NOAA Technical Memorandum
NOS MEMD 11

OLD WOMAN CREEK
NATIONAL ESTUARINE RESEARCH RESERVE

PHOSPHORUS DYNAMICS IN THE OLD WOMAN CREEK NATIONAL ESTUARINE RESEARCH RESERVE - A PRELIMINARY INVESTIGATION

Washington, D.C.
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Robert T. Heath

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Washington, D.C.

August 1987

UNITED STATES DEPARTMENT OF COMMERCE / National Oceanic and Atmospheric Administration / National Ocean Service

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REPORT TO
NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION
U.S. DEPARTMENT OF COMMERCE

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Phosphorus Dynamics in the Old Woman Creek National Estuarine Research Reserve - A Preliminary Investigation

Robert T. Heath

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U.S. DEPARTMENT OF COMMERCE
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NATIONAL OCEAN SERVICE
OFFICE OF OCEAN AND COASTAL RESOURCE MANAGEMENT
MARINE AND ESTUARINE MANAGEMENT DIVISION
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This work is the result of research sponsored by the U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service, Office of Ocean and Coastal Resource Management, Marine and Estuarine Management Division Under Contract Number NA-84-AA-CZ022

ABSTRACT

The purposes of this study were to determine the importance of phosphorus (P) inputs to the planktonic community in the Old Woman Creek National Estuarine Sanctuary (OWCNES), and to determine the effects of this wetland on the availability of phosphorus to Lake Erie phytoplankton. From June 1984 through July 1985 the growth of phytoplankton in OWCNES was not limited by the availability of phosphorus. Based on observations of the change in distribution P components and their metabolism during passage through the estuary, passage through OWCNES greatly ameliorated the availability of P to Lake Erie phytoplankton. Even during times of stagnation (i.e. relatively slow flow in the summer), a pattern was consistently observed from a relatively high proportion of soluble reactive phosphorus (SRP) at the inlet to OWCNES, to a continual decrease in SRP toward the outlet into Lake Erie. This wetland community decreased the availability of P to Lake Erie phytoplankton by increasing the proportion of particulate P and the proportion of soluble forms of phosphorus from which phosphate was slowly released, if at all. Phosphate uptake was largely dependent on metabolic activities of the plankton, rather than ion exchange adsorption to suspended sediments. The release of phosphate from phosphomonoesters was slow in comparison to the rate of phosphate uptake by seston. A preliminary study showed that anaerobic sediments could serve as a significant source of phosphate to adjacent waters, but sediments under aerobic conditions took up phosphate at a moderate rate.

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INTRODUCTION

Freshwater estuaries are wetland ecosystems that can form at the mouths of rivers entering large freshwater lakes. The flow through these systems can resemble marine estuaries if those lakes seiche frequently with a sufficient magnitude to dominate the flow of the estuary. These wetland systems can support large populations of rooted macrophytes and phytoplankton. All of the Great Lakes have "freshwater estuaries", but these are best developed on Lake Erie because the shallowness of that lake coupled with frequent wind and barometric disturbances result in frequent significant seiches. The period of oscillation for these seiches is typically 14 hours (OWCNES Management Plan 1983).

These systems may be important in determining the amount and the availability of nutrients that drain from non-point sources (e.g. agricultural fields) into the receiving lakes. It may be that nutrient inputs to the estuary are removed from availability, becoming fixed in organic matter, much as toxins are removed in marine estuaries (Odum 1971). Or it may be that the estuary acts as a net source of P (eg. through inputs from the sediments, directly or via macrophytic activities). Or it may be that P inputs equal P outputs but that the estuary processes them into a more available (or a less available) form. Because of their potential impact on the nutrient dynamics of the large lakes into which they empty, understanding the functioning of "freshwater estuaries" is essential for determining the effect of non-point source inputs to the Great Lakes and for developing large-scale watershed management plans.

Old Woman Creek National Estuarine Sanctuary is currently the only freshwater estuary in the National Estuarine Sanctuary program. Because it is situated in a relatively uninhabited agricultural watershed on the south shore of Lake Erie near Huron, OH, it represents one of the best known examples of

freshwater estuaries. Although some studies have been completed to describe the geological, chemical, faunal and floral characteristics of this freshwater estuary, (OWCNES Management Plan 1983), little is known of how it functions in terms of energy flow or nutrient dynamics. Studies to determine the functional behavior of this wetland system and its potential effect on Lake Erie must begin at the most fundamental level. If energy flow in the open water is limited by the size of the primary producer community, the first problem is to determine those factors that limit the growth of populations primary producers. In contrast with marine systems, phytoplankton growth in freshwater systems is often limited by the availability of phosphorus (Schindler 1977). Dillon and Rigler (1972) showed that in a wide variety of lakes annual chlorophyll production was correlated to the total phosphorus content--a finding consistant with the view that the phytoplankton growth is limited by P-availability. Also, when laboratory cultures Selenastrum capricornutum were cultured in water from a variety of lakes that had been filtered and amended with possible limiting nutrients, often cell growth was stimulated only in those cultures amended with phosphate (Miller, et al 1978).

Physiological and biochemical evidence taken in situ also indicated that phytoplankton growth often was P-limited. Fitzgerald and Nelson (1966) showed that many algal species are capable of producing alkaline phosphatase as an adaptive response to P-limiting conditions following depletion of internal polyphosphate stores. Heath and Cooke (1975) demonstrated that phosphatase activity appeared only after a phytoplankton bloom of Aphanizomenon flos-aquae ceased growing. Following appearance of phosphatase, the levels of phosphomonoester substrates dimished to undetectable levels, the potential productivity increased 10-fold and the size of the standing crop increased. Chow-Fraser and Dulthie (1982) recently showed that the rate of phosphate uptake

was a function of the P-nutritional status of the phytoplankton and could be used as an indicator of P-limitation. Using polyphosphate content and phosphate uptake kinetics Lean et al (1983) have recently investigated P-limitation of Lake Erie phytoplankton. They have corroborated considerable earlier work by others that phytoplankton in Lake Erie are P-limited during the stratified season but are not P-limited following overturn.

If the phytoplankton are limited in their growth by P-availability then the size of their populations are controlled by sources of available phosphorus--i.e. as orthophosphate (Fogg 1974). Phosphate can become available to these populations either from external sources or from processes within the freshwater system itself. External loading is determined by direct measurement of the amount of phosphate entering the system. Internal loading is generally determined by estimating the potential for release of phosphate from various dissolved or particulate sources within the system.

Heath and his associates have demonstrated that dissolved organic P compounds may serve as significant sources of phosphate, provided the mechanisms for release of phosphate are present. Francko and Heath (1979) identified two classes of functionally distinct complex P Compounds. A functional class of compounds is a group of compounds capable of releasing phosphate via a particular process. One class of compounds can release phosphate through the hydrolylic action of alkaline- and acid phosphatases. Boavida and Heath (1984) report that these enzymes are released by certain zooplankton as well as being attached to phytoplankton and bacterioplankton. Heath and Cooke (1975) showed that this class likely was useful in supporting cyanobacterial blooms in a eutrophic lake. The second class of compounds is a humic-iron-phosphatae complex. This class releases phosphate as the iron moiety is photoreduced (Fe III) to Fe (II) (Francko and Heath 1981, 1983). This class of photosensitive P

compounds appears to provide a minor portion of the phosphate demand in a humic acid bog--an environment in which such compounds exist in relatively large quantities (Cotner 1984).

Phosphorus apparently can also be transported from the sediments by rooted macrophytes and released in an available form. The more a body of water is dominated by macrophytes the greater may be this source of P input to the marsh community and its consequent relaese to the receiving community. Reimold (1972) showed that phosphate (traced as ^{32}P - orthophosphate) could be transported from the sediments to the leaves of Spartina in a Georgia salt marsh. Some phosphorus was released in a soluble form as the tide "washed" the leaves, but much phosphorus was not released until the leaves died and were processed by detritivores (Odum and delaCruz 1967). McRoy and Barsdate (1970) showed macrophytic pumping by Zostera in an arctic salt marsh, and Twilley et al (1977) made a similar finding in the freshwater macrophyte Nuphar. Each of these studies showed a seasonal dependence on phosphorus secretion to the surrounding water, being greatest in the summer. Whether P is released as phosphate or in some other form was not investigated in these studies.

The sediments also can release P also through the action of burrowing meiobenthic organisms (Davis, et al 1975) as well as through other processes. In estuaries the mixing and suspension of materials in anoxic pore water may contribute to the P load (Odum 1971). However, the sediments can act as a sink as well as a source of P to overlying waters (Mortimer 1971). Under aerobic conditions (greater than 0.4 ppm O_2) phosphate is sorbed to sediment particles at a rate and to an extent that is a function of Eh, pH and temperature (Ku et al 1974). Heath (1980) reported that phosphate uptake by suspended aerobic sediments proceeded at two different rates. The fast rate apparently independent of biotic activity; the slower rate was biotically mediated,

probably by bacteria attached to sediment particles. He also demonstrated that the extent of phosphate uptake was a function of living micro-organisms.

In addition to affecting the wetland community itself, processes within this freshwater estuary may also control events in the adjoining open water of Lake Erie if they act significantly to affect the availability of phosphorus to Lake Erie phytoplankton. Numerous studies have documented in a wide variety of ways that Lake Erie communities are P-limited during the growing season (Schelske 1979). Bioassays of water from the Great Lakes show that in all lakes P is a limiting nutrient, and that availability of trace elements and chelating agents synergisticity exacerbate the P limitation (Schelske et al 1978, Schelske 1979). Nitrogen availability was limiting in Lake Erie owing to the great P input to the lake (Schelske et al 1978). Using polyphosphate content of cells, phosphate turnover time and a "phosphorus deficiency index", Lean et al (1983) recently have shown that Lake Erie phytoplankton are P-limited during the stratified growing season (from late May until mid-September). Therefore processes that affect phosphorus inputs would be especially important during the stratified season.

Because phosphorus availability often limits freshwater community growth and activities, it makes sense to begin an investigation of community function by asking whether this community is P-limited or not. Also, even if P is not a limiting nutrient in OWCNES, it is important to determine whether estuarine processes affect the amount or availability of P to the Lake Erie community, especially during the P-limited season.

There were two major goals of this study:

1) to establish whether P-availability was in growth limiting supply in the open water of OWCNES freshwater estuary, and

2) to assess the overall effect of the estuary on P inputs to Lake Erie planktonic communities by examining the potential sources of P that may be or may become available to Lake Erie plankton. Particular attention was focused on determining the potential significance of dissolved organic phosphorus compounds. Also, a preliminary investigation of the effects of the sediments on P availability was conducted.

This study was designed to answer those questions essential to the formulation of management plans and also to planning future investigations of the functions of OWCNES freshwater estuary. Its scope was confined to an investigation of P-dynamics in the open water of OWCNES at several stations from the creek inlet to the mouth at Lake Erie. It was confined to the interval from June 1984 through July 1985.

METHODS

Sampling:

Samples were collected from the 0.1 m stratum in acid-rinsed autoclaved polyethylene bottles. One liter of water was collected from each of three sites on each visit:

Site 1: from the Darrow Road bridge

Site 2: at the East Island site in the channel

Site 3: midway between the S.R. 2 bridge and the outlet

At the time of collection the water temperature and the oxygen tension was recorded using a Yellow Springs Model semi-automatic temperature correcting oxygen electrode. Also, at Site 2 and Site 3 the secchi depth was recorded using a 20 cm all white secchi disk; Site 1 was sampled with a tethered bucket, precluding the routine determination of the secchi depth.

Samples were returned to the OWCNES laboratory within one hour after sampling and to the KSU laboratory within four hours after sampling.

Sediment samples were collected with an Ekman dredge. Sediment was placed into autoclaved polyethylene sample bottles. No attempt was made to keep the sample in an undisturbed condition.

Procedures performed at the OWCNES:

Water (100 - 250 mL) was filtered through two Whatman GF/C glass fiber filters. The filtered water was placed in separate bottles and returned to KSU, where it was refiltered through acid-rinsed Millipore HAWP filters having an average pore size of 0.45 um. This twice filtered water was used in tests requiring "filtered water".

Chlorophyll content of the material collected on the Whatman GF/C filters was determined by the standard 2-wavelength method (APHA 1976). To prevent formation of phaeopigments, a 1 percent slurry of magnesium carbonate was

filtered through the filter immediately following collection of seston. These filters were frozen until the extraction procedure was performed on them (generally within two days and always within one week from the time of collection). Pigments were extracted into acetone saturated with MgC_3 by grinding them with a Potter-Elvejehm teflon tissue homogenizer for 2 to 10 minutes then allowing them to stand in the dark for 12 to 16 hours. The homogenate was centrifuged at 10,000 x g. (10,000 rpm in a SS-34 rotor) in a Sorvall RC-2B refrigerated high speed centrifuge. The supernatant was carefully withdrawn with a Pasteur pipet and placed in 1 cm quartz cuvettes. The absorbance was read at 650 nm and 750 nm against acetone. The determination was corrected for phaeopigments by acidification and re-reading the absorbance at 665 nm and 750 nm. Total chlorophyll (uncorrected) and "active" chlorophyll (phaeo-corrected) were calculated from the observed absorbances.

The pH was determined with a Radiometer Model PHM-84 automatic titrator equipped with a glass/calomel pH electrode. The pH was determined to a precision of +/- 0.01 units.

Conductivity of the samples was determined with a YSI Model 32 conductance meter and read to precision of +/- 1.0 umhos.

Turbidity of the samples was determined with a Sargent-Welch Model S-83700 turbidimeter, calibrated each time with a set of nephalometric standards. Also, the absorbance of the samples at 550 nm was determined in 1 cm quartz cuvettes (at KSU).

Following completion of these measurements and procedures at the OWCNES laboratory, the samples were returned to the Kent State University laboratory within four hours after sampling. By placing samples in a thermally insulated chest, an attempt was made to maintain the sample temperature at ambient temperature during transport.

Procedures performed at the Kent State University laboratory:

Nutrient limitation bioassays were performed according to the method of Hartig and Wallen (1984). To 100 mL unfiltered samples was added 1 mL of 8.06 x 10^{-5} M KH_2PO_4, or 1 mL of 1.79 x 10^{-3} M $NaNO_3$, or both; unamended controls were also run. These amendments increased the P content by 25 ug per L, or the N content by 250 ug per L, or both. All tests were done in triplicate in 250 mL acid-rinsed Erlenmeyer flasks. These flasks were placed at randomly selected positions in an environmental chamber illuminated with cool white fluorescent lighting (150 uE m^{-1} s^{-1}) controlled on a regime: 12 hr. on, 12 hr. off. The temperature was maintained at 21°C, within 1°C. After 14 days, the total contents of each flask were collected on Whatman GF/A glass fiber filters and the total (uncorrected) chlorophyll content was determined as described above.

Total nitrogen content of freshly collected samples was determined by the method of Raveh and Avnimilich (1979). Following persulfate digestion for 1 hr. at 126°C, 1.5 atm in an autoclave, all nitrogen was released as nitrate. In turn, nitrate was reduced to ammonium by adding 1 g. Devarda alloy to 50 mL of hydrolysate. The ammonium concentration was detected by the indophenol blue method of Solorzano (1969) and compared to a set of ammonium chloride standards.

Soluble reactive phosphorus (SRP) was determined according to the method of Murphy and Riley (1962) as modified by the USEPA. Filtered water samples (2.5 mL) were placed in acid-rinsed tubes, then 0.5 mL of the molybdate reagent, the mixture vortexed, then 0.2 mL of 4 percent ascorbate solution was added and the tubes vortexed again. The tubes were allowed to stand for 10 min. before reading the absorbance at 885 nm. Each test was run in triplicate. Also, each test included a control for endogenous color: 2.5 mL filtered water, 0.5 mL molybdate reagent, and 0.2 mL distilled water was added in lieu of the ascorbate reagent. The absorbance of this "endogenous color" control was subtracted from

the average absorbance of each test. This color corrected absorbance was compared against a standard curve determined from a set of standard solutions of KH_2PO_4 run simultaneously. The set of standards included phosphorus concentrations of 500, 250, 125, 62.5, and 0.0 ug P/L; each standard solution was run in duplicate. The standard curve was determined by linear regression of the absorbance of the color of the standard solutions; in general the square of the correlation coefficient (r^2) was greater than 0.9900.

Total phosphorus (Total P) and total soluble phosphorus (TSP) were determined as above following persulfuric acid-catalysed hydrolysis of unfiltered or filtered water samples, respectively. Aliquots (2.5 mL) of water were added to 0.5 mL of 10 percent sodium persulfate and 0.1 mL of 10 M sulfuric acid. These were mixed and incubated at 2 atm, 126^{o}C for 60 min.(APHA 1976). After cooling to room temperature each sample was titrated to the phenolphthalein end-point with 10 M sodium hydroxide and back titrated with 1 M sulfuric acid. The colorimetric reaction was run as above on a 2.5 mL aliquot of this hydrolysate. Also as above, each test was performed in triplicate and the endogenous color was subtracted from the test color before comparison with a standard curve.

Phosphomonoester (PME) content of the filtered water samples was determined as the increase in SRP following incubation with calf intestinal mucosa alkaline phosphatase (essentially as Francko and Heath 1979). This enzyme specifically hydrolyses PME to release phosphate detectable as SRP. To 2.25 mL of filtered water was added 0.25 mL of a solution containing 0.5 mg calf intestinal mucosa alkaline phosphatase (Sigma Chem.Co.) per mL of 0.1 M tris- hyroxymethyl amino methane (Sigma Chem. Co.) at pH 9.0. Samples were run in two batches, in triplicate in each batch. The SRP content of one batch was determined immediately; the SRP content of the second batch was determined after incubation

for 24 h at 37°C. The SRP content was determined by comparison of the absorbance at 885 nm in 1 cm cuvettes, with a set of standards of KH_2PO_4 run in duplicate in tandem. The standards were the same as those used above. Also, to determine that the reaction was complete a set of standards of glucose-6-phosphate (500, 250, 125, 62.5 ug P/L) was run occassonally.

UV-sensitive P compounds (UVS) was determined as the increase in SRP following irradiation with low doses of ultraviolet light (Francko and Heath 1979). Filtered water samples (25 mL) were placed in 50 mL beakers and irradiated with a UV-mineral light (UltraViolet Products, Model UV-22) positioned 5 cm over the surface of the samples. The SRP content was determined at the beginning of irradiation and again after 3 hours of irradiation. UVS material was taken as the difference in the two SRP estimates.

Radiometric determination of the proportional uptake rate of phosphate was done according to Heath (1986,in press). Carrier-free acid-free ^{32}P-labelled orthophosphate (New England Nuclear) was added to 50 mL of unfiltered samples to give a final acitivity of about 800 Bq/mL (i.e. about 50,000 cpm/mL). Aliquots of 1 mL were removed after 25, 100, 200, 300, 400, and 500 seconds and filtered through Millipore HAWP filters (average pore size 0.45 um). To minimize non-particulate binding of radiolabel, the filters were presoaked in 0.5 M KH_2PO_4; following filtration of the labelled particles, the filters were washed with three 10 mL rinses of distilled water. The activity of the filters (x) was determined after the filters were air dried, placed into 5.0 mL Beckman BioGamma vials containing Scintilene (Fisher) and counted by liquid scintillation in a Beckman Model 6800 liquid scintillation microprocessor controlled spectrometer. The total activity (P_o) in 1 mL of radiolabelled water samples was determined by placing 1 mL into 7 mL of Beckman RediSolv MP, followed by liquid scintillation counting. The apparent first-order uptake rate constant was determined as the slope of the line $\ln[P_o/(P_o - x)]$ vs. time.

Rate of phosphate uptake by total seston was estimated by multiplying the apparent first-order rate constant, k, by the phosphate concentration (determined as SRP) in the water:

$$v = (k) \times (SRP)$$

Maximal rate of phosphate release from PME by alkaline phosphatase was determined using 1×10^{-3} M p-nitrophenyl phosphate (pNPP) as the substrate (Boavida and Heath 1984). To 2.5 mL aliquots of unfiltered water samples was added 0.3 mL 1×10^{-2} M pNPP and 0.3 mL tris buffer at pH 9.0. The mixture was incubated at ambient temperature for 2, 4, or 24 hours. The product p-nitrophenol was detected by its absorbance at 395 nm in 1 cm quartz cuvettes. The enzyme activity was estimated from the difference in absorbance observed at 395 nm over the interval considered. Enzyme activity was expressed as the change in absorbance per hour. This value was converted to nmol/L/hr by dividing the change in absorbance/hr by the extinction coefficient of a 1 nanomolar solution: 8.932×10^{-6}. Because the substrate concentration used was so large, this value approximates the maximal velocity of the enzyme present, V_{max}.

The "affinity constant" (k_m) for the collection of alkaline phosphatases naturally occurring in these water samples was determined also using pNPP as a model substrate. The above test was performed using a variety of substrate concentrations, ranging from 2×10^{-3} to 2×10^{-4} M pNPP. The K_m was determined using a Hanes plot, regressing the value (pNPP)/v vs. (pNPP). The slope of this line estimates the inverse of the V_{max}, and the intercept at the ordinate estimates the K_m/V_{max}. The K_m is estimated by dividing the intercept by the slope.

The actual velocity of release of of phosphate from PME was estimated from the Michaelis-Menten equation:

$$v = \frac{V_{max} \times (PME)}{K_m + (PME)}$$

where V_{max} and K_m are the values determined using pNPP substrate and (PME) was the concentration of the naturally occurring PME, measured as described above.

Determination of sediment characteristics:

Pore water was prepared by centrifuging sediment at 10,000 x g for 10 min. The supernate was filtered through acid rinsed Millipore HAWP filters. SRP, SUP, and PME content of the pore water was determined as above. To 100 mL of pore water thus obtained was added 300 mL of distilled water; this mixture was designated "diluted pore water".

Organic content of the sediment was determined as the difference between the dry weight of a sediment sample and its ash-free dry weight. Sediment was placed into pre-weighed crucibles and the sediment taken to dryness at 110° C. Drying was continued until a stable weight was obtained. Following dry-weight determination, the samples were re-weighed following incubation at 600° C in a muffle furnace for 12 hours.

Rate of phosphate uptake by suspended sediments was determined in a modified radiometric procedure. A small portion of fresh sediment was suspended in 25 mL distilled water, obtained by centrifugation as above. One mL of this slurry was in turn diluted in 25 mL of pore water at ambient temperature containing carrier-free ^{32}P-phosphate. The time at which sediment slurry was added to the radiolabelled pore water was designated as time 0. One mL aliquots were removed at timed intervals to pre-soaked Millipore filters as described above and the proportional uptake rate constant was determined as above.

Rate of phosphate release from sediments was determined on samples removed by an Ekman dredge from the three stations routinely visited. Approximately 10 g of fresh sediment (not centrifuged) was placed into 250 mL BOD (biochemical oxygen demand) bottles that had been acid-rinsed and autoclaved. The bottles were filled with "diluted pore water" and tightly stoppered. The bottles were shaken vigorously to suspend the sediments throughout the BOD bottles.

After 30 min and at daily intervals thereafter the bottles were carefully opened and the oxygen tension determined with a calibrated Orion Oxygen Electrode designed to fit into BOD bottles. After measurement of the oxygen tension, a 3 mL aliquot was removed to an acid-rinsed centrifuge tube; the solution removed from the BOD bottle was replaced with distilled water, and the bottle was capped. The tube was centrifuged at 10,000 x g for 10 min. and 2.5 mL of supernate was removed for SRP determination. The dry weight of the sediment in the BOD bottles was determined after completion of the experiment by removing a 10 mL aliquot of the sediment suspension to a pre-weighed 25 mL volumetric flask. The solution was taken to dryness over Drierite in a dessicator at 70^{o}C. The exact volume of the BOD bottle was determined by subtracting the weight of the bottle empty from the weight of the bottle filled with distilled water at 15^{o}.

RESULTS

The purposes of this study were to determine

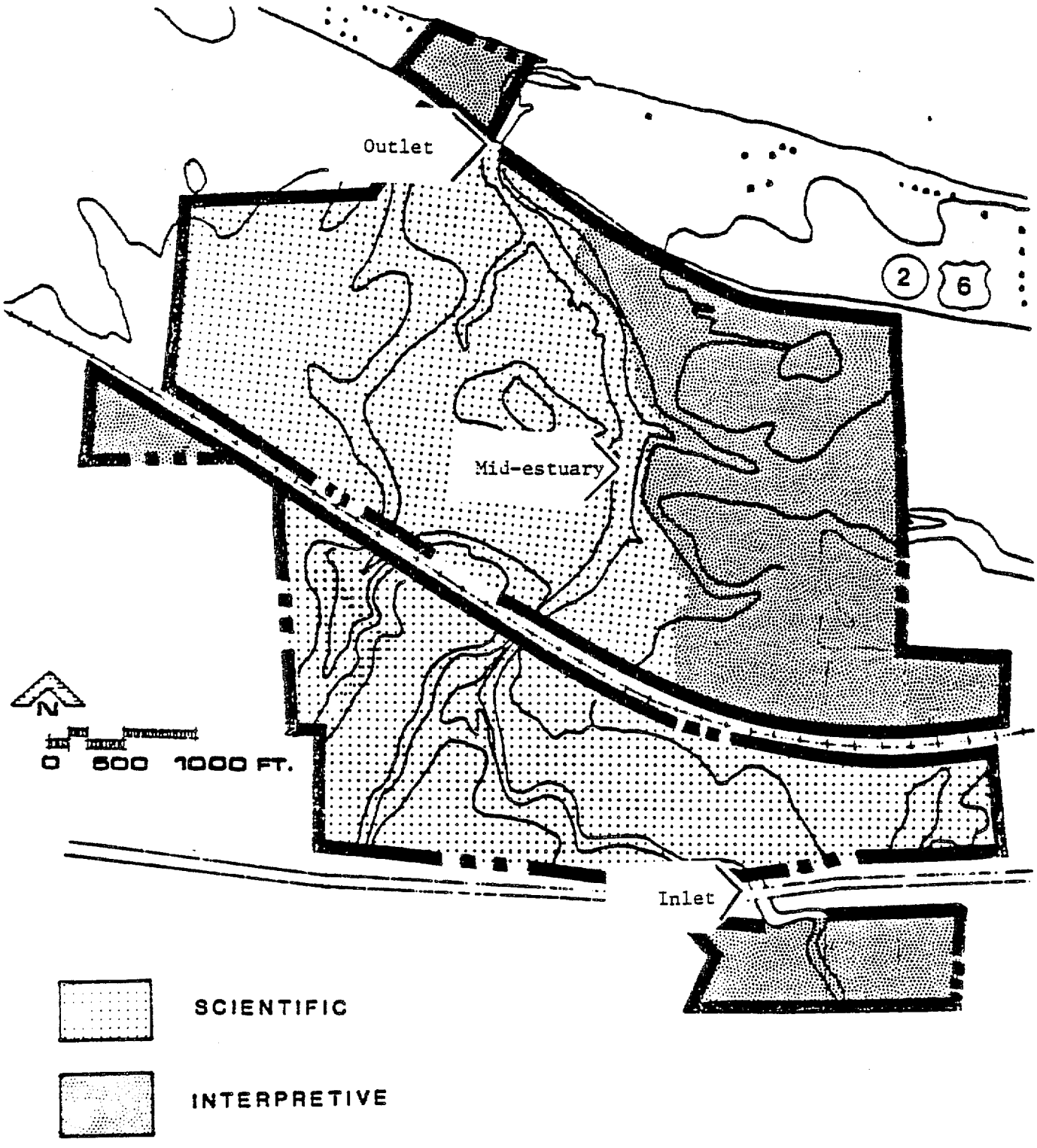
1) the impact of phosphorus inputs on the wetland planktonic community, and
2) the impact of passage through the wetland on availability of P to plankton in the receiving (Lake Erie) community.

The results of this study will be reported in three parts. The first part will provide a general description of the phosphorus composition of the samples taken throughout this study. This description will serve as a basis for presenting the results of various investigation conducted to determine whether the plankton in OWCNES are growth limited by the availability of phosphorus. The evidence collected in this study indicate that the plankton in the estuary seldom, if ever, were limited by the availability of P. However, presented in the third part of this report, is a set of findings that indicate that passage through this wetland changes the availability of P to the receiving community.

I. General description of limnological variables and phosphorus composition

This study began on 22 June 1984 and continued to collect samples through 23 July 1985. Fourteen visits were made during this interval, and each visit sampled three sites (Figure 1). Site 1, the bridge on Darrow Road, represented the "incoming water"; Site 2, the East Island site sampled within the channel, represented the "mid-estuary" condition; Site 3, the bay between the S.R.2 bridge and the outlet to Lake Erie, represented the "out going water". This wetland is a "pulsed-flow" freshwater system. It flows openly during the winter months, but storms typically close the mouth of the estuary with sand in mid- to late June, and the estuary generally remains closed until a spate re-opens it in the autumn. Over the interval studied, the mouth was closed from immediately before the study began until late October 1984. Flow continued

FIGURE 1



OLD WOMAN CREEK NATIONAL ESTUARINE SANCTUARY

through the spring and re-closed in late June. Although this system can show significant seiches resulting from large storms in Lake Erie, no such major seiches were observed during this study.

Limnological variables at the sites visited are shown in Table I. The column marked "day" refers to the day number after the start of the project. During the winter and spring periods of flow through the estuary, the temperature of the surface water was the same throughout the estuary on a given day. Even during the summer stagnation period only a slight trend toward increasing temperature from Site 1 to Site 3 was observed. The pH of the water remained relatively constant throughout the study, with only a slight trend toward an increase in pH as the water flowed through the estuary. Shown below is a summary of the average pH throughout the year and during the periods of stagnation (late spring through summer) and flow.

	annual pH (S.D.)	Stag. pH (S.D)	Flow pH (S.D.)
Site 1	7.88 (0.24)	7.95 (0.29)	7.77 (0.10)
Site 2	8.12 (0.28)	8.14 (0.19)	8.08 (0.42)
Site 3	8.12 (0.25)	8.20 (0.31)	8.13 (0.17)

Oxygen dissolved in the water was at a maximum during the winter, as expected from its increasing solubility in cold water. During the summer the oxygen concentration was lowest at Site 2, contrary to expectations based on increased productivity within the marsh community itself. Because the sites were visited early in the morning, this probably is the result of greater nighttime respiration within the marsh.

Conductivity invariably was highest at Site 1 and declined toward the mouth of the creek, both during the flow and stagnant periods. Figure 2 shows patterns typical of each period. Conductivity at Site 1 was highest during the winter and lowest during April, likely due to the agricultrual run-off during periods of snow melt. Ionic content of the water declined through the estuary.

TABLE I

LIMNOLOGICAL VARIABLES - OWCNES 1984-85

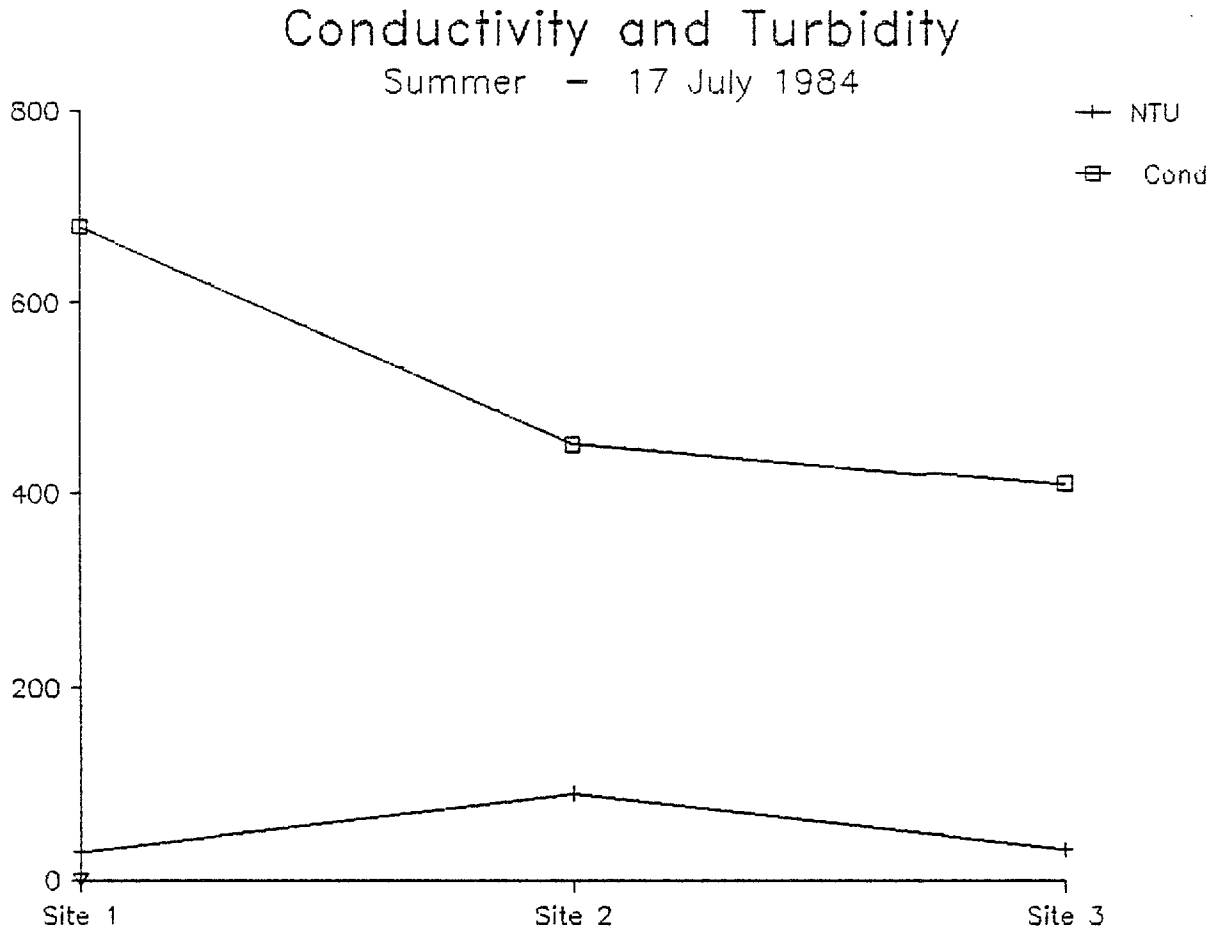
Visit	Day	Date	Secchi cm.	Temp deg C	Oxy ppm	pH	Cond umho	NTU
Site 1 Darrow Road Bridge:								
1	1	22 Jun 84	*	18.0	6.2	7.80	625	48
2	13	5 Jul 84	5	20.5	5.4	8.08	652	250
3	25	17 Jul 84	25	22.5	9.5	8.35	679	30
4	39	31 Jul 84	30	23.0	10.6	8.39	588	27
5	54	15 Aug 84	*	23.2	3.8	7.88	619	28
6	76	6 Sep 84	*	18.5	4.6	7.54	632	35
7	102	2 Oct 84	15	11.0	8.8	7.67	688	55
8	180	19 Dec 84	*	4.0	12.0	7.84	715	5
9	291	9 Apr 85	*	3.0	12.4	7.90	288	23
10	333	21 May 85	12	17.0	7.8	7.77	516	49
11	347	4 Jun 85	*	19.0	4.9	7.67	567	33
12	361	18 Jun 85	*	18.9	6.5	7.84	621	24
13	382	9 Jul 85	*	25.5	8.0	7.91	623	19
14	396	23 Jul 85	*	23.4	7.2	7.85	622	40
Site 2 East Island - in mid stream flow								
1	1	22 Jun 84	25	19.0	7.8	8.40	431	50
2	13	5 Jul 84	15	22.9	4.5	8.32	442	86
3	25	17 Jul 84	15	23.0	5.9	8.23	453	91
4	39	31 Jul 84	20	23.6	2.4	8.10	456	74
5	54	15 Aug 84	11	24.8	3.2	8.15	433	75
6	76	6 Sep 84	10	17.0	.0	7.93	434	80
7	102	2 Oct 84	20	11.5	10.2	7.99	431	44
8	180	19 Dec 84	*	3.5	14.4	7.94	336	17
9	291	9 Apr 85	23	4.5	11.4	7.96	239	87
10	333	21 May 85	12	18.5	6.4	7.70	470	76
11	347	4 Jun 85	23	20.0	8.2	8.80	460	43
12	361	18 Jun 85	26	19.1	7.8	8.40	431	50
13	382	9 Jul 85	17	26.5	5.4	7.97	519	53
14	396	23 Jul 85	20	24.5	5.6	7.95	517	49
Site 3 Outlet in Lake Erie								
1	1	22 Jun 84	23	20.0	8.5	8.50	401	32
2	13	5 Jul 84	25	23.5	4.6	8.36	405	50
3	25	17 Jul 84	35	24.2	8.2	8.22	410	32
4	39	31 Jul 84	30	23.0	.2	8.30	454	31
5	54	15 Aug 84	15	24.8	4.2	7.90	420	43
6	76	6 Sep 84	20	18.0	6.9	7.71	445	55
7	102	2 Oct 84	25	12.5	9.8	8.01	426	36
8	180	19 Dec 84	*	4.0	13.6	8.18	270	15
9	291	9 Apr 85	23	4.0	11.9	7.91	186	65
10	333	21 May 85	20	16.0	9.6	8.20	254	46
11	347	4 Jun 85	35	19.0	7.7	8.35	325	36
12	361	18 Jun 85	24	19.9	8.6	8.50	404	33
13	382	9 Jul 85	25	26.5	6.4	7.97	517	24
14	396	23 Jul 85	28	24.0	4.6	7.84	502	29

Although this decline may have been due in part to mixing with Lake Erie water flowing back into the marsh during small seiches, the paucity of such events suggests that the decline is due either to loss of ions from the water (e.g. by ion exchange adsorbtion to sediments) or to dilution by other water inputs to the wetland (e.g. ground water, precipitation, or surface water run-off from non-agricultural sites in the watershed). Turbidity was generally greatest at Site 2. Suspension of plankton and sediment were observed to be greatest at this site, except during storms, when the rapid flux agricultural run-off through Site 1 greatly elevated the turbidity of water entering OWCNES (Figure 2).

