolia areas of both impacted and control branches (furthest from direct York River influence). The similarity of soil chlorinity and interstial nutrient concentrations within comparable vegetative zones of control and impacted branches would indicate that the predominate controlling factor with regard to this parameter and possibly vascular plant distribution is tidal exchange-flushing rather than nutrient levels per se.

Benthic Infauna

A qualitative representation of benthic infauna and numerical abundance from various marsh zones is presented in Table 8. Because of the considerable processing time required for such analyses only one set of cores could be worked up within the time frame of this preliminary study. The cores were taken immediately after sewage discharge was stoped (July, 1976). The main difference between areas was the presence of blue-green's in the nutrient enriched areas and the dominance of diatoms in benthic algae mats in the control areas. No statistical comparisons can be made with regard to benthic infaunal or epifaunal composition without further study although one might expect, based on the differences in microfloral dominance, that the two areas would differ in species composition.

Table 8: Numerical Abundance (#/core) and observations on Benthic Infauna, Epifauna and Microflora in Yorktown Creek

Zone	Nematodes	Copepods	Amphipods	Oligochaetes	Arachnids	Protozoa	Dipteran larvae	Gastropods	Total	Dominant Microflora
IBTA										
top	70	26	1		6	5	3		111	Blue-green algae
bottom	114	32		15		1			162	
IBMV										
top	106	41	2				5	3	157	Blue-green algae
CBMV										
top	34	26	4			4			66	Diatoms
bottom	112	59							171	
CBHM										
top	62	37	3				15		117	Diatoms

Nitrogen Fixation Studies

Nitrogen fixation occurred in all the areas where blue-green or floating algal mats were present. Most of the mud surface cores showed only low or slight activity. One area of surface mud cores in the impacted area showed fixation for all samples taken at the site with the average being 101.46 ug N x gm dry sed^{-1} x hr^{-1} (545.33 ug N x m^{-2} x hr^{-1}). The overall average being 25.365 ug N x gm dry sed^{-1} x hr^{-1} (136.33 ug N x m^{-2} x hr^{-}) for intertidal sediments. Although nitrogen was not fixed uniformly, the rates were higher than the average, 8.9 ug N x gm dry sed^{-1} x hr^{-1}, found by Marsho, et al., (1975) and fell into the range of values (41.9 to 807 ug N x m^{-2} x hr^{-1}) for intertidal marsh sediments found by Whitney, et al., (1975). N_2 fixation for vegetation samples were low and only one sample showed activity (18.99 ug N x gm dry sed^{-1} x hr^{-1}).

High N_2 fixation occurred on intertidal sediments of the control area that were covered by mats of blue-green algae. Rates for the mat ranged from 974 to 2895 ug N x m^{-2} x hr^{-1} with an average of 1882 ug N x m^{-2} x hr^{-1}. These values are significantly higher than the values reported by Van Raalte, et al., (1974). The Yorktown rates fall in the upper range of values given by Whitney et al. (1975) of 260-8910 ug N x m^{-2} x hr^{-1}. The floating algal mats fixed nitrogen at the highest rates and the low "dark" values suggest that the principal source of fixation is algal and

Table 9: Yorktown Creek Nitrogen Fixation (June, 1976)

I. Transect XI5 (Impacted Branch)

A. Intertidal Mud

1. 54.8 ugN x g dry sed.$^{-1}$x hr^{-1}
2. 46.0 " " "
3. 203.6 " " "

B. Floating Algae (ponds)

1. Light; 2127 ugN x m^{-2}x hr^{-1}
2. Dark ; 264 " " "

II. Transect XC5 (Control Branch)

A. Blue-green mat (surface)

1. $\bar{x}$ = 1881.5 ugN x m^{-2}x hr^{-1}

B. Floating Algae (ponds)

1. 1459 ugN x m^{-2}x hr^{-1}
2. 2933 " " "

III. High Marsh Rhizomes & Mud Transect XI6

1. 0.00 ugN x g dry sed.$^{-1}$x hr^{-1}
2. 18.99 " " "

and not bacterial. There was no significant differences in fixation rates between control and impacted sites.

Based on these studies, a rough estimate for overall N_2 fixation in Yorktown is 17.54 ug N x m^{-2} x day^{-1} which is considerably higher than reported values. Marsho, et al., (1973) reports an average value of 0.6 - 0.7 ug $N.m^{-2}.day^{-1}$ and Whitney, et al. (1975) reports an average rate of 4.6 ug $N.m^{-2}.day^{-1}$. Table 9 summarizes the N_2 fixation studies.

Diel Mass Balance Study; June 15-16, 1976

Two diel mass balance studies were completed during our preliminary investigations; June 15-16, 1976 and August 4-5, 1976. We report here the results of our first study, June 1976, prior to relaxation of the sewage input.

Figure 15 illustrates the atypical nature of the tidal pattern for Yorktown Creek. Highway and culvert construction across the mouth of Yorktown Creek has obviously changed the hydrodynamic behavior of the creek-marsh system. During this study, no reverse in creek flow (marsh flooding) was observed due to tidal inundation; i.e. creek flow was unidirectional and always out of the creek system to the York River. Current velocities ranged from 30 to 120 cm x sec^{-1} in the impacted branch, 10 - 70 cm x sec^{-1} in the control branch, and 0 to 50 at the culvert. We have observed, however, creek flow reversal and marsh flooding during the course of our routine sampling. We do not know at this time, what tidal and/or creek flow conditions are necessary for inundation to occur or how frequently it may happen.

FIGURE 15; RELATIVE TIDE HEIGHTS; DIEL STUDY, JUNE 15-16, 1976.

