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ANGIOSPERM GROWTH, DISTRIBUTION, AND TISSUE NITROGEN CONCENTRATION IN A SEWAGE-IMPACTED MARSH: YORKTOWN CREEK, VIRGINIA

A Thesis Presented to The Faculty of the School of Marine Science The College of William and Mary in Virginia

In Partial Fulfillment of the Requirements for the Degree of Master of Arts

> by Mark S. Kowalski 1979

# **APPROVAL SHEET**

This thesis is submitted in partial fulfillment of the

requirements for the degree of

Master of Arts

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# ABSTRACT

Yorktown Creek, a marsh-creek ecosystem grading from salt- to fresh-water vegetation, was historically impacted (ca. 19 years) by the addition of nutrient-rich, secondarily treated sewage to the head of one of two main creek branches. Angiosperm communities in impacted and natural areas were studied to delineate effects attributable to chronic nutrient-enrichment on angiosperm production, growth form, community distribution and species composition, and aerial tissue nitrogen concentration (TNC). The communities included those dominated by <u>Spartina alterniflora</u>, <u>Typha angustifolia</u>, and <u>Spartina patens</u> - <u>Distichlis spicata</u>.

No discernible effect of enrichment was found on aerial annual net primary production of the communities. Limitation of production by interstitial salinity was suggested in some enriched areas. Differences in community distribution and species composition were observed but may be attributable to differences in topographic and hydrologic factors rather than to enrichment. Growth form of species in impacted areas was different from that of the same species in natural areas: enrichment apparently increased mean plant heights. Enrichment did not consistently increase TNC of species in impacted areas; however, it may have acted to maintain TNC at relatively higher levels at the end of the growing season.

Keywords: marsh, primary production, nutrient enrichment, salinity, tissue nitrogen, <u>Spartina</u>, <u>Typha</u>, <u>Distichlis</u>, sewage.

ANGIOSPERM GROWTH, DISTRIBUTION, AND TISSUE NITROGEN CONCENTRATION IN A SEWAGE-IMPACTED MARSH: YORKTOWN CREEK, VIRGINIA

#### INTRODUCTION

Yorktown Creek, located in southeastern Virginia near Yorktown, branches from the York River 10 km west of Chesapeake Bay. The river in this vicinity has a nominal salinity of 18-23 0/00 (Boesch, 1971) and a mean tidal range of 0.73 m (USCGS Tide Tables). The creek-marsh system encompasses 11.6 ha and lies wholly within Colonial National Historic Park. The major dominant angiosperms are <u>Distichlis spicata, Spartina alterniflora, S. patens</u>, and <u>Typha</u> <u>angustifolia</u>. The creek, 2 m wide and 0.5 m deep, bifurcates 300 m from the river confluence to form south and west creek-marsh branches separated by upland forest. Figure 1 illustrates the area's general topographic features.

The creek was chronically impacted (ca. 1957-July, 1976) by disposal at the head of the south branch of nutrient-rich wastewater from a secondary sewage treatment plant operated by the National Park Service (Wetzel et al., 1977). The discharge did not affect the western branch due to local land form. The sewage plant discharged a mean operating-day volume of  $2.7 \times 10^5$  l during the latter part of 1975 and early 1976. This volume was slightly in excess of the plant's design maximum of  $2.3 \times 10^5$  l (National Park Service, Yorktown, personal communications and operational records). FIGURE 1 YORKTOWN CREEK AND THE ASSOCIATED MARSH



Chronic discharge of domestic wastewater to natural aquatic systems can induce eutrophication. This condition frequently causes depletion of some species, appearance of others, and acceleration of the ecosystem's succession. In addition, concomitant losses can occur in water quality, economic return, and aesthetic value (National Academy of Sciences, 1969). The wastewater components primarily responsible for inducing eutrophication are organic matter and dissolved, bound nutrients (nitrogen and phosphorus in this context). Secondary wastewater treatment oxidizes much of the organic matter and is commonly in use. Tertiary treatment removes the nutrients; however, its application has been limited by the expense and technical sophistication necessary for engineered treatment systems (Rohlich and Uttormark, 1972).

Several natural systems, including salt and brackish marshes, have been proposed as sites for achieving relatively low-cost tertiary treatment. The proposal for marshes envisions several ecosystem components functioning to achieve the desired result. Components such as microbes, fauna, and sediments would remove nutrients (e.g., by release to the atmosphere or to the terrestrial ecosystem) or sequester them in relatively stable, inactive ecosystem components (e.g., by burial in sediments). Technically, the angiosperms would not achieve true tertiary treatment, for nutrients would be chemically transformed and released, not removed. Angiosperms would be used in assimilation of nutrients, storage of nutrients as tissue components, and release of nutrients as

constituents of angiosperm detritus and decomposition products (Teal and Valiela, 1973; Valiela, Teal, and Sass, 1975; Gosselink, Odum, and Pope, 1974). In their detrital form, the nutrients would enter aquatic elemental cycles along pathways less likely to immediately induce eutrophication than would their dissolved, inorganic forms.

The long-term effects of marsh nutrient-enrichment have been documented in one case (Valiela et al., 1975). The execution and environment of that study, however, were fundamentally different from the present one. Valiela et al. made topical broadcasts of sewage sludge over a four year period to four small  $(314 \text{ m}^2)$  marsh plots. It is doubtful the plots achieved a steady state response to enrichment by the conclusion of the study. Attainment of this condition in Yorktown Creek was more probable due to the longer time span over which enrichment took place (19 years). In contrast to Valiela et al., the enriched area in Yorktown Creek was larger (ca. 6 ha), spatially continuous, and diverse in environmental regimes. Furthermore, the present study was not an experimental fertilization; nutrients enriching impacted sites were transported there without direct human action. The type and disposal mode of wastes enriching Yorktown Creek represent a more common circumstance in coastal zones, at present, than the experimental design of Valiela et al. (Lynch et al., 1975).

Nitrogen is considered to be the element most likely to limit productivity of estuarine and coastal waters. Phosphorus rarely

limits these systems (Pomeroy, Smith, and Grant, 1965; Pomeroy et al., 1969; Lake, 1977). The ability to assimilate dissolved, combined nitrogen can be viewed as the major criteria in evaluating the role of marsh angiosperms in ameliorating eutrophication by nutrient-rich wastewater. Many previous studies have examined the relationship between nitrogen nutrition and marsh angiosperm production. In certain marsh environments, production may be limited by nitrogen supply (Berenyi, 1966; Tyler, 1967; Pigott, 1969; Wise, 1970; Sullivan and Daiber, 1974; Gallagher, 1975; Payonk, 1975; Valiela et al., 1975; Chalmers, Haines, and Sherr, 1976; Patrick and DeLaune, 1976). The short growth form of <u>S</u>. <u>alterniflora</u> can be changed to a taller form upon nitrogen fertilization (Gallagher, 1975; Valiela, Teal, and Deuser, 1978). Nitrogen additions can increase tissue nitrogen concentration (Tyler, 1967; Wise, 1970; Stewart, Lee, and Orebanjo, 1973; Broome, Woodhouse, and Seneca, 1975b; Gallagher, 1975; Payonk, 1975; Patrick and DeLaune, 1976). One study (Valiela et al., 1975) investigated effects of enrichment on species composition of marsh angiosperm communities. Exclusion of D. spicata was noted, although the mechanisms are obscured by lack of adequate controls and by generally inadequate sample sizes.

The two objectives of this thesis were to observe effects on angiosperms in Yorktown Creek attributable to the chronic discharge of wastewater and to evaluate whether the angiosperm communities functioned as components of a tertiary treatment system. Aside from these considerations, the results should contribute to knowledge of

primary production in marsh ecosystems. The results of the previously cited studies influenced the choice of parameters used to meet the objectives. Specifically, in both nutrient-enriched and natural sections of Yorktown Creek, measurements included:

- Aerial annual net primary production of distinct angiosperm communities;
- Growth form (plant height) of the communities' dominant species;
- 3) The species' tissue concentration of nitrogen; and
- The distribution and species composition of the angiosperm communities.

## LITERATURE REVIEW

## Yorktown Creek

The Yorktown Creek flora was first described in a Marsh Inventory Report by the Wetlands Research Section, VIMS (Silberhorn, 1974). The creek marsh was classified a "Brackish Water Mixed Community," a type considered among the highest in total environmental value (Silberhorn, Dawes, and Barnard, 1974). The percentage of total area per community was estimated by ground survey as: 25% <u>S</u>. <u>alterniflora</u>, 25% Salt Grass Meadow, 5% Saltbushes, 5% <u>S</u>. <u>cynosuroides</u>, and 40% <u>Typha</u> species. These values were estimated in a later study (Wetzel et al., 1977) as: 11% <u>S</u>. <u>alterniflora</u>, 23% high marsh (including Salt Grass Meadow, Saltbushes, and ca. 100 m<sup>2</sup> of <u>S</u>. <u>cynosuroides</u>), and 66% <u>T</u>. <u>angustifolia</u>. The discrepancy in results is due to different methods.