Table II presents both turbidity and chlorophyll data. The chlorophyll content of the water at Site 1 was invariably lower than the content of water sampled from the other sites. Generally, the water at Site 2 (mid-estuary) contained the maximum observed at a given time. Chlorophyll rapidly increased from minimum values in the winter through early spring, reaching a maximum in early June, declining somewhat through the summer and reaching a second maximum in early September. Phaeo-pigments also reached a maximum in the late summer. Figure 3 illustrates that turbidity during the summer was likely due to the presence of phytoplankton blooms, turbidity correlating well with chlorophyll content of the water. However, similar turbidity observed during the springtime flow period, was apparently abiogenic, resulting from the turbulent suspension of sediments.

Table III presents the phosphorus compositon of the water at the three sites throughout the study period. Also, the Total P, Total soluble P (TSP) and the soluble reactive P (SRP) are graphed in Figures 4 - 6. Each of these figures presents the data in two ways: in the top figure the X-axis shows the changes in calandar time after the start of the project:

FIGURE 2


Conductivity and Turbidity
Summer - 17 July 1984
800
600
400
200
0
NTU
Cond
Site 1
Site 2
Site 3

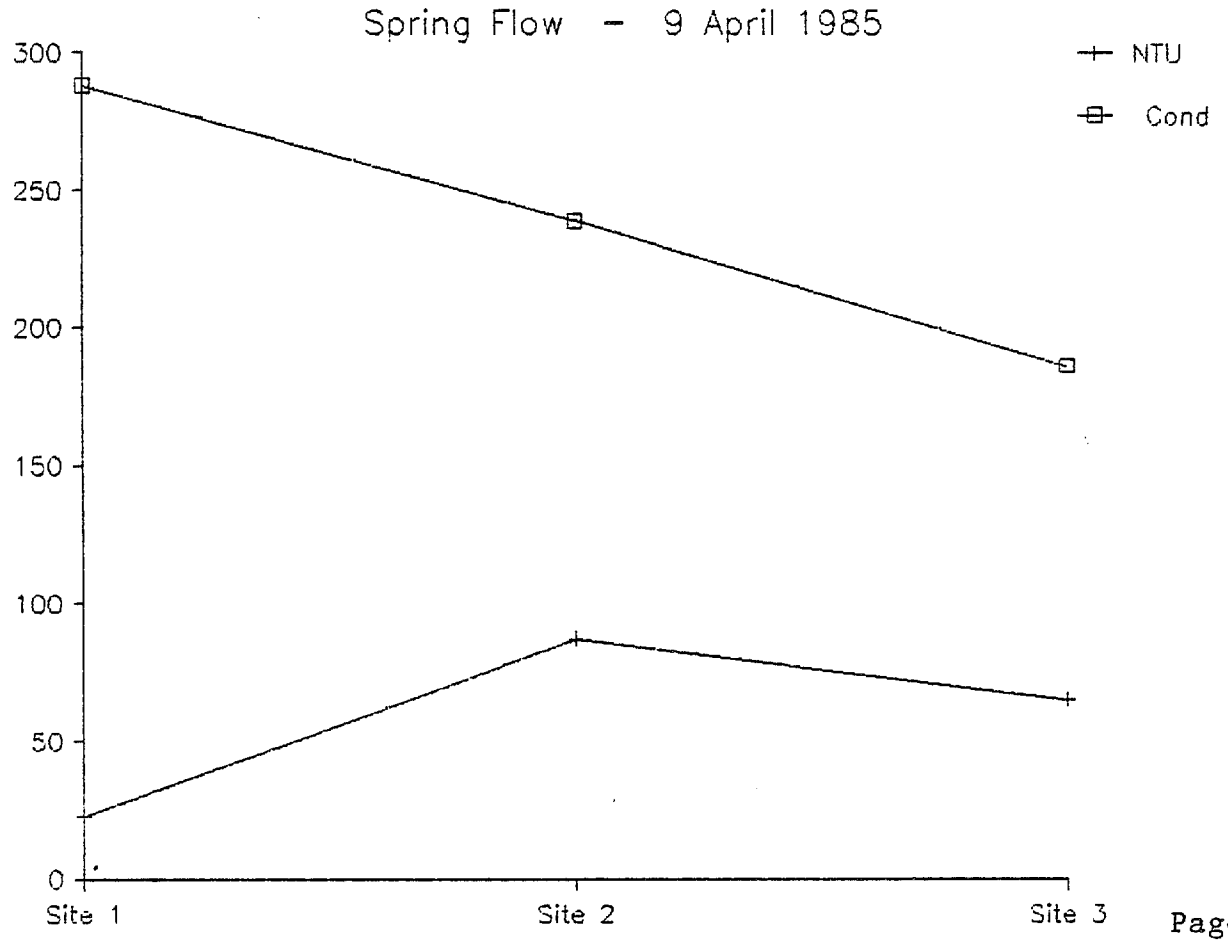
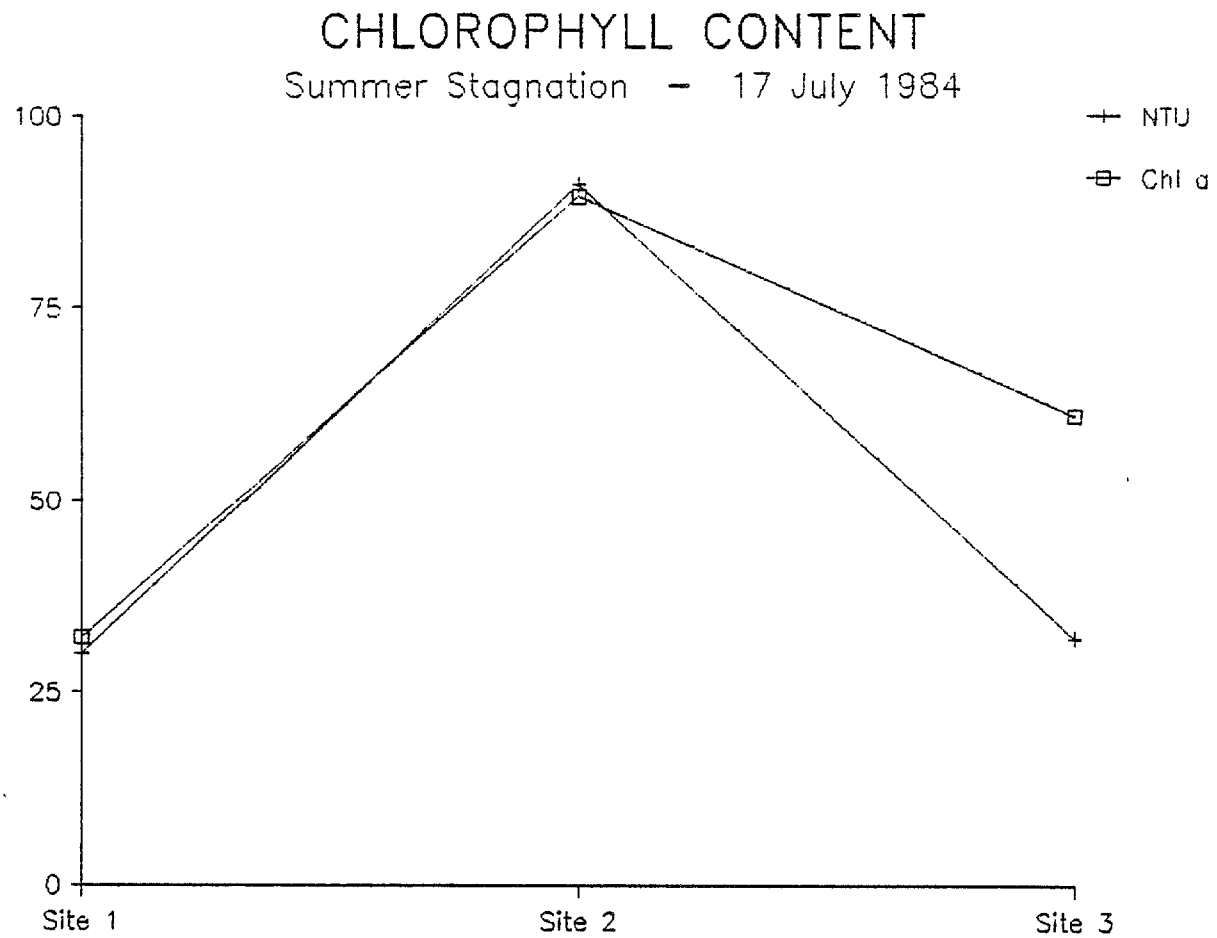

Spring Flow - 9 April 1985
300
250
200
150
100
50
0
NTU
Cond
Site 1
Site 2
Site 3

TABLE II

CHLOROPHYLLL CONTENT

date	NTU	Total chl a	Phaeo	Active chl a
Site 1 - Darrow Road				
22 Jun 84	48	5.24	1.27	3.97
5 Jul 84	250	10.01	1.12	8.89
17 Jul 84	30	32.07	.58	31.49
31 Jul 84	27	11.70	7.18	4.52
15 Aug 84	28	24.64	3.74	20.90
6 Sep 84	35	17.16	.00	17.16
2 Oct 84	55	6.29	.00	6.29
19 Dec 84	5	.92	1.57	-.65
9 Apr 85	23	.00	.00	.00
21 May 85	49	.52	1.27	-.75
4 Jun 85	33	1.84	.00	1.84
18 Jun 85	24	2.77	.00	2.77
9 Jul 85	19	26.49	4.34	22.15
23 Jul 85	40	65.30	.00	65.30
Site 2 - East Island				
22 Jun 84	50	64.68	11.41	53.27
5 Jul 84	86	82.39	9.72	72.67
17 Jul 84	91	89.49	14.81	74.68
31 Jul 84	74	21.25	4.34	16.91
15 Aug 84	75	103.49	18.26	85.23
6 Sep 84	80	143.00	30.58	112.42
2 Oct 84	44	104.68	1.82	102.86
19 Dec 84	17	3.39	1.94	1.45
9 Apr 85	87	5.54	4.05	1.49
21 May 85	76	7.08	4.49	2.59
4 Jun 85	43	231.20	.00	231.20
18 Jun 85	50	120.12	.00	120.12
9 Jul 85	53	81.62	9.06	72.56
23 Jul 85	49	96.71	.00	96.71
Site 3 - Outlet to Lake Erie				
22 Jun 84	32	44.66	.00	44.66
5 Jul 84	50	148.61	.00	148.61
17 Jul 84	32	60.98	7.22	53.76
31 Jul 84	31	24.95	4.27	20.68
15 Aug 84	43	80.70	17.66	63.04
6 Sep 84	55	100.67	29.47	71.20
2 Oct 84	36	102.39	6.48	95.91
19 Dec 84	15	4.31	3.52	.79
9 Apr 85	65	6.47	4.22	2.25
21 May 85	46	5.85	4.49	1.36
4 Jun 85	36	35.40	.00	35.40
18 Jun 85	33	154.31	.00	154.31
9 Jul 85	24	39.12	14.37	24.75
23 Jul 85	29	62.52	.00	62.52

FIGURE 3


CHLOROPHYLL CONTENT
Summer Stagnation - 17 July 1984
100
75
50
25
0
NTU
Chl a
Site 1
Site 2
Site 3

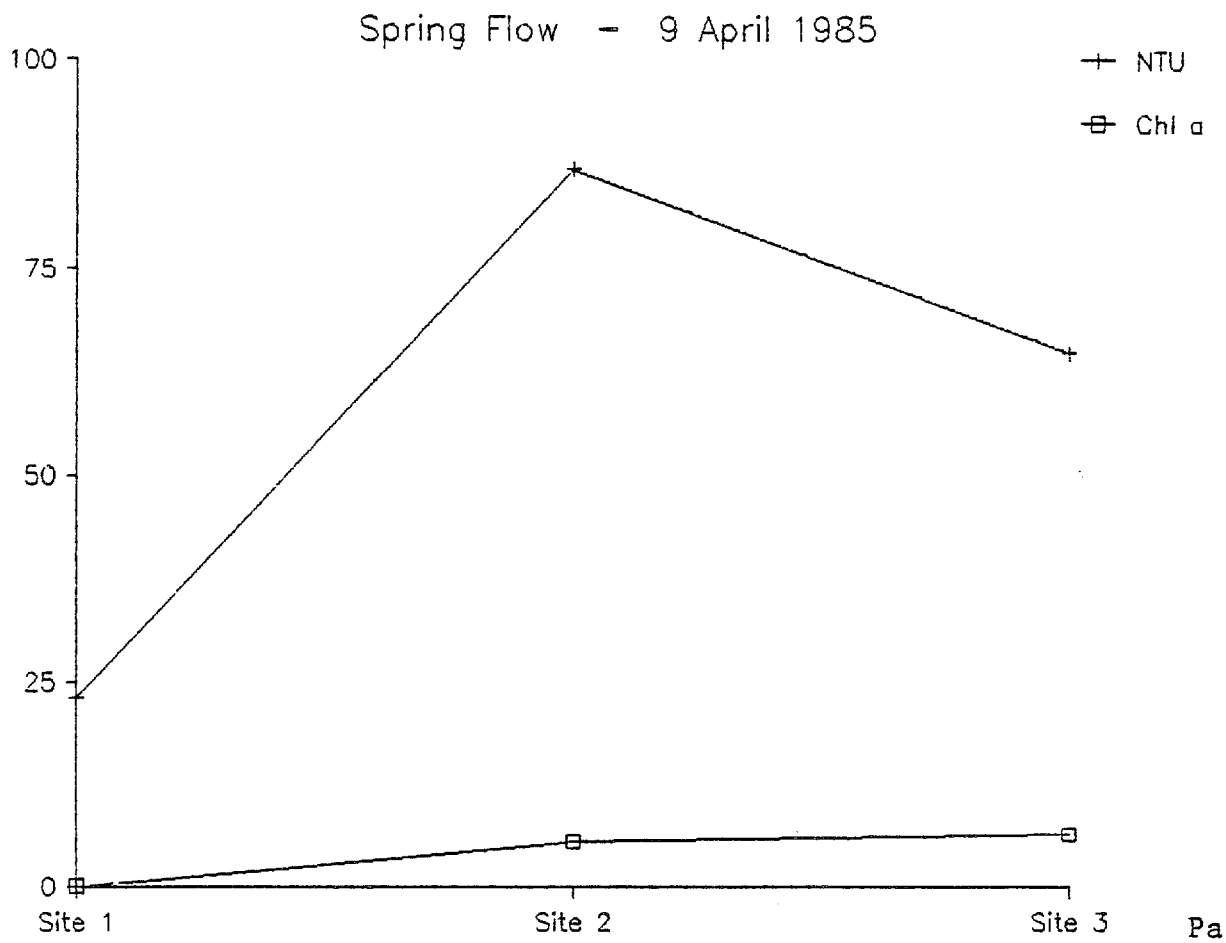

Spring Flow - 9 April 1985
100
75
50
25
0
NTU
Chl a
Site 1
Site 2
Site 3

TABLE III

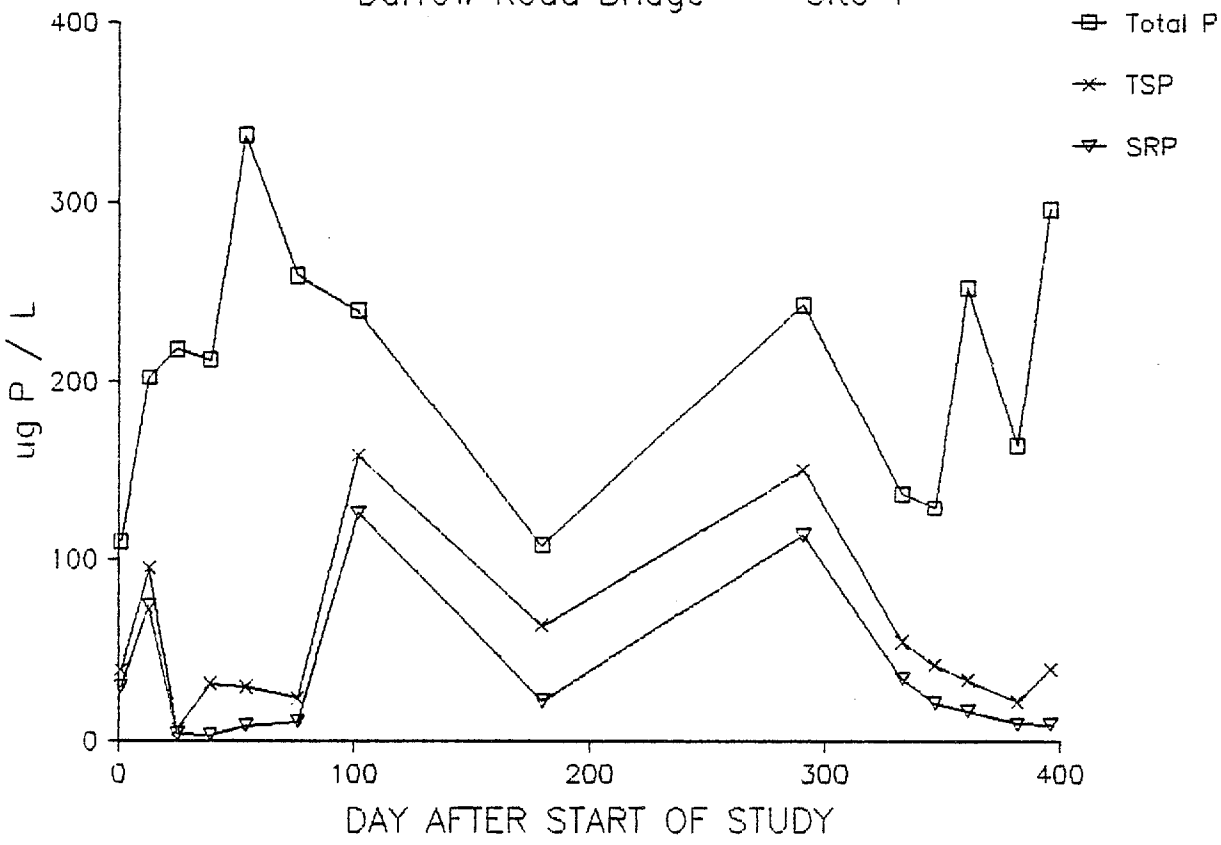
PHOSPHORUS COMPOSITION - O.W.C.N.E.S. - 1984-85

DATE	SRP ug P/L	SUP ug P/L	TSP ug P/L	Part.P ug P/L	Total P ug P/L
Darrow Road Bridge - Site 1					
22 Jun 84	29.58	8.88	38.46	71.24	109.70
5 Jul 84	74.13	21.01	95.14	107.18	202.32
17 Jul 84	3.69	2.73	6.42	211.76	218.18
31 Jul 84	3.13	27.94	31.07	181.04	212.11
15 Aug 84	8.35	20.97	29.32	307.30	336.62
6 Sep 84	10.39	13.02	23.41	235.72	259.13
2 Oct 84	125.90	31.80	157.70	82.10	239.80
19 Dec 84	22.04	41.18	63.22	44.78	108.00
9 Apr 85	113.57	36.61	150.18	92.75	242.93
21 May 85	34.05	20.23	54.28	82.54	136.82
4 Jun 85	20.88	20.48	41.36	87.59	128.95
18 Jun 85	16.32	16.82	33.14	219.46	252.60
9 Jul 85	9.49	12.09	21.58	142.29	163.87
23 Jul 85	8.97	30.23	39.20	256.61	295.81
East Island - Site 2					
22 Jun 84	7.57	2.75	10.32	198.91	209.23
5 Jul 84	19.06	3.67	22.73	221.49	244.22
17 Jul 84	11.16	10.00	21.16	309.94	331.10
31 Jul 84	12.84	36.02	48.86	313.35	362.21
15 Aug 84	9.59	22.39	31.98	462.96	494.94
6 Sep 84	3.46	12.42	15.88	294.90	310.78
2 Oct 84	3.10	21.80	24.90	205.00	229.90
19 Dec 84	9.18	48.50	57.68	54.82	112.50
9 Apr 85	24.90	70.06	94.96	196.56	291.52
21 May 85	8.38	14.13	22.51	208.35	230.86
4 Jun 85	2.32	20.31	22.63	194.64	217.27
18 Jun 85	4.04	26.74	30.78	236.03	266.81
9 Jul 85	7.72	20.26	27.98	266.87	294.85
23 Jul 85	10.27	24.12	34.39	311.21	345.60
Outlet to Lake Erie - Site 3					
22 Jun 84	2.61	.10	2.71	106.99	109.70
5 Jul 84	8.99	.63	9.62	192.70	202.32
17 Jul 84	5.56	14.06	19.62	198.56	218.18
31 Jul 84	6.80	38.89	45.69	166.42	212.11
15 Aug 84	5.33	19.46	24.79	311.83	336.62
6 Sep 84	1.73	14.90	16.63	242.50	259.13
2 Oct 84	3.60	26.60	30.20	209.60	239.80
19 Dec 84	6.79	82.61	89.40	18.60	108.00
9 Apr 85	22.85	58.86	81.71	161.22	242.93
21 May 85	6.02	10.24	16.26	120.56	136.82
4 Jun 85	2.32	11.54	13.86	115.09	128.95
18 Jun 85	8.77	20.35	29.12	223.48	252.60
9 Jul 85	4.77	22.41	27.18	136.69	163.87
23 Jul 85	1.00	31.95	32.95	262.86	295.81

FIGURE 4

PHOSPHORUS COMPOSITION

Darrow Road Bridge – Site 1



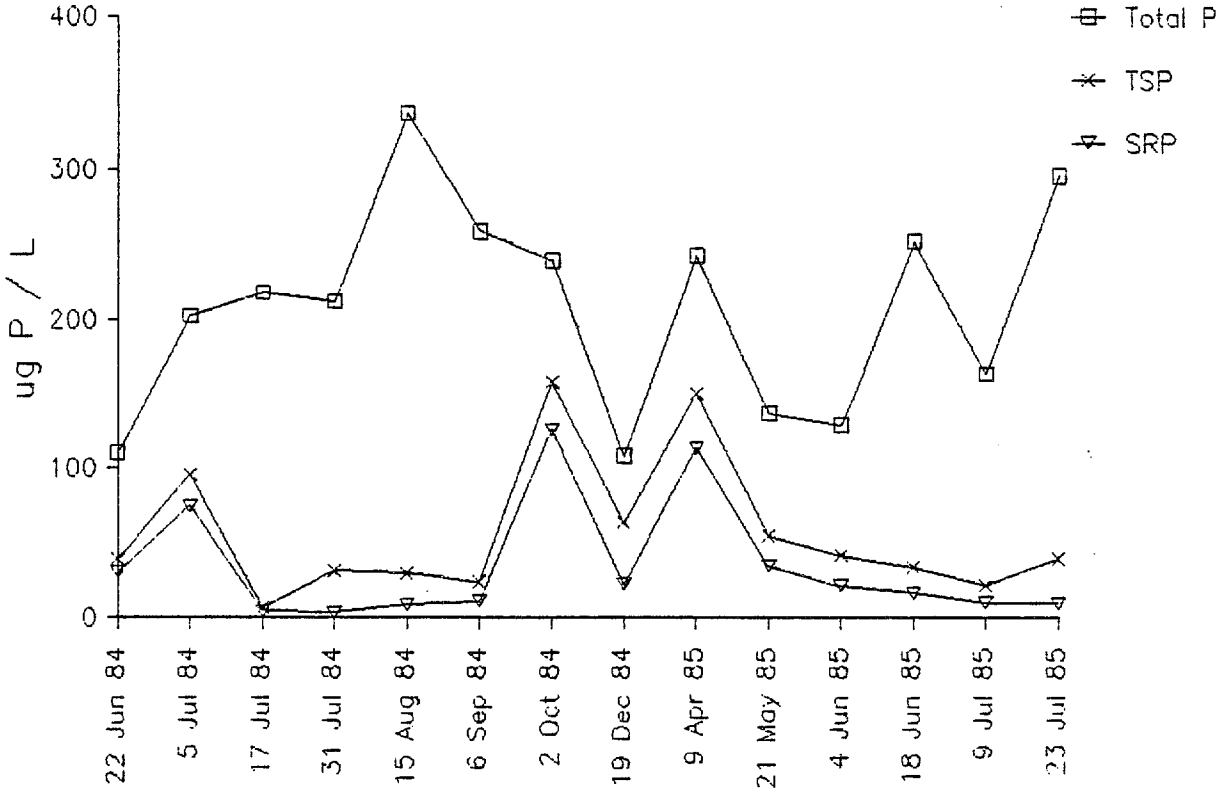


FIGURE 5

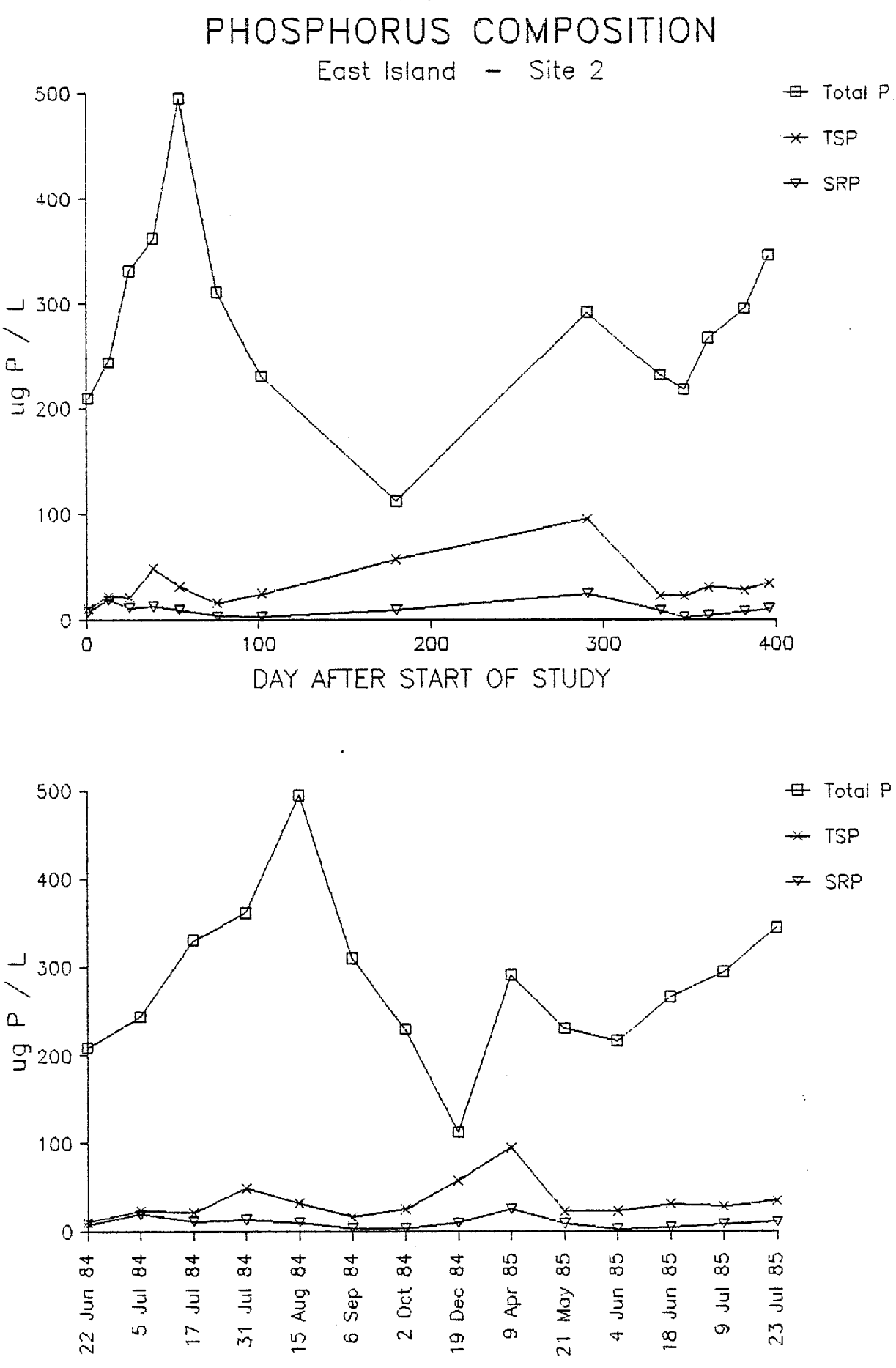
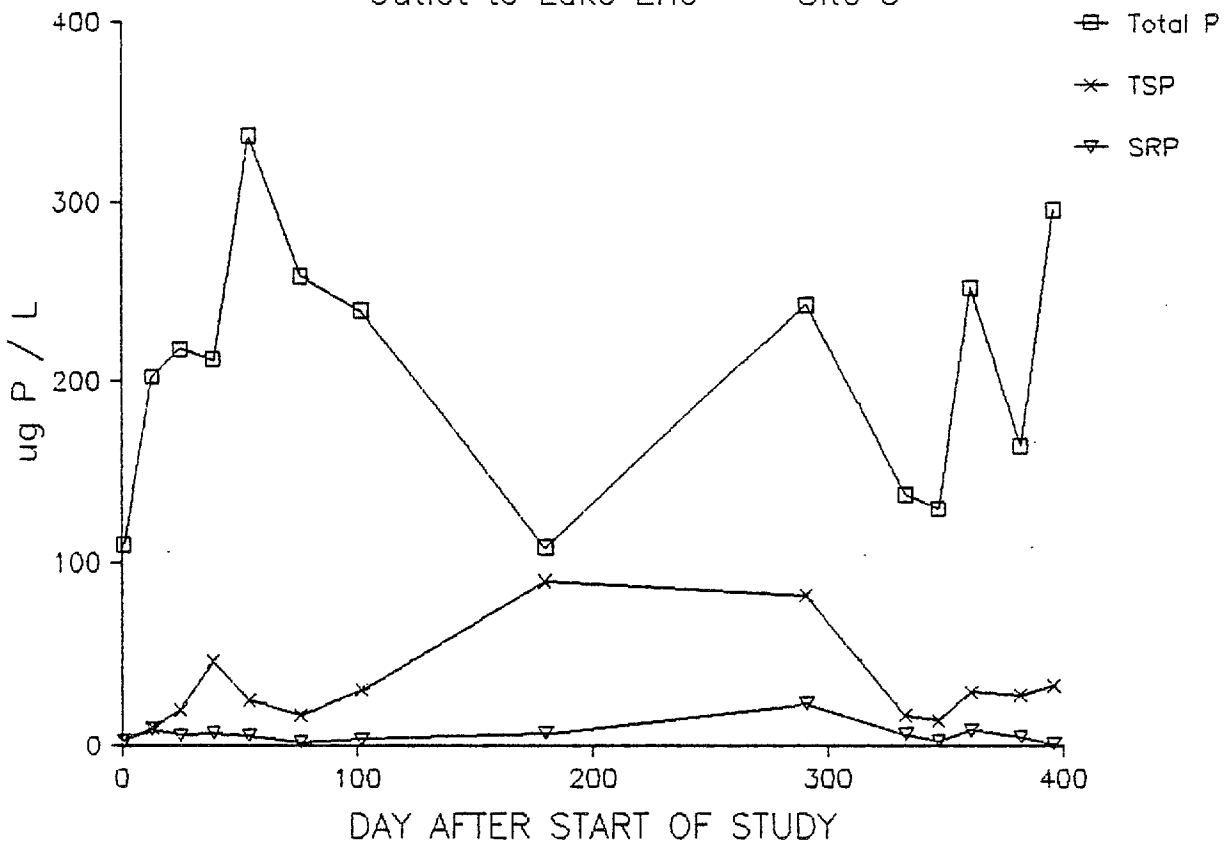
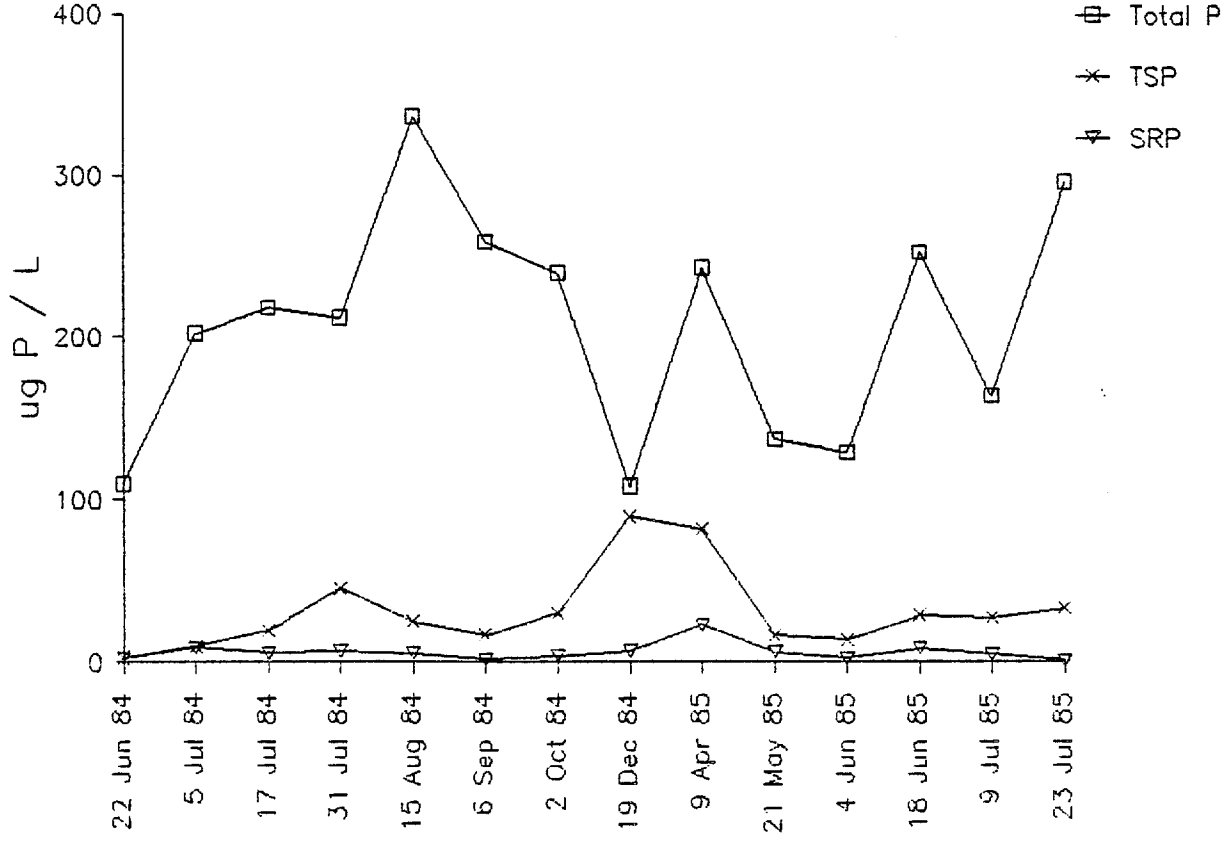