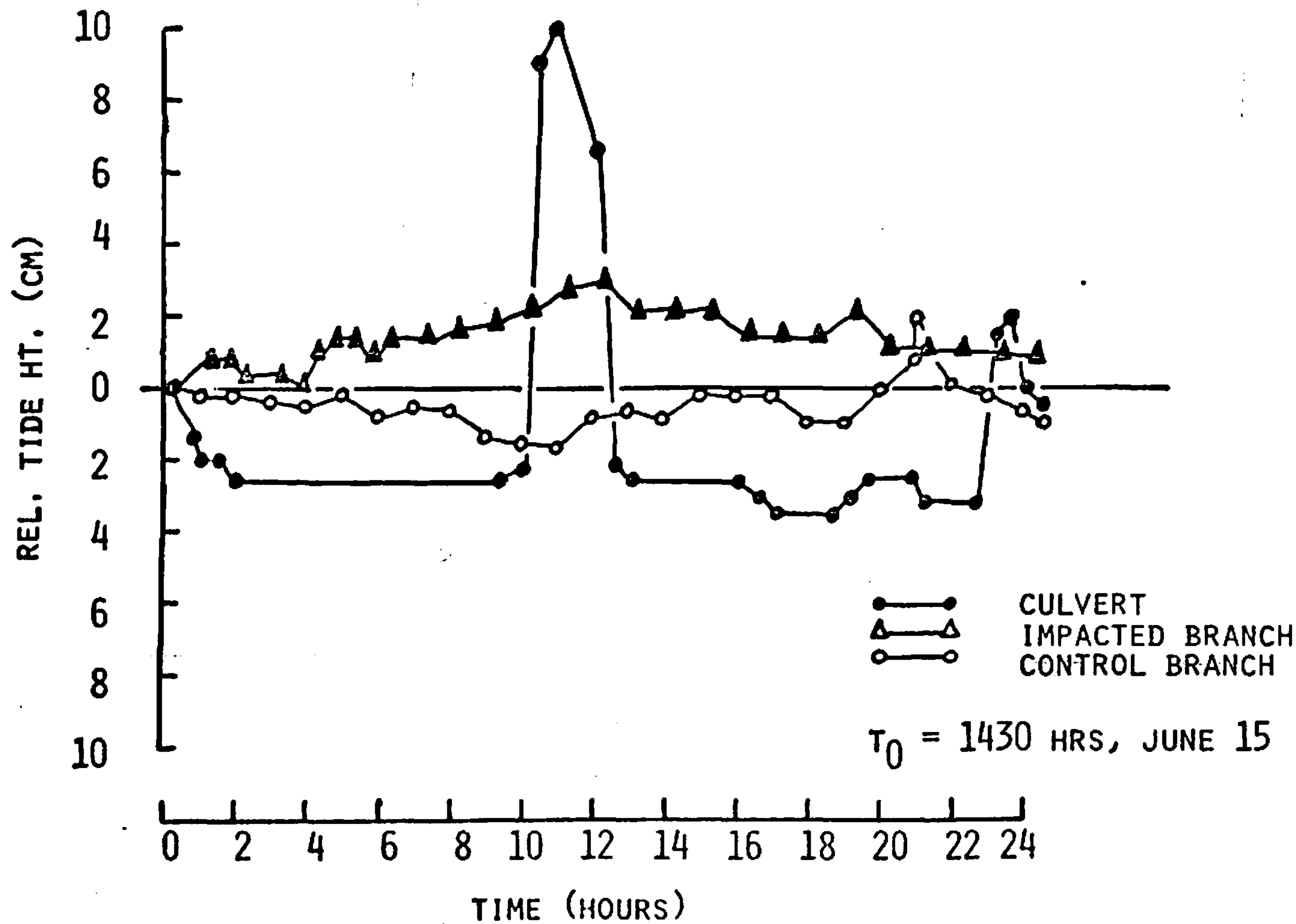


Figure 16 illustrates the diel behavior of NO_3^-, NO_2^-, NH_3 at the disposal outfall for the June 24-hour study. No apparent cyclic behavior was noted although there was a general tendency for NO_3^- and NH_3 to vary inversely with nitrate reaching maximums during the day and ammonia reaching minimums. Together, nitrate and ammonia dominated dissolved inorganic nitrogen input and accounted for over 90% of the total (see Figure 16). Contribution to total input from the sewage outfall was approximately equal for the two nitrogen species with nitrate slightly greater.

Figure 17 illustrates the diel behavior of NO_3^-, NO_2^- and NH_3 at the Yorktown Creek culvert for the June study. There was apparent cyclic behavior in the concentrations of nitrate and ammonia at the culvert station. Nitrate maximums coincided with ammonia minimums. However, as opposed to the outfall station, ammonia was always higher than nitrate and varied by a factor of 10 to 20 times greater. Ammonia-nitrogen dominated output from the creek system while nitrate-nitrogen predominated the inorganic nitrogen input. As with the outfall station, there appeared to be an inverse correlation between nitrate and ammonia concentrations. Figure 18 illustrates the linear regression and simple linear corelation between nitrate and ammonia at the culvert station.

ATP and chlorophyll _a_ were also measured at the culvert station. No apparent cyclic behavior or correlation with other measured parameters was observed. ATP varied between 2.4

FIGURE 16; DIEL NUTRIENT FLUX; JUNE 15-16, 1976. YORKTOWN SEWAGE DISPOSAL OUTFALL (STATION 12).

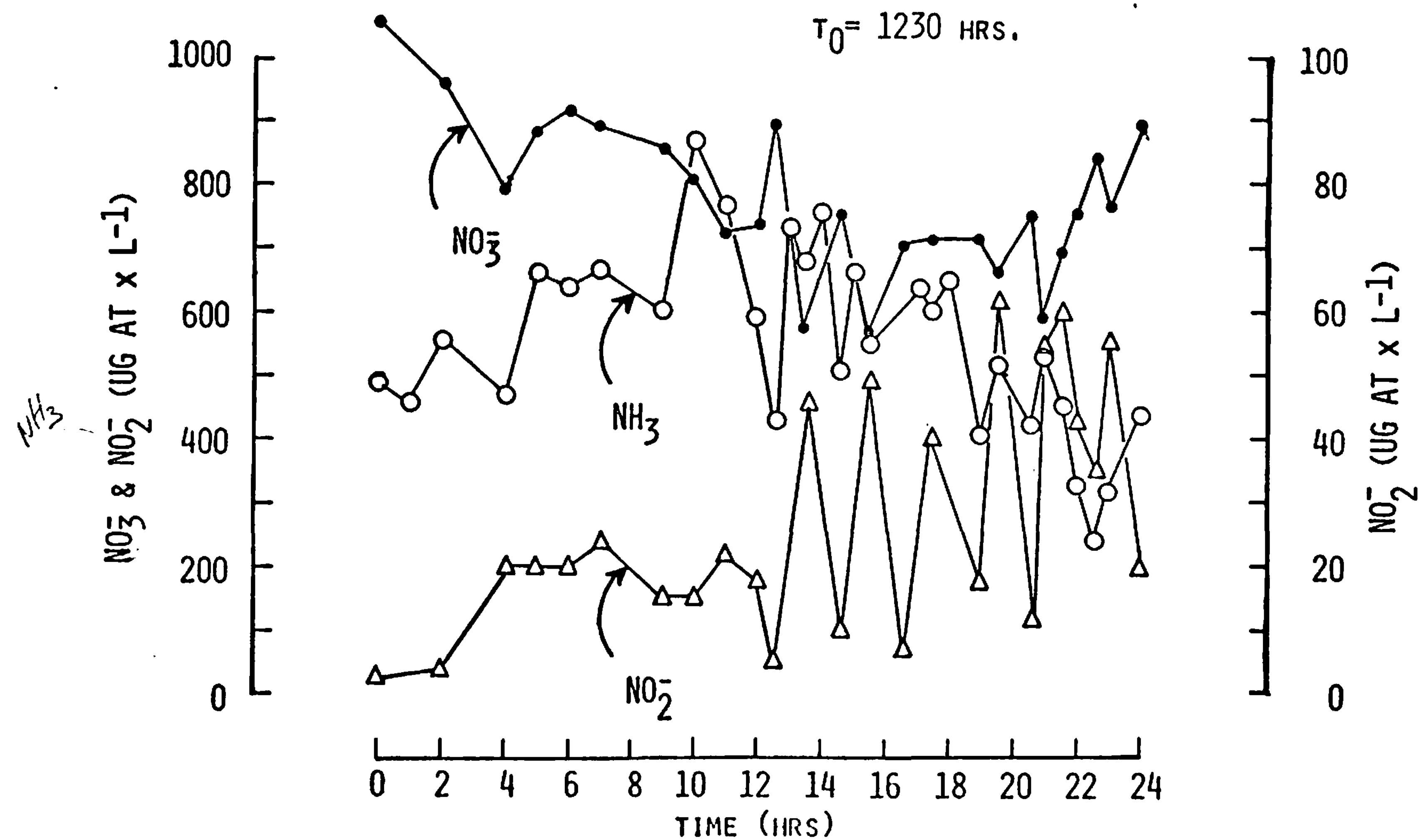


FIGURE 17; DIEL NUTRIENT FLUX, JUNE 15-16, 1976;
YORKTOWN CREEK CULVERT.

