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Contrasting Patterns of Phytoplankton Community Pigment Composition in Two Salt Marsh Estuaries in Southeastern United States

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Phytoplankton community pigment composition and water quality were measured seasonally along salinity gradients in two minimally urbanized salt marsh estuaries in South Carolina in order to examine their spatial and temporal distributions. The North Inlet estuary has a relatively small watershed with minimal fresh water input, while the Ashepoo, Combahee, and Edisto (ACE) Basin is characterized by a relatively greater influence of riverine drainage. Sampling stations were located in regions of the estuaries experiencing frequent diurnal tidal mixing and had similar salinity and temperature regimens. Phytoplankton community pigment composition was assessed by using high-performance liquid chromatography (HPLC) and multivariate statistical analyses. Shannon diversity index, principal-component, and cluster analyses revealed that phytoplankton community pigments in both estuaries were seasonally variable, with similar diversities but different compositions. The temporal pigment patterns indicated that there was a relatively weak correlation between the pigments in ACE Basin and the relative persistence of photopigment groups in North Inlet. The differences were presumably a consequence of the unpredictability and relatively greater influence of river discharge in the ACE Basin, in contrast to the greater environmental predictability of the more tidally influenced North Inlet. Furthermore, the timing, magnitude, and pigment composition of the annual phytoplankton bloom were different in the two estuaries. The bloom properties in North Inlet reflected the predominance of autochthonous ecological control (e.g., regenerated nutrients, grazing), and those in ACE Basin suggested that there was greater influence of allochthonous environmental factors (e.g., nutrient loading, changes in turbidity). These interestuarine differences in phytoplankton community structure and control provide insight into the organization of phytoplankton in estuaries.

Processes and mechanisms that determine the spatial and temporal patterns of phytoplankton have been a central focus of marine research for decades. This research has led to the identification of biotic and abiotic factors that regulate primary productivity and the development of models that describe phytoplankton growth dynamics under specific environmental conditions (4). Phytoplankton communities are multispecies communities which are highly complex in terms of their diversity and dynamics. Successional shifts in phytoplankton community structure are primarily due to changes in environmental variables (e.g., degree or type of nutrient limitation) and/or shifts in higher trophic levels (e.g., micro- versus mesozooplankton) (23, 35, 40, 54, 56). How phytoplankton community composition changes with environmental variables and/or preferential grazing by herbivores is not well understood, particularly in estuarine ecosystems. Identifying the ecological variables that regulate phytoplankton community structure is essential for facilitating the development of broad hypotheses underlying our understanding of such pervasive environmental issues as eutrophication and harmful algal blooms (53, 61).

One approach for characterizing phytoplankton communities in estuarine ecosystems is to analyze their photosynthetic and photoprotective pigments by using high-performance liquid chromatography (HPLC) (42, 43, 66). Pigments vary in their chemotaxonomic specificity. Unambiguous biomarkers and their corresponding phytoplankton classes include (i) peridinin for the *Dinophyceae*, (ii) prasinoxanthin for some *Prasinophyceae*, (iii) alloxanthin for the *Cryptophyceae*, and (iv) divinyl chlorophyll *a* for the *Prochlorophyceae* (72). Ambiguous biomarkers and some representative phytoplankton classes include (i) fucoxanthin for the *Bacillariophyceae*, *Haptophyceae*, and *Dinophyceae*; (ii) chlorophyll *b* for the *Chlorophyceae* and *Prasinophyceae*; and (iii) 19′-hexanoyloxyfucoxanthin for the *Haptophyceae* and *Chrysophyceae* (72). One major advantage of this approach over (conventional) microscopic evaluation of phytoplankton is that the HPLC technique allows rapid and cost-effective processing of numerous samples, which is needed for ecosystem-scale projects. In addition, the HPLC approach can be less subjective than microscopic approaches, especially with regard to fragile phytoplankton that are distorted by fixation or small species which are difficult to identify (22, 58).