Wetzel et al. (1977) conducted a study of Yorktown Creek centered on angiosperms, benthic microflora, nutrients in creek and interstitial waters, and nutrient mass-balance relationships. Results of the angiosperm study are reported in this thesis in full. Wetzel et al.'s other results and conclusions were:

- The system was impacted by nutrient enrichment and altered hydrology due, respectively, to sewage disposal upstream and culvert construction at the creek mouth. These factors had a confounding influence on interpretation of total system's behavior.
- Benthic microflora standing crop was not directly influenced by enrichment although an indirect effect of enhanced macrophyte shading was suggested.
- Nitrogen fixation, primarily algal in origin, occurred at high rates but was not different between impacted and natural sites.
- 4) Concentrations of dissolved inorganic nutrients (nitrogen and phosphorus) in enriched creek water varied little seasonally and were principally determined by sewage disposal. Any biological influence on nutrient dynamics was masked by high levels of nutrient input. These data indicated mixing between impacted and natural creek branches was minimal.
- 5) Yorktown Creek did not act to ameliorate enrichment due to sewage disposal, but did qualitatively change nutrient relationships. Output of dissolved inorganic nitrogen from the creek equaled or exceeded that input through disposal.

# Marsh Angiosperm Production

# General

Extensive primary production studies of marsh angiosperms have substantiated the claim that intertidal marshes are among the world's most productive habitats (Odum, 1961). Literature reviews on this subject by Keefe (1972) and Turner (1976) and an annotated bibliography of coastal marsh productivity (U.S. Department of the Interior, 1977) are available. While strictly production-oriented studies are still valuable in detailing belowground production and geographic variations in aboveground production, much more attention at present is given to environmental mechanisms of production control and maintenance.

## Environmental Factors Affecting Production

Most investigations into environmental factors affecting production must overcome one fundamental obstacle: the influence of the tides. In general, all factors potentially influencing production are modified or controlled to some degree by the occurrence of tidal flooding (Gray and Bunce, 1972). Initial work in this area, although concerned with angiosperm zonation and distribution rather than production <u>per se</u>, was interpreted solely in terms of the direct (e.g., submergence) or indirect (e.g., soil salinity, moisture, and air capacity) influence of the tides (Johnson and York, 1915; Reed, 1947; Bourdeau and Adams, 1956; Keith, 1958; Adams, 1963; Ranwell et al., 1964; Kerwin and Pedigo, 1971). While the importance of the tidal factor is recognized, an attempt to

quantify it is a complicated multivariate problem. Investigations into other factors affecting production have tried, with some success, to surmount the tidal factor by several approaches: by correlative field studies utilizing many different sites and by <u>in</u> situ and laboratory manipulations of the factor of interest.

# Salinity

Although some marsh angiosperms are true (obligate) halophytes, most dominants on Atlantic and Gulf Coast marshes are not. The negative effect of saline soil on these species is established under both field and laboratory conditions. The effect has been shown for S. alterniflora in culture (Adams, 1963; Gosselink, 1970; Halvorso and Singer, 1974), in correlative field studies (Broome et al., 1975a; Mendelssohn, 1973), and by in situ manipulation of salinity regimes (Nestler, 1977). The effect is documented for <u>S</u>. patens only in culture (Adams, 1963; Berenyi, 1966; Seneca, 1972; Gosselink, 1970; Wise, 1970; Halvorso and Singer, 1974), although Palmisano (1970) found a lower growth rate at low salinities. Palmisano's results may be attributable to genetic adaptation to high salinity conditions. S. patens is genetically divergent and well-adapted to local microenvironments (Silander, 1979). D. spicata may be the only obligate halophyte among the angiosperms considered here. Adams (1963) and Palmisano (1970) both found its best growth at moderate salinities (10 O/oo), with production declining around this optimum. However, Barbour and Davis (1970) reported the species to be an

intolerant halophyte (best growth at very low salinities) and attributed the conflicting results to different culture methods.

#### Nitrogen and Phosphorus Nutrition

Soil salinity does not account for all local variations in marsh angiosperm production (Nestler, 1977). Aside from direct tidal influence, mineral nutrition of angiosperms is a source of additional growth-limiting factors. The most widely studied factors are nitrogen and phosphorus. Initial research in this area attempted to correlate various soil and water standing stocks of nitrogen and phosphorus with measures of angiosperm growth (Boyd and Hess, 1970; Broome et al., 1975a,b; Nixon and Oviatt, 1973; Mendelssohn, 1973). This approach yielded few significant results. The major reasons for this seem clear:

- Environmental standing stocks of nutrients have little bearing on a plant's nutritional status. The parameter of importance is rate of nutrient supply.
- 2) Active nutrient assimilation may not occur throughout the growing season. Nitrogen uptake, in particular, is almost completed by late spring (Boyd, 1970; Squiers and Good, 1974; Mason and Bryant, 1975; Chalmers et al., 1976; Boyd, 1978). Thus, correlations derived from data collected later in the growing season may only be chance reflections of past events.

3) Due to the methods of chemical analysis and conceptual gaps in understanding nutrient assimilation, the measured size of a nutrient stock may have little relationship to how much of that nutrient is physiologically available.

An alternative approach to study nutrient and production relationships involves <u>in situ</u> or laboratory manipulation of nutrient regimes. Two culture studies attributed a limitation on <u>S</u>. <u>patens</u> growth to nitrogen availability (Berenyi, 1966; Halvorso and Singer, 1974). <u>In situ</u> experiments have fertilized angiosperm stands with sewage sludge, liquid sewage or organic waste effluent, and inorganic nitrogen and/or phosphorus compounds. Results are reviewed separately for <u>S</u>. <u>alterniflora</u> and other marsh angiosperms.

The short and tall growth forms of <u>S</u>. <u>alterniflora</u> are often found in mono-form stands in separate locales. The highly productive tall-form occurs in low-elevation areas contiguous, or nearly so, with non-stagnent waters (the low marsh environment). The short-form typically occupies higher elevated meadows formed interior to low marsh. Both forms may be subject to nitrogen limitation of production (short-form: Sullivan and Daiber, 1974; Broome et al., 1975b; Gallagher, 1975; Valiela et al., 1975; Chalmers et al., 1976; tall-form: Patrick and DeLaune, 1976). Phosphorus and nitrogen may limit production on sandy sediments (Broome et al., 1975b). However, the sediment characteristic of well-established marshes is a fine-grained, silt-clay type. In these soil conditons, the literature evidence for nitrogen limitation of tall-form production

is equivocal. The characteristics of the three fertilization studies in question are listed in Table 1. Although differences in experimental design are evident, these are probably less important than local nutrient regimes and situation of study sites. Wolaver et al. (in preparation) have recently shown incoming tidal water is gradually depleted in inorganic nitrogen as it crosses the tall-form zone. Thus, conflicting results (Effect) in Table 1 may be ascribed to plants' success or failure to fulfill nitrogen requirements for optimum growth from the tidal supply. This would be dependent on local dissolved nitrogen concentrations, degree of nitrogen depletion of flooding waters due to 'up-tide' activity, and local tidal hydrodynamics.

The other species considered in this discussion are those found at the highest elevations affected by tidal flooding, i.e., the high marsh environment. Production of this vegetation may be nutrient-limited, although the degree and nature of this is not entirely clear from the available literature. Valiela et al. (1975) and Payonk (1975) reported increased <u>S. patens</u> production after fertilization with organic wastes. This might be attributable to relaxation of nitrogen limitation in light of other studies. Wise (1970) found <u>S. patens</u> and a <u>S. patens-D. spicata</u> mixture increased production after application of inorganic nitrogen. Berenyi's (1966) field fertilization studies indicated <u>S. patens</u> production was nitrogen and (to a lesser degree) phosphorus limited. Culture

# TABLE 1

# NITROGEN LIMITATION OF TALL-FORM

# S. ALTERNIFLORA PRODUCTION

The characteristics of three fertilization studies conducted on fine-grained, silt-clay sediments are listed. Rates in units of kg N ha<sup>-1</sup> yr<sup>-1</sup>. Effect is the statistically significant increase of dry weight yield over controls expressed as a percent of control yield.