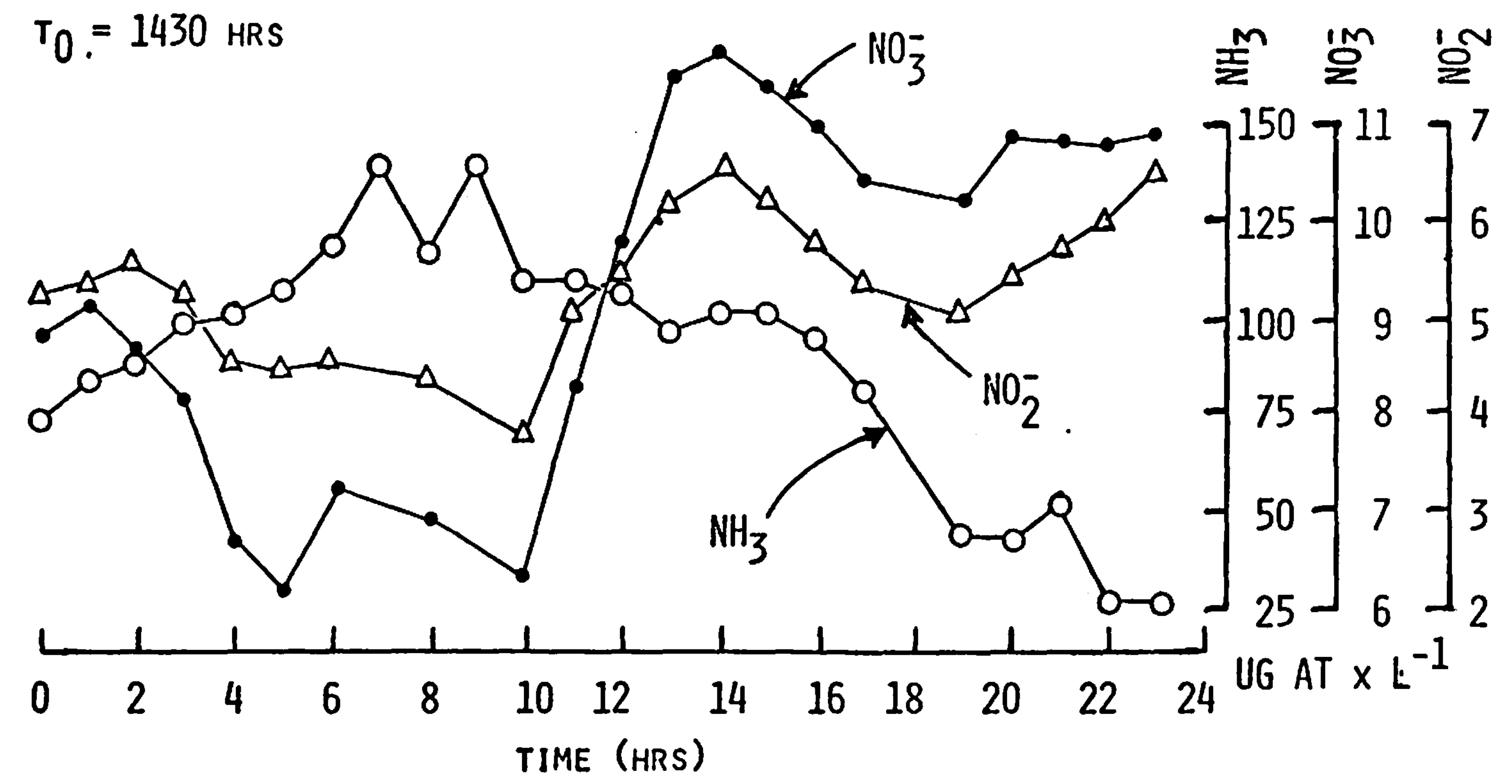
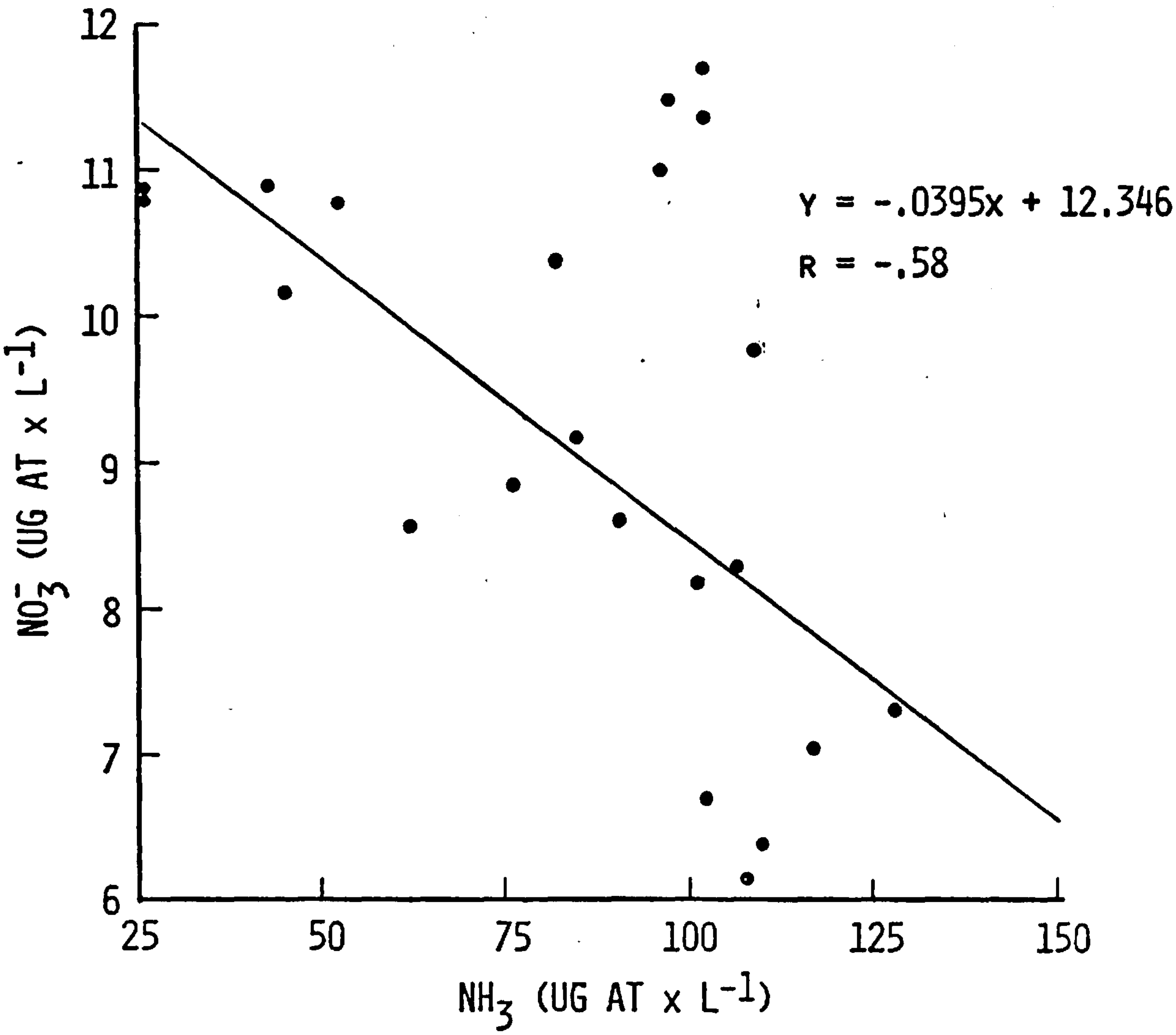


FIGURE 18; SIMPLE LINEAR REGRESSION AND CORRELATION OF AMMONIA VS. NITRATE CONCENTRATION; DIEL STUDY JUNE 15-16, 1976, YORKTOWN CREEK CULVERT.



and 4.0 ug ATP x l^{-1} ($\bar{x}$ = 3.00 $\pm$ 0.49) and chlorophyll a ranged from approximately 1.0 to 6.00 ug x l^{-1} ($\bar{x}$ = 2.49 $\pm$ 1.82).