PHOSPHORUS COMPOSITION
East Island - Site 2
Total P
TSP
SRP
ug P / L
500
400
300
200
100
0
0
100
200
300
400
DAY AFTER START OF STUDY
Total P
TSP
SRP
ug P / L
500
400
300
200
100
0
22 Jun 84
5 Jul 84
17 Jul 84
31 Jul 84
15 Aug 84
6 Sep 84
2 Oct 84
19 Dec 84
9 Apr 85
21 May 85
4 Jun 85
18 Jun 85
9 Jul 85
23 Jul 85

FIGURE 6

PHOSPHORUS COMPOSITION

Outlet to Lake Erie – Site 3





day 0 - 22 June 1984 - stagnation begins

day 100 - 16 September 1984 - end of summer

day 180 - 3 December 1984 - channel flow begins

day 200 - 25 December 1984 - winter flow

day 300 - 4 April 1985 - early spring flow

day 358 - 1 June 1985 - stagnation begins

day 400 - 13 July 1985 - mid summer

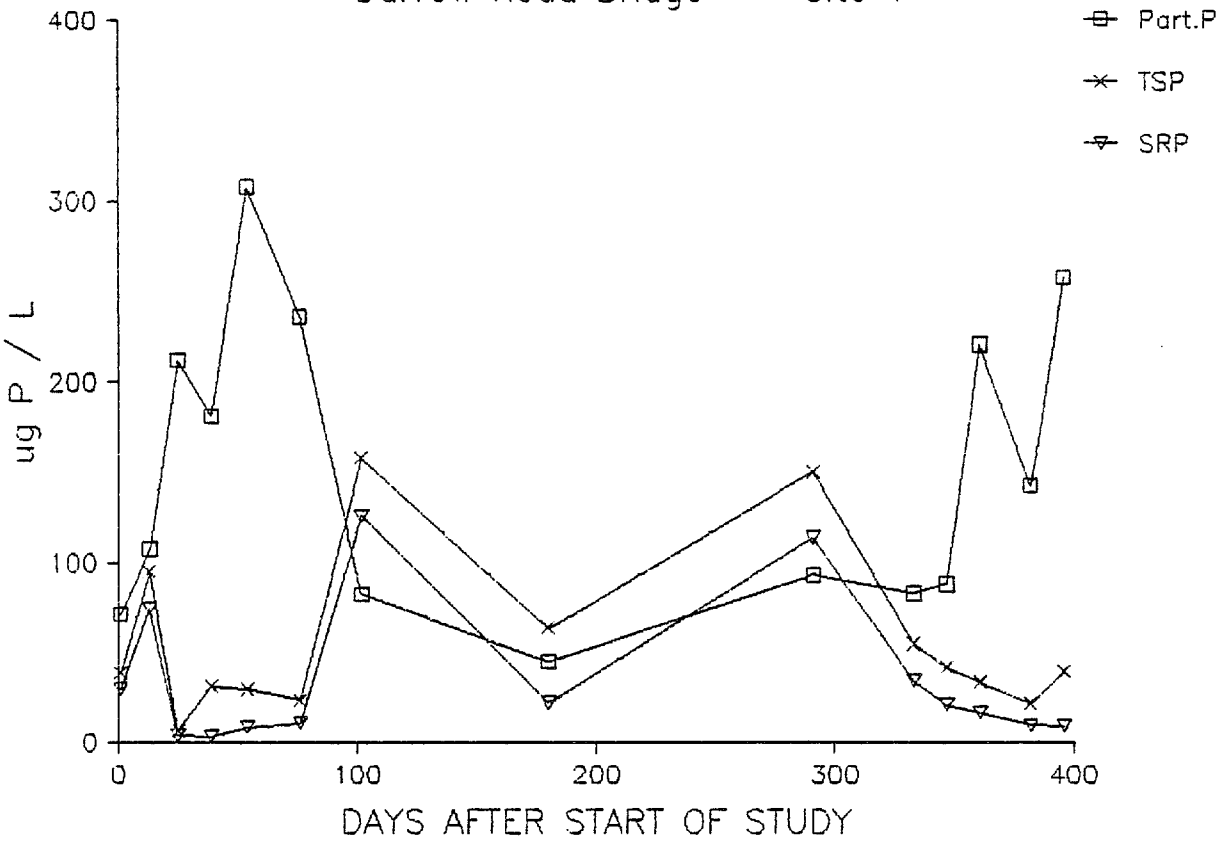
The range between Total P and TSP represents the amount of particulate P, and the range between TSP and SRP represents the amount of P compounds dissolved in the water other than phosphate. The second graph in Figures 4 - 6 presents the data on a visit-by-visit basis. The seasonal events are best considered by examining the top graph, because its axis is in "real time"; the lower graph presents a better view of the data, but it also provides an unrealisitc view of the events during the periods of open flow.

At each sample site the total phosphorus content of the water reached its maximum values late in the growing season, then declined as the channel opened and particulate material was carried into Lake Erie. The Total P content reached a minimum in the winter, then increased as the spring rains contributed agricultural run-off to the estuary. Particulate P and soluble P tended to vary inversely at each site (Figures 7 - 9). During the periods of flow through the estuary the soluble P composition predominated, as particulate P reached its minimum in December. From early spring through the end of the summer the soluble P content declined at each site, it then increased from late August until October (as particulate P decreased) and remained high throughout the winter. Soluble P at Site 1 differed from that at the other sites in that it was predominately SRP, while SRP represented a relatively small portion of the TSP at Sites 2 and 3. SRP rose to very high concentrations (greater than 100 ug/L) at Site 1 during the winter flow period; however the highest concentrations of

FIGURE 7

PARTICULATE AND SOLUBLE P

Darrow Road Bridge – Site 1



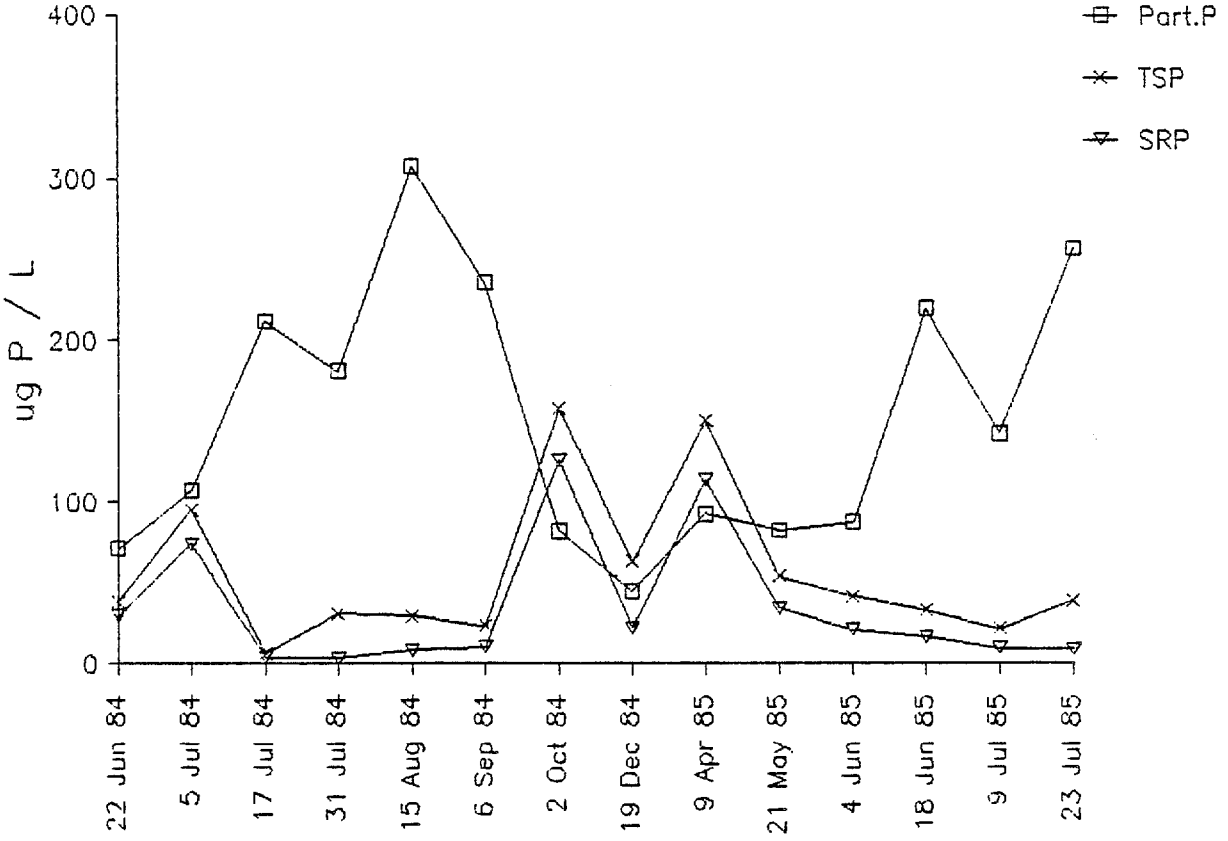


FIGURE 8

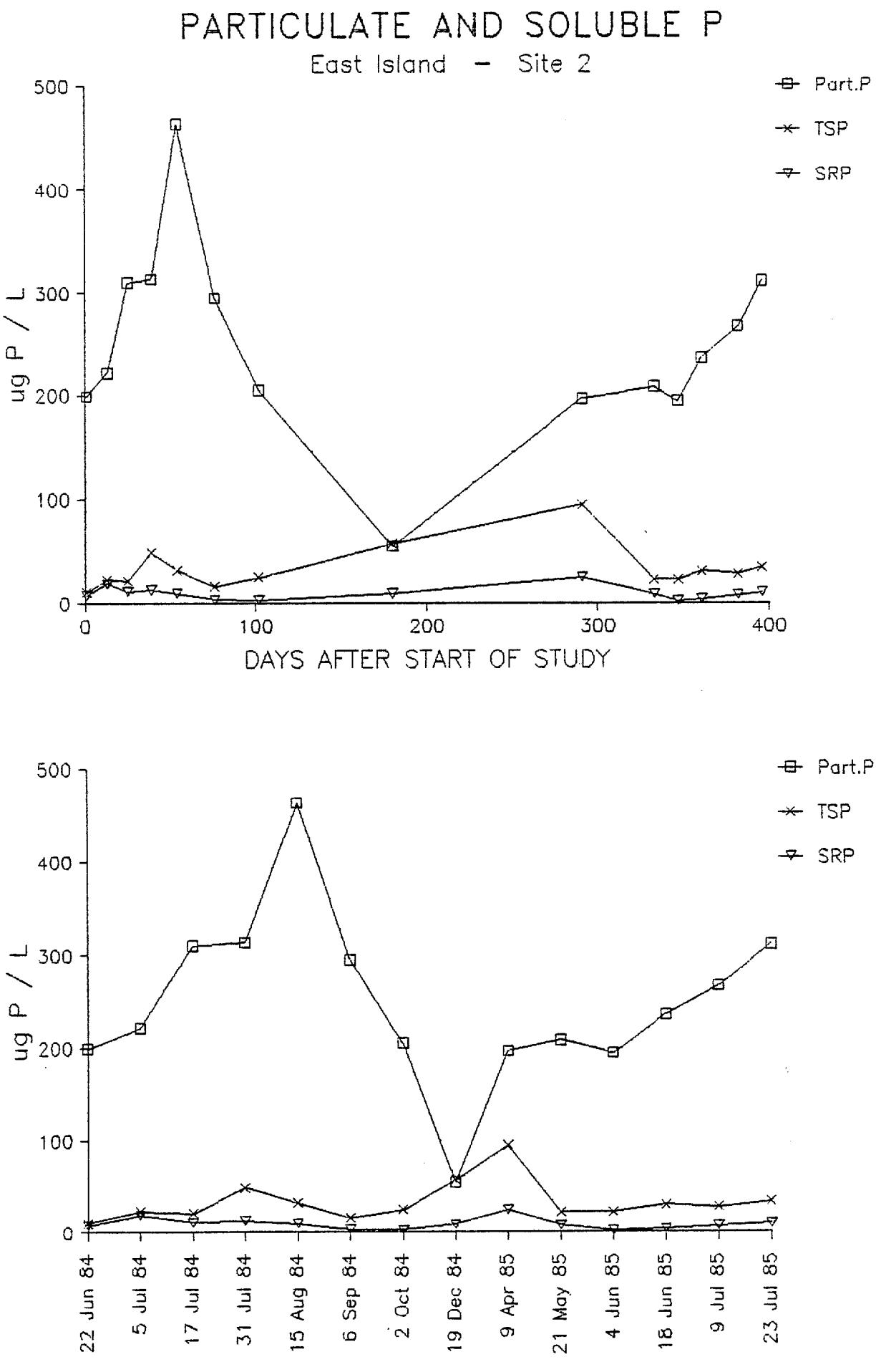
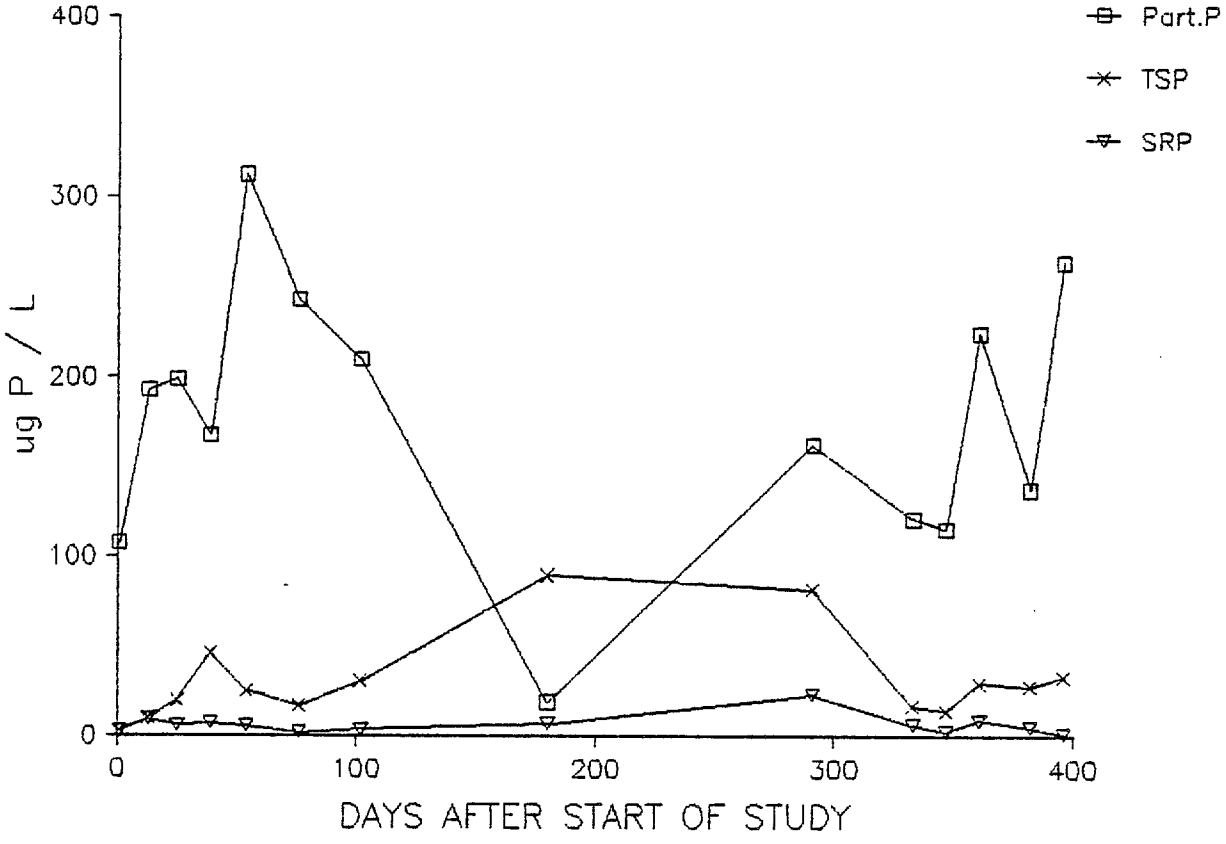
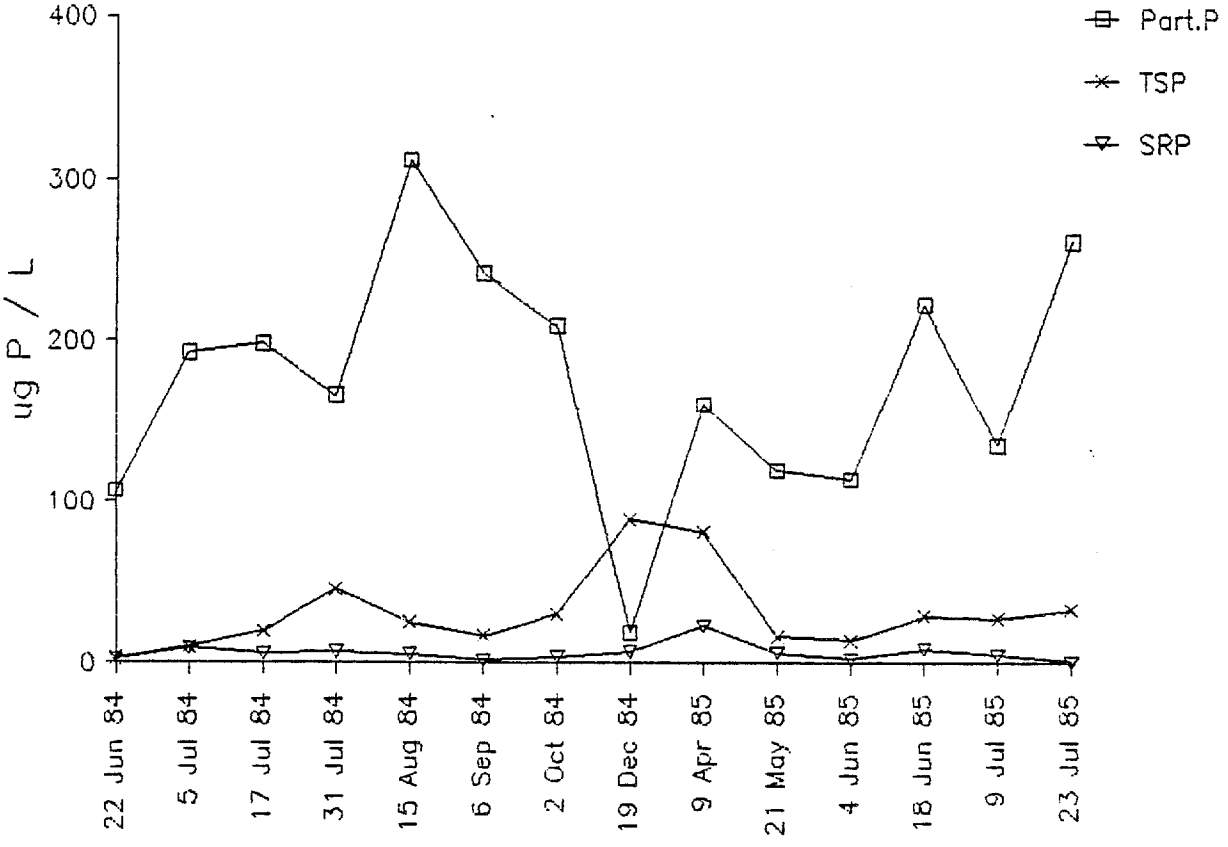

PARTICULATE AND SOLUBLE P
East Island - Site 2
Part.P
TSP
SRP
ug P / L
500
400
300
200
100
0
0
100
200
300
400
DAYS AFTER START OF STUDY
Part.P
TSP
SRP
ug P / L
500
400
300
200
100
0
22 Jun 84
5 Jul 84
17 Jul 84
31 Jul 84
15 Aug 84
6 Sep 84
2 Oct 84
19 Dec 84
9 Apr 85
21 May 85
4 Jun 85
18 Jun 85
9 Jul 85
23 Jul 85

FIGURE 9

PARTICULATE AND SOLUBLE P

Outlet to Lake Erie - Site 3





SRP in the were seen during the late spring run-off period. It was only during this early springtime that SRP concentrations higher than 20 ug/L were observed at Sites 2 and 3.

The relationship between the sampling sites is illustrated in a series of figures compiled from data taken during the summer stagnation period (Figures 10 and 11), during the period winter flow through the estuary (Figure 12) and then during the early and late springtime (Figures 13 and 14). This series of figures together emphasize that even during the period of flow the amount of particulate P is greatest at the mid-estuary site. Also, a general trend observed was a decreasing TSP and SRP content in the water as flow progressed through the estuary.

This general presentation of the limnological variables and phosphorus composition on an annual basis indicates that study of OWCNES should be divided in two "limnological seasons": a period of open flow through the estuary from the late autumn through late spring, and secondly a period of relative stagnation when lake wave activity closes the open flow through the mouth into Lake Erie. The period of open flow is characterized by non-biogenic turbidity and relatively low conductivity. Total P reaches a minimum during this period, apparently as substantial quantities of particulate P are carried into Lake Erie. The phosphorus content of the water is largely in dissolved compounds, especially during the early spring run-off period. SRP reaches its maximum then declines throughout the growing season at all sites. The growing season occurs during the period of relative stagnation. The water is turbid, but this turbidity is in large part due to blooms of phytoplankton. The ionic content of the water is high. Total P increased during this period, due almost solely to the rapid increase of particulate P suspended in the water. This period is characterized by a continually decreasing content in TSP and SRP as the particulate P increases.

FIGURE 10

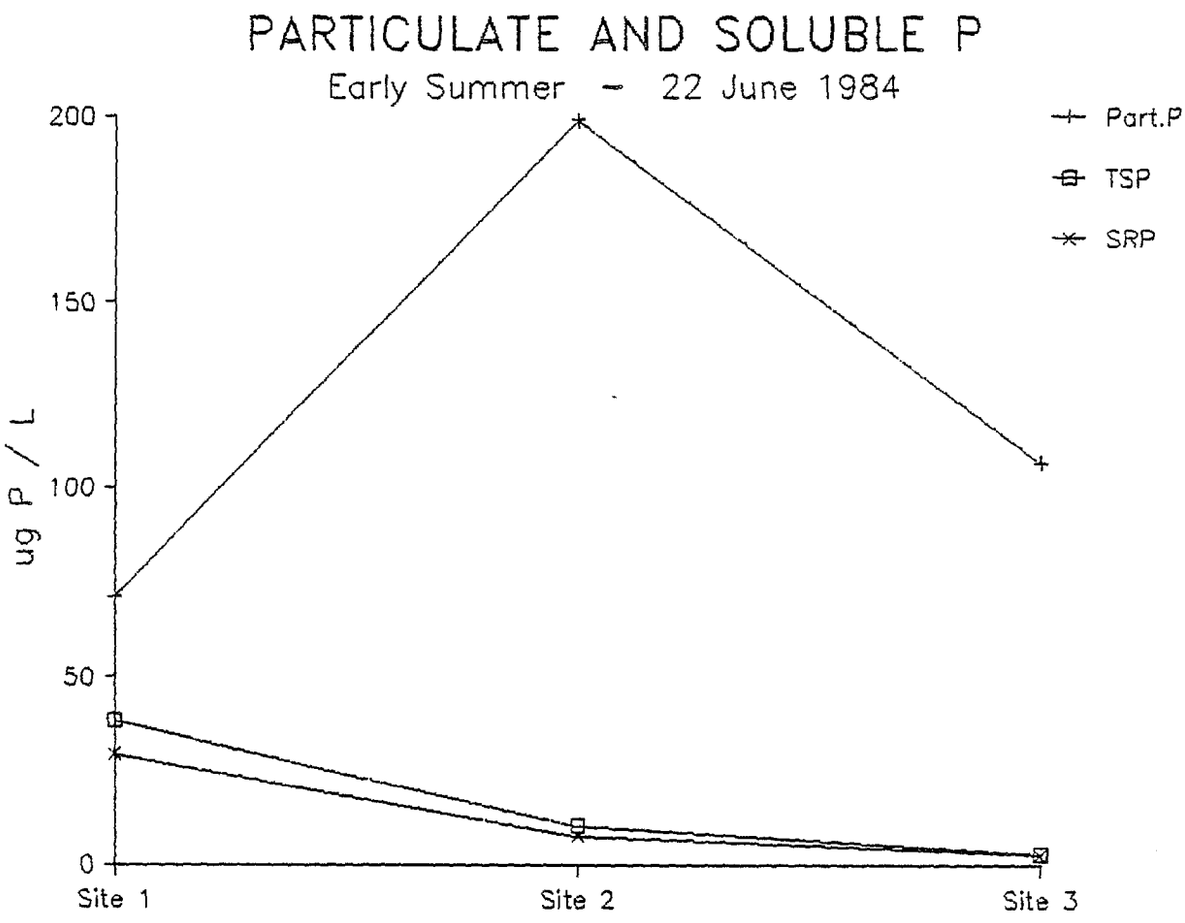

PARTICULATE AND SOLUBLE P
Early Summer - 22 June 1984
ug P / L
200
150
100
50
0
Site 1
Site 2
Site 3
Part.P
TSP
SRP

FIGURE 11

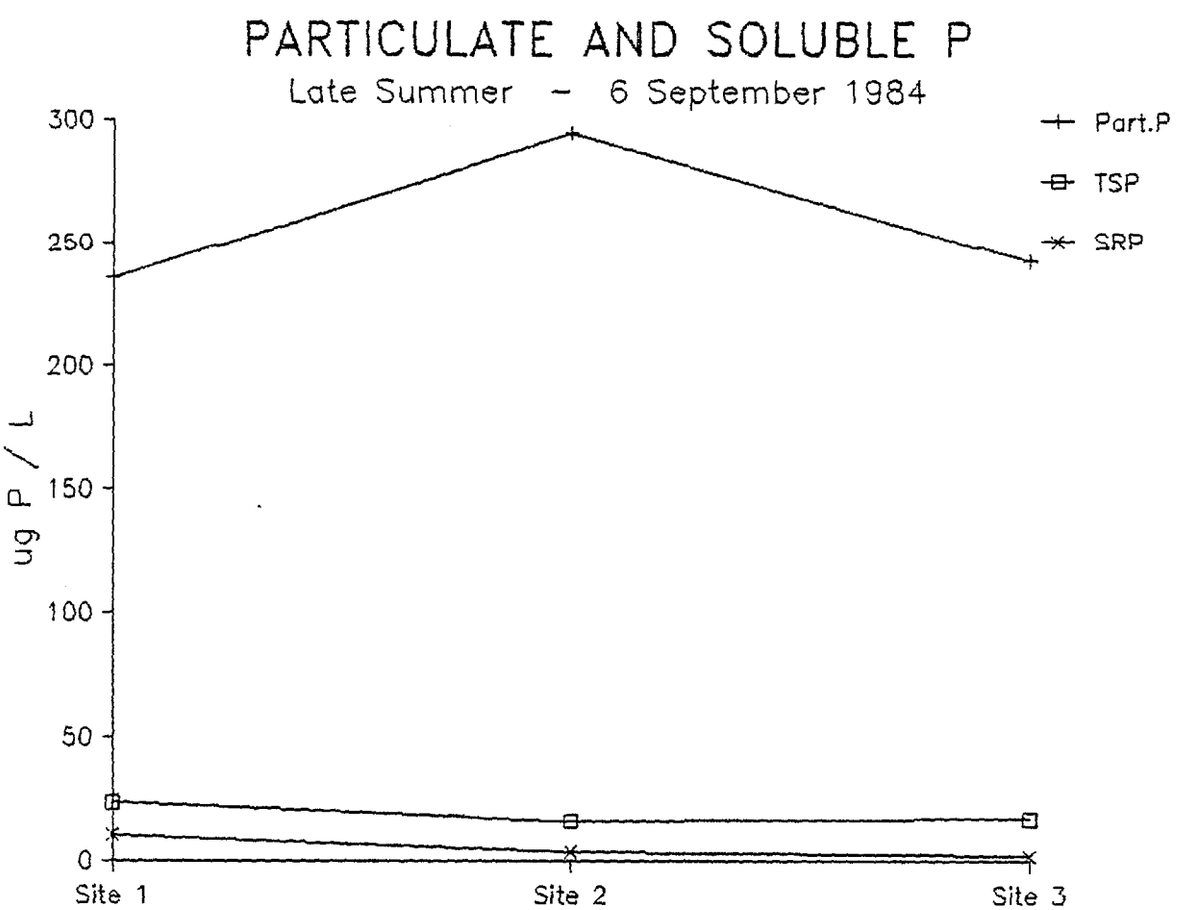

PARTICULATE AND SOLUBLE P
Late Summer - 6 September 1984
Part.P
TSP
SRP
ug P / L
300
250
200
150
100
50
0
Site 1
Site 2
Site 3

FIGURE 12

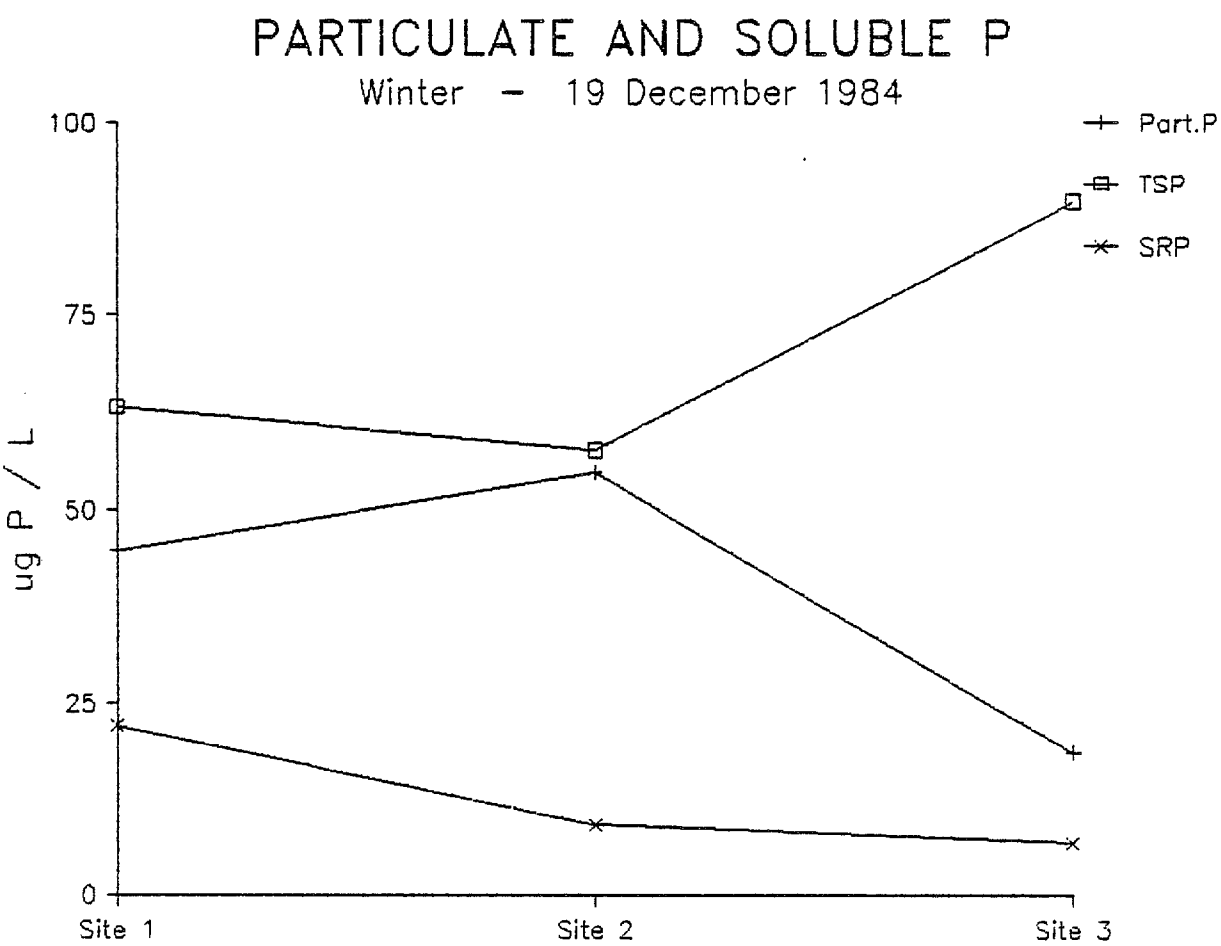

PARTICULATE AND SOLUBLE P
Winter - 19 December 1984
Part.P
TSP
SRP
ug P / L
100
75
50
25
0
Site 1
Site 2
Site 3