Investi- gator:	Patrick and DeLaune (1976)	Gallagher (1975)	Broome et al. (1975b)						
Site:	Barataria Bay, Louisiana	Oakdale Marsh, Sapelo Is., Ga.	Oak Is., N.C. Marsh stands A and B						
Fertilizer:	(NH4)2SO4	NH4NO3	(NH4)2SO4						
Treatment Rate:	0,200	0,200	A: 0, 168, 336, 672 B: 0, 168, 336, 672						
Fertilizer Placement:	8-10 cm belowground	25 cm belowground	surface broadcast						
Time of Fertili- lization:	15 May 1973	16 July 1971	A: 18 May, 30 June 3 August, 1971 B: 1 May, 22 June, 26 July, 1972						
Time of Harvest:	September 1973	22 September 1971	A: 4 October 1971 B: 20 September, 1972						
Sample, Ea. Treatment:	1 m <sup>2</sup> x 4	$0.25 \text{ m}^2 \times 3$	A: 0.25 m <sup>2</sup> x 3 B: 0.25 m <sup>2</sup> x 3						
Effect:	15 %	none	A: none B: none						

studies yielding positive results for nitrogen limitation of <u>S</u>. <u>patens</u> production have already been noted (Berenyi, 1966; Halvorso and Singer, 1974). In contrast, Gallagher (1975) found no increase in yield of <u>Juncus roemerianus</u> 10 weeks after nitrogen fertilization.

#### Nitrogen Concentration in Marsh Angiosperm Aerial Tissues

Burkholder (1956) made the first extensive investigation of tissue concentration of nitrogen in a marsh plant (<u>S. alterniflora</u>). The study of tissue nitrogen concentration (TNC) was later spurred, in part, by Gerloff and Krombholz's (1966) work in determining threshold TNC values below which fresh-weight yield was sub-optimal for freshwater emergent angiosperms. They established a strong correlation between TNC and nitrogen availability in aqueous culture medium. The implication was TNC could be used to evaluate the plant's environmental nutritional status.

There is now a substantial literature base of aerial TNC determinations for most marsh angiosperms. Several general conclusions may be drawn. First, nitrogen uptake and tissue concentration are greatest early in the growing season (Boyd, 1970; Squiers and Good, 1974; Mason and Bryant, 1975; Chalmers et al., 1976; Boyd, 1978). Second, TNC decreases over the growing season although, third, tissue nitrogen on an areal basis continues to increase (Boyd, 1970; Squiers and Good, 1974; Mason and Bryant, 1975; Boyd, 1978).

The implications of Gerloff and Krombholz's work are not substantiated in field studies in salt and brackish marshes. The objections noted earlier to correlations between nutrient standing stocks and measures of growth are also relevant here. Mendelssohn and Marcellus (1976) found no significant correlation between naturally occurring stocks of environmental nitrogen and S. alterniflora TNC. In contrast, Gossett and Norris (1971) reported a significant positive correlation of environmental nitrogen and TNC of a fresh-water marsh plant, water-hyacinth (Eichornia crassipes). In situ fertilization studies have led to conflicting reports. Tyler (1967), Wise (1970), Stewart et al. (1973), Broome et al. (1975b), Gallagher (1975), Payonk (1975), and Patrick and DeLaune (1976) found increased aerial TNC for some salt marsh angiosperms in nitrogen fertilized stands. However, the significant results of Broome et al. for S. alterniflora growing on sandy, fertilized soils were tempered by non-significant results for tall-form plants on fertilized, finegrained soils. Although Gallagher found TNC for fertilized shortand tall-form S. alterniflora was higher than controls at 10 weeks following treatment, similar results were not found for fertilized J. roemerianus. The short-form differences had disappeared one year following fertilization. Chalmers et al. (1976) saw no consistent differences in short-form TNC between control and sewage sludge treated stands over a one year span. Despite these conflicts, the majority of evidence indicates marsh angiosperms will optimize aerial tissue nitrogen concentration in the presence of a sufficient supply of usable nitrogen. The conflicting findings may arise from several

sources: interaction of nitrogen assimilation with other environmental factors (Boyd, 1978); translocation of belowground tissue nitrogen to aerial tissues; and failure of fertilizers to become available to plants either through excessive leaching by tidal waters, low soil permeability, denitrification, storage in microbial biomass, or insufficient microbial decomposition of organic forms not directly assimilable by plants.

# Nutrient Flux in Salt and Brackish Marshes

The nutrient mass-balance (flux) properties of marshes deserve consideration if marshes are to be utilized as tertiary treatment systems. In this context, net flux for an ecosystem is simply the annual difference in amount of elemental material input to and output from the system. Use of flux measurements reduces complex processes of elemental recycling and external exchange to three situations: the ecosystem may represent to its external surroundings either an elemental source, sink, or 'counterpoise'. Bender and Correll (1974) summarized mass-balance studies of nitrogen and phosphorus for two undisturbed, regularly flooded marshes on the York River, Virginia. Net annual flux of both elements was into the marsh. However, this import was dominated by particulate forms not directly usable by autotrophs. Considering only forms directly available to autotrophs, there was little if any net loss of material to the marsh. The authors concluded these marshes served to maintain estuarine productivity through processes of mineralization of particulate organic material and export of inorganic, combined nutrient species

utilizable by estuarine autotrophs. Woodwell and Whitney (1977) made similar conclusions concerning the phosphorus budget of a regularly flooded marsh ecosystem, Flax Pond, N.Y. Although the annual budget was balanced overall, a net export of inorganic phosphorus and a net import of organic (including particulate) forms occurred. In contrast, Wolaver et al. (in preparation) recently reported another marsh on the York River is a sink for all inorganic, bound forms of nitrogen and phosphorus. Valiela et al. (1978b) reported Great Sippewissett Marsh on Cape Cod to be a net exporter of nitrogen; however, when their data on nitrogen input via fresh groundwater is subtracted from the overall flux, nitrogen flux derived from precipitation and tidal exchange shows a net import of all species to the marsh. This approach is commensurate with Bender and Correll's, Woodwell and Whitney's, and Wolaver et al.'s measurements, since either their sites were not appreciably affected by groundwater input or their results are corrected for it. In a study of an irregularly flooded, low salinity marsh on the Patuxent estuary, Maryland, Heinle and Flemer (1976) found a net annual export of nitrogen and phosphorus (principally of dissolved species) derived from precipitation and tidal exchange.

In summary, generalizations about natural nutrient flux properties of marshes are not clear. Bender and Correll, Wolaver et al., and Valiela et al. found a net marsh import of nutrients. Woodwell and Whitney saw no net phosphorus flux at their site. Heinle and Flemer's site exported considerable quantities of nitrogen

and phosphorus. Some evidence supports Bender and Correll's contention that marshes are important remobilizers of nutrients and act to maintain estuarine productivity through release of inorganic, bound nutrients.

Since the processes resulting in marsh nutrient flux are not predictable under altered nutrient regimes from a priori consideration of their undisturbed operation, flux studies incorporating a pre- and post-perturbation approach are desirable. Unfortunately, none are presently available for nutrient-enriched marshes. Results of several marsh fertilization experiments may lend insight to the ability of marshes to retain added nutrients. Bender and Correll (1974) enriched plots in two brackish Maryland marshes with inorganic phosphorus and nitrogen. No net accumulation of added nitrogen was found in either low or high marsh plots. Phosphorus was transiently bound in low marsh but was eventually lost as orthophosphate during ebb tides. High marsh was found deficient in phosphorus as evidenced by phosphorus binding in the upper few centimeters of sediment and detritus. In conjunction with study of nutrient limitations to angiosperm production, Valiela, Teal, and Sass (1973), Gallagher (1975), and Chalmers et al. (1976) estimated losses and retentions of supplemental nitrogen added to marsh plots. Valiela et al. sampled ebb-tide water from control and treated plots on successive tides following fertilization. An estimated 6-20% of added nitrogen was lost to tidal exchange within six tides, following which losses became negligible. The implied high retention

efficiency is questionable, as chemical analyses did not include organic forms which comprised 60% of fertilizer (sewage sludge) nitrogen. Gallagher accounted for 53 and 36% of supplemental nitrogen in increased aerial tissue nitrogen stock of short form S. alterniflora 10 weeks and one year following fertilization, respectively. In contrast, Chalmers et al. estimated increased tissue nitrogen of short form S. alterniflora accounted for only 3% of their fertilizer nitrogen after one year, while 51% appeared in soil-associated components. These differences may be attributable to the fertilizers' qualitative properties. Gallagher made a subsurface, pulse application of ammonium nitrate at the rate of 200 kg N ha<sup>-1</sup>yr<sup>-1</sup>. These nitrogen forms (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>) are directly assimilable by vascular plants (Haynes and Goh, 1978). Chalmers et al. applied a pulverized sewage sludge fertilizer twice monthly at a rate of 840 kg N ha<sup>-1</sup>yr<sup>-1</sup>. Only about 2% of this nitrogen appeared as directly assimilable forms (Chalmers et al., 1976). Thus, availability of nitrogen to angiosperms in Chalmers et al.'s study depended in large part on microbial processes of mineralization and remobilization, while in Gallagher's study the path was direct. This could also have been a factor in the failure of Chalmers et al. to find consistent differences in TNC between control and fertilized plots as described earlier.