Table 10 summarizes our mass balance calculations for the June study. Failure of two current meter/recording devices prevents us from making precise water volume transport calculations. The figures presented in Table 10 are thus approximate and may be as much as 50% in error. We were unable to obtain transport data in one of the creek branches and one of the three culvert discharge pipes. The one discharge pipe that we were unable to monitor for transport was minor compared to the other two and on the occasions when we were able to measure current velocities, transport through the pipe was generally less than 10% of the total culvert discharge. This error and errors associated with cross-sectional area measurements probably put our estimate of total systems discharge within 20%. Calculation of input from the sewage outfall was taken as the average annual daily discharge (from plant operation records) times the mean 24 hour concentration of the nutrient.

Based on the above, for the June 15-16 study, the sewage discharge input accounted for approximately 50% of the total output or export from the creek-marsh system in terms of inorganic nitrogen and 20% for phosphorous. As mentioned, the creek system was continuously flowing out of the marsh and therefore only exported material. Export from the creek system exceeded outfall input for all nutrient species. The

Table 10: Summary of Nutrient Mass-Balance Calculations, June 15-16, 1976 Diel Study*

		NO_3^-	NO_2^-	NH_3	PO_4^{-3}
I. Sewage outfall					
($\bar{x}$ daily discharge = $2.68 \times 10^2 m^3$)	$\bar{x}$ (μg at x l^{-1})	720.	38.3	489.	56.2
	s $\bar{x}$	27.	11.1	7.5	1.8
	n	12	12	15	15
	daily output (gm)	2704.	144.3	1835.	468.
	% EN	57.7	3.1	39.2	-
	EN (kg)	4.68			
II. Culvert (Yorktown Creek mouth)					
discharge 15-16 June = $7.86 \times 10^3 m^3$	daily output (gm)	995.4	573.2	10227.	2258.
	% EN	8.4	4.9	86.7	-
	EN (kg)	11.8			

* EN = $\left[NO_3^- + NO_2^- + NH_3\right]$

major change noted was, while nitrate dominated outfall input, ammonia accounted for nearly 90% of total nitrogen export (Table 10).

DISCUSSION

Vascular Plant Study

The vascular plant sampling design was intended to determine production-nutrient relationships within the major marsh communities (high, low, and *Typha sp*. marsh), while minimizing differences in species composition and hydrography between comparable sites in the three experimental areas. Late winter (1975-1976) field observations of dead plant material were made to assess areas of generally similar species composition. In the absence of quantitative hydrographic data, a somewhat arbitrary decision was made for sampling site placement. Comparable sites were placed equidistant up/down-stream from the creek branching point and equidistant inland such that species composition would be similar on the basis of the late winter survey. The 1976 end of growing season survey indicated no strictly similar areas of species composition between areas. Further, creek flow measurements indicated hydrographic differences between sampling areas. The placement of the sites therefore included only the major macrophytic communities of each strata without reference to our initial criteria. Elevation and tidal inundation studies at each site are needed to further quantify hydrological relations for macrophyte distribution and species composition.

The production results for Yorktown Creek are within the range of results reported in the literature (Table 11). Among high marsh sites, greater production is shown in CBHM. This is surprising in light of several studies showing standing crop and production increases for halophytes receiving N and N/P fertilization (Gosselink, 1970; Broome, et al., 1973; Sullivan and Daiber, 1974; Valiela and Teal, 1974). Other factors such as tidal inundation, marsh elevation and soil salinity may have an overriding effect on available nutrients in limiting production for the Yorktown marsh.

There were no apparent seasonal changes in creek water nutrients that could be attributed to uptake due to vascular plant growth. During the major growth period, March to May, NO_3^- concentrations decreased while NO_2^-, NH_3, Urea and PO_4^{-3} concentrations increased in the impacted and mixed branches and all measured nutrient species in the control branch increased in total concentration (See Fig. 14). From these data, it would seem measures of creek water nutrient concentrations are poor indicators for identifying active periods or mechanisms of nutrient exchange processes. We attempted to resolve this problem, at least in part, by calculating a relative measure of nutrient change as creek water traversed a section of marsh. The relative percent change was calculated as:

Table 11: Net Primary Production of Vascular Plants in Various Marsh Systems

Location	Species	NPP g/m^2	Reference
Georgia	*S. alterniflora*	643.2-1098.0	Smalley, 1958
N. Carolina	*S. alterniflora*	610-1300	Marshall, 1970
N. Carolina	*S. alterniflora*	650	Williams, Murdock; 1969
Virginia	*S. alterniflora*	695-1570	Wass, Wright; 1969
Virginia	*S. patens*	805	Wass, Wright; 1969
Virginia	*D. spicata*	360	Wass, Wright; 1969
N. Carolina	*S. patens*	1300	Waits, 1967
N. Carolina	*S. patens/D. spicata*	1320	Waits, 1967
New York	*S. patens*	424-547	Udell, et al., 1969
New York	*D. spicata*	523-773	Udell, et al., 1969
Great Britain	*T. angustifolia*	1445	Mason, Bryant 1975

$$\%\Delta = \pm\ (Xd-Xu\ /\ Xu)_t \times 100$$

where $\%\Delta$= Relative change (%)

Xd= concentration of given nutrient at downstream station

Xu= concentration of given nutrient at upstream station

t= month of collection

According to the sign convention, a '+'$\%\Delta$ would indicate a downstream increase in the given nutrient concentration and a '-' $\%\Delta$ would indicate a downstream decrease. Three marsh traverses $\%\Delta$'s were calculated:

Impacted Traverses using Xu=station 11 and Xd=station 6;
Mixed Traverses using Xu=station 6 and Xd=station 2;
Control Traverses using Xu=station 17 and Xd=station 14.

Figures 19 and 20 illustrate the seasonal changes in $\%\Delta$ for the three traverses. It is apparent from the figures the differences in behavior between impacted and control traverses. In the control marsh-creek branch, NO_3^- is always reduced while NO_2^-, NH_3, Urea, and PO_4^{-3} show variable behavior but consistently increase in concentration downstream during the early growing season. In the impacted area, NO_3^- behavior is variable while NO_2^-, NH_3, Urea and PO_4^{-3} consistently decrease in concentration downstream. This suggests a fundamental change in processes controlling nutrient flux in the impacted branch compared to the control branch. If vascular plants, either through active metabolic uptake or by modification of soil-nutrient relations during growth, influence the observed behavior, the data suggests a

FIGURE 19; RELATIVE CHANGE IN DISSOLVED NUTRIENT CONCENTRATIONS BY CREEK BRANCH.