When an HPLC approach is used to characterize phytoplankton communities, however, precise identification and quantification of phytoplankton classes in water samples are often difficult because (i) the pigment compositions of many algal

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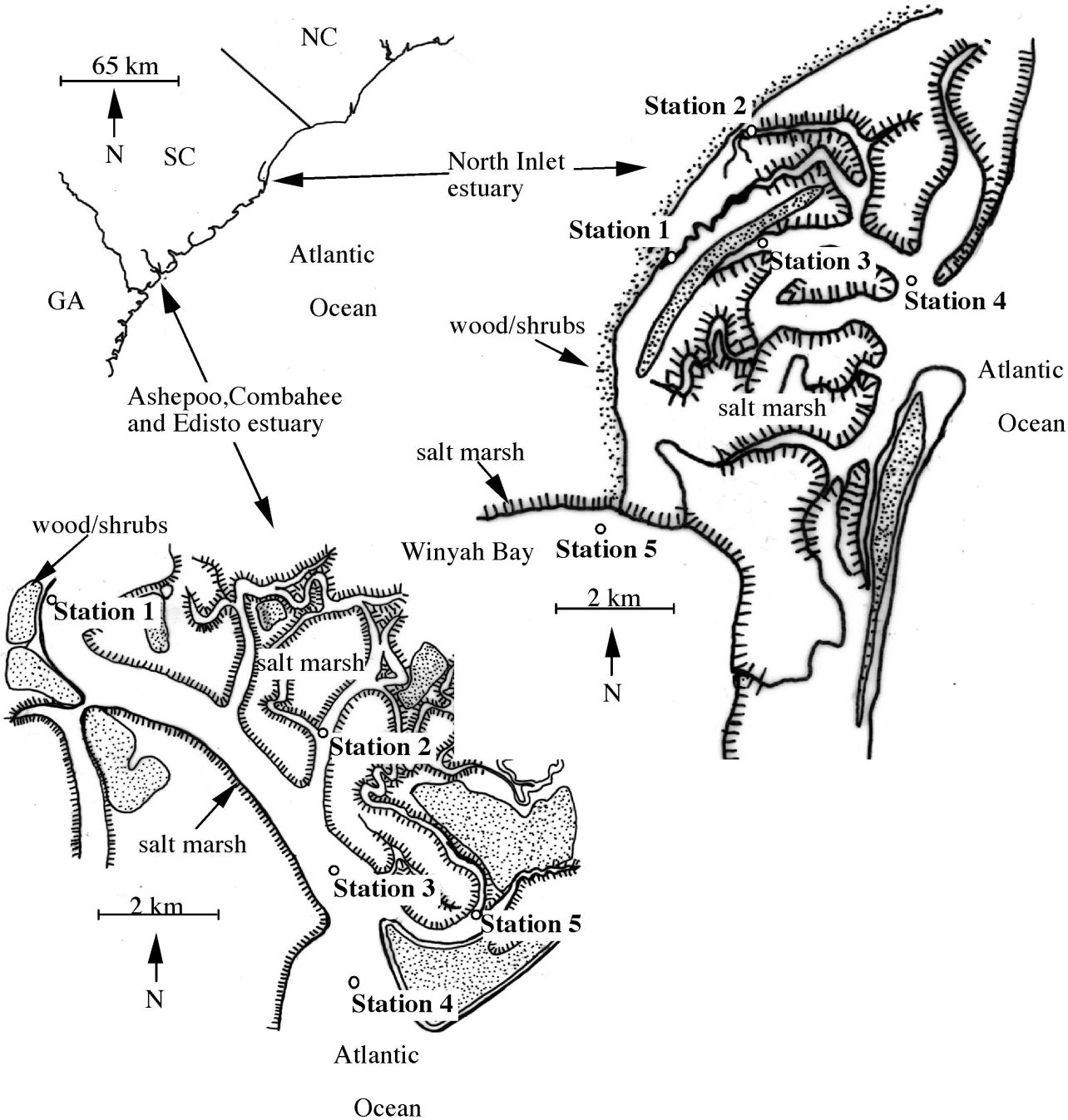

FIG. 1. Map showing sample stations at two sites, the NI and ACE Basin estuaries. Stations 1 to 4 represent a salinity gradient ranging from mesohaline stations upstream to oligohaline stations downstream. Station 5 served as a reference sample site in both estuaries.