#### Summary: The Use of Salt Marshes as Tertiary Treatment Systems

There are several implications in this literature review for using marshes as tertiary treatment systems. The variable nutrient

flux properties of marshes indicate that a careful site-by-site evaluation would be needed if this resource management plan was implemented. Since some marshes are natural exporters of inorganic nutrients, it cannot be expected all marshes are potentially useful sites. As marshes are bio-physical systems and must obey both biological and physical laws concerning limiting rates and functions, they cannot be expected to have an unlimited capacity to immobolize dissolved nutrients. Valiela et al.'s statement (1973) that "Salt marsh systems are flexible enough to be able to use as much nitrogen as is available regardless of the mechanism of supply" is certainly indefensible as a generalized statement of salt marsh nutrient behavior or of the response of natural ecosystems to nutrient enrichment. The ability of angiosperms to assimilate supplemental nutrients has been demonstrated to be highly dependent on qualitative properties of the supplement, time of year, the plant's location within the marsh, nutritive potential of the substrate, and the existence of other growth-limiting factors.

# METHODS AND MATERIALS

#### Field Sampling Design

The creek-marsh system was divided into three blocks based on <u>a</u> <u>priori</u> knowledge of environmental nutrient regimes: 1) a nutrient-impacted south branch, 2) an unimpacted (control) west branch, and 3) a mixed branch formed by the confluence of the south and west branches. Sampling sites were established within major community types of each block as recognized from initial field surveys. These communities were high marsh and <u>T</u>. <u>angustifolia</u> for the control and impacted branches and high marsh and monospecific <u>S</u>. <u>alterniflora</u> for the mixed branch. Table 2 lists the abbreviations to be used in reference to these sites. Figure 2 illustrates the location of each site. To reduce intracommunity variability, the high marsh and <u>S</u>. <u>alterniflora</u> sites were demarcated as 5x20 m staked areas. Permanently staking <u>Typha</u> sites proved unfeasible.

Staked sampling sites were divided into 100 relocatable squares, each 1 m<sup>2</sup> (Figure 3a). Each square was assigned a permanent sample number which, used with a random number table, identified squares for clipped biomass sampling. Records kept of previously clipped squares were used to avoid recutting. Brass rings physically defining the

# TABLE 2

# ABBREVIATIONS OF YORKTOWN CREEK SAMPLING SITES
## LOCATION AND ORIENTATION OF SAMPLING SITES

Sampling sites not drawn to scale.



DESIGN OF STAKED SAMPLING SITES IN YORKTOWN CREEK

A. PHYSICAL SIZE AND SAMPLE IDENTIFICATION



B. WALKWAYS AND QUADRAT PLACEMENT



quadrat area to be clipped within a square were always placed in the same respective corner of a square (Figure 3b). This arrangement resulted in uncut walkways running the length and breadth of each staked site. <u>Typha</u> samples were selected by a random toss into virgin stands in the general areas identified in Figure 2.

#### Aerial Annual Net Primary Production

The method of Smalley (1958) and peak live biomass (PLB) were used to estimate aerial annual net primary production (NPP). Smalley's method utilizes changes in live and dead standing crop between successive samplings to achieve a better estimate of NPP than a PLB sample alone (Linthurst and Reimold, 1978). The following algorithm was used to compute NPP by Smalley's method:

	Sign of Bi Over	omass Change Time ∆t		NPP Over	Time ∆t
	<u>Live (L</u> )	Dead (D)	NP	P=	
1.	+	+	1.	ΔL + ΔD	
2.	+	-	2.	۵L	
3.		-	3.	0	
4.		+	4a.	ΔL + ΔD	iff  ∆D > ∆L
			4b.	0	

Production is summed over all time intervals to calculate NPP. The method requires that sampling intervals are small (ca. one month) to minimize errors due to disappearance of dead material. Since some intervals in this study were unavoidably large, PLB was also used as an estimator of NPP. Three high marsh sites (IBHM, MBHM, and CBHM), two <u>Typha</u> sites (IBTA and CBTA), and one <u>S</u>. <u>alterniflora</u> site (MBSA) were harvested using 0.1 m<sup>2</sup> circular, 0.25 m<sup>2</sup> rectangular, and 0.1 m<sup>2</sup> circular quadrats, respectively. Size and shape of quadrats were optimized in terms of statistical variability and processing-time requirements in July, 1976 by the technique of Wiegert (1962). Vegetation was clipped 1-2 cm above the marsh surface. Standing dead and live and any ground litter were collected and placed in plastic bags. Samples were replicated 6-8 times. Samples were separated into live by species and non-specific dead fractions in the laboratory. Samples not immediately processed were stored at  $-20^{\circ}$ C. Fractions were dried to constant weight at 105-110°C and subsequently weighed to the nearest 0.1 g.

### Live Plant Height

Four species dominant in the marsh (<u>D</u>. <u>spicata</u>, <u>S</u>. <u>alterniflora</u>, <u>S</u>. <u>patens</u>, and <u>T</u>. <u>angustifolia</u>) were subsampled from the fresh harvest material for live plant height. Subsamples were obtained from every sample in which these species occurred as live material. In general, the subsampling proceeded by hand mixing of a sorted live species fraction, laying material out in a parallel, single-shootlayer row, and 'randomly' choosing individuals for measurement until 10 measurements were made. Individuals of some species occasionally numbered less than 10 in a sample - resulting from either growth form (large biomass per individual, e.g., <u>Typha</u>) or spotty occurrence in the particular area. In either case, as many individuals as possible

were measured to a total of 10. The datum recorded was length of the plant to the nearest centimeter from the clipped shoot end to the tip of the longest leaf as the plant was straightened by hand.

Data were grouped and statistically pooled by species, sampling date, and sampling site. Pooled data sets were tested for deviation from normality by a chi-squared ( $\chi^2$ ) goodness-of-fit method ( $\alpha$ =0.05, Snedecor and Cochran, 1967). All were assumed normally distributed, as 86% of the sets showed no difference from the normal distribution according to the  $\chi^2$  tests (unreported).

Effect of sampling sites upon mean intra-specific plant heights was evaluated by monthly comparisons between different sites. Data sets compared were first tested for homogeneity of variances ( $\alpha$ =0.05) by a variance ratio (F) test, Bartlett's test, or Cochran's test depending on sample size equality (Guenther, 1964). Mean height comparisons were made by two-sample t-tests or by Cochran's t' approximation ( $\alpha$ =0.05) depending on homogeneity of variances (Guenther, 1964).

#### Tissue Nitrogen Concentration

Analysis of TNC was made on leaf and stem tissues of three dominant species subsampled from the dried live species fractions collected for production analysis. The whole aerial portion of <u>S</u>. <u>patens</u> was analyzed. Tissue was ground with a Wiley mill to pass a 40 mesh screen and stored at  $-20^{\circ}$ C. Portions of subsamples were

ashed at 550°C for 4 hours to determine tissue concentration on an ash-free dry weight (AFDW) basis. The means of triplicate determinations are used to report tissue AFDW concentrations.

TNC was analyzed using a dry combustion technique that employs a Fisher Model 1200 Gas Chromatograph. Approximately 10 mg of sample, 100 mg of cupric oxide, and 20 mg of fine copper strips were sealed in a 10 ml glass ampul under a helium atmosphere. The ampuls were heated to 550°C for 4 hours to combust the sample. Under these conditions, the equilibrium of N<sub>2</sub> and nitrogen-oxide gas concentrations heavily favored N<sub>2</sub>; this was verified experimentally. The gases evolved in combustion were introduced to the chromatograph through an air-tight ampul crushing apparatus and a valve train which allowed purging of atmospheric N<sub>2</sub> from the crushing apparatus and mixing of carrier and combustion gases. Sample runs were calibrated by standardization with acetanilide (Fisher Co.).