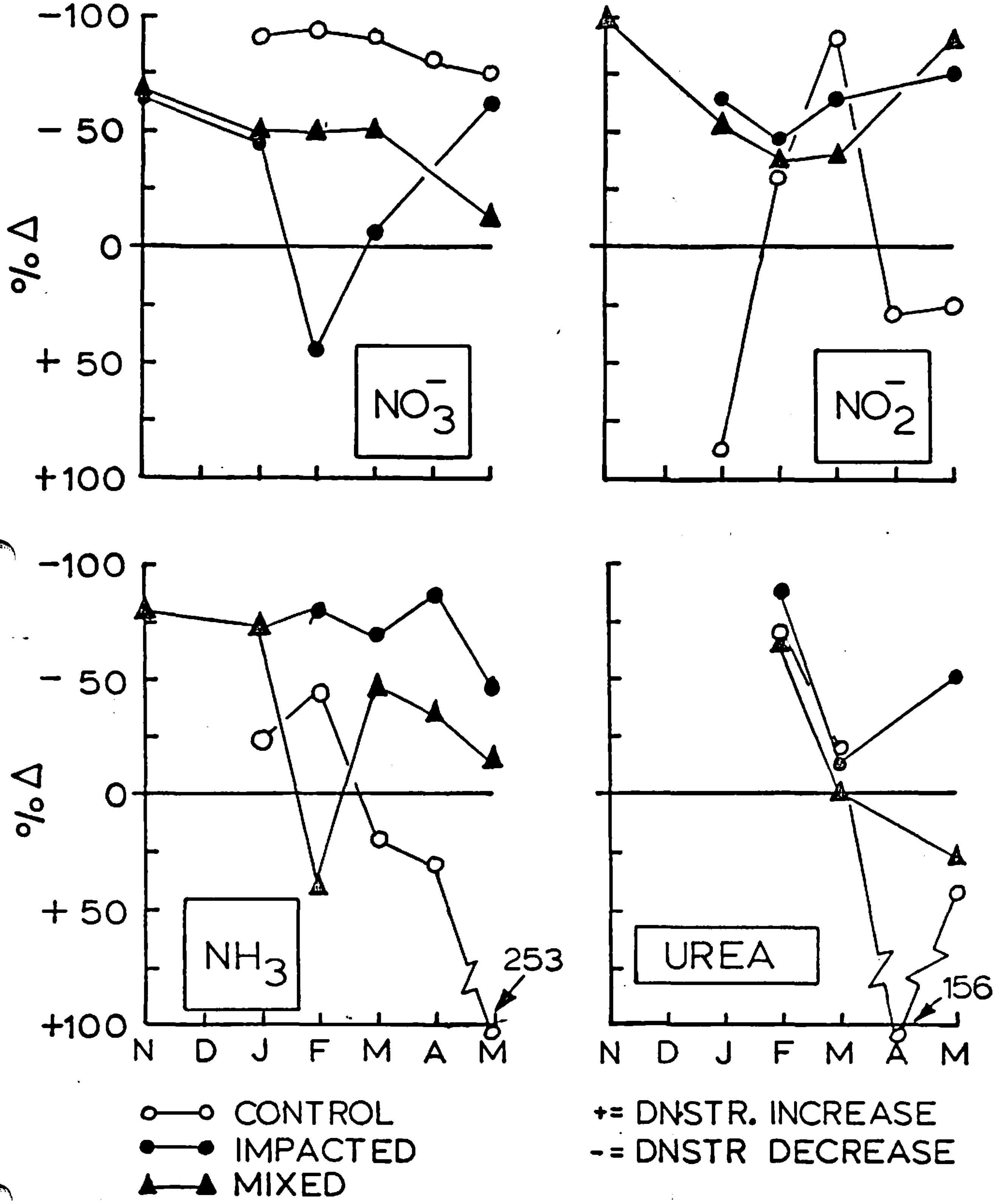
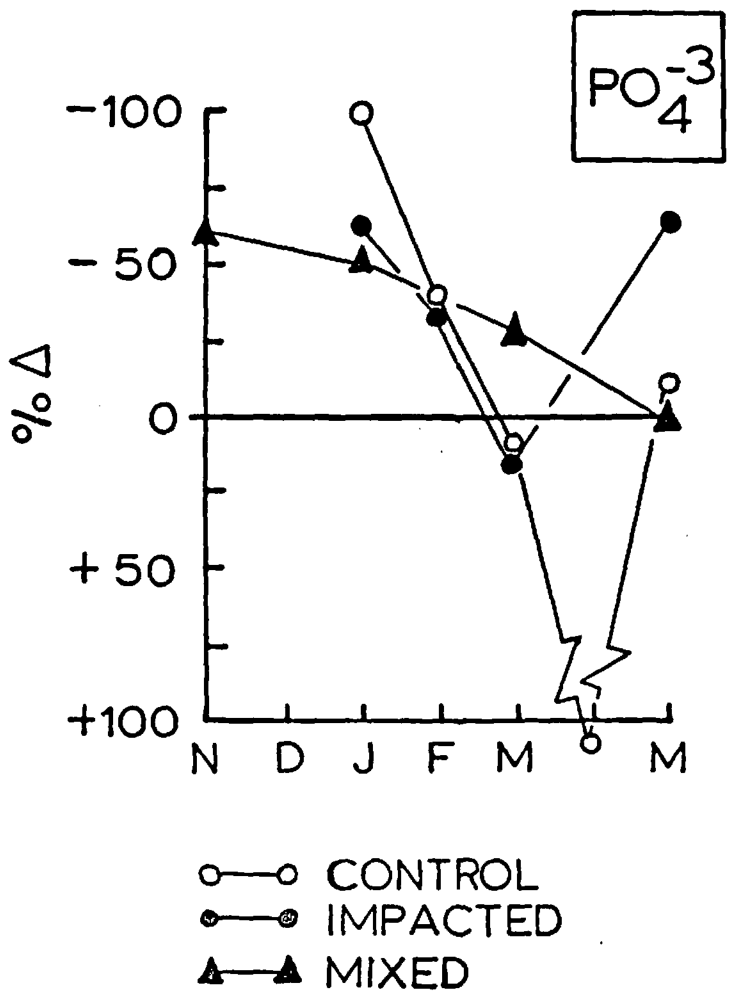


FIGURE 20; RELATIVE CHANGE IN DISSOLVED NUTRIENT CONCENTRATIONS BY CREEK BRANCH.



shift in nitrogen preference for uptake; i.e. NO_3^- in the control area (low nitrogen levels) and NO_2^- and NH_3 in the impacted branch (high nitrogen levels). Attributing this directly to vascular plant growth of course is tenuous. In all probability, the result is the product of combined effects due to changes in sediment soil nutrient relationships, either physically or biologically mediated, soil microflora and vascular plants.

Although annual production in the impacted and control marshes was approximately equal, plant growth characteristics were different. For the Typha areas, the growing season and initiation of plant flowering occured approximately a month earlier in the impacted marsh. Growth form was also different. In the enriched marsh areas, stem lengths were significantly greater than in comparable un-enriched areas, while biomass on an areal basis was similiar (See Table 4). These data suggest and support the above contention that there has been a fundamental shift in nutrient exchange processes and perhaps in plant nutrient metabolism.

It was apparent from aerial photography and ground surveys that a spatial successional sequence also exists in each branch of Yorktown Creek. A typical successional sere sequence is followed (Waisel, 1972). Proceeding upstream, a Spartina-Distichlis sere is followed by a Typha sere which blends gradually into a woody upland sere (including a hardwood climax at the extreme upstream reaches). The spatial successional sequence may reflect temporal succession, although no definitive conclusions can be drawn without further study. If a temporal sequence does exist, the available evidence (greater species richness, more freshwater species, and encroachments of Typha in CBHM) would indicate that the control branch is further advanced on such a successional sequence.