classes are not known, (ii) symbionts within phytoplankton often possess pigments (6, 30, 67, 68), (iii) heterotrophic or mixotrophic protists often retain photosynthetic pigments from their prey (34, 36), and (iv) pigment composition varies depending on the light regimens, nutrient concentrations, and physiological status of the phytoplankton (17, 19, 24, 33, 59). Nonetheless, an HPLC pigment approach can be a valuable monitoring tool for determining relationships between environmental variables and the absolute or relative biomasses of major phytoplankton classes as determined by shifts in pigment concentrations (2, 26, 37, 51, 52, 55, 57, 71, 72).

In this study, we used pigment concentrations as a composite indicator of phytoplankton community composition for the purpose of examining the effects of riverine drainage (nutrient loading) on tidally dominated salt marsh estuaries. Our working hypothesis was that the estuarine phytoplankton community composition and biomass differed in different estuaries as a function of the importance of allochthonous inputs. We

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TABLE 1. Characteristics of water in the NI and ACE Basin estuaries during the study period (April 1999 to October 2000)

Estuary	Station	Salinity (ppt)		Temp (°C)		Silicate concn (μM)		Phosphate concn (μM)		Ammonia concn (μM)		Nitrate concn (μM)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
NI	1	29.2 ± 10.8 (10)[a]	1.0–36.7	24.7 ± 4.4 (11)	18.0–30.0	28.4 ± 21.9 (7)	0.0–48.6	0.3 ± 0.2 (8)	0.0–0.5	3.5 ± 3.1 (6)	0.1–8.0	0.4 ± 0.3 (11)	0.0–0.9
	2	32.5 ± 3.9 (10)	26.0–37.0	25.7 ± 4.7 (10)	18.8–31.0	36.9 ± 47.7 (4)	0.0–100.0	0.2 ± 0.1 (8)	0.0–0.4	1.3 ± 1.1 (6)	0.1–3.0	0.2 ± 0.2 (11)	0.0–0.6
	3	33.5 ± 2.9 (10)	28.0–37.0	25.4 ± 4.5 (11)	19.1–31.0	7.7 ± 6.3 (7)	0.0–16.6	0.2 ± 0.2 (8)	0.0–0.5	1.4 ± 0.5 (6)	0.7–2.0	0.2 ± 0.2 (11)	0.1–0.5
	4	33.9 ± 2.5 (10)	29.0–37.0	26.3 ± 4.7 (11)	20.6–32.2	5.2 ± 3.7 (7)	0.0–9.3	0.2 ± 0.1 (8)	0.0–0.3	0.9 ± 0.8 (6)	0.0–2.1	0.2 ± 0.1 (11)	0.0–0.4
ACE Basin	1	23.4 ± 7.9 (11)	6.1–32.3	26.236 ± 4.882 (11)	19.4–32.2	26.9 ± 22.2 (7)	0.0–58.4	0.8 ± 0.2 (8)	0.4–1.0	1.1 ± 1.0 (6)	0.0–2.7	3.3 ± 3.0 (11)	0.0–7.7
	2	29.4 ± 4.2 (11)	21.2–35.1	26.064 ± 4.783 (11)	19.3–32.2	19.5 ± 14.1 (7)	0.0–32.5	0.7 ± 0.3 (8)	0.2–1.0	1.1 ± 0.6 (6)	0.5–2.2	1.1 ± 1.0 (11)	0.0–3.2
	3	30.9 ± 3.6 (11)	23.5–35.5	25.891 ± 4.625 (11)	19.3–32.0	13.8 ± 9.8 (8)	0.0–25.4	0.5 ± 0.3 (8)	0.1–0.9	1.0 ± 0.9 (6)	0.0–2.4	0.9 ± 1.0 (11)	0.0–2.6
	4	32.6 ± 2.1 (11)	29.3–36.0	25.418 ± 4.487 (11)	19.1–31.0	5.7 ± 4.1 (7)	0.0–9.8	0.4 ± 0.2 (8)	0.1–0.7	1.1 ± 1.0 (6)	0.0–2.7	0.4 ± 0.8 (11)	0.0–2.8

[a] The numbers in parentheses are numbers of samples.