The results were grouped by botanical species and tissue type. A hierarchial, mixed model was adopted for each group's observations. The general form of the model is:

where:

- yijk = the (ijk)<sup>th</sup> observation, considered as an independently
  distributed normal variate;
  - µ = the population mean for the species and tissue considered;

 $\beta_i$  = the fixed effect of sampling date and site;

- $\gamma_{ij}$  = the random effect of samples taken within a date and site, distributed normally with mean = 0 and variance =  $\sigma_{\gamma}^2$ ;
- $\varepsilon_{ijk}$  = the random effect (error) of replicate determinations within a sample, distributed normally with mean = 0 and variance =  $\sigma_z^2$ .

Hierarchial, mixed model analyses of variance (ANOVA) were performed on the grouped observations using the model to determine overall significance of the date and site term ( $\beta_i$ ) and to estimate variance components,  $\sigma_{\gamma}^2$  and  $\sigma_{\varepsilon}^2$ . <u>A priori</u> mean comparisons within groups were made to test effects of sampling site and date. Evaluation was made by a t statistic of the form:

t = 
$$\begin{bmatrix} \Sigma c_i (y_{i..} / n_{i..}) \end{bmatrix} \times \begin{bmatrix} MS_{\varepsilon} (\Sigma c_i^2 / \Sigma n_{ij}) \end{bmatrix}^{-1/2};$$

degrees of freedom =  $\sum_{i j} \sum_{j} (n_{ij} - 1);$ 

where:

( $y_{i..}/n_{i..}$ ) = the mean estimate for the i<sup>th</sup> group;  $c_i$  = the i<sup>th</sup> group comparison coefficient;  $MS_{\epsilon}$  = the ANOVA replication mean square;  $n_{ij}$  = the total replicates for the (ij)<sup>th</sup> sample. These tests are regarded as conditional, as the t statistic formula assumes  $\gamma_{ij}$  is fixed. The tests were considered significant at the 95% confidence level.

#### Community Structure and Distribution

Marsh floral ground surveys were conducted on three occasions to establish principal features of distribution and dominance within the salt marsh and the downstream reaches of the <u>Typha</u> areas. These surveys were qualitative in nature in that they relied on visual estimates of species dominance. No attempt was made to enumerate minor species. The surveys are descriptions of distinguishing features of the communities' structure. The data were used in conjunction with topographic surveys to map zonal distributions.

### Interstitial Salinity

Soil salinity was determined by chlorinity titration of interstitial water squeezed from sediment cores 5 cm in depth (Strickland and Parsons, 1972). Cores were taken by hand from the sampling sites on five occasions in 1977. Data were analyzed by a randomized complete block ANOVA (sampling dates blocked) and an <u>a</u> <u>posteriori</u> sequential Studentized-Newman-Kuels (SNK) test of mean differences ( $\alpha$ =0.05, Snedecor and Cochran, 1967).

#### Surface Elevation

Sampling site surface elevations were measured with a leveling instrument (Nikon Auto-Level AE) and surveyor's pole. Measurements

were not taken on <u>Typha</u> sites. A nearby benchmark (USCGS #S1949) was used as an elevation reference. Twelve readings were taken along the long axis of each site. Two sample t-tests were performed to test for differences in mean site elevation ( $\alpha$ =0.05).

#### RESULTS

#### Aerial Annual Net Primary Production

NPP is reported for each site in Table 3. The appendix summarizes data used in calculating NPP by Smalley's method. Figure 4 illustrates the distributional properties of the PLB samples. No statistical test for evaluating production differences is available for the results obtained from Smalley's method. The PLB distributional properties (Figure 4) invalidate statistical tests based on the normal distribution. A qualitative inspection of the figure reveals no gross differences in mean PLB among similar community types.

Table 4 identifies the contribution of dominant species PLB to the total site PLB. One or two species in all cases contribute greater than 89% of site PLB. Four sites are nearly monospecific (CBTA, IBTA, MBSA, and CBHM) and two are mixtures of <u>S</u>. <u>patens</u> and <u>D</u>. <u>spicata</u> (IBHM and MBHM). PLB occurred in mid-August in all sites and for all species except for <u>S</u>. <u>patens</u> in CBHM, where PLB occurred 22 July 1976. Since PLB of <u>S</u>. <u>alterniflora</u> in CBHM occurred in August, the species' contribution to site PLB is not defined in the context of Table 4.

# SUMMARY OF NPP ESTIMATES FOR YORKTOWN CREEK

Site	PLB	Smalley
IBTA	721.5 <u>+</u> 111.9	1506.3
CBTA	734.4 <u>+</u> 108.4	1316.2
MBSA	726.2 <u>+</u> 70.7	927.4
IBHM	577.2 <u>+</u> 58.0	598.0
MBHM	538.4 <u>+</u> 63.8	677.2
СВНМ	560.4 <u>+</u> 151.7	997.0

All values in units of grams dry weight per square meter.

PLB: estimate by method of peak live biomass (mean  $\pm$  mean standard error).

Smalley: estimate by the method of Smalley.

## DISTRIBUTIONAL PROPERTIES OF PLB SAMPLES

A modified box-and-whisker diagram is used to show the distributional properties of the observations comprising the peak live biomass sample in each site. The diagrams indicate the value of the following sample statistics:



Below the diagrams are the sampling site, the sampling date on which peak live biomass was observed, and the values of sample size (n), mean  $(\bar{x})$ , median (m), and mean standard error  $(s_{\bar{x}})$ . Units are grams dry weight per square meter.



# CONTRIBUTION OF DOMINANT SPECIES TO SAMPLING SITE PEAK LIVE BIOMASS

The peak live biomass of each site's dominant species is given in column 3 in units of grams dry weight per square meter. If species' PLB occurred during the same sampling as site PLB, the species' contribution to site PLB is indicated in column 4 as a percent of site PLB. A star (\*) indicates site and species PLB occurred at different samplings. Peak live biomass occurred in all sites except CBHM during mid-August. CBHM peak live biomass occurred 22 July 1976.

Site	Species	PLB	% Site
IBTA	<u>T. angustifolia</u>	651.6	90
CBTA	<u>T. angustifolia</u>	682.9	93
MBSA	<u>S. alterniflora</u>	726.1	100
IBHM	<u>S. patens</u>	353.2	61
	<u>D. spicata</u>	221.0	38
MBHM	<u>S</u> . <u>patens</u>	247.6	46
	<u>D</u> . <u>spicata</u>	265.4	49
СВНМ	<u>S. patens</u>	531.7	95
	<u>S. alterniflora</u>	94.4	*

The dynamics of live and dead standing crops are shown in Figures 5 and 6. With the exceptions of CBTA and CBHM, there is an inverse relationship between live and dead standing crops over the growing season. The exceptional increase in dead crop between May and July in CBTA while the live crop is increasing is probably due to sampling error. Live crop has a greater increase in the period February-May in the impacted branch sites than in comparable control branch sites. These differences disappear in August. The amount of dead biomass in CBHM and IBHM is much greater relative to MBHM. Given the equality of production between these sites, this indicates the rate of dead material disappearance was greater in MBHM.

### Aerial Live Plant Heights

Table 5 summarizes plant height results and intra-specific mean comparisons between sites by month. In most cases, species in nutrient-enriched sites (IBTA, IBHM, and MBSA) are significantly longer than the same species in control branch sites (CBTA, CBHM). Notable exceptions occur in the early (May, <u>T. angustifolia</u>) and late (September-October, <u>S. alterniflora</u>, <u>D. spicata</u>, <u>S. patens</u>) growing season. MBHM species never had a significantly greater mean height than CBHM species.

## Tissue Nitrogen Concentration

Mean estimates and comparisons between sampling months and sites of tissue nitrogen concentration appear in Tables 6-8. Some results are evident for which no statistical tests were necessary. Leaf tissues in all cases have higher TNC than corresponding stem tissues

## DYNAMICS OF STANDING CROPS IN HIGH MARSH SITES

Vertical bars indicate mean plus/minus standard error.



# DYNAMICS OF STANDING CROPS IN MBSA, IBTA, AND CBTA SITES

Vertical bars indicate mean plus/minus standard error



## PLANT HEIGHTS OF DOMINANT SPECIES

Mean comparisons were made within a month's sample for a particular species. Underlining indicates a significant difference (p=95%) between the underlined values. A star (\*) indicates no data were available.