FIGURE 13

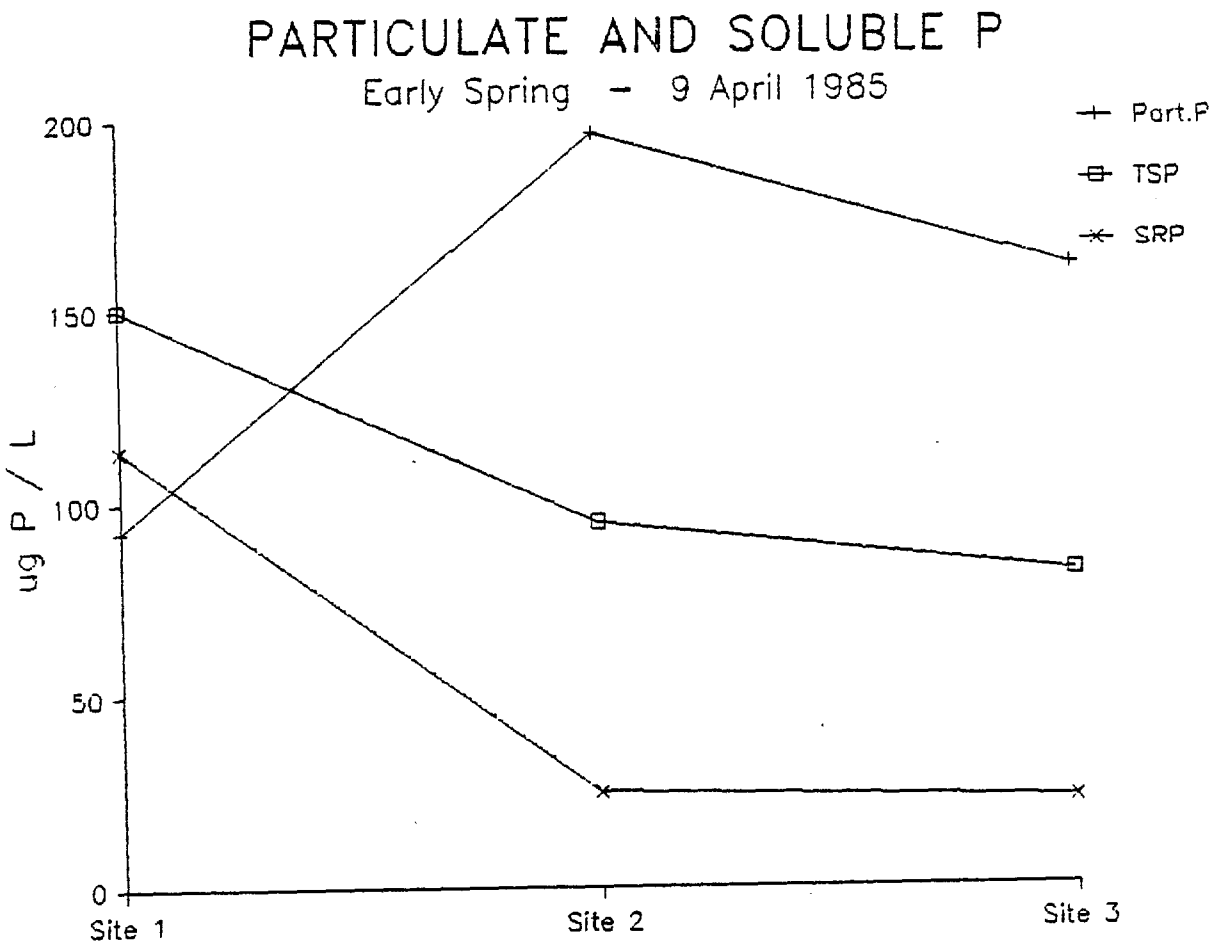
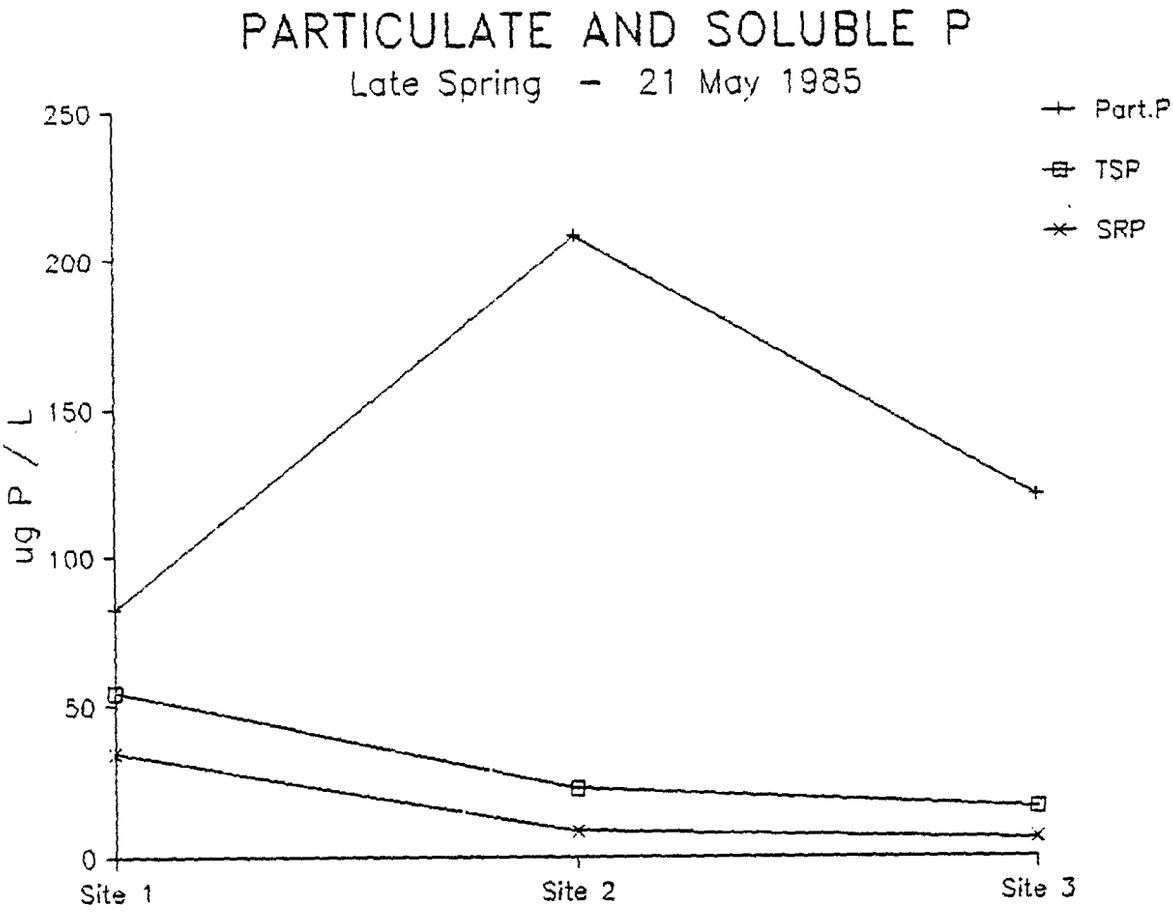

PARTICULATE AND SOLUBLE P
Early Spring - 9 April 1985
Part.P
TSP
SRP
200
150
100
50
0
ug P / L
Site 1
Site 2
Site 3

FIGURE 14


PARTICULATE AND SOLUBLE P
Late Spring - 21 May 1985
Part.P
TSP
SRP
250
200
150
100
50
0
ug P / L
Site 1
Site 2
Site 3

II. Examination for limitation of planktonic growth by P availability

Phosphorus limitation of the growth of freshwater communities can be examined by bioassay techniques as well as by examining the physiological conditions of the biota at the time of collection. Also, the chemical characteristics of the water can be useful in determining whether the community may be P-limited. In this study each of these approaches was used. Nutrient addition bioassays were conducted during the height of the growing season. Biochemical responses such as the alkaline phosphatase specific activity and the rate of phosphate turnover relative to the particulate P present were used to characterize the physiological status of the phytoplankton. Finally, the chemical characteristics of the collected were used as evidence of P-limitation. None of these criteria is paramount in making a decision of P limitation, but their results taken together are useful in interpreting the nutritional status of the planktonic community with respect to its phosphorus resources. The body of the evidence indicates that planktonic growth was seldom, if ever, limited by the availability of P.

Nutrient addition bioassays were conducted on samples collected during the height of the growing season. Also, at this time the amount of SRP approached its minimum concentrations. Because the standing crop reached its maximum and because of the relative paucity of nutrients, I reasoned that a nutrient limitation that markedly affected algal standing crop would most likely be found at this time. Although nutrient limitations could be sought at other times of the year, their significance to activities in OWCNES would be questionable. After addition of nutrients, triplicate tests were incubated for 14 days at ambient temperature. The chlorophyll content of the total contents was then determined and the results of the various treatments were compared.

The means of each treatment were compared to the mean of the untreated control and tested for significant increase over the control. Determination of

significant differences ($p<0.05$) was done using Dunnet's method (1955), calculating a q' statistic and comparing it with tabulated critical q' (Zar 1974). The determination as conducted was one-tailed (i.e. we only looked for significant increases over the control). Of the samples collected from Site 2 on 17 July 1984, only the sample amended with nitrate significantly increased in its growth (Figure 15). Samples collected from all sites on 30 July 1984 showed that although there was a tendency for a decrease in growth of the control toward the outlet and an apparent tendency for an increase in growth in nitrate-amended treatments (Figure 16), only the differences in the nitrate-amended samples from Site 1 differed significantly from the control (Table IV).

Physiological variables examined were the phosphate turnover time and the alkaline phosphatase specific activity. The phosphate turnover time is the inverse of the proportional uptake rate and it indicates the time that it would take to remove all of the available phosphate from solution to particles, if it continued to move at the steady-state rate. Table V shows that the turnover times were generally longer than 100 min. at Site 1 and seldom less than 20 min. at any of the sampling sites. Only at Sites 2 and 3 in June 1985 were phosphate turnover times observed to be less than 10 min.

Jones (1972) has suggested that the magnitude of the specific activity (nmol phosphate released/L/min per ug Chl a/L resulting in a unit of nmol P released/min/ug Chl a) may serve as an indicator of P-limitation stress. Enzyme activity can also be scaled for the amount of particulate P, yielding a unit of specific activity that represents throughput of the standing particulate P if throughput was exclusively due to phosphatase: nmol P released/L/min per nmol particulate P/L, giving a unit in 1/min, i.e. proportion of particulate P moved from soluion to particles via phosphatase. Figures 17 to 19 show that the specific activity of alkaline phosphatase in the OWCNES community was always

FIGURE 15

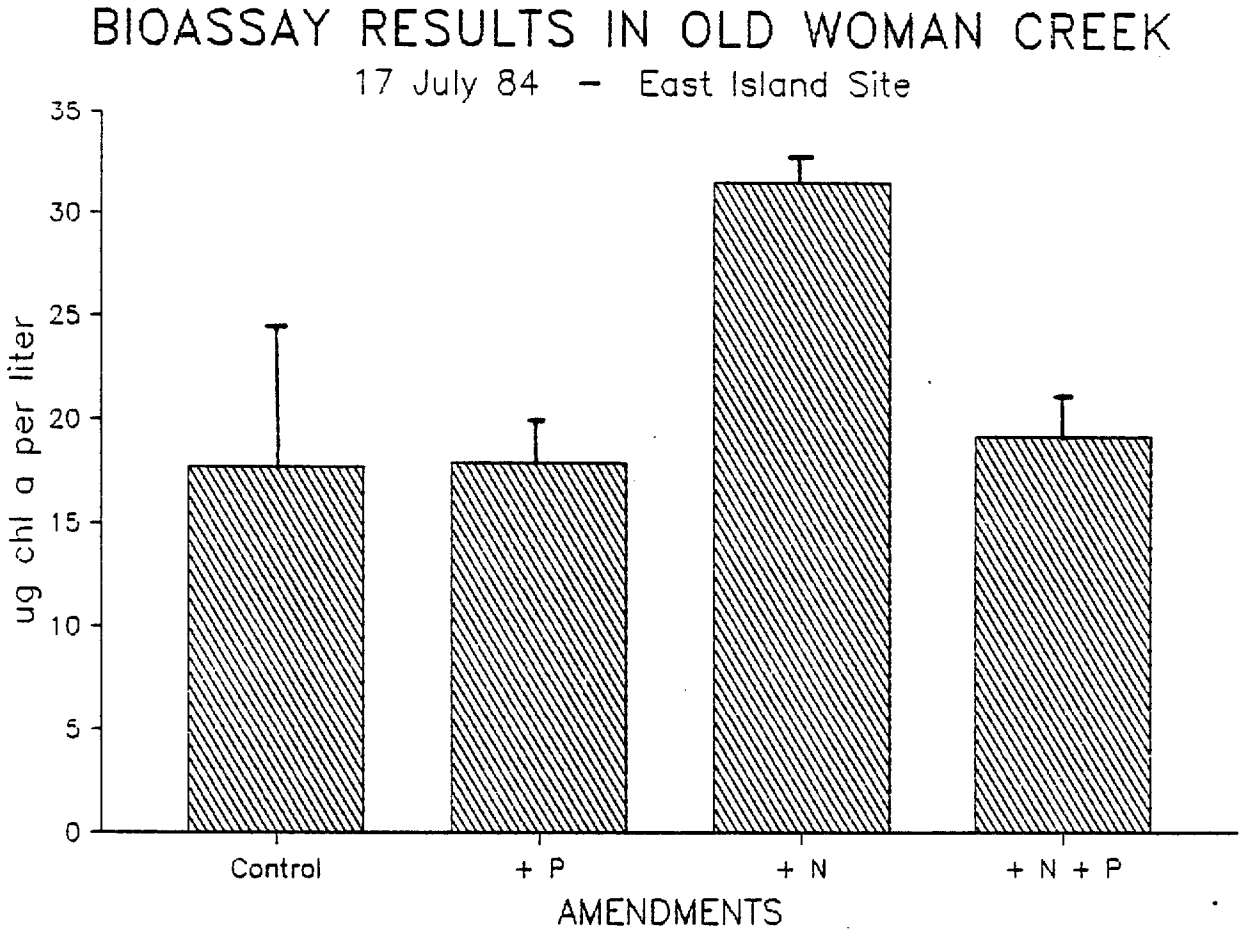

BIOASSAY RESULTS IN OLD WOMAN CREEK
17 July 84 - East Island Site
ug chl a per liter
35
30
25
20
15
10
5
0
Control
+ P
+ N
+ N + P
AMENDMENTS

FIGURE 16

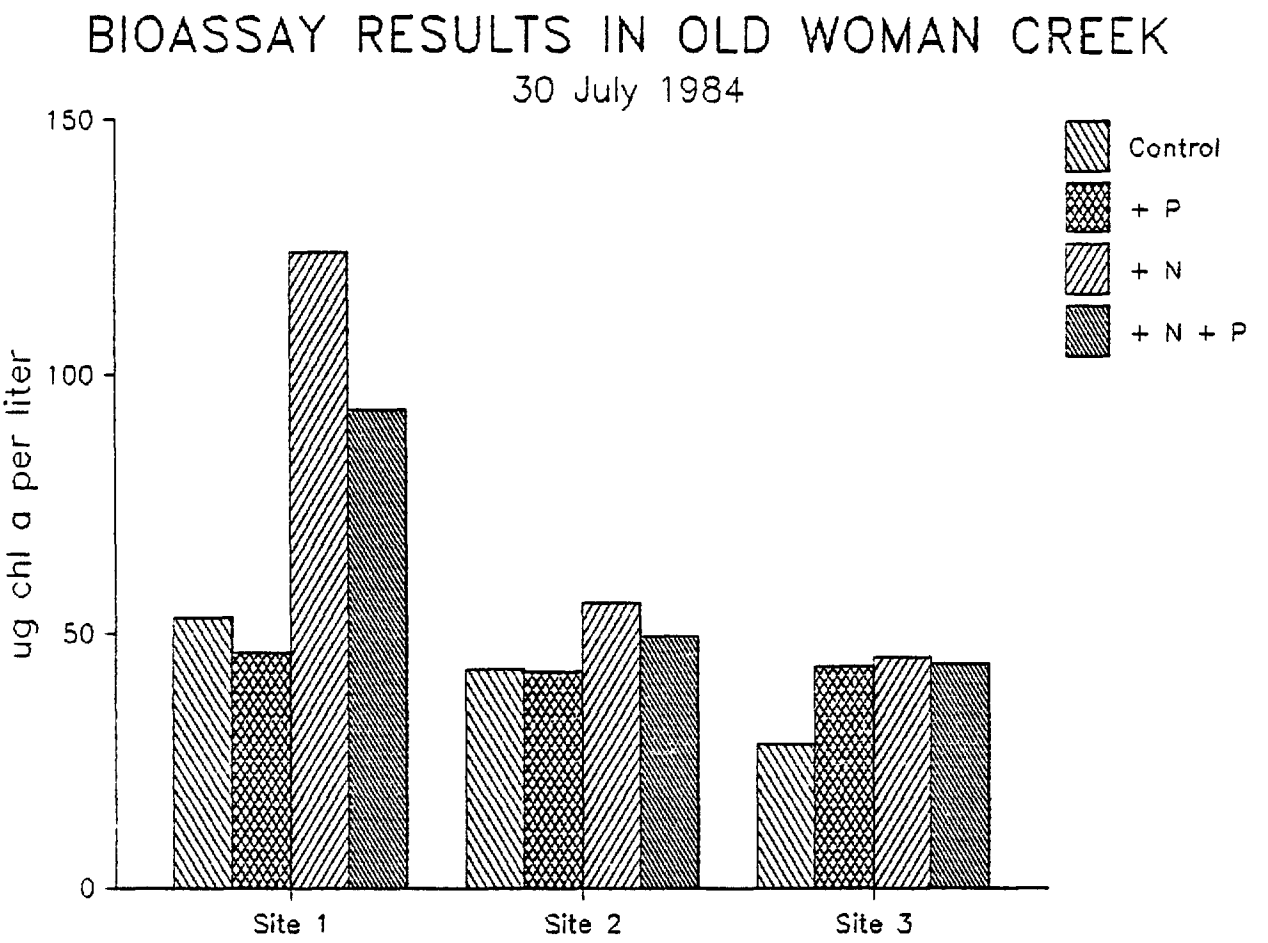

BIOASSAY RESULTS IN OLD WOMAN CREEK
30 July 1984
ug chl a per liter
150
100
50
0
Site 1
Site 2
Site 3
Control
+ P
+ N
+ N + P

TABLE IV

NUTRIENT ADDITION BIOASSAYS

30 July 1984

	Chlorophyll content (mg/L) 14 days after nutrient addition: Control	+ P	+ N	+ N + P
SITE 1 : DARROW ROAD BRIDGE				
	46.20	53.13	130.90	93.17
	53.90	33.88	116.27	100.10
	59.29	51.59	123.58	86.24
Average	53.13	46.20	123.58	93.17
S.D.	6.58	10.70	7.32	6.93
Std. Error		7.19	31.91	18.57
q'		.96	2.21	2.16
			*	*
SITE 2 : EAST ISLAND				
	40.04	42.35	40.04	43.89
	43.89	30.03	65.45	53.90
	44.66	54.67	62.37	50.82
Average	42.86	42.35	55.95	49.54
S.D.	2.48	12.32	13.87	5.13
Std. Error		6.49	9.34	4.19
q'		.08	1.40	1.59
SITE 3 : OUTLET TO LAKE ERIE				
	23.87	33.11	39.27	39.27
	29.26	56.98	42.35	50.82
	31.57	40.81	54.67	41.58
Average	28.23	43.63	45.43	43.89
S.D.	3.95	12.18	8.15	6.11
Std. Error		9.55	9.00	7.95
q'		1.61	1.91	1.97

* Signifies that observed value is significantly different from the control flask ($P < 0.05$). One-tailed Dunnet test.

TABLE V

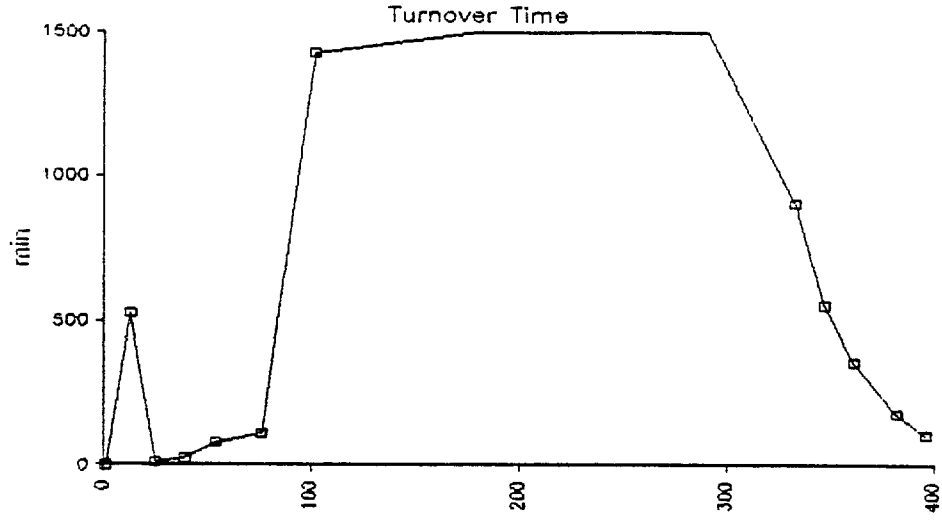
PHOSPHATE TURNOVER TIME AND
PHOSPHATASE SPECIFIC ACTIVITY

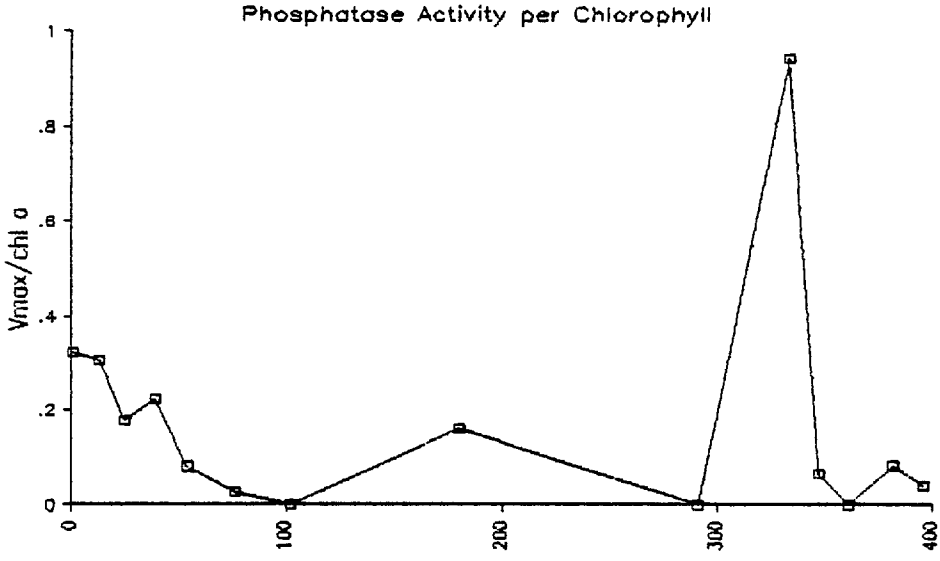
Date	T min	Vmax/chl a nmol/min/ug chl	Vmax/PartP 1/day
Site 1 - Darrow Road Bridge			
22 Jun 84	*	.325	1.065
5 Jul 84	526.3	.307	1.279
17 Jul 84	12.8	.178	1.207
31 Jul 84	23.3	.223	.643
15 Aug 84	78.7	.081	.290
6 Sep 84	111.1	.027	.088
2 Oct 84	1428.6	.002	.008
19 Dec 84	2000.0	.162	.149
9 Apr 85	10000.0	.000	.000
21 May 85	909.1	.946	.266
4 Jun 85	555.6	.065	.061
18 Jun 85	357.1	.000	.000
9 Jul 85	178.6	.084	.697
23 Jul 85	106.4	.040	.454
Site 2 - East Island			
22 Jun 84	*	.000	.000
5 Jul 84	217.4	.026	.433
17 Jul 84	172.4	.078	1.005
31 Jul 84	128.2	.083	.253
15 Aug 84	60.2	.028	.277
6 Sep 84	31.4	.014	.311
2 Oct 84	14.3	.005	.123
19 Dec 84	1428.6	.022	.061
9 Apr 85	238.1	.054	.068
21 May 85	294.1	.069	.105
4 Jun 85	8.7	.036	1.894
18 Jun 85	17.1	.047	1.074
9 Jul 85	68.5	.036	.494
23 Jul 85	105.3	.024	.331
Site 3 - Outlet to Lake Erie			
22 Jun 84	*	.070	1.300
5 Jul 84	131.6	.014	.487
17 Jul 84	13.5	.061	.838
31 Jul 84	29.9	.059	.392
15 Aug 84	29.2	.027	.307
6 Sep 84	26.3	.019	.359
2 Oct 84	18.9	.003	.076
19 Dec 84	1428.6	.017	.179
9 Apr 85	833.3	.131	.235
21 May 85	238.1	.087	.188
4 Jun 85	24.8	.079	1.081
18 Jun 85	8.5	.070	2.167
9 Jul 85	43.7	.048	.613
23 Jul 85	31.1	.039	.415

* Variable not determined

FIGURE 17

SITE 1 DARROW ROAD BRIDGE





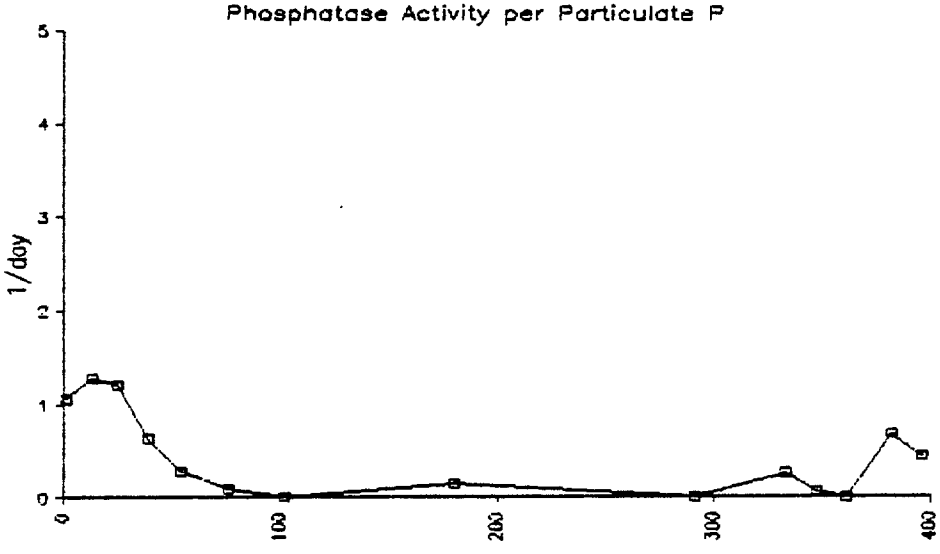
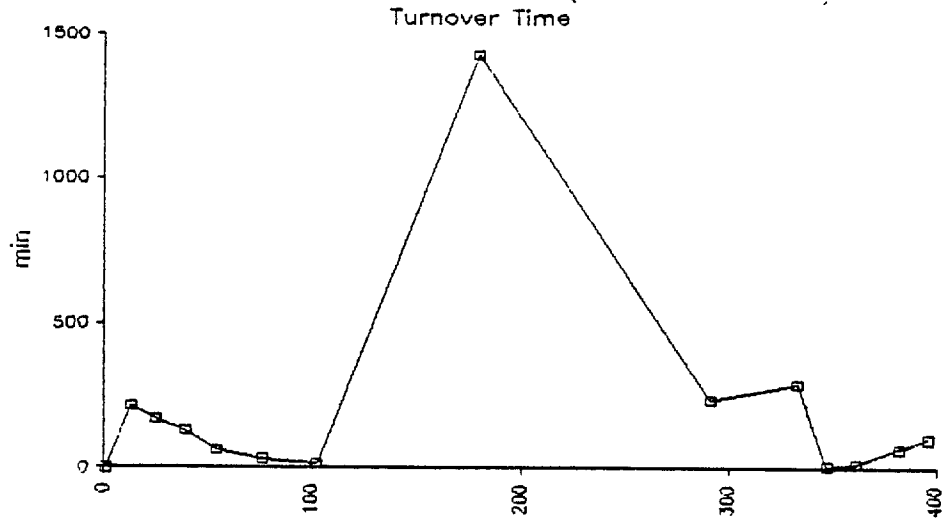
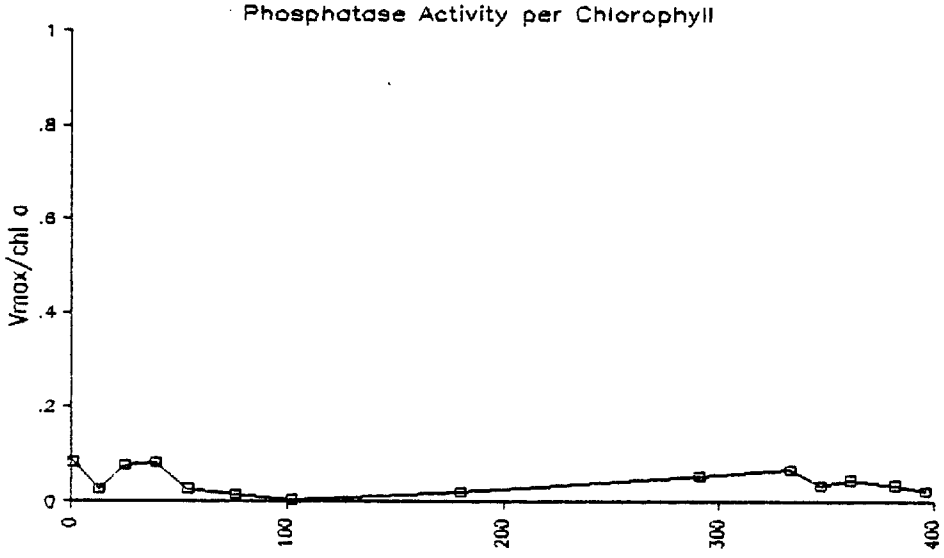


FIGURE 18

SITE 2 MID-ESTUARY (EAST ISLAND)





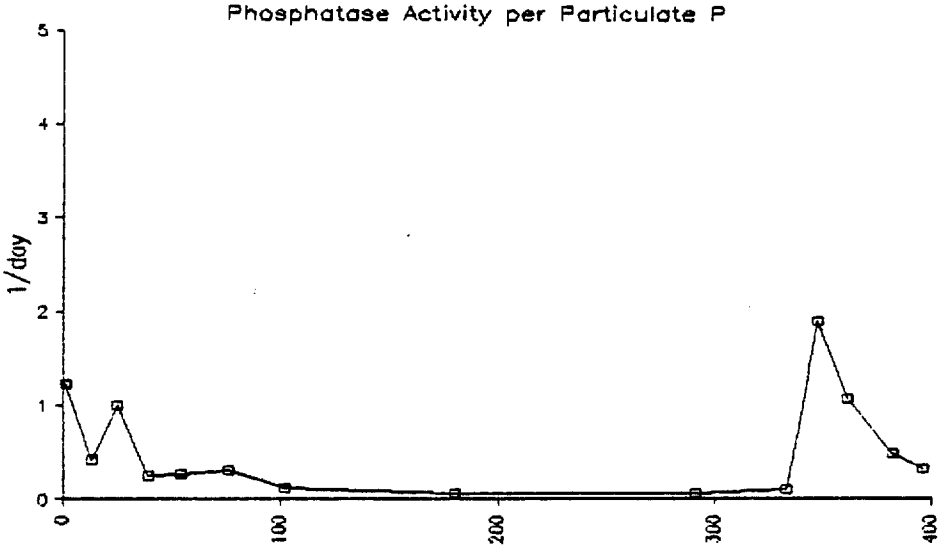
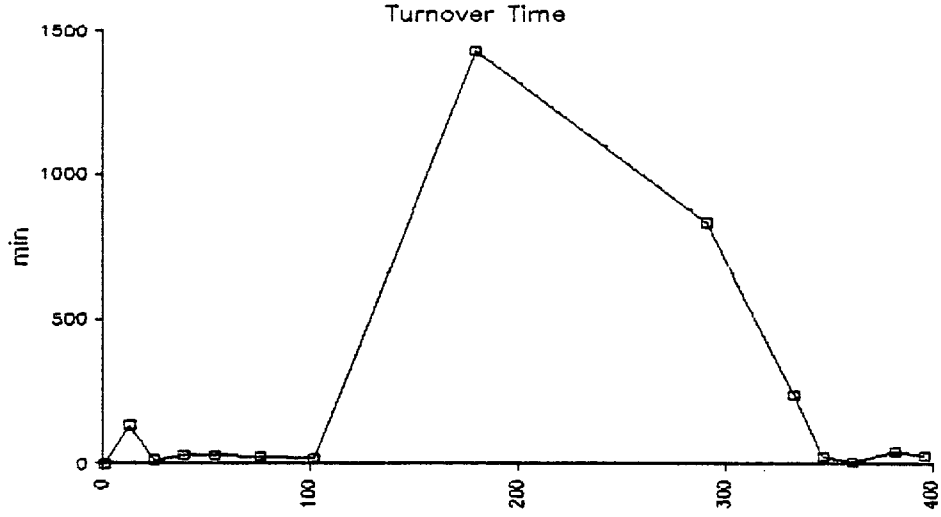
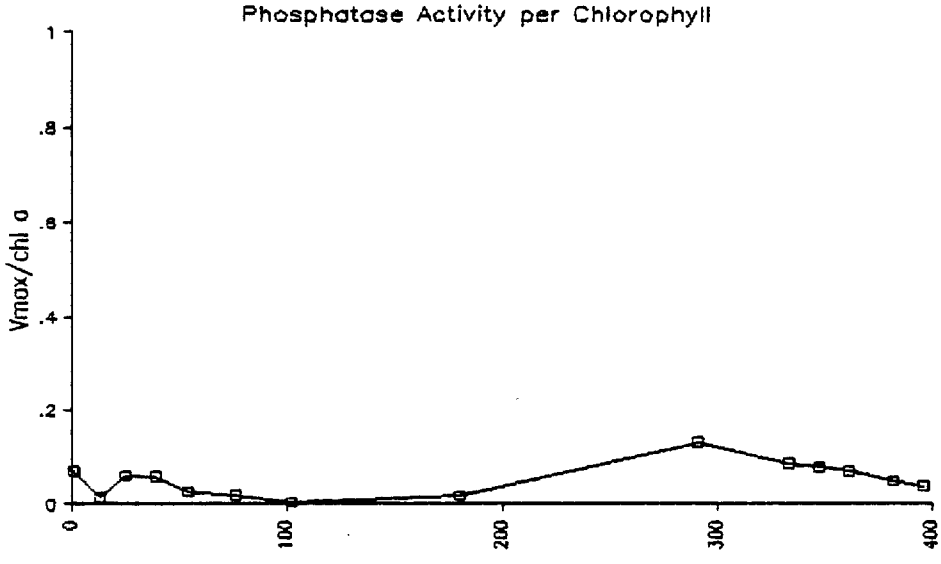
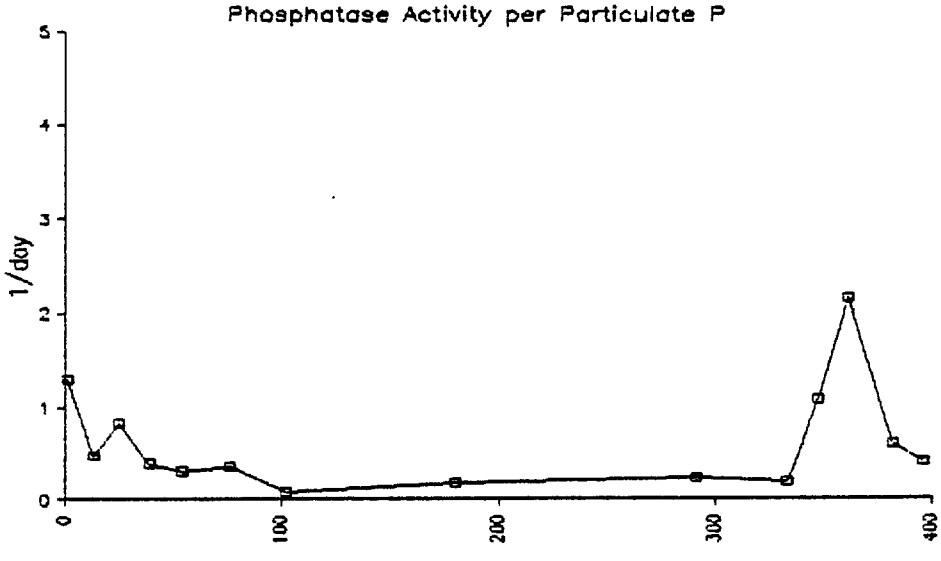


FIGURE 19

SITE 3 OUTLET TO LAKE ERIE







low, but increased in the spring to reach its highest levels in June. Phosphatase specific activities tended to decrease during passage through the OWCNES.

Chemical observations of the SRP concentration and N:P ratios (Figure 20 and Table VI) supported these direct biological and biochemical indicators of P limitation. Figure 20 shows that the SRP seldom was less than 5 ug/L in OWCNES, suggesting that there was always a sufficient supply of available P. Table VI shows that the total N: total P ratio was greater than 28 in the springtime. However, the materials were largely in a dissolved form at this time. The particulate N: particulate P ratio was considerably lower at each of the sites examined, indicating that the particles were much lower in nitrogen content than the water as a whole.