A final, notable feature of vascular plant distribution in the Yorktown marsh is the absence in the control branch of D. spicata as a dominant species (Figure 5). The seeds of the three dominant species in the Yorktown Creek system (S. alterniflora, S. patens, and D. spicata) show a significant, positive-percent germination response to a low temperature thermoperiod (4 C for approximately 4 weeks) after ripening (Amen, et al., 1970; Broome, et al., 1973). However, the percent germination response of D. spicata can also be increased by high substrate nitrate levels (~2 M), with or without a preceeding low temperature period (Amen, et al., 1970). Conceivably, a germination response to nitrate levels could increase D. spicata seedling production, thereby increasing adult biomass early in the growing season. In areas of great spatial competition between D. spicata and S. patens, such a response could result in a D. spicata dominated area which otherwise might have been mixed species or a monospecific S. patens area.

Further quantification of hydrographic conditions in the marsh, and laboratory studies of the germination response of all three species of plants to inorganic nutrient levels are needed.

Benthic Microflora Study

No significant differences between impacted and control branches in the high marsh or mixed vegetation zones of Yorktown Creek were observed prior to the onset of vascular plant growth. Recent studies have indicated that chlorophyll a concentrations in the sediment increase with the addition of nitrogen during the period March-May (Sullivan and Daiber, 1975). They report values of 155 mg/m^2 during this season; values in Yorktown Creek were nearly double this value (Table 5). However, the chlorophyll a values in Yorktown Creek were inversely correlated with the nutrient gradient revealed by the water chemistry data. The highest chlorophyll a concentrations were found at the creek mouth, with levels decreasing upstream in both arms. This would suggest inhibition or micro-autotrophic growth due either to nutrient inhibition or shading by living and dead macrophytes.

The lowest levels of chlorophyll a were found in the IBTA zone where the largest amount of dead macrophytes remained standing. In May, after macrophyte growth was well-established, chlorophyll a in the control branch sediment was significantly higher in all but the CBTA zone. Standing dead macrophytes in the *Typha* zones of both branches evidently produced a shading effect prior to the growing season. During the active growing season, March to May, chlorophyll a was reduced in all marsh vegetation zones.

Macrophyte shading has been shown to limit sediment chlorophyll a production during the summer months (Estrada, et al., 1974; Sullivan and Daiber, 1975). Also, Estrada, et al.,(1974) found that fertilization with N and P increased vascular plant growth enough to significantly decrease sediment chlorophyll a.

In the high marsh in June, chlorophyll a levels were significantly higher in the IBHM zone. Apparently, nutrients are not limiting during the summer (Estrada, et al.,1974; Sullivan and Daiber, 1975), suggesting that this difference is the result of a lag in macrophyte growth in the control arm. If this is the case, it is conceivable that micro-autotrophic growth is equal in each branch during the winter when macrophyte growth is essentially zero. During the growing season, the increased availability of nutrients in the impacted branch may cause decreased chlorophyll a production compared to the control branch, due to a greater shading effect. The situation may then be reversed, as macrophyte growth in the control branch reaches some critical level, while nutrient input apparently enables some increased production in the impacted branch despite enhanced shading.

The difference between high and low tide chlorophyll a samples would indicate that a significant amount of sediment chlorophyll a is lost at high tide, possibly through transport to the estuary by the water column, and/or through predation by organisms entering the marsh to feed at high tide. The initially high levels of water column chlorophyll a at high tide (from the June 24 hour study) supports this contention, but the erratic fluctuations thereafter yield little conclusive evidence.

The results of such a short study must be stated with caution, since a full year of study is desirable before any final statement of effects can be shown. Nevertheless, a summary of preliminary findings indicate that:

1) Chlorophyll a in the control arm of this brackish marsh exceeds levels found in the high salinity marshes of Virginia's eastern shore, in early spring (Rizzo, unpublished data).

2) There is no apparent difference in chlorophyll a concentrations between arms of Yorktown Creek before initiation of the macrophyte growing season.

3) After two months of macrophyte growth, there is significant lowering of chlorophyll a levels in the impacted branch, probably due to increased shading.

4) Higher micro-autotrophic growth may occur in the impacted branch later in the summer, as shading increases in the control branch, and/or due to altered nutrient, temperature, or carbon dioxide interactions in the impacted branch.

5) Sediment chlorophyll over a tidal cycle, and to some extent, water column chlorophyll a, indicate that the primary production of the micro-autotrophic community enters the adjoining estuary via the dendritic marsh creek system at high tide.

Further research would examine the relationship between microautotrophs and nutrient fluxes, between impacted and control branches regarding both standing crop (chlorophyll a) and productivity (carbon dioxide fixation), and to determine what annual impacts may result from high nutrient levels.

Water Nutrient Studies

It has been suggested by various investigators that tidal marshes act as sinks, or buffers, in the nutrient cycles of estuaries, particularly with regard to phosphorus cycling (Ho et al., 1970; Pomeroy et al., 1972). Tidal marshes have also shown the capacity to retain additional nutrients (Valiela and Sass, 1973; Sullivan and Daiber, 1974). The results of the water chemistry study completed to date do not allow conclusive evidence for or against this hypothesis.

The exchange of phosphate between water and sediments has been described as the result of two processes (Pomeroy, et al., 1965); first, surface sorption occurs followed by incorporation of phosphate into the crystal lattice of the sediment, and secondly, phosphate exchange may be biologically controlled. Since biological exchange was found to be trivial in undisturbed sediments by Pomeroy, et al., (1965), it is generally not considered. The process of phosphate exchange tends to maintain a concentration of approximately of 1 uM phosphate in the water column (Pomeroy et al., 1965). The water nutrient data from the control branch of Yorktown Creek supports Pomeroy's work. Phosphate concentrations in the control branch were consistently near 0.5 uM from Nov.-May and showed little variation. The phosphate values in the mixed and impacted branches were considerably higher than 1 uM (in the range of 5-30 uM), suggesting that the sediments were saturated (Heinle and Flemer, 1976) and exchange rates minimized. Variability in phosphate concentrations can probably be attributed to variation in nutrient loading and/or dilution. This consideration is especially important in view of the fact that we have little knowledge on the nature (time behavior) of nutrient input to the creek and little knowledge of water residence time.

In both the mixed and control branches NO_3^-, NO_2^-, and urea concentrations peaked in March or April, and declined in May. Import of inorganic nitrogen species in May, June, July, and August has been observed coincident with the largest observed macrophyte growth (Heinle and Flemer,1976).

Total nitrogen and N/P ratios measured in Yorktown Creek agree with the pattern found for NO_3^-, NO_2^-, and urea, but because of the anomalous behavior of ammonia, make interpertation difficult. A precipitous decrease in ammonia was observed from Nov.-Feb. followed by a slow rise through May. We concur with Heinle and Flemer (1976) that due to nutrient loading by treated sewage the ecosystem may have been changed to such an extent that "normal" relationships are no longer operating. As mentioned, we believe measures of water column nutrient concentrations are poor indicators of rate controlling processes in such systems, i.e. saturated conditions.

Our initial studies suggest that quantitative evidence concerning the seasonal functioning of the Yorktown Creek-marsh system in nutrient cycling and mass-balance properties requires an expanded program of experimental studies regarding soil-water, soil-microflora and soil-plant nutrient relations.