			Ν	lean He <sup>.</sup>	ight (cn	ı)	
Species	Month	IBTA	CBTA	IBHM	MBHM	CBHM	MBSA
T. angustifolia	5/76	122	120	*	*	*	*
	8/76	<u>182</u>	<u>143</u>	*	*	*	*
<u>S. patens</u>	5/76	*	*	<u>35</u>	<u>31</u>	*	*
	8/76	*	*	<u>38</u>	<u>35</u>	33	*
	9-10/76	*	*	<u>35</u>	<u>28</u>	34	*
<u>D. spicata</u>	5/76	*	*	<u>28</u>	<u>25</u>	*	*
	8/76	*	*	<u>37</u>	<u>34</u>	*	*
	9-10/76	*	*	31	33	32	*
<u>S. alterniflora</u>	4/76	*	*	*	<u>20</u>	*	<u>30</u>
	5/76	*	*	*	<u>33</u>	*	<u>47</u>
	7/76	*	*	*	*	44	<u>59</u>
	8/76	*	*	*	44	<u>51</u>	<u>69</u>
	9-10/76	*	*	*	*	63	73

## LEAF TISSUE NITROGEN CONCENTRATION

		% 1	(AFDW)
Species	Site	May	August
<u>T. angustifolia</u>	IBTA CBTA	2.11 A <u>2.37</u> B	2.22 A <u>1.33</u> B
<u>D. spicata</u>	IBHM MBHM	1.78 A 1.41 B	1.68 1.62
<u>S. alterniflora</u>	MBSA MBHM CBHM	1.31 A 1.31 A 1.81 B	$\frac{1.17}{0.98}$

Significant differences (p=95%) in % N between sites within a species and month (column sections in table) indicated by symbols (A,B). No symbol or identical symbol indicates non-significance.

Significant differences (p=95%) in % N between months within a species and site (table rows) indicated by underlining.

A dash (-) indicates no data available.

STEM TI	SSUE	NITROGEN	CONCENTRATION

		% N (AFDW)	)
Species	Site	May	August
<u>T</u> . <u>angustifolia</u>	IBTA CBTA	$\frac{1.20}{1.04}$	<u>0.47</u> 0.32
<u>D. spicata</u>	IBHM MBHM	<u>0.64</u> A <u>0.92</u> B	<u>0.88</u> A <u>0.73</u> B
<u>S</u> . <u>alterniflora</u>	MBSA MBHM CBHM	<u>1.01</u> A 1.28 B <u>1.44</u> B	<u>0.62</u> A B

Significant differences (p=95%) in % N between sites within a species and month (column sections in table) indicated by symbols (A,B). No symbol or identical symbol indicates non-significance.

Significant differences (p=95%) in % N between months within a species and site (table rows) indicated by underlining.

A dash (-) indicates no data available.

# S. PATENS TISSUE NITROGEN CONCENTRATION

Site	May	<u>% N (AFDW)</u> August	October
IBHM	<u>1.09</u>	<u>1.01</u> A	0.66
MBHM	-	<u>0.84</u> B	0.59
СВНМ	1.16	<u>0.97</u> A	0.69

Whole aerial portion of  $\underline{S}$ . <u>patens</u> analyzed.

Significant differences (p=95%) in % N between sites within a month (table columns) indicated by symbols (A,B). No symbol or identical symbol indicates non-significance.

Significant differences (p=95%) in % N between months within a site (table rows) indicated by underlining.

A dash (-) indicates no data available.

when examined by months. The highest TNC values were found in  $\underline{T}$ . <u>angustifolia</u> leaf tissue. The lowest TNC also occurred in that species' stem tissue.

Significantly lower TNC was found in most cases late in the growing season (August or October) for a specific tissue. The exceptions are associated with nutrient-enriched sites (IBHM, MBHM, and IBTA). No significant seasonal change was found for leaf tissue of <u>D</u>. <u>spicata</u> in IBHM and of <u>T</u>. <u>angustifolia</u> in IBTA (Table 6). A seasonal increase occurred in <u>D</u>. <u>spicata</u> leaf tissue in MBHM and stem tissue in IBHM (Tables 6 and 7).

<u>I</u>. angustifolia leaf tissue shows significant differences in TNC between the impacted and control branch sites in both May and August (Table 6). In May, TNC of leaf tissue is higher in the control branch. This difference between sites is reversed in August. Stem tissue of <u>D</u>. <u>spicata</u> exhibits a similar pattern, although a mixed branch site replaces the control branch site (Table 7). This is also the case for <u>S</u>. <u>alterniflora</u> stem tissue with the impacted branch site replaced by a mixed branch site (Table 7). Two comparisons of leaf tissue which were significant in May (<u>S</u>. <u>alterniflora</u>, CBHM>MBSA; <u>D</u>. <u>spicata</u>, IBHM>MBHM) were not significant in August (Table 6).

#### Community Structure and Distribution

Table 9 lists the angiosperms encountered in clipped samples collected between February and October, 1976. The tabulation is not

# SPECIES OCCURRENCE IN PRIMARY PRODUCTION SAMPLES

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

intended to be an exhaustive compilation of the Yorktown Creek flora. A total of 18 species occurred in samples from the six sites. Number of species in high marsh sites was greatest in the control branch (7) and the lowest in the impacted branch (3). One species (<u>Cyperus</u> <u>erythrorhizos</u>) noted in CBHM is not common in salt and brackish marshes (Hotchkiss, 1970).

Figure 7 illustrates zonal distribution and species composition of the angiosperm communities at the end of the 1976 growing season. Although somewhat idealized, the boundaries as shown in the figure between areas of different species composition are quite distinct in the field. Certain species tend to fringe upland areas ( $\underline{T}$ . <u>angustifolia</u>) and to parallel ditches and creeks ( $\underline{S}$ . <u>alterniflora</u>). A peculiar aspect of the distribution is the lack of  $\underline{D}$ . <u>spicata</u> as a dominant component of the control branch high marsh community. The species does occur far upstream in the impacted branch high marsh.

#### Surface Elevation

Table 10 summarizes the surface elevations of the four staked sampling sites and the results of mean comparisons between sites. The CBHM and IBHM sites were not significantly different although all other comparisons were. Greatest mean elevation was found at the MBHM site (54.9 cm), least at the MBSA site (46.4 cm), and intermediate elevations at the CBHM and IBHM sites (51.9 and 52.5 cm, respectively).

## VASCULAR PLANT DISTRIBUTION IN 1976

Key to species:

- 1. <u>Spartina alterniflora</u>
- 2. Distichlis spicata
- 3. <u>Spartina</u> patens
- 4. <u>Scirpus</u> robustus
- 5. <u>Spartina cynosuroides</u>
- 6. Borrichia frutescens
- 7. <u>Aster tenuifolis</u>
- 8. Typha angustifolia
- 9. <u>Kosteletzkya</u> virginica
- 10. <u>Pluchea purpurascens</u>
- 11. Cyperus erythrorhizos
- 12. <u>Aster subtulatus</u>
- 13. Polygonum punctatum



# STAKED SAMPLING SITE ELEVATIONS

Site:	MBHM	MBSA	CBHM	IBHM
Mean elevation (cm):	54.9	46.4	<u>51.9</u>	52.5
Mean standard error:	•55	.40	.88	.40

Datum reference is NOAA mean sea level. Underlining indicates significant differences (p=95%) in mean elevation.

## Interstitial Salinity

The interstitial salinities observed during 1977 at the sampling sites and the results of mean comparisons are summarized in Table 11. Significant differences in mean interstitial salinity were found for all sites excepting the pair of <u>Typha</u> sites. The mixed branch sites were highest, followed by the other high marsh sites, with the lowest salinities occurring in the <u>Typha</u> sites. Salinity range was greatest in MBHM and IBHM (17.0 and 15.0 °/oo, respectively) and least in CBHM (8.9 °/oo).

# SUMMARY OF 1977 INTERSTITIAL SALINITY OBSERVATIONS

Source	D.F.	M.S.	F
Dates (Blocks)	4	132.8	
Sites (Treatments)	5	111.3	13.38*
Error	20	8.3	
Total	29		
	· · · · · · · · · · · · · · · · · · ·		

# Randomized Complete Block ANOVA

## SNK Test of Mean Differences

Site:	IBHM	MBHM	CBHM	MBSA	IBTA	CBTA
Mean (º/oo):	<u>11.9</u>	<u>18.0</u>	<u>9.1</u>	<u>14.9</u>	6.6	6.1
Minumum:	4.7	10.6	4.2	7.8	1.4	1.4
Maximum:	19.7	27.6	13.1	21.7	14.0	14.0
Range:	15.0	17.0	8.9	13.9	12.6	12.6

Underlining indicates significant differences (p=95%) in mean interstitial salinity.