III. Effect of OWCNES marsh on phosphorus availability to plankton

Even though phosphorus inputs to the OWCNES do not appear to control planktonic growth in the sense of providing the limiting nutrient, they do eventually flow into a community known to be P-limited. Therefore regarding the "performance" of the OWCNES ecosystem, it is important to ask whether phosphorus inputs to the system are altered during transport through the estuary such that P-availability to the plankton of the receiving community is affected. The approach used in this study was to compare the composition of the incoming water (Site 1) with the composition of the water within the estuary (Site 2) and the water at the outlet to Lake Erie (Site 3). The "effect" of the marsh on the P-availability is the qualitative difference between the inputs and the outputs.

Earlier in this report I have presented some of the physical and chemical characteristics useful in this comparison. OWCNES is a turbulent flow-through system. Even when the flow is slowed by blockage at the mouth, many particles are suspended in the water column. Generally the mid-estuary site (Site 2) showed the greatest turbidity, both during the times of high flow

FIGURE 20

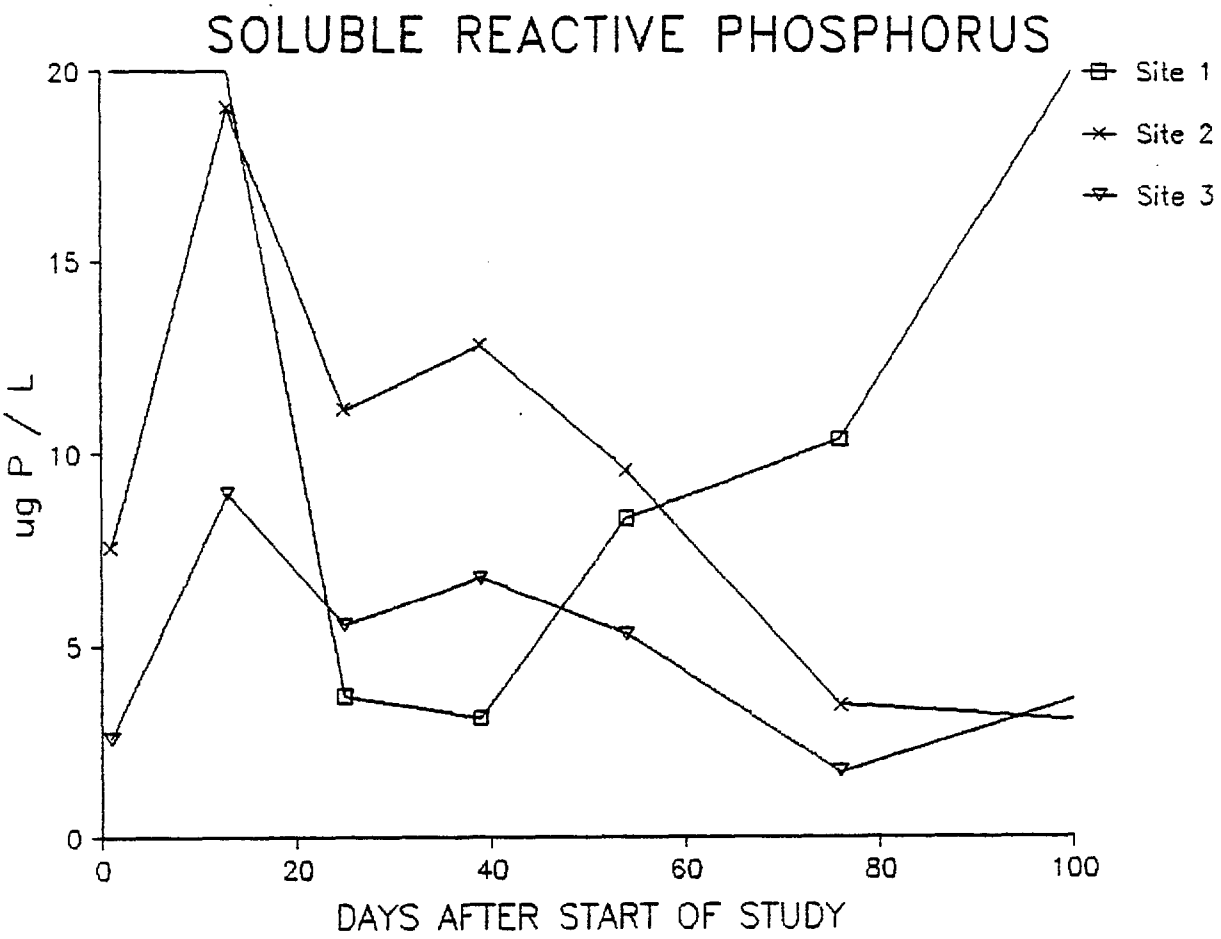

SOLUBLE REACTIVE PHOSPHORUS
Site 1
Site 2
Site 3
ug P / L
20
15
10
5
0
0
20
40
60
80
100
DAYS AFTER START OF STUDY

TABLE VI

NITROGEN TO PHOSPHORUS RATIOS
9 April 1985

TOTAL P umol/L	TSP umol/L	TOTAL N umol/L	TSN umol/L	TOTAL N:P	SOLUBLE N:P	SESTON N:P
Site 1: Darrow Road Site						
6.13	4.84	404.10	404.01	65.92	83.47	.07
Site 2: East Island - Mid-Estuary						
9.40	3.06	323.56	285.11	34.42	93.17	6.06
Site 3: Outlet to Lake Erie						
7.84	2.64	224.06	196.73	28.58	74.52	5.26

and times of relative stagnation (Figure 2). During open flow these particulate materials are carried into Lake Erie. In the growing season the particulate content correlated well with the chlorophyll content of the water samples, suggesting that much of this turbidity was biogenic. However, during the non-growing season of open flow, similar turbidities were observed without the presence of chlorophyll, indicating that much of the turbidity then was due to suspended sediments.

Soluble phosphorus compounds predominated during the winter months, especially at the inlet site (Figures 7 - 14), and especially during the early spring run-off. As the growing season progressed there was a continual decrease in the amount of soluble P and a continual increase in the amount of particualte P. The increase in particluate P greatly exceeded the decrease in soluble P on a mole-for mole basis, suggesting that P may come from sources other than the inlet stream (e.g. from the sediments within the wetland). The concentration of particulate P was always highest at the mid estuary site (Site 2). Early in the growing season (Figure 10) the soluble P was predominantly SRP at all sites, but as the growing season progressed, SRP declined more rapidly and to a greater extent than did other soluble P compounds. Except for the winter sample, there was always a lower concentration of SRP and TSP in the waters leaving the estuary than entered it. The winter samples showed that there was a greater concentration of TSP in the outflow to Lake Erie than there was in the water entering OWCNES at that time.

Several questions were addressed:

A) Was the SRP taken up by biota or was it sorbed to non-living particulate material?

B) Could any of the soluble unreactive P (SUP = TSP - SRP) release phosphate under natural conditions, and if so, was it a nutritionally significant source of P to the planktonic community?

C) Did the sediments act as a potential source or a potential sink for incoming phosphorus compounds?

A. Phosphate Uptake

The rate of phosphate uptake was determined radiometrically as previously described (Heath 1986). Shown in Figure 21, the straight line rising from the origin indicates that the sum of all processes removing phosphate from solution to particles appeared to conform to first-order kinetics. Also, a frequent finding was the "break" in the line after several hundred seconds. Because this procedure traced the uptake of phosphate at steady state (or nearly steady state) this break does not indicate a cessation of uptake, rather it likely indicates that there were two classes of uptake processes. The first class took phosphate up into a compartment that reached saturation within several hundred seconds (i.e. input equaled output of radiolabelled phosphate after a brief period); the second class of processes took phosphate up into a compartment with much greater capacity. Because the purpose of these investigations was to determine the rate of phosphate uptake by all processes, only the original line was considered further. The slope of this line was k, the proportional uptake rate. That is, a k proportion of the available phosphate moved from solution to particles per minute. The inverse of k is T, the turnover time, discussed above.

In addition to determining the phosphate uptake rate under ambient conditions alone, I also determined the proportional phosphate uptake rate following addition of a metabolic inhibitor: potassium cyanide or tri-chloro-cyano- carbonyl- phenylhydrazone (CCCP). Addition of cyanide greatly inhibited the rate of transfer of phosphate to particles (Figure 22). However, because it is an anion present in concentrations three to four orders of magnitude above the anion in question (10^{-4} M KCN vs. 10^{-7} to 10^{-8} M phosphate), it is not clear whether its effect resulted from inhibition of metabolic activity or merely

FIGURE 21

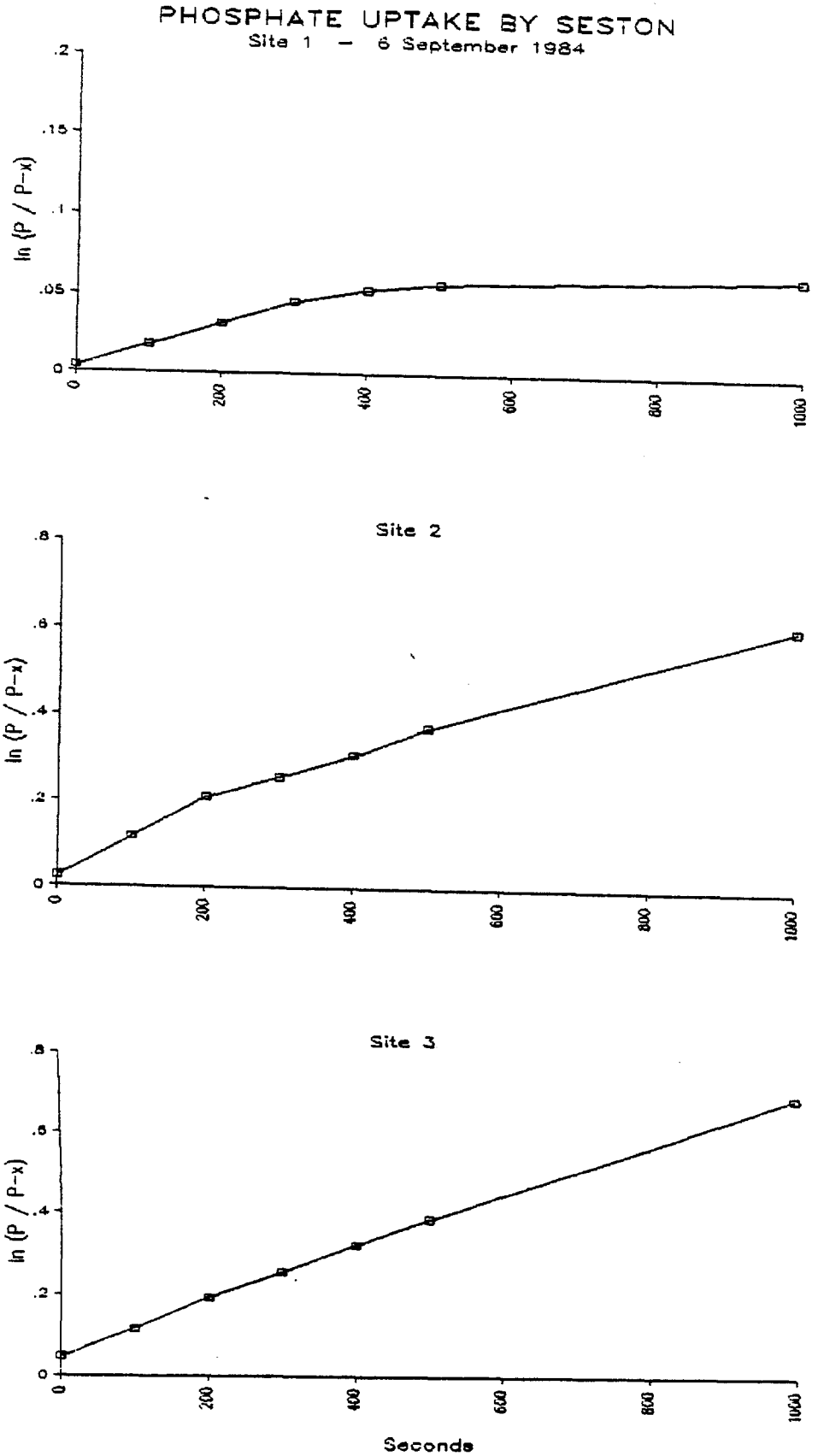
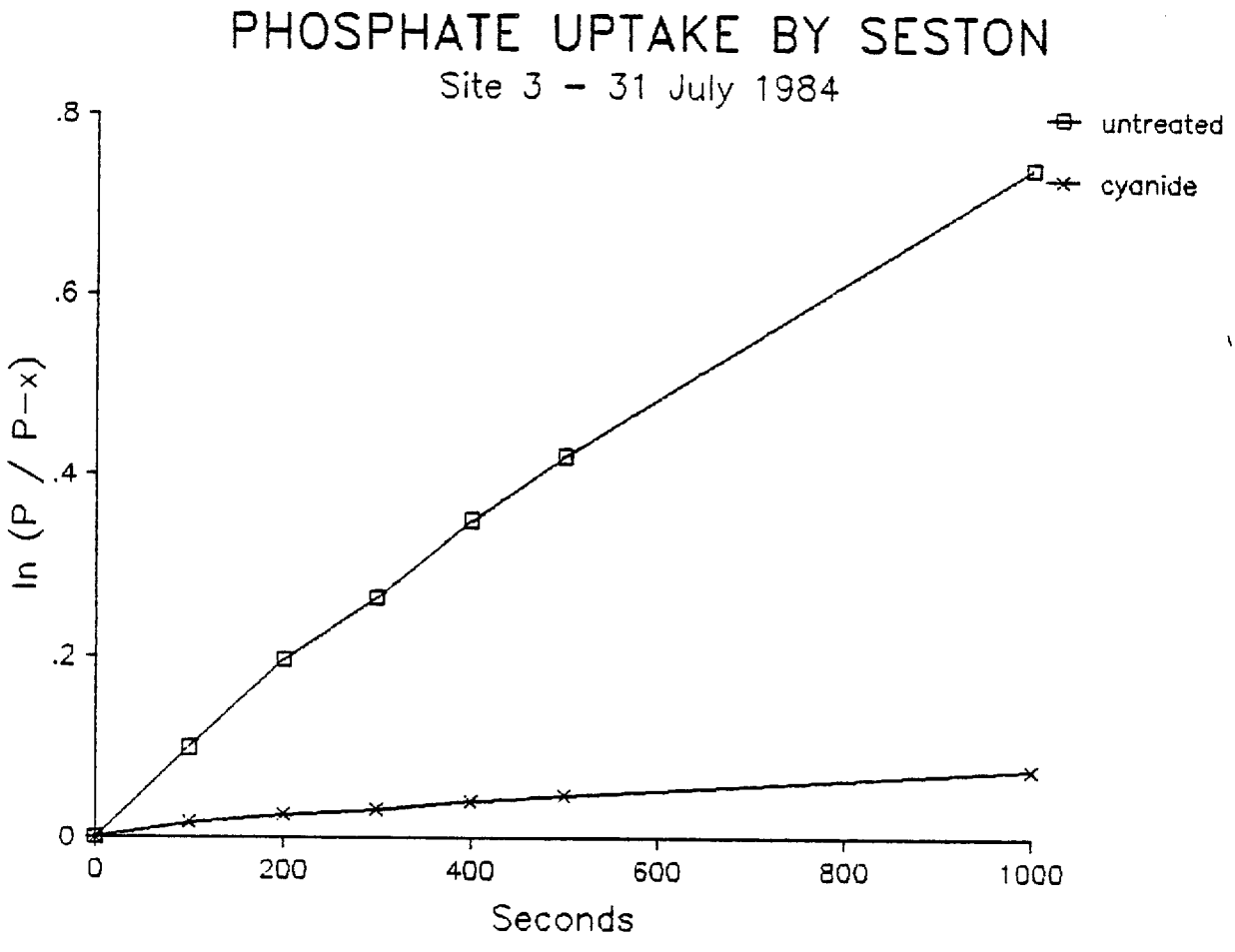

PHOSPHATE UPTAKE BY SESTON
Site 1 – 6 September 1984
ln (P / P–x)
.2
.15
.1
.05
0
0
200
400
600
800
1000
Site 2
ln (P / P–x)
.8
.6
.4
.2
0
0
200
400
600
800
1000
Site 3
ln (P / P–x)
.8
.6
.4
.2
0
0
200
400
600
800
1000
Seconds

FIGURE 22


PHOSPHATE UPTAKE BY SESTON
Site 3 - 31 July 1984
untreated
cyanide
ln (P / P-x)
.8
.6
.4
.2
0
0
200
400
600
800
1000
Seconds

competition for cationic sites on which to sorb. CCCP, a potent proton translocator known to inhibit metabolic activity in concentrations of approximately 1×10^{-7} M, also had a pronounced effect on the uptake of phosphate (Figure 23). Phosphate uptake was very sensitive to temperature variations (Figure 24), showing a Q_{10} of 2.34 up to 20 degrees and a precipitous decline above 20 degrees, reminiscent of enzyme-mediated processes. These results indicate that much of the phosphate uptake was dependent on metabolic activity.

The velocity of phosphate uptake was calculated according to the first order equation, giving uptake rate in units of nmol/L/min:

$$v = k \text{ (available phosphate)}.$$

There is a considerable controversy concerning the detection of "available phosphate" as SRP. Rigler (1966) used a radiometric bioassay procedure to show that SRP may greatly overestimate the amount of biologically available phosphate. Especially in very P-limited systems where phosphate reached barely detectable concentrations, SRP and the available phosphate detected by his bioassay differed by an order of magnitude. Whether these discrepancies arose because of some hidden problem with the bioassay, or from a hydrolysis of labile compounds by the molybdenum blue colorimetric procedure for determining SRP remains unresolved. However, in systems having SRP of greater than 5 ug/L (161.3 nmol/L), he seldom noticed a significant discrepancy between SRP and his bioassay estimate. Also, Shapiro (1972) used an extraction method (extracting phosphomolydate into iso-butanol) to separate phosphate from arsenate and silicate; he found that SRP was a reliable estimate of phosphate. Francko and Heath (1979) showed that greater than 90 percent of the SRP co-chromatographed with authenic ortho-phosphate in two independent chromatographic procedures in which the materials were chromatographed at approximately ambient conditions, to prevent hydrolysis of SUP to SRP. Recently Nurnberg (1984) used a bioassay to

FIGURE 23

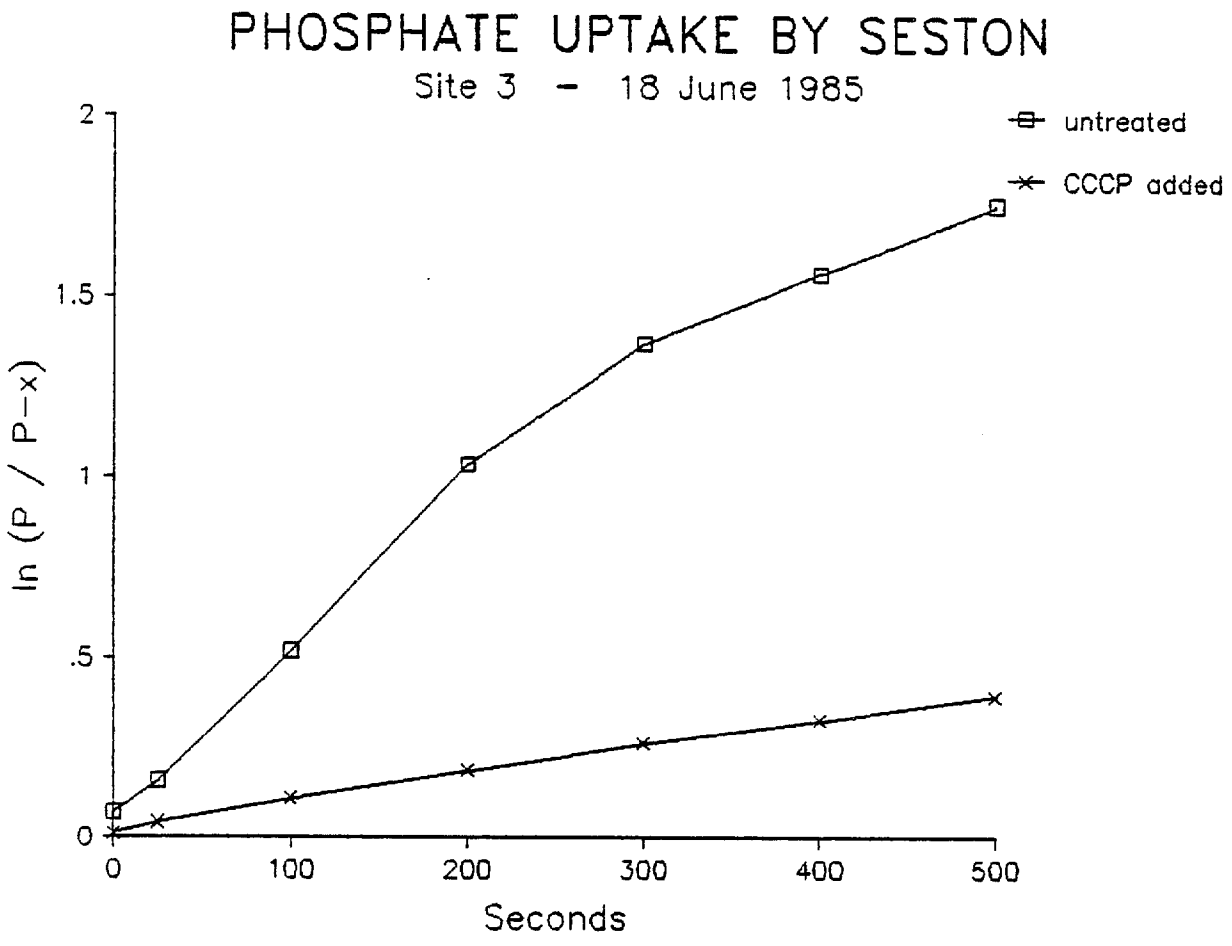
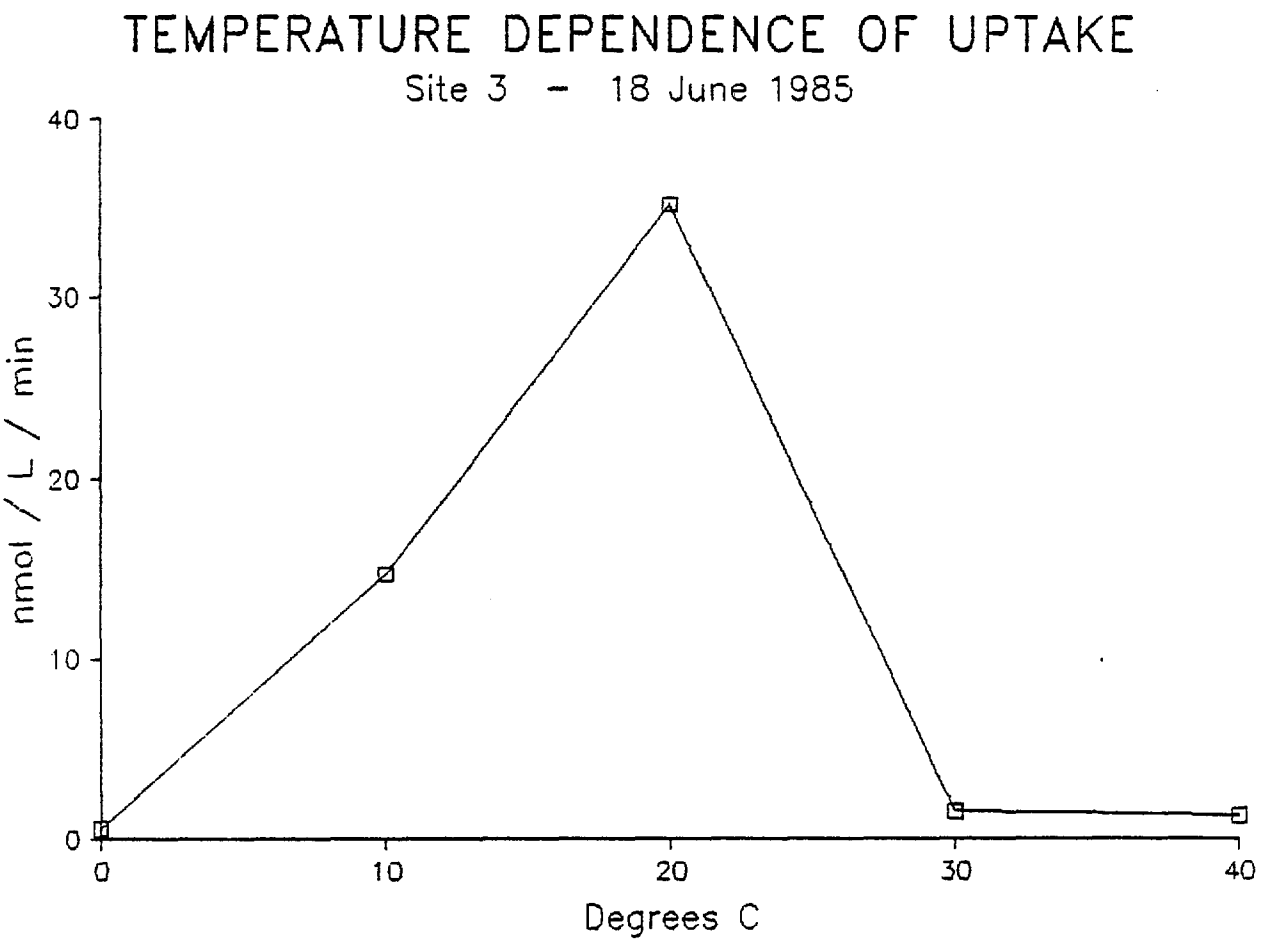

PHOSPHATE UPTAKE BY SESTON
Site 3 - 18 June 1985
untreated
CCCP added
ln (P / P-x)
2
1.5
1
.5
0
0
100
200
300
400
500
Seconds

FIGURE 24


TEMPERATURE DEPENDENCE OF UPTAKE
Site 3 - 18 June 1985
nmol / L / min
0
10
20
30
40
Degrees C

estimate biologically available phosphate. She has shown that except in anoxic waters from acid bog lakes with great amouns of ionic iron, SRP provided a reliable estimate of available phosphate.

Because of the increasing recent evidence that SRP often is a reliable indicator of available phosphate, and because SRP concentrations in OWCNES usually were in the range that even the Rigler bioassay showed correspondence between SRP and bioassay-available P, I used SRP to estimate "availale phospate" throughout this study. The foregoing discusson notwihstanding, this estimate should be regarded as an upper estimate of the available phosphate, and so the calcualted velocities of uptake are maximum possible estimates.

Table VII presents the data regarding the uptake of phosphate observed throughout this study, and Figures 25 - 27 present these data on a seasonal basis from Sites 1 - 3, respectively. Several general statements can be made from these observations. At each of the sites, phosphate uptake was fastest in the summer and slowest in the winter, as would be expected from the observed temperature sensitivity of up[take. In general, phosphate uptake was more sensitive to metabolic inhibitors during the summer than during the winter. Transect profiles (Figures 28 - 31) from selected dates throughout the study indicate that phosphate uptake velocity tended to increase during passage through the marsh. Also, the uptake velocity was relatively insensitive to cyanide or CCCP at the inlet, and became progressively more sensitive to these metabolic inhibitors during passage through the marsh.

These findings indicate that entering phosphate is taken up by bacterioplankton and phytoplankton in the marsh, and that this uptake into living cells increases progressively during passage through the marsh. This appears to be most notable during periods of relative stagnation (Figures 28 and 29). During periods of rapid flow through the estuary, the uptake appears to be dominated by processes not affected by metabolic inhibitors (Figures 30 and 31).

TABLE VII

VELOCITY OF PHOSPHATE UPTAKE
BY ACTIVE (CYANIDE-SENSITIVE) PROCESSES

Date	SRP nmol/L	k uptake 1/min	k CN^- 1/min	Active Vel. nmol/L/min	Ratio Active:Total
Site 1 - Darrow Road Bridge					
22 Jun 84	954.25	*	*	*	*
5 Jul 84	2391.43	.0019	.0029	2.39	-.53
17 Jul 84	119.04	.0781	.0056	8.63	.93
31 Jul 84	100.97	.0430	.0039	3.95	.91
15 Aug 84	269.37	.0127	.0029	2.64	.77
6 Sep 84	335.18	.0090	*	3.02	*
2 Oct 84	4061.53	.0007	.0005	.81	.29
19 Dec 84	711.01	.0005	*	.36	*
9 Apr 85	3663.77	.0001	*	.37	*
21 May 85	1098.45	.0011	.0007	.44	.36
4 Jun 85	673.59	.0018	.0018	.00	.00
18 Jun 85	526.48	.0028	.0017	.58	.39
9 Jul 85	306.15	.0056	*	1.71	*
23 Jul 85	289.37	.0094	.0055	1.13	.41
Site 2 - East Island in Mid-Stream					
22 Jun 84	244.21	*	*	*	*
5 Jul 84	614.88	.0046	.0017	1.78	.63
17 Jul 84	360.02	.0058	.0014	1.58	.76
31 Jul 84	414.22	.0078	.0018	2.49	.77
15 Aug 84	309.37	.0166	.0070	2.97	.58
6 Sep 84	111.62	.0318	*	3.55	*
2 Oct 84	100.01	.0697	.0067	6.30	.90
19 Dec 84	296.15	.0007	*	.21	*
9 Apr 85	803.27	.0042	*	3.37	*
21 May 85	270.34	.0034	.0016	.49	.53
4 Jun 85	74.84	.1144	.0191	7.13	.83
18 Jun 85	130.33	.0585	.0457	1.67	.22
9 Jul 85	249.05	.0146	*	3.64	*
23 Jul 85	331.31	.0095	.0099	-.13	-.04
Site 3 - Outlet to Lake Erie					
22 Jun 84	84.20	*	*	*	*
5 Jul 84	290.02	.0076	.0024	1.51	.68
17 Jul 84	179.37	.0741	.0010	13.11	.99
31 Jul 84	219.37	.0335	.0023	6.84	.93
15 Aug 84	171.95	.0343	.0089	4.37	.74
6 Sep 84	55.81	.0380	*	2.12	*
2 Oct 84	116.14	.0528	.0058	5.46	.89
19 Dec 84	219.05	.0007	*	.15	*
9 Apr 85	737.14	.0012	*	.88	*
21 May 85	194.21	.0042	.0011	.60	.74
4 Jun 85	74.84	.0404	.0115	2.16	.72
18 Jun 85	282.92	.1182	.0981	5.69	.17
9 Jul 85	153.88	.0229	*	3.52	*
23 Jul 85	32.26	.0322	.0211	.36	.34

FIGURE 25

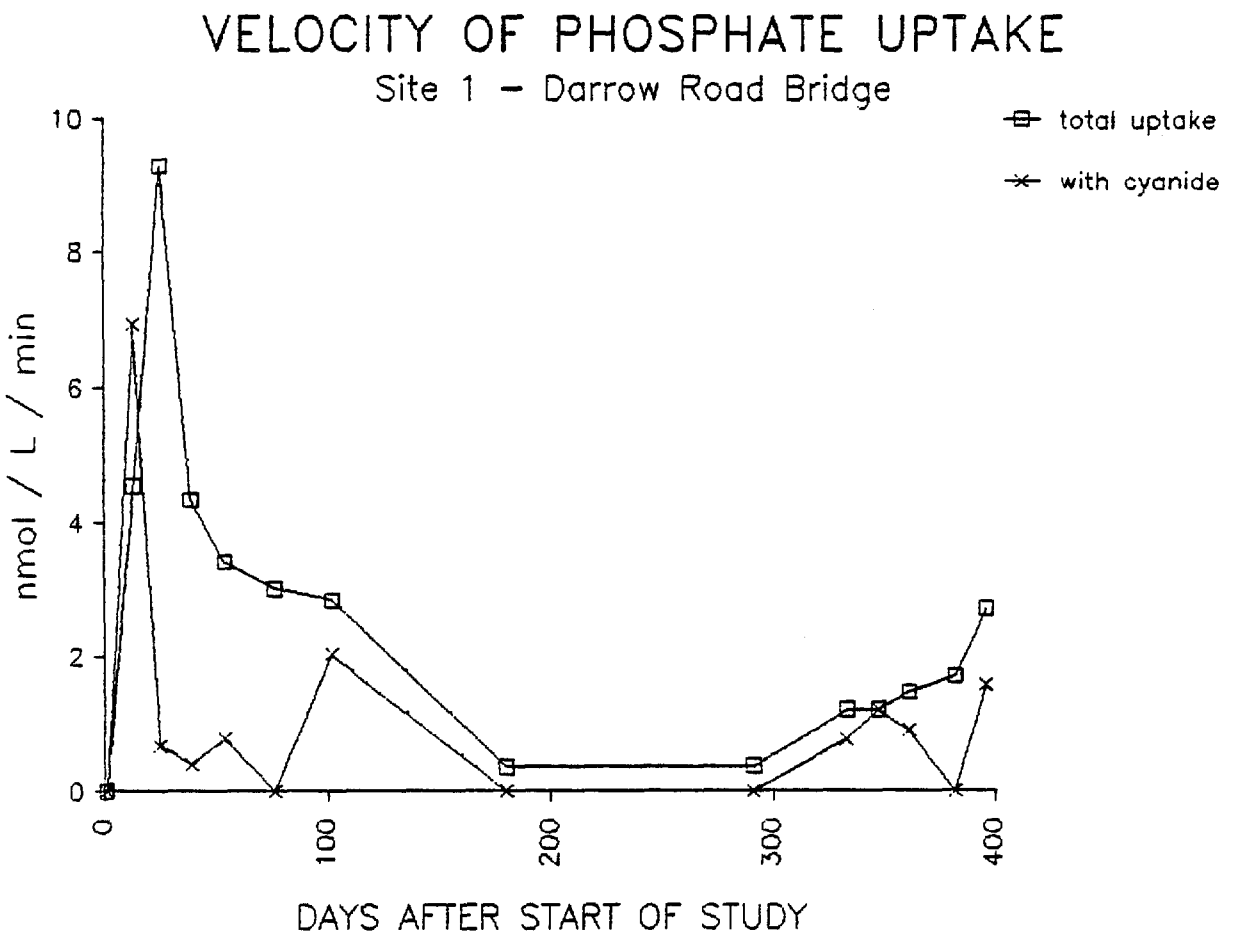

VELOCITY OF PHOSPHATE UPTAKE
Site 1 – Darrow Road Bridge
total uptake
with cyanide
nmol / L / min
10
8
6
4
2
0
0
100
200
300
400
DAYS AFTER START OF STUDY