Nitrogen Fixation Studies

The rates of fixation found in Yorktown Creek are generally greater than or equal to the averages found in the literature for east coast marshes. The rates were higher than the average found by Marsho, et al., (1975) of 8.9 ngN/gm dry sed./hr. and fell into Whitney, et al.(1975) range of values (41.9-807) for intertidal marsh sediments. The rates for the intertidal blue-green algal mat in the control branch, with an average of 1,882 ug/M^2/hr., is significantly higher than the values found by Van Raalte, et al., (1974). The Yorktown rates fall into the upper range given by Whitney, et al., (1975), of 260-8910 ug N/m^2/hr. The floating mats of algae fixed nitrogen at the highest rates and again fall into the upper range of the values found by Whitney et al., (1975). The low "dark" values suggest that the source of fixation was algal not bacterial.

There was no significant difference in fixation rates between control and impacted sites. These rates, 219.6 and 212.7 ngN/m^2/hr., were in fact higher than the average value determined by Van Raalte et al., (1974) of 100 ngN/cm 2/hr. for control areas. They were comparable to Van Raalte et al., (1974) control values of May and June 1972. The interstitial NH_4^+-N values ranged from 1.89 to 12.69 ug-at N^{-1} which are probably not high enough to inhibit nitrogen fixation (Van Raalte et al.,1974; Stewart, 1969). The surface tidal pool NH_4^+-N values range from 2.72 and 13.4 ug-at N/l for control ponds to 65.3 to 85.9 ug-at N/l for impacted ponds. The levels in the impacted ponds may be high enough to cause inhibition (Van Raalte, et al.;1974) but apparently had little affect on the nitrogen fixing activity of floating algae. The algae covering the ponds and the blue-green algal mats fix a great deal of nitrogen, but intertidal sediments in the S. alterniflora, S. Patens, and Typha angustifolia zones do not have much activity unless algae is present. The algal contribution is probably highly seasonal. The mat's abundance, composition, and nitrogen fixing activity probably vary greatly with temperature. Although the surface area covered by blue-green algae is relatively large in certain areas (particularly Site 4 Control), a patchy distribution may restrict their importance. The rhizomes of plants indicated low activity, but their widespread abundance may provide a more consistent source of combined nitrogen. Thus 17.47 mg N/m^2 day may be an overestimate for the contribution of nitrogen fixation considering the total area of the marsh.

Further study is needed to obtain a better estimate of average rates of fixation, especially for the intertidal sediments. The abundance, extent of converge, and nitrogen fixing activity of the blue-green algal mats needs to be monitored to determine difference between control and impacted areas and to determine seasonal affects. Further water nutrient samples (interstitial and tidal pool) need to be taken in conjunction with fixation studies to determine affects of NH_4^+-N levels on fixation.

Diel Mass-Balance Study: June 15-16,1976

Studies of the mass-balance nutrient properties (net exchange) of natural marsh-estuarine ecosystems have been attempted by several investigators in marshes of the U.S. East and Gulf coast to clarify the role of these systems in the mineral-nutrient cycling of estuaries (Alexrad, 1974; Awrand and Daiber, 1973; Grant and Patrich, 1970; Henile and Flemer, 1976; Windom, et al., Stevenson, et al., 1976; Woodwell, et al., 1976). The results of these studies has led to divergent opinions concerning both the qualitative and quantitative nature of net nutrient exchange. Valiela et al., (1973) in Massachusetts, Sullivan and Daiber (1974) in Delaware, Marshall (1970) in North Carolina,and Gallagher (1975) in Georgia have found indications that nitrogen is limiting to marsh production and that nitrogen fertilization will increase production of above ground material. Based on these studies and results, marshs have been proposed by some investigators as efficient "processes" for sewage disposal. One study (Valiela et al., 1973) indicated that greater than 80% of added nitrogen and phosphorous in the form of sewage sludge was retained by the marsh. In contrast, and supported by our limited studies, other investigators have indicated that certain marsh types, especially lower salinity and brackish water systems are "exporters" of nutrients naturally and will on enrichment become quickly saturated and export any added nutrients (Bender and Correl, 1974; Stevenson, et al., 1976).

The diel mass-balance study of Yorktown Creek, draining ca. 35 marsh acres, exported all forms of measured nutrient species. Diel nutrient behavior was not entirely obvious although NO_3^- and NH_3 tended to vary inversely at the creek mouth. Qualitatively NO_3^- dominated sewage input and NH_3 export. Alexrad (1974) reported net import of NO_3^- for all times of year in a marsh-creek system geographically near Yorktown and on the York River (Ware Creek). NH_3 was exported during most of year except fall. It would appear that both the Yorktown system and Ware Creek have similar over-all dynamics, i.e., NO_3^- utilization (uptake) and NH_3 release-export. The principal finding was, however, that the Yorktown Creek-marsh ecosystem did not effectively retain added nitrogen and phosphorous. We concur with both the finding of Stevenson, et al., (1976) and the opinions of

Bender and Correll (1974) that these systems do not act as buffers for ameliorating the effects of added nutrients quantitatively but may qualitatively change nutrient relationships.

SUMMARY

Yorktown Creek, an oligohaline marsh-creek ecosystem with mixed vegetation, has been historically impacted (ca. 19 years) by the addition of secondarily treated domestic sewage to the head of one of the two main creek branches. Road and culvert construction at the mouth of the creek system has resulted in gross alterations of marsh elevation and hydrology. Tidal flooding and exchange with the adjacent York River has changed significantly compared to natural patterns. Total systems behavior, especially as it might deviate from unperturbed natural systems behavior, reflects the interaction of high levels of nutrient loading and alterations in tidal-marsh hydrodynamics. The general objective of our studies included in this report was to characterize the system prior to relaxation of the domestic sewage input. The principal areas of study were vascular plants, benthic microflora, water nutrients, and nutrient mass-balance relationships.

Annual production by the three dominant vascular plant associations, *Typha angustifolia*, *Spartina patens*-*Distichlis spicata*, and *Spartina alterniflora*, is comparable for the particular species to other natural marsh systems. Distribution and zonation of the three appears controlled in large

part by the hydrology of the system although there is the suggestion that high soil nutrient levels, especially NO_3^-, may influence competitive, density-dependent species interactions and result in atypical associations and species dominance. This result is noted in the *Distichlis spicata-Spartina patens* and *S. patens-S. alterniflora* associations. Certain minor and typically freshwater species were also eliminated from the enriched areas.

Plant population growth characteristics within comparable marsh habitats differed for the same species growing in nutrient enriched and un-impacted areas. This was particularly true of the dominant vascular plant for the system *T. angustifolia*. The initiation of new year's growth and flowering in the high nutrient zones preceeded the control areas by approximately a month. Growth form was significantly different for nearly all intraspecific comparisons. Species growing in the nutrient impacted areas were typically less dense but had significantly higher individual plant biomass. All these results suggest radically different nutrient exchange processes controlling component behavior between nutrient enriched and un-enriched marsh areas. Since phosphorous is probably not a limiting nutrient in this as well as similar marsh systems, changes in component behavior can perhaps be linked with processes controlling or influencing nitrogen dynamics. These processes are in all likelihood soil-sediment related and result from geochemical and biological interactions.

From a holistic viewpoint, it would appear that the temporal succession of the nutrient enriched branch has been slowed compared to the control branch by reducing species richness and invasion. This result is attributed to the nutrient loading since impacts associated with changes in hydrology would have equally affected both enriched and control areas.