#### DISCUSSION

Although Yorktown Çreek was a highly appropriate setting to study the use of a natural marsh ecosystem as a tertiary sewage treatment facility, the effects of nutrient-enrichment on the angiosperms are not clear for most comparisons from a simple inspection of the results. Several general factors are responsible for this difficulty. Perhaps the most important of these is the lack of knowledge concerning direct, interactive, and hierarchial effects of various environmental variables on marsh angiosperm ecology. In comparison to manipulative field experiments, this study was at a disadvantage by the inability to control natural between-site variation of factors not of primary interest. This problem in experimental design at best complicates interpretation of the data in terms of nutrient-enrichment and at worst makes certain comparisons entirely meaningless.

The live plant height results present the most demonstrable effect of enrichment on the angiosperms. Greater mean height was associated in most cases with nutrient-enriched sites. Nutrient enrichment can increase angiosperm height: Halvorso and Singer (1974) found <u>S</u>. <u>patens</u> height was more than doubled in 28  $^{\circ}$ /oo Hoagland's full nutrient culture over that in a 28.2  $^{\circ}$ /oo sea water

culture. Since micronutrients are plentiful in sea water, the differences they found may be attributable to an effect of supplemental nitrogen or phosphorus. Between-site differences for T. angustifolia heights were not observed in the May sample. This may be an indication that control branch Typha was not then subject to nutrient deficiency. However, many Typha individuals in the impacted branch were noted to have mature infloresences at that date; this was not the case in the control branch. Field observations one month later found both branches about equal in the proportion of flowering individuals. The earlier onset of flowering in the impacted site could account for the lack of difference in May lengths due to energy and resource demands of producing the reproductive structures. Boyd (1970) reported inflourescence and fruit formation of T. latifolia accounted for up to 125.7% of net dry matter production during the period May-June. In mid-June, seed head tissue nitrogen and phosphorus represented 30.1 and 39.0%, respectively, of total tissue nitrogen and phosphorus.

The conflicting or lack of differences throughout the growing season in plant heights of species in CBHM and MBHM may be due to a soil salinity effect. Nestler (1977) reported a negative correlation of <u>S. alterniflora</u> height and soil salinity. The mean and range of salinity in MBHM was nearly twice that in CBHM. However, as soil salinity was sampled in 1977, the values reported may not be representative of those during production sampling in 1976. The cessasion of sewage disposal in July, 1976, may have reduced water

transport in the impacted branch by as much as 10% (Wetzel et al., 1977). The manner in which this affected soil salinity regimes is unknown.

The lack of differences in plant heights in September-October for salt marsh species could be due to several factors:

- The small amounts of live biomass could have biased the sample through sub-sampling error;
- Preferential mortality of some size classes could have destroyed previous differences;
- 3) Further growth of remaining material could have occurred and been enhanced by nutrients remobilized from decomposing dead crop.

The limited literature concerning the elemental composition of tissues of marsh angiosperms suggests differences between tissues are common (Boyd, 1970; Boyd, 1978). In this study, differences in TNC between stem and leaf tissues were evident without statistical analysis. These results probably reflect differences in structure and function of the tissues.

With some exceptions, TNC decreased over the growing season. This trend has also been found in previous studies (see LITERATURE REVIEW). TNC of species in nutrient-enriched sites was not consistently greater than TNC for the same species in control branch sites. However, examination of the exceptions to the TNC seasonal trend and of between-site comparisons of TNC (Tables 6-8) provides
some evidence supporting the hypothesis that TNC is maintained at relatively higher levels over the growing season where assimilable, combined nitrogen is abundant. For example, an increase or no decrease of TNC between May and August was found for leaf and stem tissue of <u>D</u>. <u>spicata</u> in IBHM, for leaf tissue of <u>D</u>. <u>spicata</u> in MBHM, and for leaf tissue of <u>T</u>. <u>angustifolia</u> in IBTA. TNC always decreased between May and August for all species and tissues in control branch sites. In May, TNC was significantly greater in control branch sites in four of seven comparisons between the impacted or mixed branch sites and the control branch sites. None of the comparisons favored the enriched sites over the control sites. However, in August, of six similar comparisons, the control sites are higher in TNC in only one case, while two comparisons show greater TNC in enriched sites.

The NPP estimates (Table 3) are within the range of literature PLB results for similar stands (Table 12). In several instances the estimates from Smalley's method appear to be in error. For example, the mid-season increase in dead crop (Appendix) in CBTA is unsupported in the literature. The dead crop increase between August and October in CBHM is not balanced by decreased live crop and presumably not accountable by production so late in the growing season. Errors of this type can seriously bias the method of Smalley (Turner, 1976). Preference is given overall to the PLB estimates. Even though this method underestimates NPP, it can serve for comparative purposes if biomass mortality during the growing season is assumed to be

## TABLE 12

#### LITERATURE ESTIMATES OF NPP FOR SPECIES

## DOMINANT IN YORKTOWN CREEK

Location	Species	NPP (g·m <sup>-2</sup> ) Reference			
N. Carolina	S. alterniflora	610-1300	Marshall, 1970		
N. Carolina	<u>S. alterniflora</u>	650	Williams and Murdock, 1969		
Virginia	<u>S</u> . <u>alterniflora</u>	695-1570	Wass and Wright, 1969		
New York	<u>D</u> . <u>spicata</u>	523-773	Udell et al., 1969		
Virginia	<u>D</u> . <u>spicata</u>	360	Wass and Wright, 1969		
N. Carolina	<u>S. patens/D. spicata</u>	939	Waits, 1967		
Virginia	<u>S. patens</u>	805	Wass and Wright, 1969		
N. Carolina	<u>S</u> . <u>patens</u>	720	Waits, 1967		
New York	<u>S</u> . <u>patens</u>	424-547	Udell et al., 1969		
Great Britain	<u>T</u> . <u>angustifolia</u>	1445	Mason and Bryant, 1975		
Virginia	<u>S</u> . <u>alterniflora</u>	726	This study		
Virginia	<u>S. patens/D. spicata</u>	538-577	This study		
Virginia	<u>S</u> . <u>patens</u>	560	This study		
Virginia	<u>T</u> . <u>angustifolia</u>	721-734	This study		

Estimate of NPP is peak live biomass.

proportionately equal in relation to live biomass between compared sites.

Visual inspection of the distributional properties of the PLB estimates (Figure 4) indicates no between site differences exist for Typha and high marsh sites. No comparative site existed for the monospecific S. alterniflora (MBSA) site. Its production was within the normal range for mid-Atlantic states (Table 12). It is important to note species composition differences in high marsh sites result in the NPP estimates being commensurable only on a very general level (i.e., non-specific high marsh). Production differences between monospecific stands of different high marsh species are well documented (Keefe, 1972). Attempting a detailed accounting of high marsh production differences -if they existed- would be meaningless here given our limited present understanding of intra-specific differences in production, mechanisms controlling production, and long-term interactions of species in mixed stands. However, the lack of a clear nutrient enrichment effect on Typha and high marsh production is surprising in light of literature evidence for nutrient-limitation of marsh angiosperm production. The results suggest production of the nutrient-enriched high marsh sites could have been limited by interstitial salinity. The negative effect of soil salinity on high marsh production is documented in the literature. The mean interstitial salinity was 2.8 <sup>0</sup>/oo higher in IBHM and almost twice as high (17 O/oo) in MBHM compared to CBHM (8.9)

<sup>0</sup>/oo). Salinity range was nearly double that of CBHM in both MBHM and IBHM. This explanation does not hold for the <u>Typha</u> sites where interstitial salinity did not differ between sites.

The <u>Typha</u> sites were characterized by dense vegetation bordering (impacted branch) or intermingled with (control branch) unvegetated, unconsolidated sediments. As unvegetated areas were inaccessible, samples were taken only from vegetated stands (as opposed to the staked sites, where the experimental design dictated what areas were sampled irregardless of their vegetative cover). This selectivity lead to estimation of stand productivity, which overestimates mean areal productivity of each <u>Typha</u> block. Although a quantitative estimate is not available, aerial photography and ground surveys indicated the percentage of <u>Typha</u> coverage was much greater in the impacted branch. Undoubtably, this would result in a higher areal productivity for the impacted branch <u>Typha</u>-dominated area. The cause of this phenomena is unknown. The equality of stand productions suggests limitation by space rather than available nutrients.