FIGURE 26

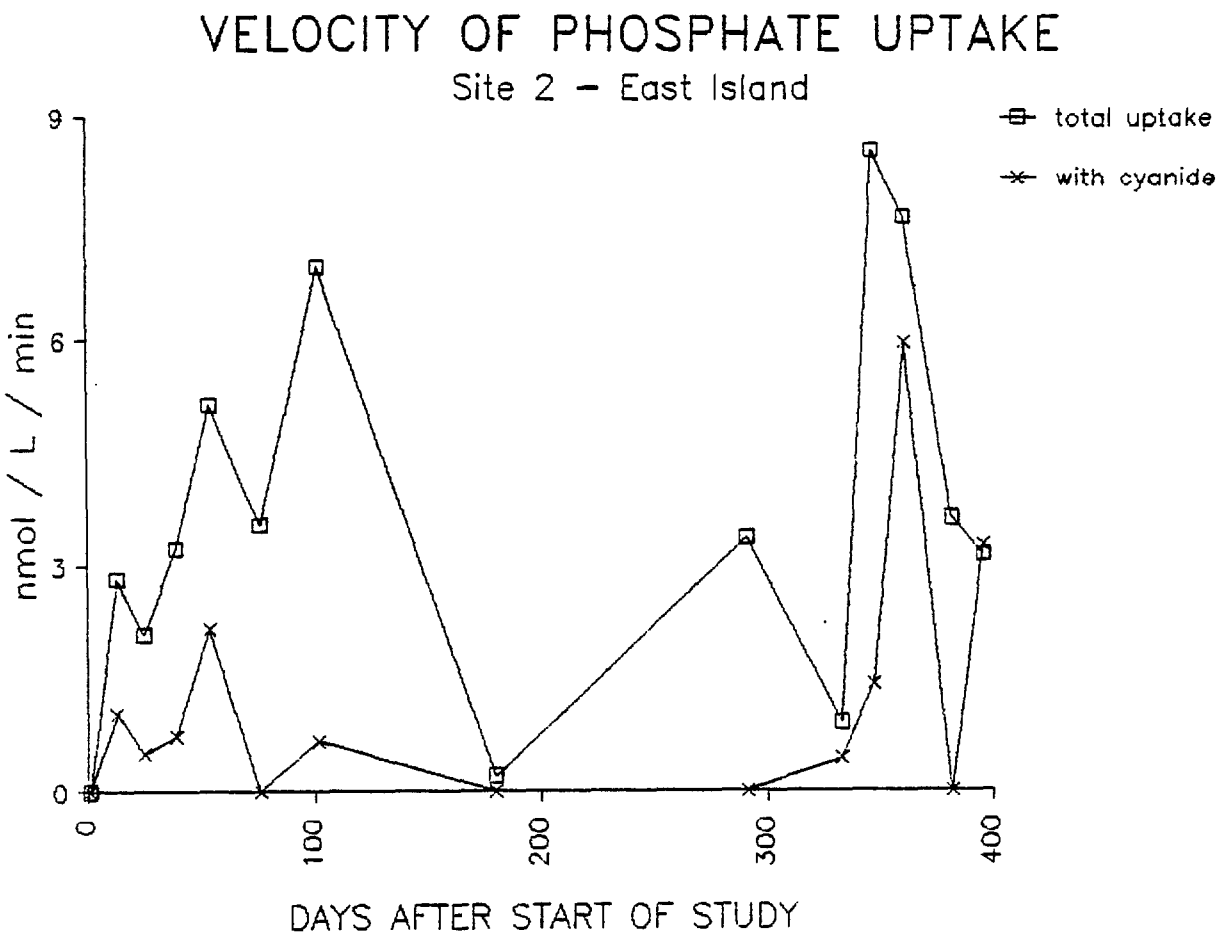

VELOCITY OF PHOSPHATE UPTAKE
Site 2 – East Island
total uptake
with cyanide
nmol / L / min
9
6
3
0
0
100
200
300
400
DAYS AFTER START OF STUDY

FIGURE 27

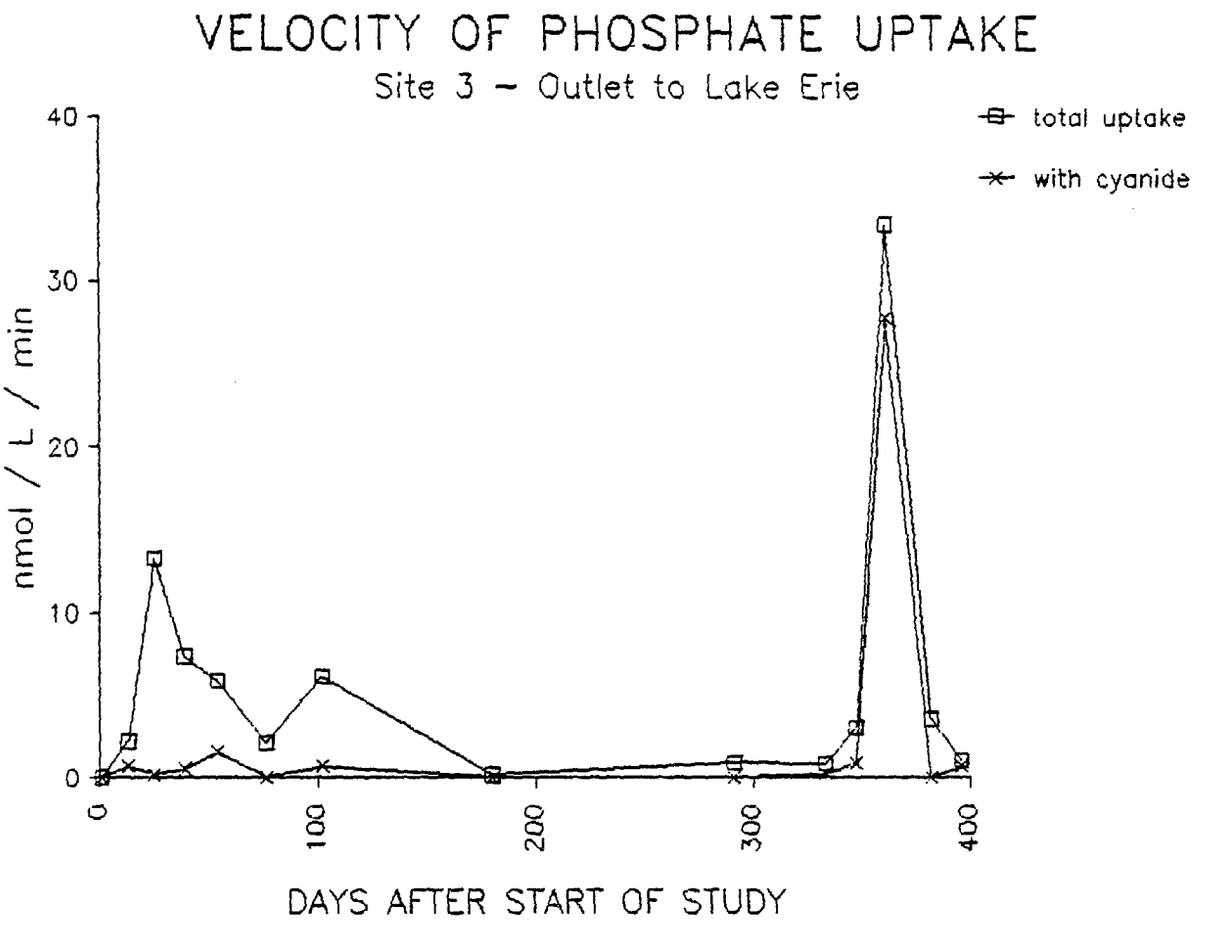

VELOCITY OF PHOSPHATE UPTAKE
Site 3 – Outlet to Lake Erie
total uptake
with cyanide
nmol / L / min
40
30
20
10
0
0
100
200
300
400
DAYS AFTER START OF STUDY

FIGURE 28

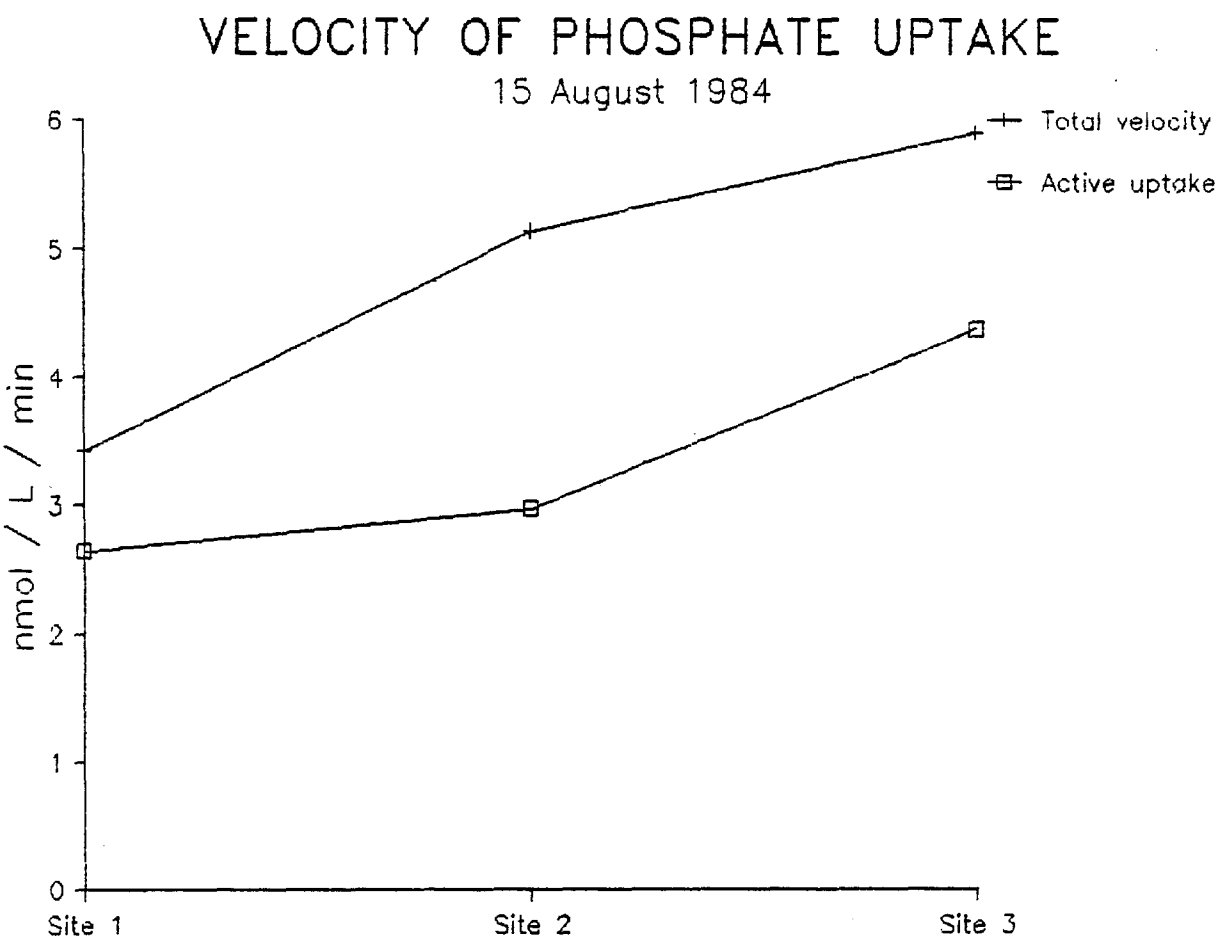

VELOCITY OF PHOSPHATE UPTAKE
15 August 1984
Total velocity
Active uptake
nmol / L / min
0
1
2
3
4
5
6
Site 1
Site 2
Site 3

FIGURE 29

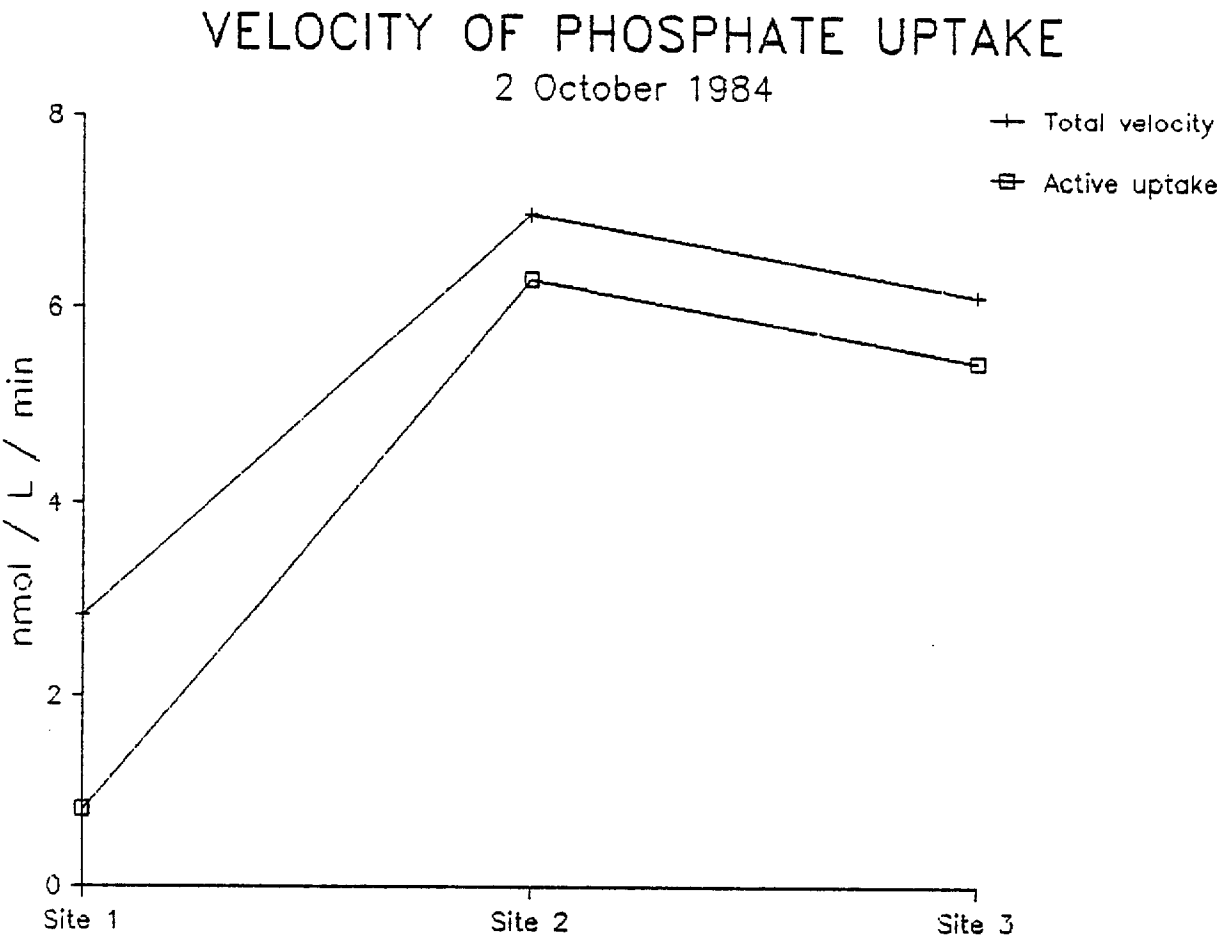

VELOCITY OF PHOSPHATE UPTAKE
2 October 1984
Total velocity
Active uptake
nmol / L / min
8
6
4
2
0
Site 1
Site 2
Site 3

FIGURE 30

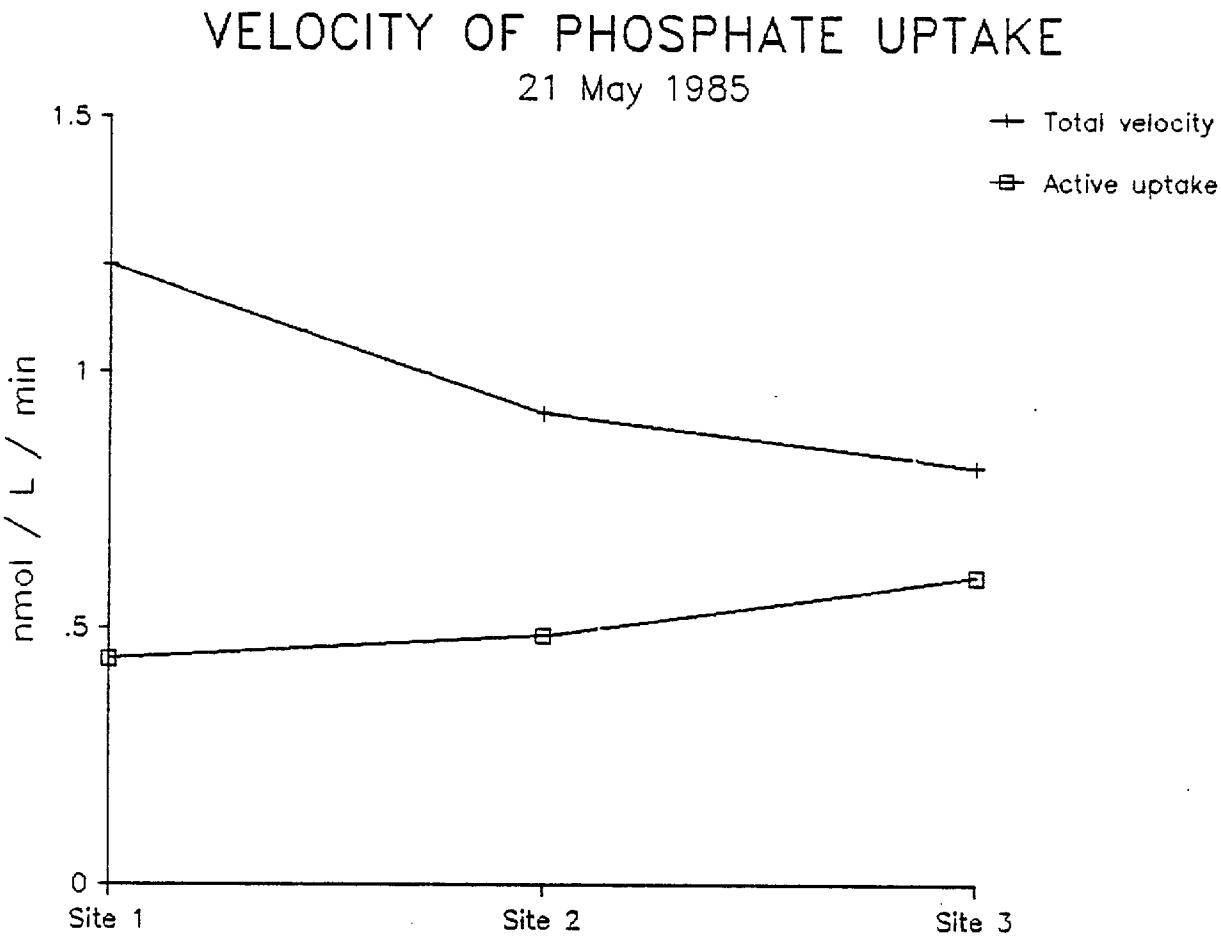
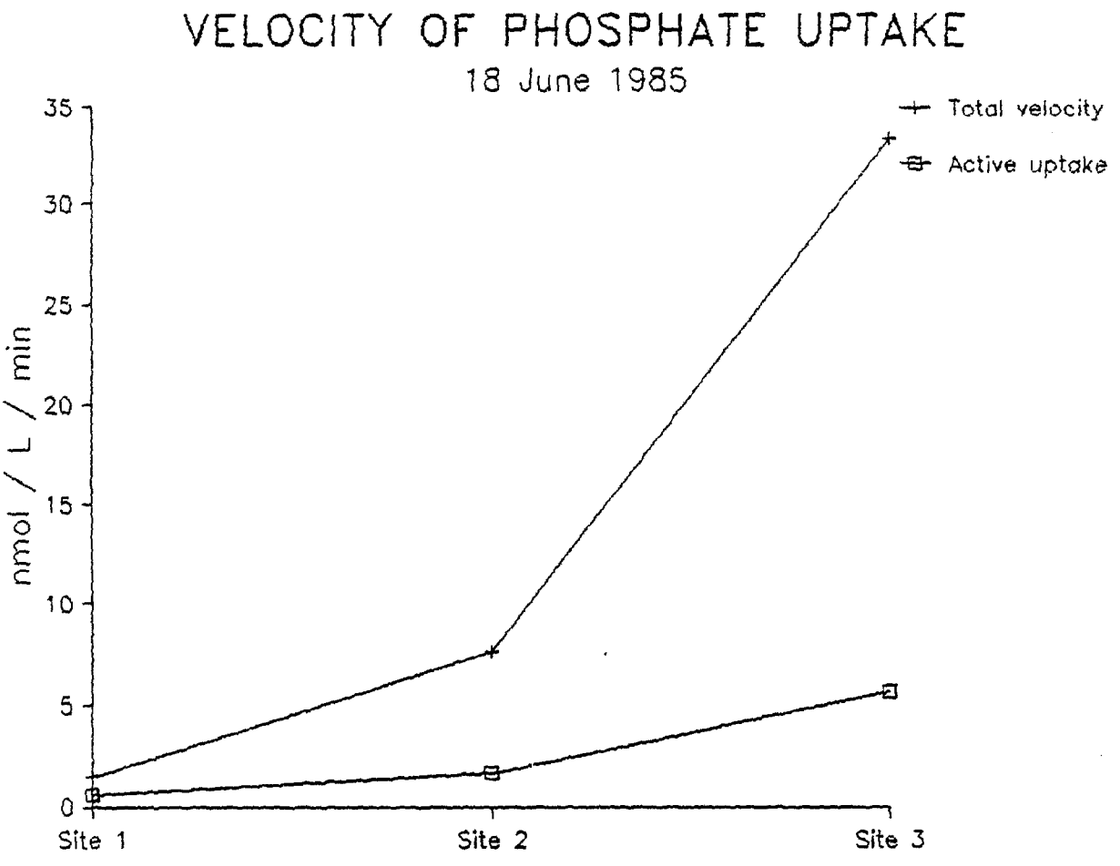

VELOCITY OF PHOSPHATE UPTAKE
21 May 1985
Total velocity
Active uptake
nmol / L / min
1.5
1
.5
0
Site 1
Site 2
Site 3

FIGURE 31


VELOCITY OF PHOSPHATE UPTAKE
18 June 1985
Total velocity
Active uptake
nmol / L / min
35
30
25
20
15
10
5
0
Site 1
Site 2
Site 3

B) Significance of dissolved organic compounds (DOP)

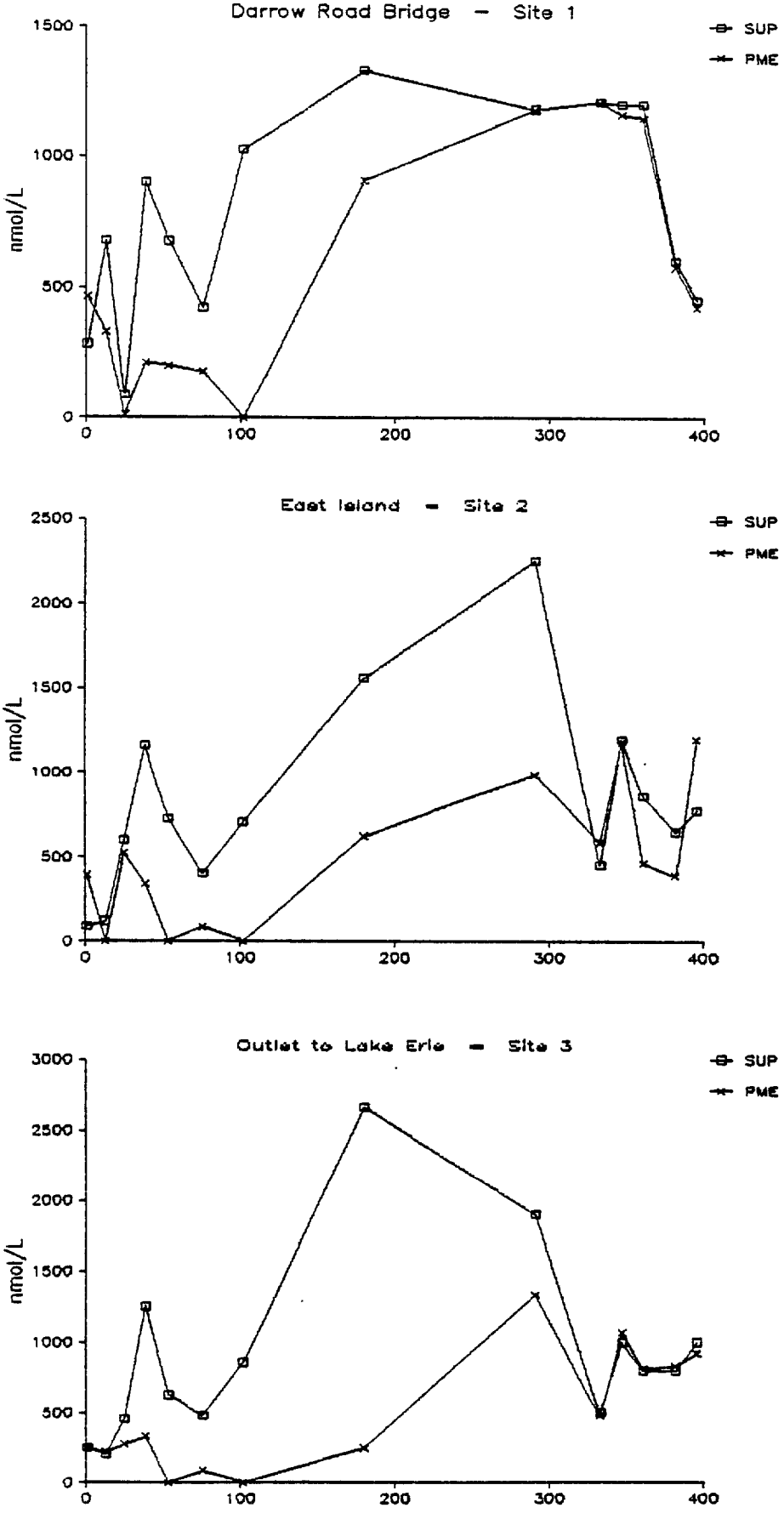
The concentration of classes of phosphorus compounds known to yield phosphate under certain conditions was determined. Francko and Heath (1979) identified two classes of complex P compounds which could release phosphate under certain conditions. Without those conditions the P in these compounds was not available to growing cells. One class of compounds predominated in bog lakes that have a high content of humic-iron complexes. Phosphate associated with these complexes can be released through a photo-dependent process in which the iron is photoreduced from Fe III to Fe II (Francko and Heath 1982, 1983). No such compounds were observed at OWCNES during this study.

Phosphomonoesters (PME) comprise the other class of complex P compounds from which phosphate can be released. Algal and bacterial cells cannot directly take up PME under normal physiological conditions. However, phosphomonoester hydrolase (i.e. phosphatase) can catallyze the hydrolysis of these compounds, releasing P as phosphate (Fernley 1971). Algae and bacteria produce phosphatase exoenzymes that generally remain attached to the cell membrane with their active sites directed toward the periplasmic space. Zooplankton also synthesize their own phosphatases (perhaps as digestive enzymes) that they release directly to the water in a soluble form (Boavida and Heath 1984). As noted above, some phosphatase activity appears to be constitutively produced, but under P-limitation greater quantities of these enzymes are produced adaptively, increasing the specific activity of these enzymes ten- to hundred-fold.

PME were detected at all sites in OWCNES and represented between zero and 100 percent of the soluble unreactive P (SUP) present at various times during the year (Figure 32). At each site the SUP increased during the autumn and was at a maximum in the winter and early spring, declining thereafter and reaching its lowest concentrations in the mid-summer. PME reached its maximum values in the early spring at each site and declined to undetectable

FIGURE 32

SUP and PME



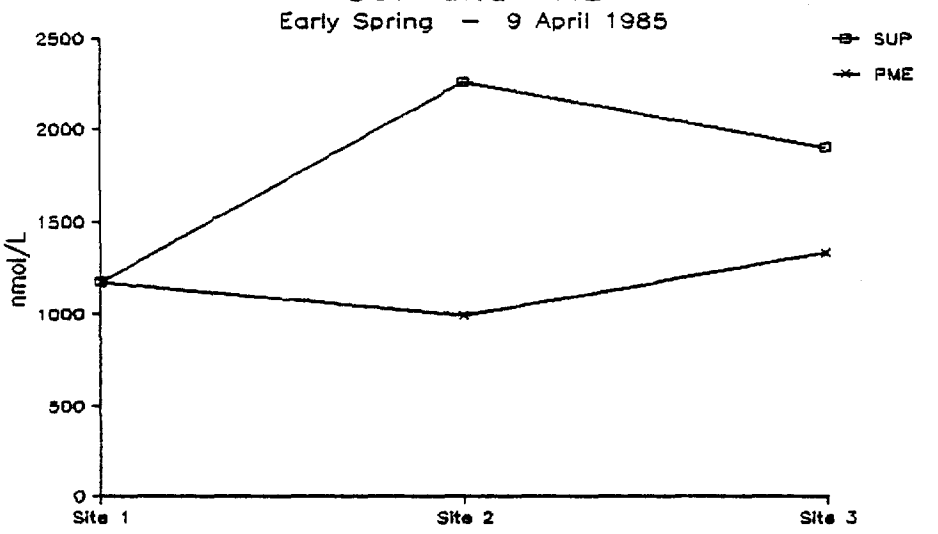
concentrations in the late summer, and then increased throughout the fall and winter. Also, the proportion of SUP represented by PME was at its greatest in the spring and at its least in the late summer. Both at times of relative abundance and relative paucity of SUP and PME there were not evident trends in the relative PME composition of SUP during passage through the estuary, except that often the lowest relative amounts of PME occurred at Site 2, within the estuary (Figure 33). Identification of other classes of SUP was not attempted.

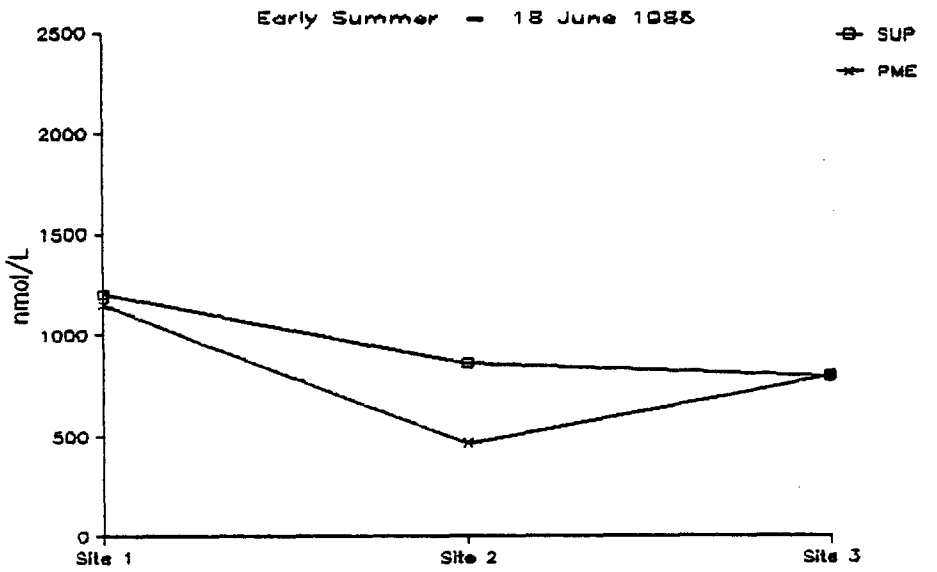
Generally, SRP exceeded PME at Site 1 (inlet), but at Sites 2 and 3 SRP was generally in lower concentrations than PME. Figure 34 presents these data as seasonal profiles at each station, and Figure 35a and 35b presents the same data as transect profiles on selected dates. Both SRP and PME were at maximum annual concentrations in the early spring, declined rapidly through the spring, reaching minimum concentrations in the late summer. Generally, the content and relative composition of SRP decreased during passage through the estuary. The amount of PME remained unchanged in early spring until early summer (Figure 35a); in the summer there was a net increase in the concentration of PME during passage through this wetland (Figure 35b).

Modest amounts of phosphatase activity were detected in OWCNES. Figure 36 presents the phosphatase activity as V_{max} at ambient pH and temperature throughout the year at each site. Enzyme activity reach its maximum during the late spring and early summer at each site; minimum activities occurred during the winter. During times of rapid flow through the estuary the enzyme activity remained unchanged during passage through the estuary. However, during relative stagnation the enzyme activity increased progressively from Site 1 through Site 3 (Figure 37).