The benthic microflora of marsh ecosystems contribute significantly to total production (25-30% of vascular plant production) in U.S. East Coast marsh-estuarine systems. Our initial studies of chlorophyll a and N_2 fixation indicate extreme spatial heterogeniety. Chlorophyll a, prior to the beginning of vascular plant growth, decreased with increasing water column nutrient concentrations. Following the period of maximum vascular plant growth (March-May), chlorophyll a in all marsh sediments was reduced and remained so for the duration of the study. Highest chlorophyll a concentrations were observed during early March in the mixed vegetation zones and was significantly higher than values reported for similar marshes. The chlorophyll data suggests that benthic algae may be inhibited at high nutrient levels and enhanced with moderate enrichment. Nitrogen fixation was variable, appeared to be primarily algal, and fell within the upper range of values reported for marshes of the east coast and was higher than values reported for most.

Water nutrient concentrations in the enriched creek branches showed little seasonal dynamics or behavior that could be attributed to other system's components. The dominant factor relative to measured concentrations was sewage disposal input. Normal behavior, or at least observable dynamics, were masked by the high levels of input. Relative changes in specific nutrients as water traversed different marsh zones illustrated the anomalous behavior in the enriched areas. Causal explanation cannot be offered to explain the observed behavior without further study.

The results of our mass balance study, completed prior to relaxation of the sewage input, indicated that the marsh system exported considerable quanities of nutrients. For this study, considering the probable measurement errors, export from the Yorktown Creek equaled or exceeded nutrient input by the sewage disposal plant. Qualitatively, output was significantly different than input for nitrogen. There was both a qualitative and quantitative shift from NO_3^- to NH_3 as creek water traversed the system (i.e. from outfall to creek mouth). There was no evidence to suggest that this marsh-creek ecosystem effectively acted as a sink for excess nutrients.

Sewage disposal into the head of the southern creek branch was stopped July, 1976. Continuing study after this period using an experimental components approach should allow us to further evaluate the perturbation effects of the long term nutrient loading and identify those components controlling systems recovery.

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APPENDIX A

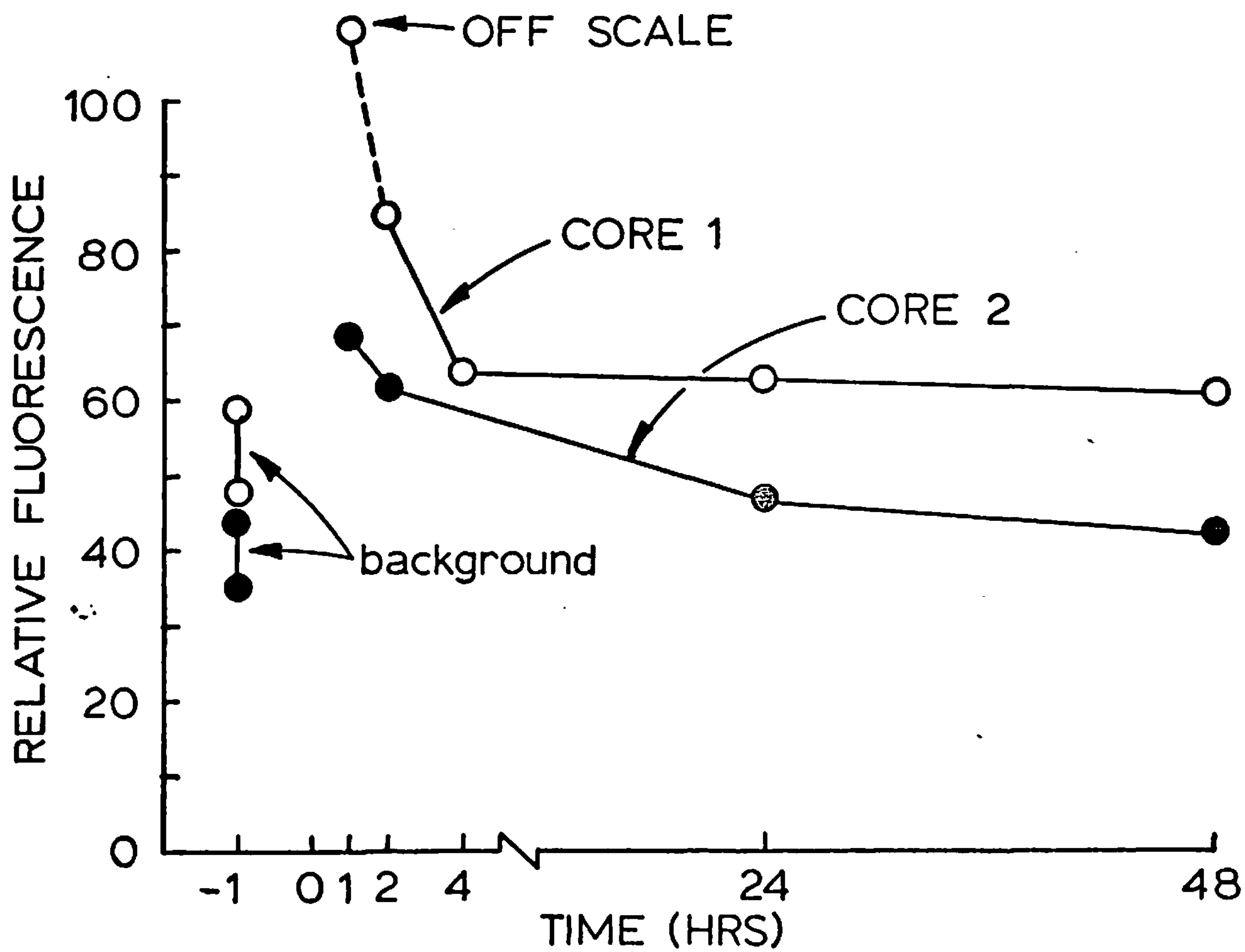
Water Residence Study

An attempt to determine water residence time of the creek-marsh system and determine the degree of mixing between the impacted and control branches was made. Rhodamine B dye was used for this tracer study. The general procedure was to release two liters of Rhodamine B (1:100 dilution stock) at the creek head over a two minute interval. The dye was released one to two hours before low water. Water samples were collected at one minute intervals from 0.5 to 5 hours after release at the Route 238 culvert (Fig. 1), approximately .5 miles from the point of injection. Samples were returned hourly to the lab and immediately processed on a Turner fluorometer with filters and wavelength appropriate for Rhodamine B. A total of six tracer studies were done varying both dye dilution and sampling frequency. None of the studies proved successful. Floating markers were then used to estimate average current speed in the impacted branch. From these data, a low water residence time for the impacted branch was calculated to be 70 minutes.

Neither the time interval in which samples were taken, the time interval between samples, nor the initial dilution used (dilution detection limit was $1{:}10^6$ stock dye) appeared to be responsible for the degative dye results. Conceivably, soil-sediment processes could have taken the dye out of solution before the dye plug reached the sampling point. To test this hypothesis, a 9 x 5 cm core of marsh soil was placed in a glass jar and distilled water added to a final volume of 2 liters. A magnetic rod was used to stir this mixture over the experimental interval. Soluble fluorescence was obtained by centrifuging a 10 ml sample of the mixture and reading the decanted supernatent on the fluorometer. After background levels were read, Rhodamine B was added to the jar in a concentration estimated to read 2-3x background. Two cores were thus prepared and followed for 48 hours.

The results (Figure A) show a marked decrease in soluble relative flourescence within several hours after dye injection. While tracing the fate of the dye was beyond the scope of the experimental design, the results suggest soil-sediment processes were a factor in the negative results obtained in the field.

FIGURE A: LABORATORY RHODAMINE B INCUBATIONS.



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