Several features of the high marsh communities' species composition and distribution are worth comment. First is the absence of <u>D</u>. <u>spicata</u> in the control branch as a dominant (Figure 7, Table 3). Differences between CBHM and IBHM in mean interstitial salinity were minor, although range of salinity was quite different. Mean salinity in both sites was very close to the optimum for <u>D</u>. <u>spicata</u> production reported by Adams (1963) and Palmisano (1970). Elevation of the two sites was not statistically different. Yet, <u>D</u>. <u>spicata</u>

accounted for 38% of IBHM peak biomass and only 0.6% of CBHM PLB. The species was also dominant in MBHM, where salinity mean and range and elevation were significantly greater than the other two sites. These observations raise questions for further research into interspecific angiosperm competition and the true nature of  $\underline{D}$ . spicata halophytism. An interesting aside to this discussion is the observation by Amen, Carter, and Kelly (1970) that the percentgermination response of D. spicata can be increased by placing the seeds on a substrate high in nitrate concentration. It is widely assumed seeds of marsh angiosperms are used almost exclusively in pioneer exploitation of bare substrate. Thereafter, reproduction occurs asexually by extension of runners or by shoot differentiation from root-rhizome stock. However, the extent to which these species actually utilize sexual reproduction to maintain stocks in prevegetated areas or to invade such areas has never been studied to my knowledge. The possibility that the zonation of D. spicata in Yorktown Creek was caused in part by an enrichment effect on seed germination points to the need for greater perception and study of the reproductive ecology of marsh angiosperms in general.

The second notable feature of zonation and distribution again brings forth our lack of knowledge concerning interspecific competition among marsh angiosperms. The control branch high marsh was dominated by <u>S. patens</u> (95% of PLB) yet contained the greatest number of minor species (Figure 7, Table 9). As noted earlier, few differences existed in soil salinity and elevation between CBHM and

IBHM. The least number of species among high marsh sites was found in IBHM. The question arises whether these differences are the result of long-term competition, natural variability, or the result of influence of some unmeasured parameter. Certainly, the greater salinity range in IBHM could have caused a "pulsed stability" (Odum, 1971) in the species structure of IBHM but a conclusion cannot be drawn with such limited information.

The construction of roads and culverts across the creek mouth has undoubtably had a major impact on the distribution and species composition of angiosperm communities in Yorktown Creek. Historical renditions of the area indicate a road was built across the creek mouth in the Colonial era. This activity has altered the hydrology of the marsh to the extent that most "flooding tides" in the creek no longer have the commonly accepted meaning. On numerous occasions a purely hydrostatic tide was observed in the creek (i.e., no reversal of creek flow). Flooding of the marsh by estuarine tidal water was occasioned only by spring and storm tides. The bedlam created by these phenomena in marsh hydrology is quite evident in the elevation and soil salinity results. For example, the site with highest elevation (MBHM) had the highest soil salinity. High salinity in high elevation areas is usually ascribed to the effects of evaporation on infrequently inundated soil (Nestler, 1977); however, this explanation does not hold in MBHM. The site was noted to be inundated as frequently, if not more, as the other high marsh sites. The relatively low amount of dead biomass in MBHM also indicates the

site was frequently inundated. It appears that soil salinity was heavily influenced by the distance from the site to the creek mouth and secondarily by the site elevation. Thus, differences in species composition between high marsh sites might be tentatively ascribed to road construction activities.

#### SUMMARY

1. Marsh angiosperm communities in Yorktown Creek were studied to assess effects upon them of nutrient enrichment resulting from chronic disposal of secondarily treated sewage wastes. Topography was such that similar communities existed in enriched and natural nutrient regimes. A lack of well-founded synecological understanding of these communities, the nature of the study site, and the experimental design contributed to ambiguity in some result's interpretation.

2. Production of high marsh and <u>Typha</u> communities was unaffected by enrichment. Production of an enriched <u>S</u>. <u>alterniflora</u> community was within the natural range found in previous studies. The effect of release from nutrient limitation may have been nullified in the high marsh communities by continued production limitation by interstitial salinity.

3. Differences in growth form as measured by aerial live plant length were attributed to enrichment. The negative effect of interstitial salinity on plant length may have overcome positive enrichment effects in one high marsh site. 4. No consistent differences were found in aerial tissue nitrogen concentration between enriched and natural nutrient regime sites. However, support was found for the hypothesis that a species aerial TNC is maintained at relatively higher levels over the growing season where assimilable, combined nitrogen is most abundant. The hypothesis that aerial TNC decreases with time (i.e., population maturity) was also supported. In all species, leaf TNC was greater than stem TNC.

5. The distribution and species composition of marsh angiosperm communities are the gross, visible attributes of the subtle integration of effects of many variables on angiosperm ecology. Not surprisingly, the effect on them of nutrient enrichment is not clear from this study. If questions concerning these angiosperm community attributes are to be answered, further research is needed in marsh angiosperm reproductive ecology, interspecific competition, and physiological ecology.

6. Wetzel et al. (1979) showed the Yorktown Creek system did not ameliorate the environmental impact of upstream sewage disposal. The lack of angiosperm response to enrichment in terms of dry matter production and tissue nitrogen concentration undoubtedly was a significant factor in the system response. It must be concluded the angiosperms were not useful components of a natural tertiary treatment system.

#### APPENDIX

## CALCULATION OF NET PRIMARY PRODUCTION (NPP)

BY THE METHOD OF SMALLEY (1958)<sup>1</sup>

	BIOMASS (g/m <sup>2</sup> )					NPP (g/m <sup>2</sup> )	
<u>SITE</u>	DATE	DEAD	LIVE	ΔD	ΔL	Δt	<u>Σt</u>
MBSA	2/28/76 4/15/76 5/20/76 7/21/76 8/17/76 9/29/76 2/28/77	509.3 244.2 140.5 205.6 235.9 900.5	(0)2 77.0 289.9 631.5 726.1* 528.4 0.0	-265.1 -103.7 65.1 30.3 664.6	- 77.0 212.9 341.6 94.6 -197.7 -528.4	77.0 212.9 341.6 159.7 0.0 136.2	0.0 77.0 289.9 631.5 791.2 791.2 927.4
					TOTAL MBSA		927.4
IBHM	2/28/76 5/24/76 8/17/76 10/5/76 2/28/77	917.7 701.8 940.0 1299.8	(0) <sup>2</sup> 225.1 577.2* 339.8 0.0	- -215.9 238.2 359.8		225.1 352.1 0.8 20.0	0.0 225.1 577.2 578.0 598.0
					TOTAL IBHM		598.0
СВНМ	2/28/76 7/22/76 8/17/76 10/5/76 2/28/77	- 792.8 517.5 1141.2 1174.1	(0)2 560.4* 472.8 285.7 0.0	- -275.3 623.7 32.9	- 560.4 -87.6 -187.1 -285.7	560.4 0.0 436.6 0.0	0.0 560.4 560.4 997.0 997.0
					TOTAL CBHM		997.0
MBHM	2/27/76 4/13/76 5/20/76 8/17/79 9/29/76 3/03/77	766.1 524.3 401.4 406.4 392.0 799.6	0.0 175.2 353.3 538.4* 273.6 0.0	-241.8 -122.9 4.7 -14.1 407.6	175.2 178.1 185.1 -264.8 -273.6	- 175.2 178.1 189.8 0.0 134.0	0.0 175.2 353.3 543.1 543.1 677.1
					IUIAL MB	ויות	0//.1

BIOMASS (g/m <sup>2</sup> )						NPP (g/m <sup>2</sup> )	
SITE	DATE	DEAD	LIVE	ΔD	ΔL	<u>∆t</u>	<u>Σt</u>
IBTA	2/28/79 5/24/79 8/24/79 10/6/76 2/28/77	936.1 552.6 2000.2 1524.3	(0)2 448.5 721.5* 58.7 0.0	- -383.5 1447.6 -475.9	- 448.5 273.0 -662.8 -58.7	448.5 273.0 784.8 0.0	0.0 448.5 721.5 1506.3 1506.3
					TOTAL IB	TA	1506.3
CBTA	2/28/76 5/24/76 7/22/76 8/25/76 10/6/79 2/28/77	- 884.0 1406.0 726.2 1491.1 1228.3	(0) <sup>2</sup> 237.2 522.8 734.4* 29.2 0.0	- 522.0 -679.9 765.0 -262.8	237.2 285.6 211.6 -705.2 -29.2	- 237.2 807.6 211.6 59.8 0.0	0.0 237.2 1044.8 1256.4 1316.2 1316.2
					TOTAL CB	TA	1316.2

 $1 \, \text{See}$  Methods and Materials for explanation of method.

<sup>2</sup>Not sampled, visual estimate

\*Peak Live Biomass

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