FIGURE 33

SUP and PME





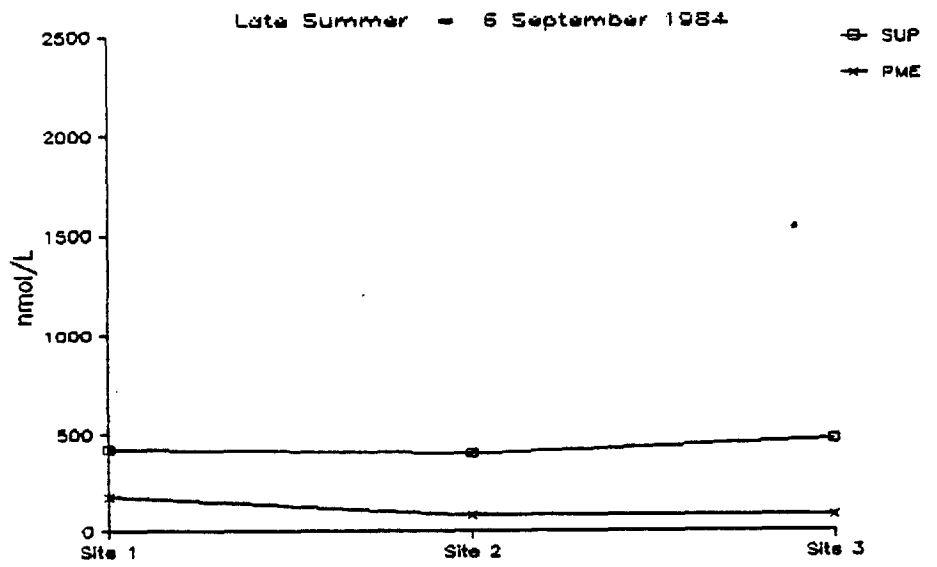
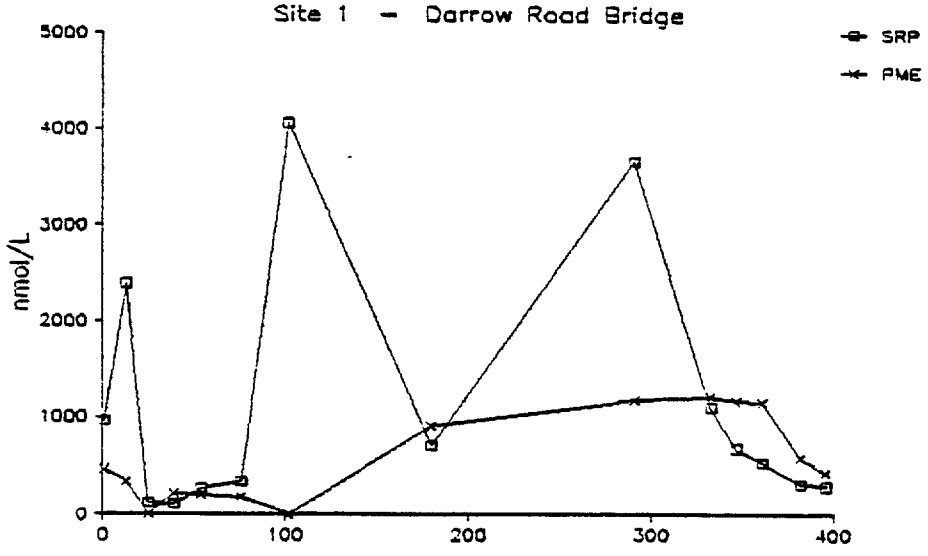
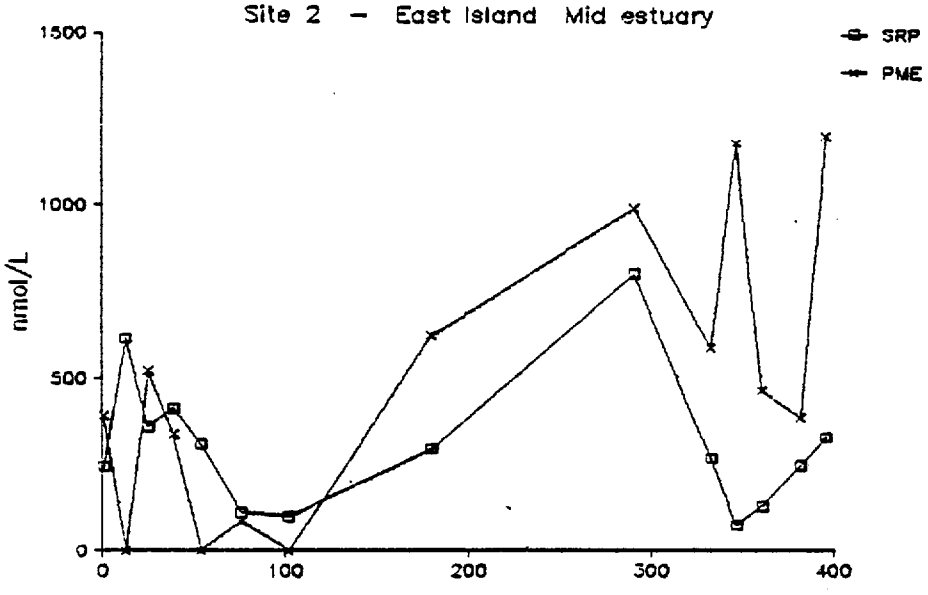


FIGURE 34

SRP and PME





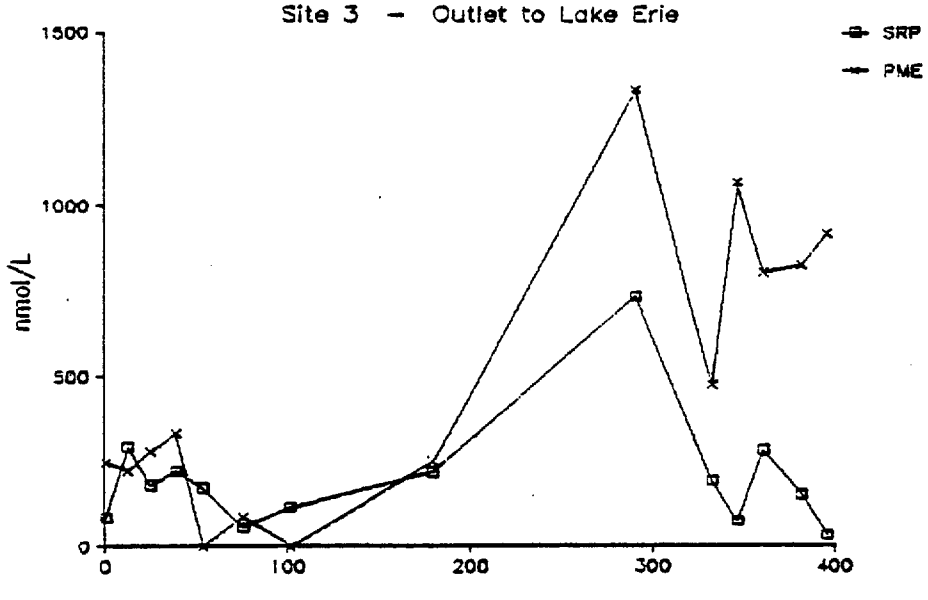


FIGURE 35a

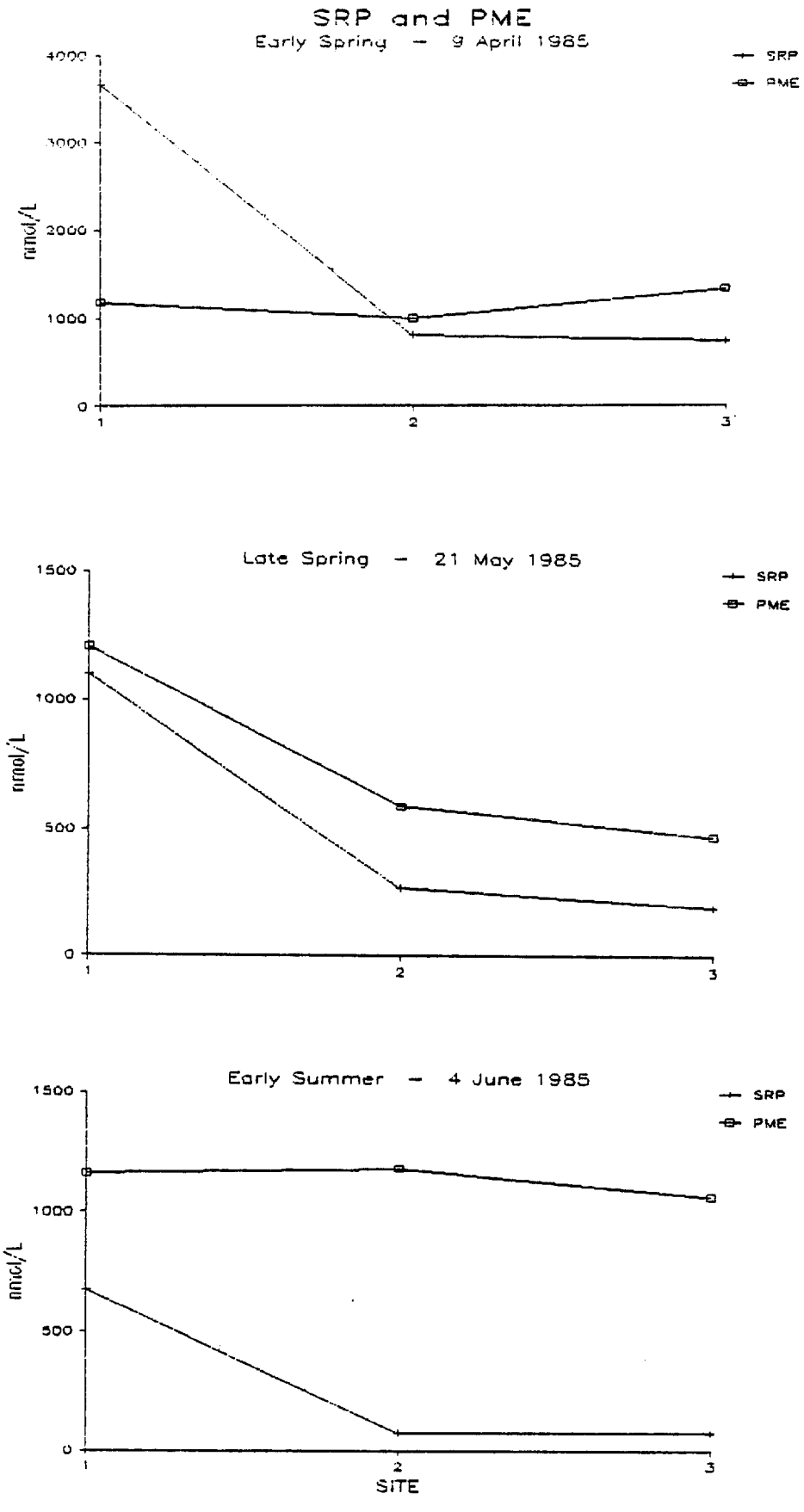

SRP and PME
Early Spring – 9 April 1985
SRP
PME
nmol/L
4000
3000
2000
1000
0
1
2
3
Late Spring – 21 May 1985
SRP
PME
nmol/L
1500
1000
500
0
1
2
3
Early Summer – 4 June 1985
SRP
PME
nmol/L
1500
1000
500
0
1
2
3
SITE

FIGURE 35b

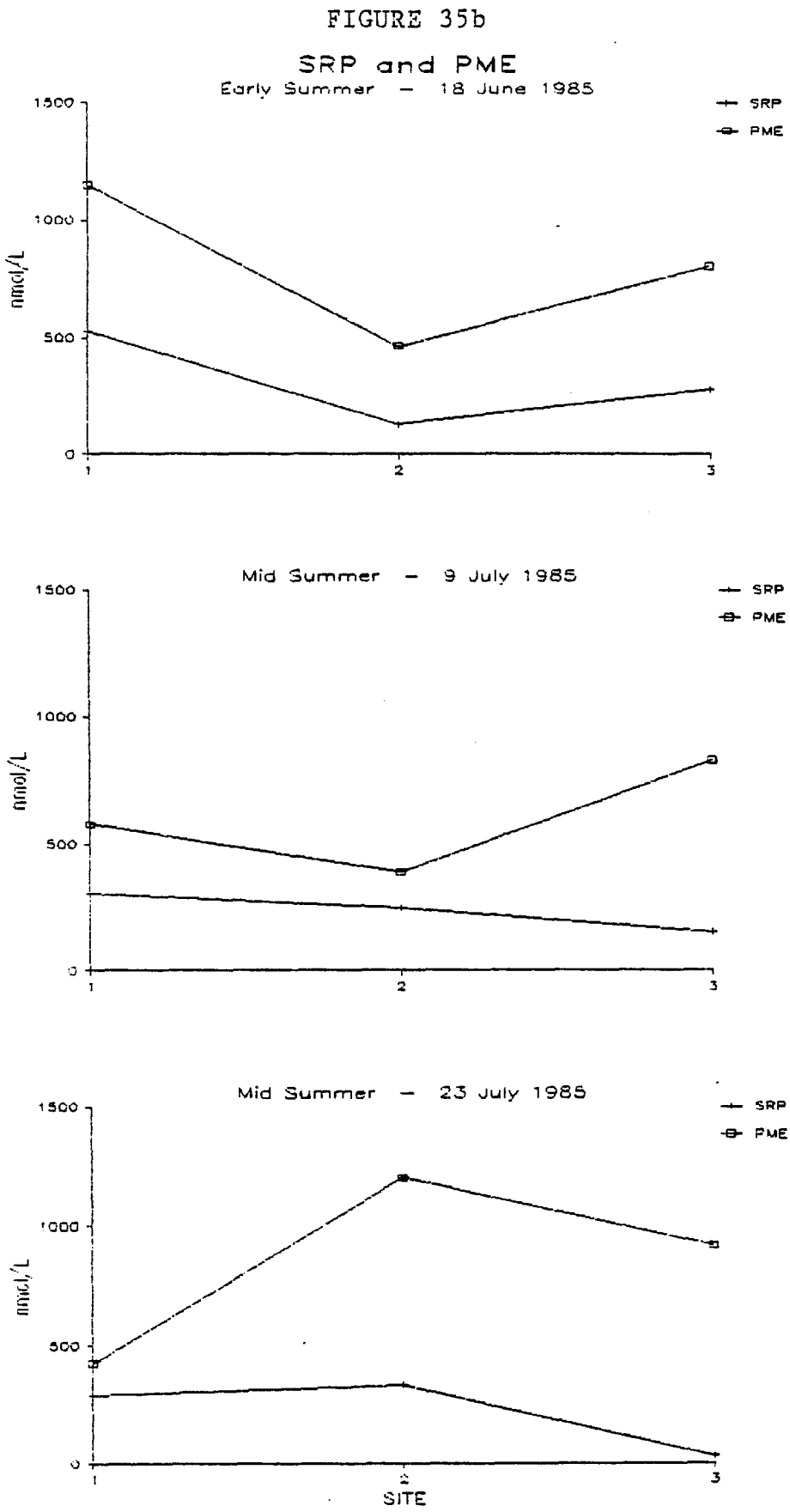

SRP and PME
Early Summer – 18 June 1985
SRP
PME
1500
1000
500
0
nmol/L
1
2
3
Mid Summer – 9 July 1985
SRP
PME
1500
1000
500
0
nmol/L
1
2
3
Mid Summer – 23 July 1985
SRP
PME
1500
1000
500
0
nmol/L
1
2
3
SITE

FIGURE 36

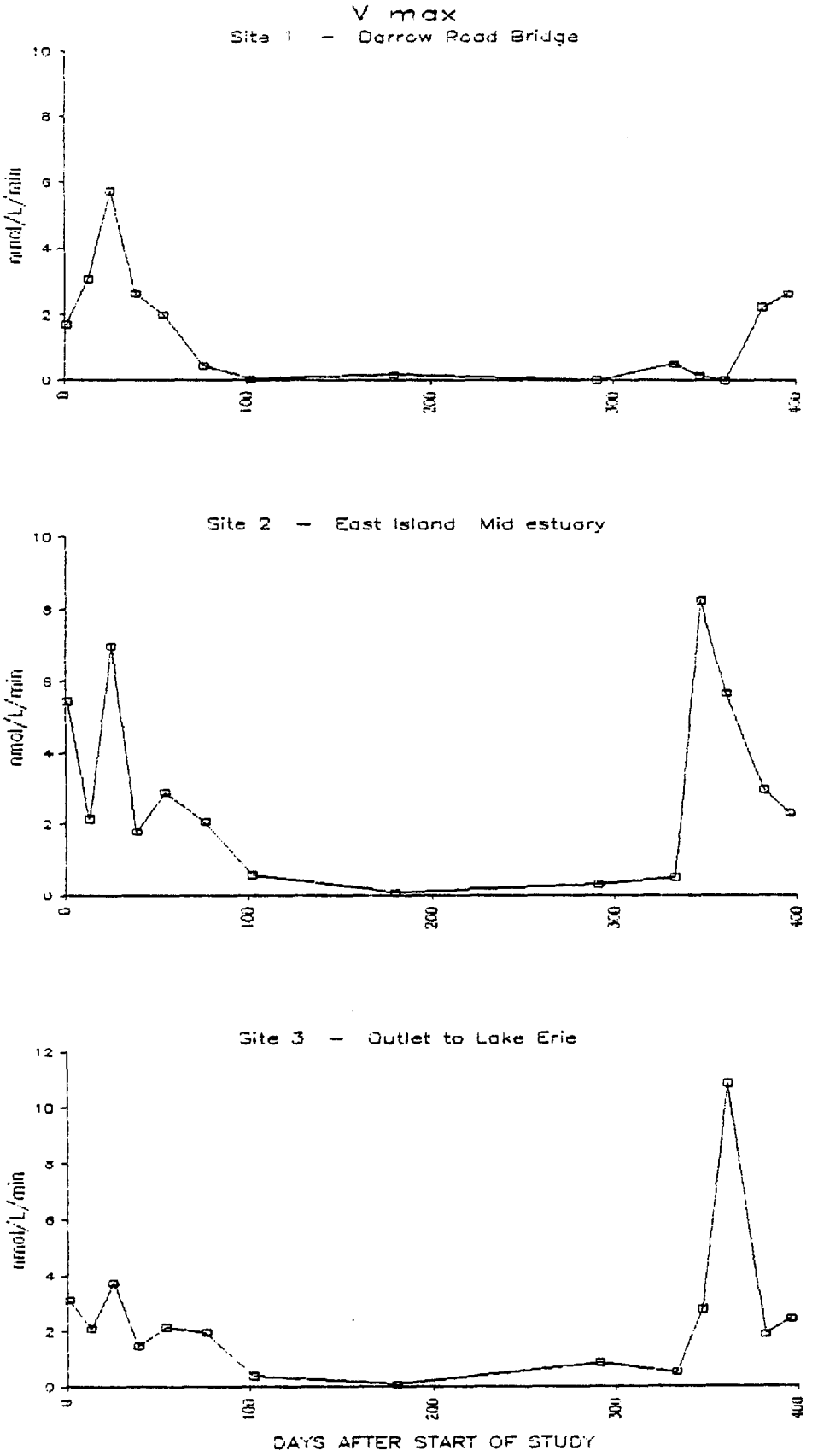
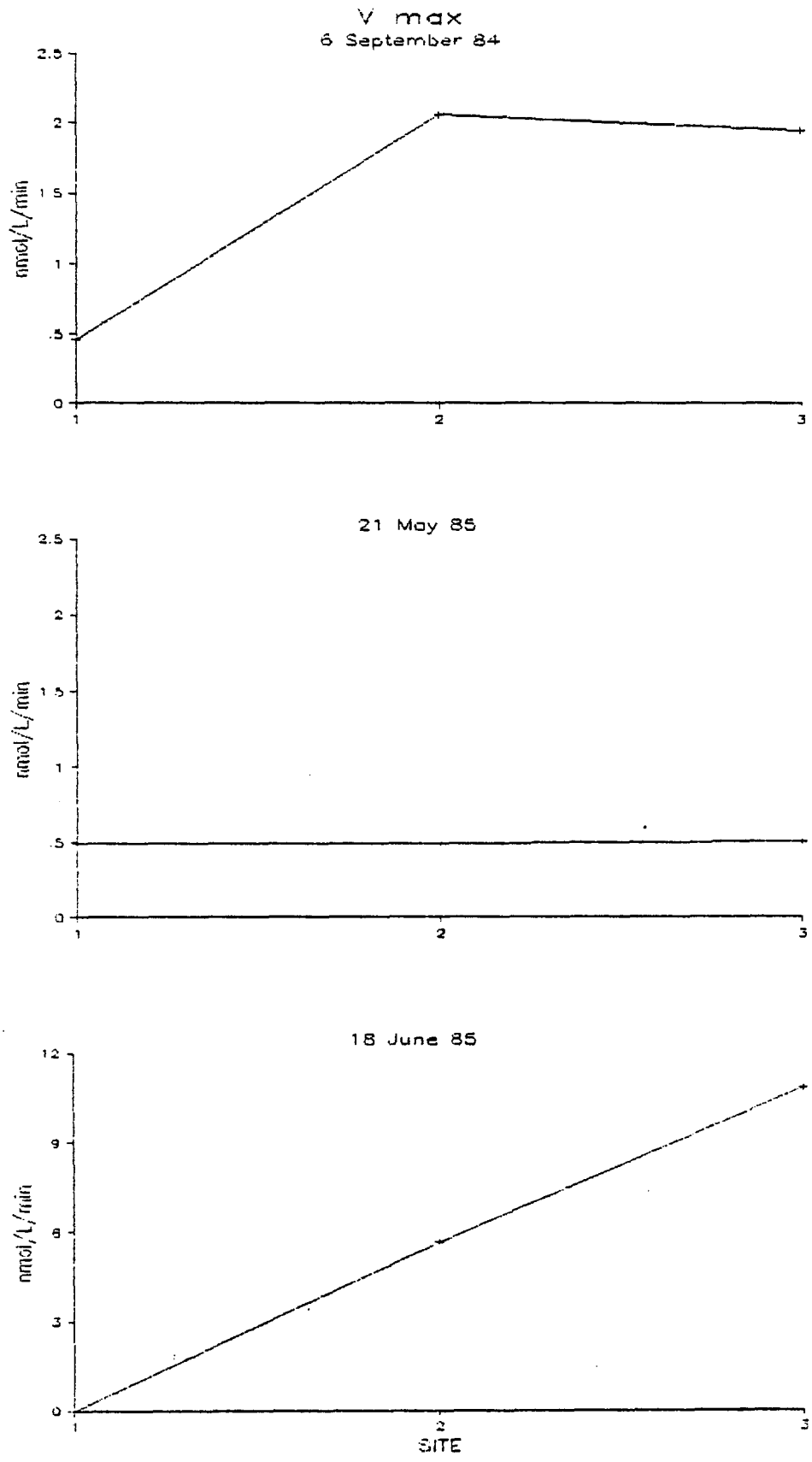

V max
Site 1 – Darrow Road Bridge
nmol/L/min
Site 2 – East Island Mid estuary
nmol/L/min
Site 3 – Outlet to Lake Erie
nmol/L/min
DAYS AFTER START OF STUDY

FIGURE 37


V max
6 September 84
nmol/L/min
2.5
2
1.5
1
.5
0
1
2
3
21 May 85
nmol/L/min
2.5
2
1.5
1
.5
0
1
2
3
18 June 85
nmol/L/min
12
9
6
3
0
1
2
3
SITE

PME is nutritionally significant only when phosphatase is present to catalyze the release of phosphate from it; conversely, phosphatase is nutritionally beneficial to organisms producing it only when the substrate PME occur in the environment. The topics of greatest interest are

i) determination of the velocity of hydrolytic release of phosphate from PME, and

ii) comparison of this rate of release with the phosphate demand of the plankton (i.e. the rate of phosphate uptake by plankton).

The velocity of hydrolytic release of phosphate was calculated from Michaelis - Menten kinetics using the routinely determined V_{max} and PME substrate concentrations. The K_m was not routinely determined in this study, but it was determined on six occassions to be 3.92×10^{-4} M, with a standard deviation of 0.642×10^{-4} M. This average value for the Michaelis coefficient was used for the calculation of all velocities. In turn, these velocities of phosphate release were compared with the velocity of phosphate uptake determined on the same day at the same site (shown above in Table VII).

Table VIII reports the results of these calculations. The column headed as "P'tase vel" presents the calculated velocity of release of phosphate from PME in units of nmol/L/min. The column headed "percent uptake by P'tase" presents that fraction of the sestonic phosphate demand (i.e. uptake rate) satisfied by the release of phosphate from PME; the fraction of release is expressed as a percent. These findings indicate that the rate of release of phosphate from PME was slow at all times investigated and never satisfied greater than two percent of the sestonic phosphate demand.

The velocity of release of phosphate from PME was fastest within the estuary in the late spring to early summer and was at very low rates during the remainder of the year (Figure 38). There was a tendency for the velocity of release to increase progressively through the wetland, except during the

TABLE VIII

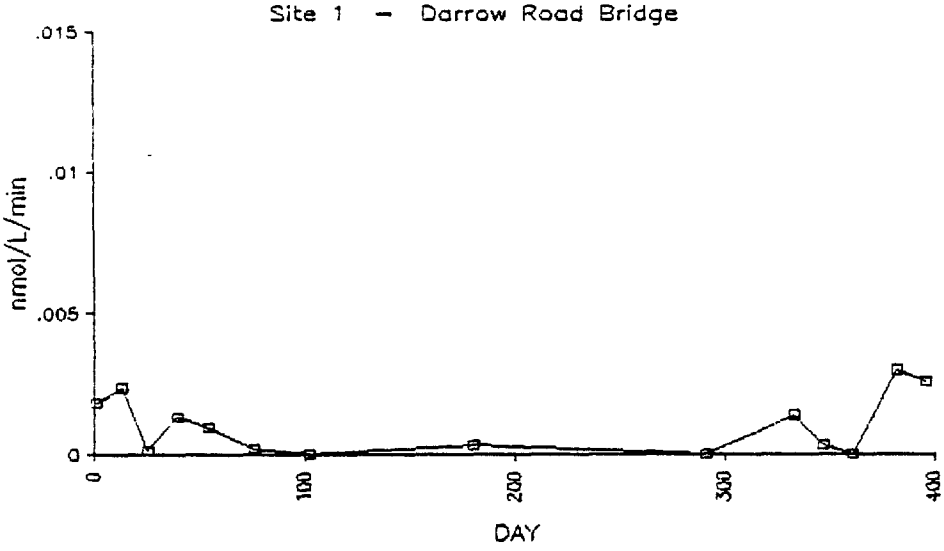
PHOSPHATASE ACTIVITY

Date	PME nmol/L	V max nmol/L/min	P'tase vel nmol/L/min	Percent uptake by P'tase
Site 1 - Darrow Road Bridge				
22 Jun 84	464.54	1.70	.00183	*
5 Jul 84	330.99	3.07	.00236	.052
17 Jul 84	10.00	5.72	.00013	.001
31 Jul 84	214.53	2.61	.00130	.030
15 Aug 84	201.95	2.00	.00094	.027
6 Sep 84	178.72	.46	.00019	.006
2 Oct 84	.00	.01	.00000	.000
19 Dec 84	908.44	.15	.00031	.088
9 Apr 85	1179.75	.00	.00000	.000
21 May 85	1207.81	.49	.00138	.114
4 Jun 85	1162.33	.12	.00032	.026
18 Jun 85	1147.17	.00	.00000	.000
9 Jul 85	576.16	2.22	.00297	.173
23 Jul 85	424.54	2.61	.00257	.094
Site 2 - East Island in Mid-Stream				
22 Jun 84	391.64	5.46	.00496	*
5 Jul 84	.00	2.15	.00000	.000
17 Jul 84	523.26	6.98	.00847	.405
31 Jul 84	338.73	1.77	.00139	.043
15 Aug 84	.00	2.88	.00000	.000
6 Sep 84	86.13	2.06	.00041	.012
2 Oct 84	.00	.57	.00000	.000
19 Dec 84	625.52	.07	.00011	.052
9 Apr 85	993.29	.30	.00069	.020
21 May 85	589.71	.49	.00067	.073
4 Jun 85	1183.30	8.26	.02263	.264
18 Jun 85	466.48	5.68	.00615	.081
9 Jul 85	388.73	2.95	.00266	.073
23 Jul 85	1202.65	2.31	.00643	.204
Site 3 - Outlet to Lake Erie				
22 Jun 84	245.18	3.12	.00177	*
5 Jul 84	220.66	2.10	.00108	.049
17 Jul 84	277.76	3.73	.00240	.018
31 Jul 84	331.96	1.46	.00113	.015
15 Aug 84	.00	2.15	.00000	.000
6 Sep 84	86.13	1.95	.00039	.018
2 Oct 84	.00	.36	.00000	.000
19 Dec 84	250.02	.07	.00004	.028
9 Apr 85	1334.92	.85	.00263	.297
21 May 85	476.16	.51	.00056	.069
4 Jun 85	1067.16	2.79	.00689	.228
18 Jun 85	805.90	10.85	.02027	.061
9 Jul 85	829.10	1.88	.00361	.102
23 Jul 85	919.73	2.44	.00521	.502

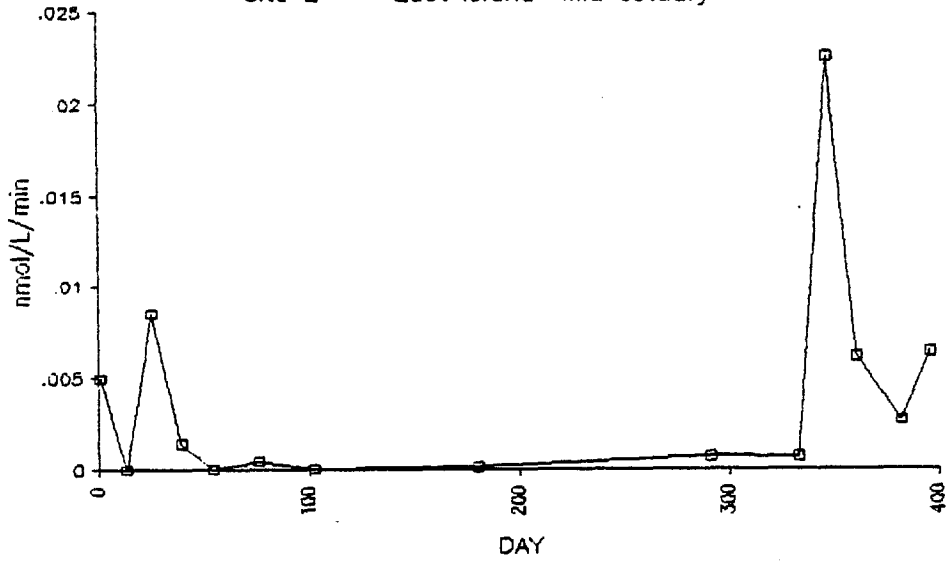
FIGURE 38

PHOSPHATASE ACTIVITY

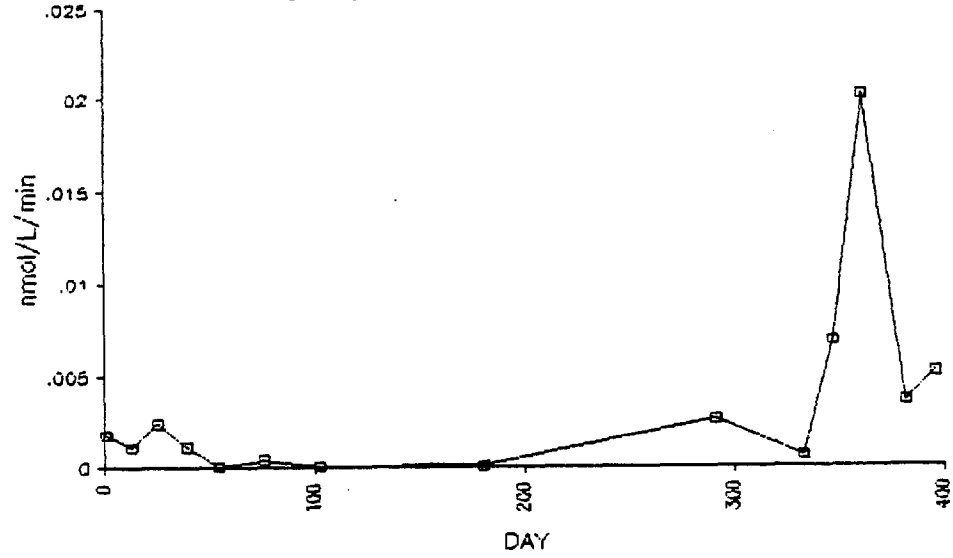
Site 1 – Darrow Road Bridge



Site 2 – East Island Mid estuary



Site 3 – Outlet to Lake Erie



springtime period of rapid flow (Figure 39). These findings taken together indicate that the wetland was a consumer of SRP and also had a certain tendency to produce PME. PME was not a nutritionally significant source of P to phytoplankton in the estuary.

C) Sediments as potential source and sink of phosphate

A preliminary investigation of the sediments at the three study sites indicated that the sediments change in character from Site 1 to Site 3. The sediment at Site 1 was a gray-brown clay type, at Site 2 a dark gray-black, and at Site 3 it had a sandy consistency. Table IX shows that the ash content at Sites 1 and 2 did not significantly differ ($P < 0.05$). However, the ash content of sediment at Site 3 was significantly lower that that at Site 2. That is, the sediment increased in its organic content at sites progressively toward the outlet into Lake Erie.

In contrast with this finding, the DOP content of the pore water, shown as SUP in Table IX, decreased progressively toward the outlet, as did the SRP and the Total P content of the pore water. Figure 40 shows that the SRP content of pore water at Sites 1 and 2 was similar, and considerably greater than the SRP composition of pore water at the outlet to Lake Erie. DOP content of pore water was greatest at Site 1 and least at Site 3. The phosphomonoesters were in barely detectable quantities in Site 1 pore water, but accounted for about half of the DOP at in the pore water of Sites 2 and 3.

The composition of the pore water tended to become more similar to the overlying water at sites progressively closer to the outlet into Lake Erie. Table X shows that the composition of the pore water was 4 - 6 times higher in concentration of Total Soluble Phosphorus compounds and in SRP than the overlying water at Site 1. At Site 2 the difference in concentration of TSP in the pore water vs. the overlying water was somewhat diminished, but SRP remained seven-fold more concentrated in the pore water. The concentrations of TSP and

FIGURE 39

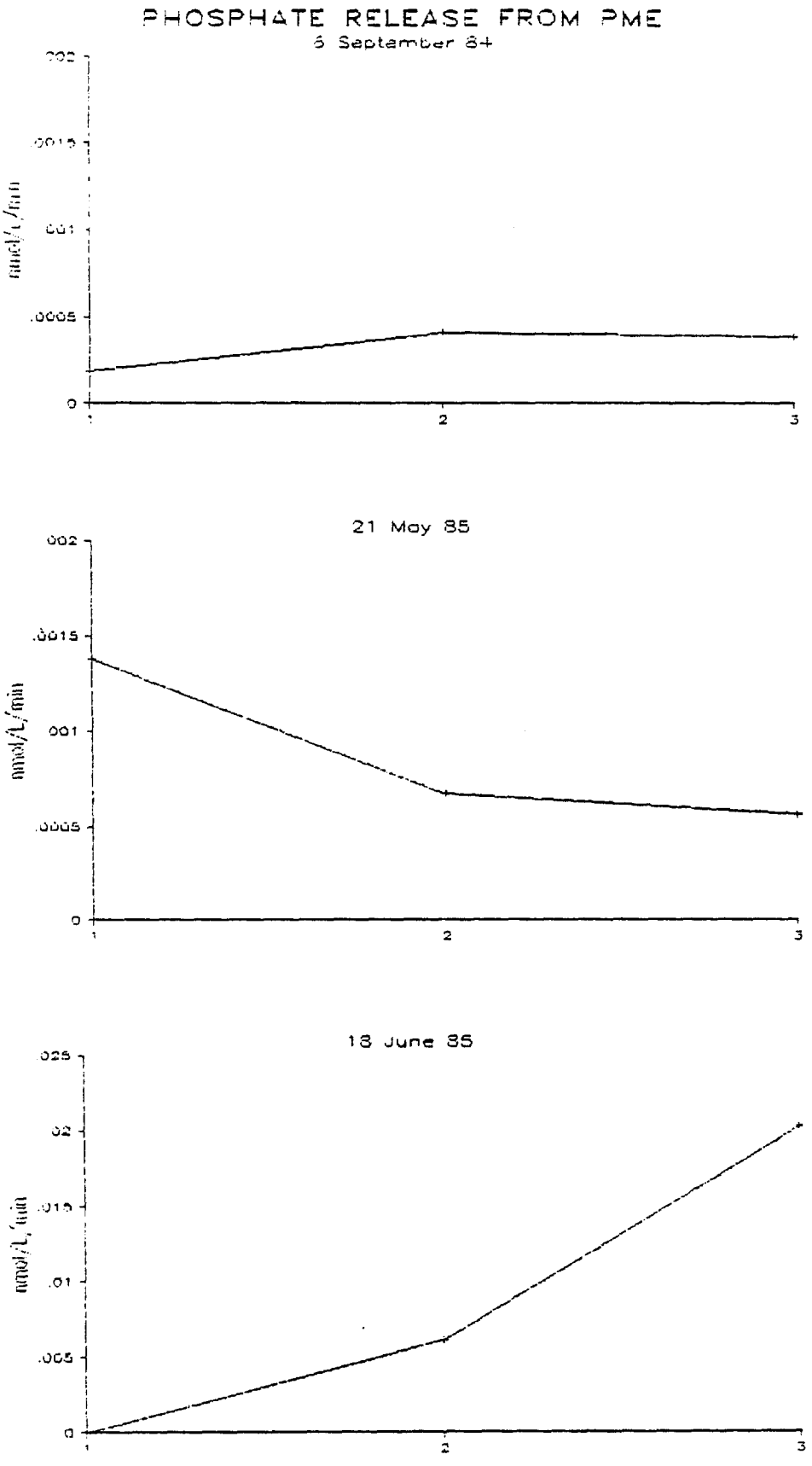

PHOSPHATE RELEASE FROM PME
6 September 84
.002
.0015
.001
.0005
0
nmol/L/min
1
2
3
21 May 85
.002
.0015
.001
.0005
0
nmol/L/min
1
2
3
18 June 85
.025
.02
.015
.01
.005
0
nmol/L/min
1
2
3

TABLE IX

SEDIMENT AND PORE WATER CHARACTERISTICS

9 July 1985

	PORE WATER COMPOSITION			SEDIMENT CHARACTERISTICS	
	SRP nmol / L	SUP nmol / L	PME nmol / L	Ash percent	Organic Content percent
SITE 1	1853.5	1280.64	50.00	90.89	9.11
SITE 2	1739.0	697.42	338.71	90.82	9.18
SITE 3	269.4	655.48	218.06	88.44	11.56

FIGURE 40

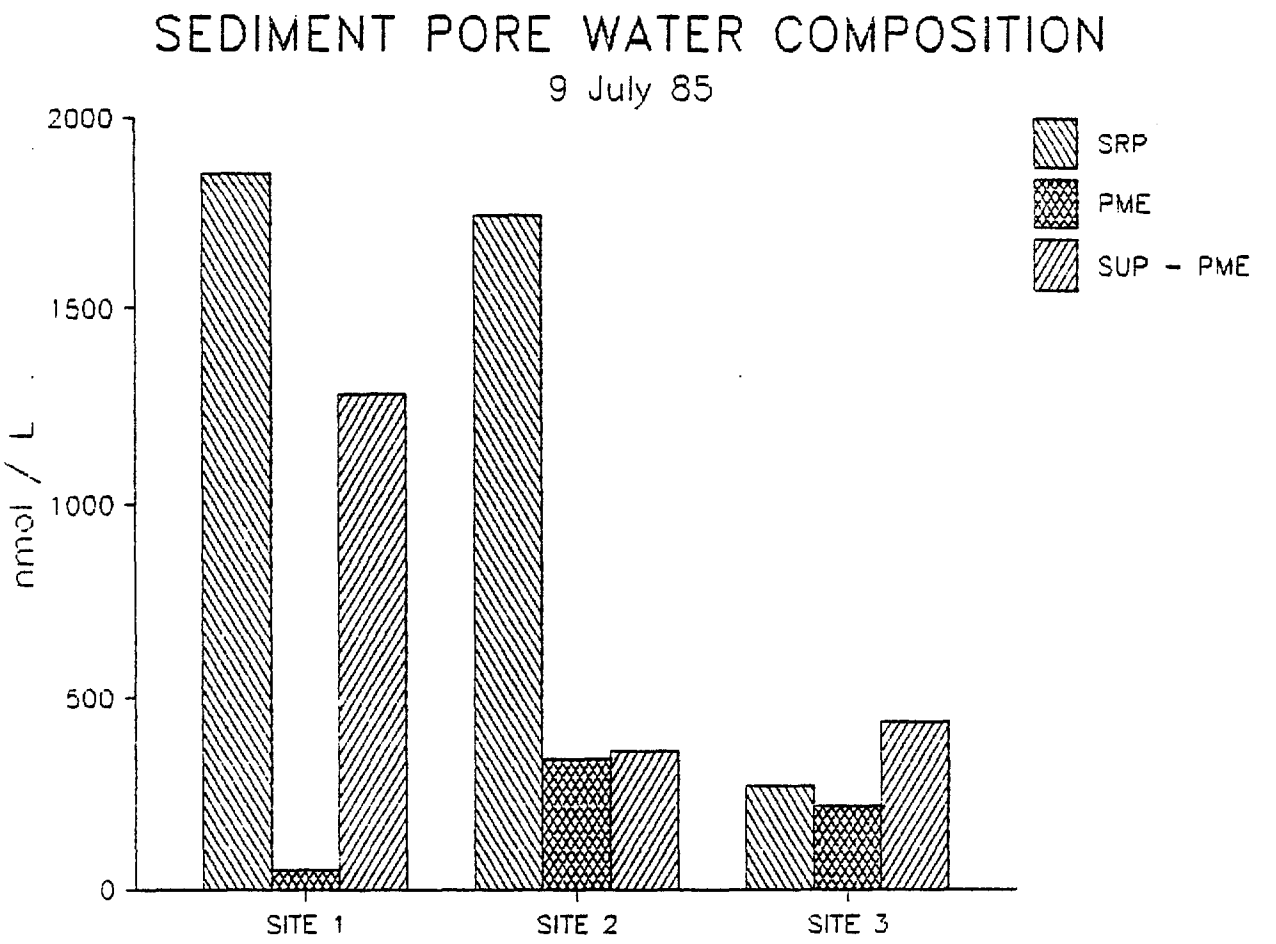

SEDIMENT PORE WATER COMPOSITION
9 July 85
2000
1500
1000
500
0
nmol / L
SRP
PME
SUP - PME
SITE 1
SITE 2
SITE 3

TABLE X

PHOSPHORUS COMPOSITION OF PORE WATER AND OVERLYING WATER

	TOTAL SOLUBLE PHOSPHORUS			SOLUBLE REACTIVE PHOSPHORUS			PHOSPHOMONOESTERS (P M E)		
	nmol / L		pore/over	nmol / L		pore/over	nmol / L		pore/over
	PORE	OVER	RATIO	PORE	OVER	RATIO	PORE	OVER	RATIO
SITE 1	3134.10	696.10	4.50	1853.50	306.10	6.06	50.00	576.10	.09
SITE 2	2436.00	902.60	2.70	1739.00	249.00	6.98	338.70	388.70	.87
SITE 3	924.90	876.80	1.05	269.40	153.90	1.75	218.10	829.00	.26

SRP in the pore water from sediment at Site 3 were virtually the same as that of the overlying water. The PME composition of the overlying water was greater than the pore water composition from each of the sites sampled.

No attempt was made in this study to observe changes in pore water composition annually. It is likely that changes in inputs to OWCNES as well as annually varying parameters such as temperature and flow rate of the overlying water would affect the activities of the sediment and the transition between the sediment and the overlying water, thereby changing the composition of the pore water. Figure 41 indicates that the concentration of SRP in pore water increased in Site 2 sediments from early June until mid August 1985. At this same time the SRP content of the overlying water increased, too. However, the SRP content of the overlying water was always about seven-fold less than that of the pore water at the same site.

Under aerobic conditions the sediments collected from each site took up phosphate. Table XI shows the rate of uptake of phosphate at each of the three sites on the same day. To provide a basis of comparison, the rate of uptake has been scaled for the amount of sediment present in the flasks; uptake is expressed as the rate per gram of sediment suspended. Using this as a basis of comparison, the sediments at Site 2 were slowest in the uptake of phosphate, and the sediments at Site 3 took up phosphate the fastest. It could be reasoned that a better procedure for scaling uptake rate would be to scale it for surface area (i.e. nmol / L / mm^2), because uptake is a surface-dependent phenomenon. However, the sandy nature of the sediments at Site 3 and the clay nature of the sediments at the other sampling sites, suggests that the surface area per gram of sediment would be least in Site 3 sediments. Therefore, scaling the uptake rate for surface area likely would not change the observations reported here: aerobic sediments at the outlet took up phosphate more rapidly than sediments at the other sampling sites, in mid August 1985.

FIGURE 41

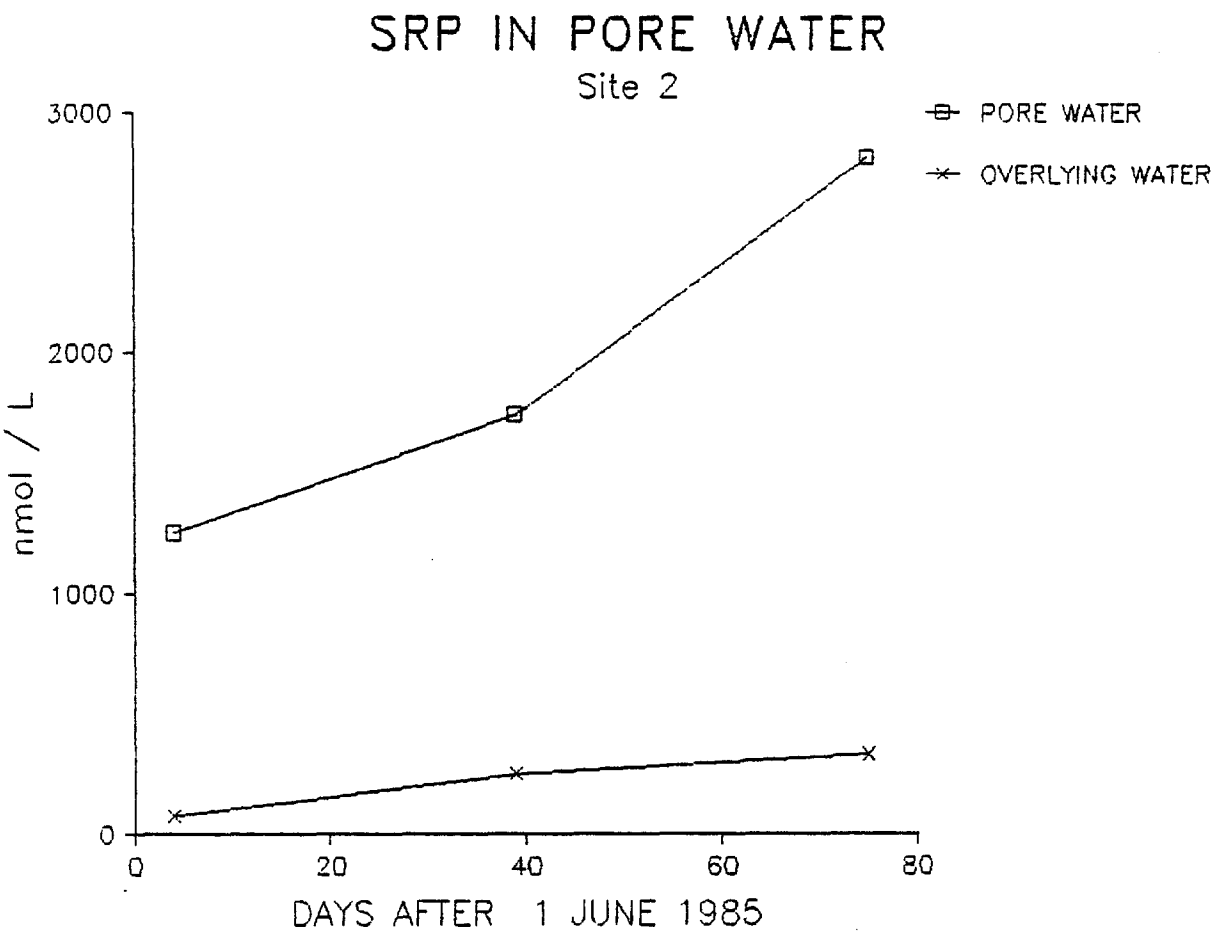

SRP IN PORE WATER
Site 2
PORE WATER
OVERLYING WATER
3000
2000
1000
0
nmol / L
0
20
40
60
80
DAYS AFTER 1 JUNE 1985

TABLE XI

SEDIMENT UPTAKE OF PHOSPHATE

14 August 1985

	S R P	Velocity of Phosphate Uptake			Active/Total
	nmol/L	nmol/L/min per g. sediment			percent
		TOTAL	+CCCP	"ACTIVE"	
SITE 1	3663.00	103.37	93.30	10.10	9.80
SITE 2	2812.60	50.45	37.30	13.20	26.20
SITE 3	2961.70	184.20	88.20	96.00	52.10

The uptake of phosphate by aerobic sediments apparently became more dependent on biotic activities at sites progressively toward the outlet. Using CCCP in low concentrations (1×10^{-6} M) as an inhibitor of metabolic activities of sediment-associated microbiota, Table XI shows that phosphate uptake at Site 1 was essentially insensitive to this inhibitor, suggesting that virtually all of the uptake by these sediments is a geochemical phenomenon (e.g. sorption to surface ions of iron, manganese, and aluminum). At Site 2 about 25 percent of the uptake was inhibited by CCCP, and at Site 3 greater than 50 percent of the uptake was inhibited by this drug. These findings indicate that the microbiotic community inhabiting the sediments became progressively more important to the uptake of phosphate.

To determine the capacity of sediments to release phosphate under anaerobic conditions, 20 g. (wet weight) portions of collected sediments were resuspended in 400 mL of diluted pore water (pore water diluted 1:3 with distilled water) and placed in stoppered B.O.D. bottles. Both the oxygen tension and the SRP concentration of the suspension in th bottle was observed for 10 days. The oxygen tension at the beginning of the experiment was 8.5 mg/L, near-saturated at ambient temperature (22° C). Table XII shows that after 30 minutes in contact with the sediment slurry, much of the oxygen had been consumed. The apparent oxygen consumption rate of the sediment at Site 2 was considerably greater than that from Sites 1 or 3. However, after 48 hours, the oxygen tension was equal or less than 0.45 mg./L in each of the samples and continued to decrease to 0.20 mg./L in each at day 10 of this study. No attempt was made to discriminate between biological and chemical oxygen demand in this preliminary study.

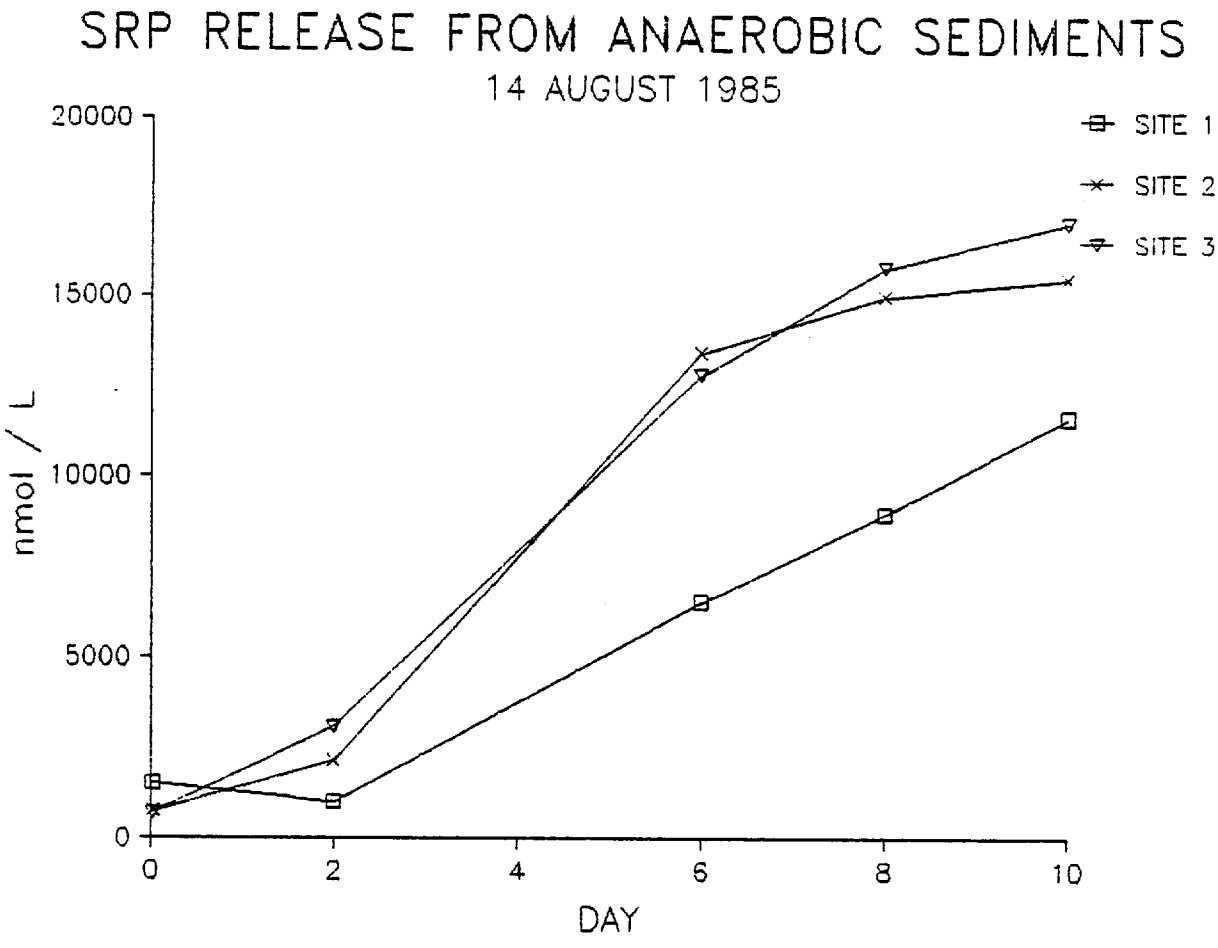
Figure 42 shows that the sediments at each site released considerable quantities of SRP into the surrounding water, following the consumption of oxygen initially present. The release of SRP from sediments of Sites 2 and 3

TABLE XII

OXYGEN CONSUMPTION BY SEDIMENTS

DAY	Site 1 Oxygen mg. / L	Site 2 Oxygen mg. / L	Site 3 Oxygen mg. / L
.02	2.99	.41	3.10
2.00	.45	.40	.35
6.00	.30	.30	.29
8.00	.21	.20	.20
10.00	.20	.20	.20

FIGURE 42


SRP RELEASE FROM ANAEROBIC SEDIMENTS
14 AUGUST 1985
SITE 1
SITE 2
SITE 3
20000
15000
10000
5000
0
nmol / L
0
2
4
6
8
10
DAY

were comparable in rate and extent. The sediments from Site 1 released SRP at a rate approximately half that of the sediments at the other sampling sites, but the extent of release was comparable, indicating that considerable releases of phosphate are possible from anaerobic sediments at all sites examined.

DISCUSSION

Rational development of plans for the management of freshwater communities depends upon identification of those resourses that limit their growth. It is through constraint and control of these resources that the community can be managed. Freshwater planktonic communities often are limited in their growth by the availability of phosphorus (Schindler 1977). Whether P-availability limits planktonic populations within this freshwater estuary is unknown. Besides its implications for the development of a management plan, future scientific studies also depend on determing whether the populations are P-limited or not. If they are P-limited the next studies should examine extensively the possible sources of P and determine ways of maintaining the input of available P in acceptable quantities. On the other hand, if these planktonic populations are not P-limited such a study would be relatively unimportant. Instead, future studies should investigate the way in which the estuary processes its P-inputs before they enter Lake Erie, where planktonic populations are known often to be P-limited. For these reasons the primary purposes of this study were to determine (1) whether the planktonic community of OWCNES was growth-limited by phosphorus availability, and (2) whether passage of P-inputs to the estuary ameliorates availability to populations in the receiving community.

1. Planktonic populations in OWCNES were not growth-limited by P-availability

Phosphorus limited growth of freshwater communities is characterized by barely detectable quantities of phosphate (as SRP), atomic N:P ratios that are greater than 22 during the springtime (Chiaudani and Vighi, 1979), and physiological responses such as a rapid "turnover time" of phosphate (Lean et al. 1983) and high specific activities of enzymes adaptively produced in response to P-limitation (Fitzgerald and Nelson1966; Heath and Cooke 1975). Also, nutrient-addition bioassays should yield additional growth when available

phosphate is added to samples of P-limited planktonic communities (Schelske 1972 ; Hartig and Wallen 1985). None of these criteria is paramount in making a decision of P limitation, but their results taken together are useful in interpreting the nutritional status of the planktonic community with respect to its phosphorus resources. All of these criteria were examined at appropriate times during this study. The body of evidence indicates that the planktonic community was rarely, if ever, limited in its growth by the availability of phosphorus.

Nutrient addition bioassays consistently failed to yield more chlorophyll when phosphate was added, but did respond when nitrate was added in some cases. Such bioassays may fail to respond to additions of available limiting nutrients because the biota do not adapt well to laboratory conditions and so do not grow despite addition of appropriate nutrients, yielding "false negative" results. However, the fact that some responses were noted argues against this possibility. These observations indicate that P-availability did not limit the growth of these populations during that part of the growing season when phosphate was seen it its least concentrations in the wetland.

Physiological variables also indicated that the phytoplankton were not limited in their growth by P availability. Very short phosphate turnover times are characteristic of severely P-limited systems. Rigler (1964) reported turnovers times of less than 5 min, and we have found turnover times shorter than 2 min in an Ohio acid bog lake (Cotner 1984). Lean et al. (1983) have observed that when the Lake Erie phytoplankton community was P-limited the turnover time was always less than 20 min. Judging by this criterion, only during July 1984 and June 1985 may the community have been P-limited in its growth, when the phosphate turnover time dropped to 8.5 min.

When phytoplankton become P-limited some are capable of adaptively producing alkaline phosphatase, an attached exo-enzyme (Fitzgerald and Nelson

1966; Kuenzler and Paras 1970). Jones (1972) has suggested that the magnitude of the specific activity (nmol phosphate released/L/min per ug Chl a/L resulting in a unit of nmol P released/min/ug Chl a) may serve as an indicator of P-limitation stress. Enzyme activity can also be scaled for the amount of particulate P, yielding a unit of specific activity that represents throughput of the standing particulate P if throughput was exclusively due to phosphatase: nmol P released/L/min per nmol particulate P/L, giving a unit in 1/min, i.e. proportion of particulate P moved from soluion to particles via phosphatase. In East Twin Lake (Portage Co., Ohio) at the height of a bloom of cyanobacteria severely P-limited, Heath and Cooke (1975) observed a large increase in the phosphatase specific activity. They observed a specific activity of 19.24 nmol P released/min/ug Chl a, and a throughput fraction of 0.0477 particulate P/min or 68.68 particulate P per day. By contrast, the specific activity of alkaline phosphatase in the OWCNES community was always low, but increased in the spring to reach its highest levels in June. Specific activities tended to decrease during passage through the OWCNES. These low specific activities are similar to the specific activities observed by Pettersson (1980) in communities not limited by P availability. Only incoming plankton (i.e. at Site 1) in June 1984 and May 1985 indicate phosphatase specific activities approaching those reported by Pettersson in communities temporarily P-limited.

The results from chemical analysis are equivocal. Although it is common for P-limited systems to have SRP concentrations less than 1 ug/L, the SRP seldom was less than 5 ug/L in OWCNES, suggesting that there was always a sufficient supply of available P. Chiaudani and Vighi (1979) showed in 26 Italian lakes that atomic ratios of total N:total P greater than 22 was indicative of P-limitation and a ratio of less than 11 was indicative of N-limitation. The ratios observed in this study ranged from 66 at Site 1 to 28 at Site 3, indicating P-limitation at all sites but becoming progressively less

severe during passage through the estuary. However, if the particulate materials represent the content of the plankton, the particulate N: particulate P ratio (0.07 at Site 1 and 5.26 at Site 3) indicated that the plankton would be relatively nitrogen poor and would not be P-limited. Gibson (1971) noted that such low N:P ratios likely indicate N-limitation and not P-limitation. Chemical analyses alone at best give some information about the structure of available nutrients, but they can not convey the performance of living populaions of planktonic organisms. Especially in a complex system in which flow is a major parameter and particulate matter is both planktonic organic matter and inorganic material suspended in the water column, it is difficult to determine the significance of structural measurements whose basis is derived from lentic systems and from laboratory algal cultures.

None of these four approaches for assessing P limitation is sufficient in itself to affirm or deny P limitation. The results from all taken together support the view that the planktonic community of OWCNES was not growth limited by the availability of phosphorus; none of these gave a strong indication of P limitation at any time during the study. These findings indicate that because P availability is not growth limiting, management practices directed toward limiting the input of P are not likely to have noticable effects on the size of phytoplankton standing crops. Also, increased inputs of P, e.g. in agricultural runoff from upstream activities in the watershed are not likely to affect the size of phytoplanktonic populations.

II. The estuary diminishes the availability of phosphorus to Lake Erie planktonic communities

Many studies focusing on the planktonic community of Lake Erie have concluded that during the majority of the growing season, this community is limited in its growth by the availability of P. Lean et al (1983) showed that following thermal stratification, the plankton of the central and eastern basins

of Lake Erie were P limited during the growing season (April - October). Hartig and Wallin (1984) extended this finding to the western basin of the lake, showing that P was the major limiting nutrient from May until September. These observations supprot the view that planktonic populations of Lake Erie are responsive to inputs of available P, and could be controlled by management practices aimed at reducing P inputs.

It is important to determine whether wetlands such as the OWCNES alter the availability of P inputs. It is conceivable that the quantity of the input could be diminished if the wetland acted as a "filter", trapping inputs and keeping them from entering the lake. Biggs and Howell (1984) have recently shown the various ways in which estuaries can act as a sediment trap, directly trapping solid inputs either from the river or from the receiving body of water. Schemel, et al (1984) have shown that incoming dissolved materials (DIC, DIN, and DIP) are taken up largely by biota and removed from the water following sedimentation as detritus. It is also conceivable that estuarine deltas could increase the output nutrients by increasing the size of littoral regions at the mouth of a river. The ensuing vegatation could in turn release to the surrounding water nutrients that otherwise would remain in the sediments (Twilley, et al 1977).

Besides serving quantitatively to remove - or supply - nutrients that otherwise would enter the receiving community, estuaries can serve qualitatively as "tranformers", altering the availability of incoming nutrients before they enter the receiving body of water. Even if estuaries release as much P or N as they receive from their supplying stream or river, if they alter nutrient availability to plankton in the receiving community their long-term influence on the receiving community must be considered potentially important. Dissolved inputs could be sorbed to inorganic suspended solids, especially under aerobic conditions common to shallow flow-through esturaries

(Mortimer 1971); or inorganic nutrients could be assimilated by bacterioplankton and phytoplankton (Schemel, et al 1984) and in this way "transform" dissolved forms to particulate forms, that presumably would be less nutritionally available to plankton in the receiving community. Alternatively, large zooplankton populations apparently can release much particluate material as dissolved inorganic nutrients (Peters and Lean 1973). Also, readily available dissolved nutrients could be converted to less available dissolved organic forms following uptake and release by bacterioplankton or phytoplankton (Fogg 1975).

Because a comprehensive phosphorus budget was beyond the scope and budget of this project, it is not possible to make quantitative statements regarding the significance of OWCNES as a "filter", trapping phosphorus inputs and preventing them from entering Lake Erie. The observations presented in the results section of this report (Table III) show that the total phosphorus content of entering and exiting waters is approximately equal, indicating that any "filtering" activities by this wetland are not obvious at first glance.

This study focused on determining qualitative changes in the availability of phosphorus inputs. The "performance of the estuary" was determined as the difference between the material at Site 1 and Site 3. That is, the importance of the estuary was assessed as those qualitative alterations that occurred during passage through the estuary.

OWCNES reduced the amount of readily available P significantly in two ways. Soluble inputs were converted to particulate matter probably both by biogenic and abiotic processes. Also, incoming water was often dominated by SRP, while water at the outlet was predominated by dissolved organic forms. Although some of these organic compounds were phosphomonoesters (PME) that can release phosphate by known processes, the release rates were apparently very slow relative to the planktonic phosphate demand. The other forms of phosphorus may be completely refractory.

Phosphate is most likely the only form of phosphorus that is directly assimilated by bacterioplankton and phytoplankton under natural conditions (Fogg 1975). Although recent studies indicate that some organic compounds may be assimilated directly from culture media by active processes dependent on specific receptor sites on the cell surface (Ruben, et al. 1977), the relatively low affinity of these receptors for their receptands suggests that they are not of great importance in nature. Therefore "readily available P" was taken to be only phosphate and was measured as SRP in this study. Considerable controversy continues regarding the suitability of SRP as a reliable estimate of phosphate. Rigler (1966, 1968) showed in a radiometric bioassay that SRP apparently overestimated the phosphate that was available to phytoplankton, especially when the concentration was less than 3 ug PO_4-P/L. Rigler believed that certain DOP compounds could be easily hydrolysed and that these were the source of the errors encountered. However, Shapiro (1973) using an extraction method, showed that SRP detected only phosphate, unless silcates and arsenates were in unusually high quantities. Also, Francko and Heath (1979) showed that greater than 95 percent of the SRP chromatographed as authentic ortho-phosphate in two independent chromatographic systems. Recently, Nurnberg and Peters (1984) indicated that SRP usually corresponded well to the "biologically available P" in a modified bioassay procedure. For these reasons I believe that SRP provided a reliable estimate of the phosphate present in the water samples studied here, with the caveat that they may provide slight (ca. 10 percent) overestimates at the lowest concentrations observed.

In all seasons SRP was highest in concentration at the inlet to the wetland (Site 1) and diminished during subsequent passage through the OWCNES. Figures 10 - 14 show that in all seasons the soluble phosphorus compounds that entered the wetland were predominantly SRP but decreased in amount and proporion of total P toward the outlet at Site 3. As the growing season progressed, the

amount of particulate P increased at all sites (Figures 7 - 9), but it was highest at Site 2 and was considerably higher at the outlet than in the incoming waters during the time of predominant growth (Figure 10). Also, while the relative content of SRP decreased during pasage through the estuary, the relative content of DOP (Total soluble P minus SRP) increased.

This study suggests that phosphate was largely taken up by biota. Uptake in the open water was very sensitive to metabolic inhibitors (cyanide and trichloro - cyanocarbonyl - phenylhydrazone, CCCP), shown in Figures 22 and 23. Also, phosphate uptake was sensitive to temperature in a fashion sugesting that it is dependent on metabolic activity (Figure 24). The proportion of phosphate utake sensitive to metabolic inhibitors was highest in the period May - July at all sites (Table VII). In general, the fraction of uptake that was sensitive to these inhibitors increased from Site 1 toward Site 3 (Figures 28 - 30, but not in Figure 31). Sediments taken from each site were able to sorb phosphate at a moderate rate under aerobic conditions, and their phosphate uptake was also sensitive to metabolic inhibitors. Sediment from Site 1 was only slightly sensitive to CCCP, while about half of the phosphate uptake by sediments from Site 3 was sensitive to this inhibitor (Table XI), suggesing that sediment uptake of phosphate became more dependent on biogenic activities, perhaps by microbiota attached to sediment particles.

The concentration and relative proportion of DOP tended to increase during passage through the wetland. The nutritional significance of these compounds is unknown, but the results of this study indicate that even if phosphate can be released from them, the release rate is considerable slower than the rate of uptake of phosphate in the same sample. Francko and Heath (1979) identified two classes of compounds that can release phosphate to freshwater communities. One of these classes was never observed in samples from OWCNES, but the other class - phosphomonoesters (PME) - was seen at all sites.

The relative proportion of PME apparently represented all or nearly all of the DOP detected in the early spring through early June. In contrast with the marked decrease in SRP through the estuary toward the outlet, the concentration of PME remained relatively unchanged in concentration through early summer (Figure 35a), but then increased in concentration at Sites 2 and 3 through the summer (Figure 35b), reaching maximum amounts in the late winter (Figure 32).

PME compounds are nutritionally useful only when phosphate can be released from them, a hydrolytic process catalysed by acid- or alkaline phosphatase enzymatic activity. That activity was observed at all sites only during the period May - August (Figure 36). From this activity and the K_m of the enzyme the actual velocity of release of phosphate can be calculated and compared with the phosphate uptake rate. Table VIII shows that PME apparently were not nutritionally significant sources of phosphate, never satisfying more than 2 percent of the planktonic phosphate demand. Heath (1986) has recently reported similar findings for a wide variety of freshwater systems. In reservoirs, eutrophic lakes, and an acidic bog lake he reported that phosphate release from PME never satisfied more than 10 percent of the phosphate demand of the same sample. From these findings I conclude that PME compounds are a form of P much less readily available to plankton than SRP. Even in the presence of phosphatase in May and early June substantial quantities of PME persisted but diminished as the season progressed, a finding consistent with the view that PME are available to plankton but only slowly. The DOP that predominated at the end of the season (i.e. SUP minus PME) had an unknown composition. Whether its P was available to plankton is also unknown, but the fact that it increased as the season (Figure 32) progressed suggests that it did not turnover rapidly.

Taken together, these results indicate that the wetland of OWCNES diminished the potential impact of inputs from this agricultural watershed by supporting a broad shallow basin in which phytoplankton and bacterioplankton can

grow. In this way much of the available P that entered OWCNES as SRP was removed from solution, being taken up largely by plankton but also in part by suspended inorganic solids. The living cells may release organic phosphorus compounds during normal vital processes or upon cell death and lysis (Fogg 1975). In these ways the wetland acts as a "transformer", transforming available P into less available forms: either as particles that may settle out of the euphotic zone of the receiving community or as dissolved phosphorus compounds that are only slowly available, if available at all as source of phosphate.

III. Implications for future studies.

The performance of estuaries in affecting the availablility of nutrients to the receiving community covers many possibilities, and distinguishing between each possibility requires a major research effort. This study compared qualitative differences in the phosphorus composition, rather than quantitative differences. This study was done as a preliminary study and as such an implicit aim was to identify questions for future studies. The availability of P does not seem to be an important factor in controlling the performance of the wetland plankton, i.e. P is not a growth-limiting nutrient.

The most important finding of this study is that the wetland appears significantly to alter the availability of P inputs. It has the important function of ameliorating the effects of agricultural activities in the watershed. Future studies should focus on further understanding this function. A quantitative understanding of the effects of the estuary is essential to a more precise knowledge of its performance. Even though the concentration of total P at the outlet was similar to that measured at the main inlet, without a comprehensive P budget it is premature to conclude that this estuary does not act as a "filter" removing P inputs.

This study observed that the wetland acts as a "transformer", effectively exchanging dissolved organic forms for SRP. The mechanisms involved

can at best be hinted at from this study. Future studies should attend to understanding the sources of DOP and their significance to OWCNES plankton and especially to Lake Erie plankton. A major amount of DOP that increased as the season progressed was of an unknown composition. Future studies should aim to characterize this material and determine its nutritional significance to plankton, by determining whether phosphate can be released from it, at what rate and under which conditions.

This study made only a cursory observation of the sediments. It showed that the sediments can act as a sink for phosphate under aerobic conditions, as occur when the sediments are suspended by the turbulence induced by winds and internal flows. Also, the sediments can act as a source of SRP under anoxic conditions as would occur in the undisturbed layers of the sediment. A spate or a seiche could then release substantial quantities of SRP from the sediment. Future studies should focus on the performance of the sediments in taking up and releasing available phosphorus compounds.

CONCLUSIONS

The purposes of this study ware to determine the importance of phosphorus inputs to the planktonic community in the OWCNES, and to determine the effects of this wetland on the availability of phosphorus to Lake Erie phytoplankton. Also, a preliminary investigation of sediment activities was performed to determine whether the sediments can act as a sink or as a source of available P. From the data presented, this study draws the following conclusions.

I. The growth of phytoplankton in OWCNES was not limited by the availability of phosphorus:

a) Nutrient addition bioassays, conducted at the height of the growing season when the lowest quantities of SRP were observed, showed no significant increase in growth in planktonic samples amended with potassium phosphate, sodium nitrate, or both (one-tailed Dunnett q' test). Addition of nitrate to incoming waters (sampled at Site 1) stimulated growth, suggesting that the incoming phytoplankton were growth limited by N availability in mid-summer.

b) The phosphate turnover time was seldom less than 20 minutes, contrary to observations made in P-limited communities, where the turnover time is often less than 5 minutes.

c) The specific activity of alkaline phosphatase remained 10 to 100 times lower than the specific activity of that enzyme when adaptively produced by phytoplankton in P-limited systems.

d) The concentration of soluble reactive phosphorus (SRP) seldom declined below 5 ug P / L; whereas SRP often reaches undetectable levels (less than 1 ug / L) in P-limited communities.

e) The particluate N:P ratio was less than 10; whereas it is often greater than 20 in P-limited systems.

II. OWCNES greatly ameliorated the availability of phosphorus to Lake Erie phytoplankton.

This conclusion is based on observations of the change in distribution phosphorus components and their metabolism during passage through the estuary. Even during times of stagnation (i.e. relatively slow flow in the summer), a pattern was consistently observed that demonstrated a shift from a relatively high proportion of SRP at the inlet to OWCNES, to a continual decrease in SRP toward the outlet to Lake Erie, both in absolute and relative terms. This wetland community decreased the availability of P to Lake Erie phytoplankton by increasing the proportion of particulate P and the proportion of soluble forms of phosphorus from which phosphate was slowly released, if at all.

a) During the growing season total P suspended in the water increased at each site even though the amount of SRP decreased

b) As the growing season progressed the proportion of particulate-P increased.

c) The amount and the proportion of soluble-P at each site was greatest in the early spring and declined through the growing season.

d) At all times the concentration of SRP declined from inlet toward outlet (Site 1 through Site 3)

e) During the winter the soluble-P content of the water increased during passage through the OWCNES (in both absolute and proportional terms). At all other times the content of soluble P declined through the estuary.

f) No UV-sensitive compounds were observed to release phosphate.

g) Phosphomonoesters reached a maximum in the spring and declined thereafter.

h) The release of phosphate from phosphomonoesters was slow in comparison to the rate of uptake of phosphate by seston, seldom satisfying more than one percent of planktonic phosphate demand.

i) Phosphate uptake was largely dependent on metabolic activities of the plankton, rather than ion exchange adsorption to suspended sediments. The proportion of uptake that was sensitive to a metabolic inhibitor (CCCP) increased during passage through the estuary.

III. The sediments can act as both a P-sink and a P-source.

a) Under aerobic conditions, as occur during turbulent suspension into the water column, sediment samples took up phosphate at a moderate rate.

b) About one-half of the rate of uptake was sensitive to CCCP, a metabolic inhibitor, swuggesting that uptake of phosphate by sediments occurs both by ion-exchange sorption to surfaces, as well as uptake by biota associated with the sediment.

c) Uptake of phosphate by sediments from Site 1 was least dependent on metabolic activities, and uptake of phosphate by sediments from Site 3 was most dependent on metabolic activities.

d) Under anaerobic conditions, as occur in sub-surface sediments, phosphate was released over a period of days.

e) Pore water from freshly collected (anaerobic) sediments contained 6 to 10 times the SRP concentration of the overlying water.

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