TABLE 2. Light attenuation coefficients and concentrations of suspended and organic solids in the NI and ACE Basin estuaries during the study period (April 1999 to October 2000)

Estuary	Station[a]	Light attenuation coefficient (K_d)	Concn of suspended solids (μM)	Concn of organic solids (μM)
NI	1	0.8 ± 0.3 (5)[b]	12.2 ± 08.2 (9)	4.0 ± 0.7 (6)
	2	0.7 ± 0.1 (5)	21.1 ± 14.6 (10)	6.0 ± 1.1 (6)
	3	0.6 ± 0.1 (5)	21.0 ± 14.7 (10)	6.3 ± 0.9 (6)
	4	0.9 ± 0.4 (5)	22.9 ± 16.7 (10)	6.8 ± 2.1 (6)
ACE Basin	1	1.1 ± 0.2 (5)	40.6 ± 33.7 (10)	9.3 ± 2.8 (7)
	2	0.9 ± 0.4 (5)	30.8 ± 24.7 (10)	7.4 ± 1.6 (6)
	3	1.0 ± 0.2 (5)	32.8 ± 26.3 (10)	7.8 ± 1.7 (6)
	4	1.1 ± 0.4 (5)	33.0 ± 29.4 (10)	8.2 ± 3.2 (6)

[a] Station 1 is in a salt marsh, and station 4 is in a coastal zone.
[b] The values are means ± standard deviations. The numbers in parentheses are numbers of samples.

tested this hypothesis by examining phytoplankton from two estuaries (Fig. 1) that have similar physical properties (e.g., salinity, water temperature, and tidal exchange) but differ in terms of the influence of the freshwater drainage basin.

The Ashepoo, Combahee, and Edisto (ACE) Basin estuary receives 10-fold more freshwater input (2.7×10^9 m^3 year^{-1} [65]) than the North Inlet (NI) estuary (2.5×10^8 m^3 year^{-1} [44]), which presumably results in significantly higher nutrient loads. The volumes of the tidal prism are about 4.6×10^8 m^3 (calculated from the data of Marshall [39] and Orlando et al. [50]) in the ACE Basin and 11.3×10^6 m^3 in NI (48), which result in residence times from tidal exchange of about 0.45 and 0.20 day, respectively. Although the freshwater input into the ACE Basin is 10-fold greater than that into NI, the freshwater input into the ACE Basin has little effect on the average residence time due to the large volume (ca. 4×10^8 m^3). However, extreme flow conditions can significantly affect the residence time and nutrient loading in the ACE Basin. Daily flow on the Edisto River, which accounts for the majority of flow into the ACE Basin, has varied by 2 orders of magnitude, from a low of 7.3×10^5 m^3 day^{-1} to a high of 1.7×10^7 m^3 day^{-1} (65).

Within each estuary, we obtained samples at stations that varied in terms of proximity to the forest wetland-water margin and consequently varied in terms of the freshwater influence and tidal flow. Our objectives were (i) to determine the spatial and temporal distributions of phytoplankton communities, (ii) to identify relationships between HPLC pigments and environmental variables (e.g., nutrients), and, by using this information, (iii) to improve our understanding of environmental regulation of phytoplankton community composition in salt marsh estuaries with different nutrient loading characteristics.

MATERIALS AND METHODS

Physical and biological characteristics of the NI and ACE Basin estuaries. The two tidally dominated, well-mixed, shallow (range of depths, 1 to 5 m) estuaries studied are located approximately 100 km apart (Fig. 1) and were similar in terms of salinity, temperature, and tidal range and frequency at the time of sampling. Both locations are National Estuarine Research Reserve System sites and are free from human development activities. The major differences between the estuaries are related to freshwater drainage. The ACE Basin estuary receives relatively high freshwater input (the area of its drainage basin is 7,987 km^2), mainly from the Edisto River, one of the longest free-flowing blackwater

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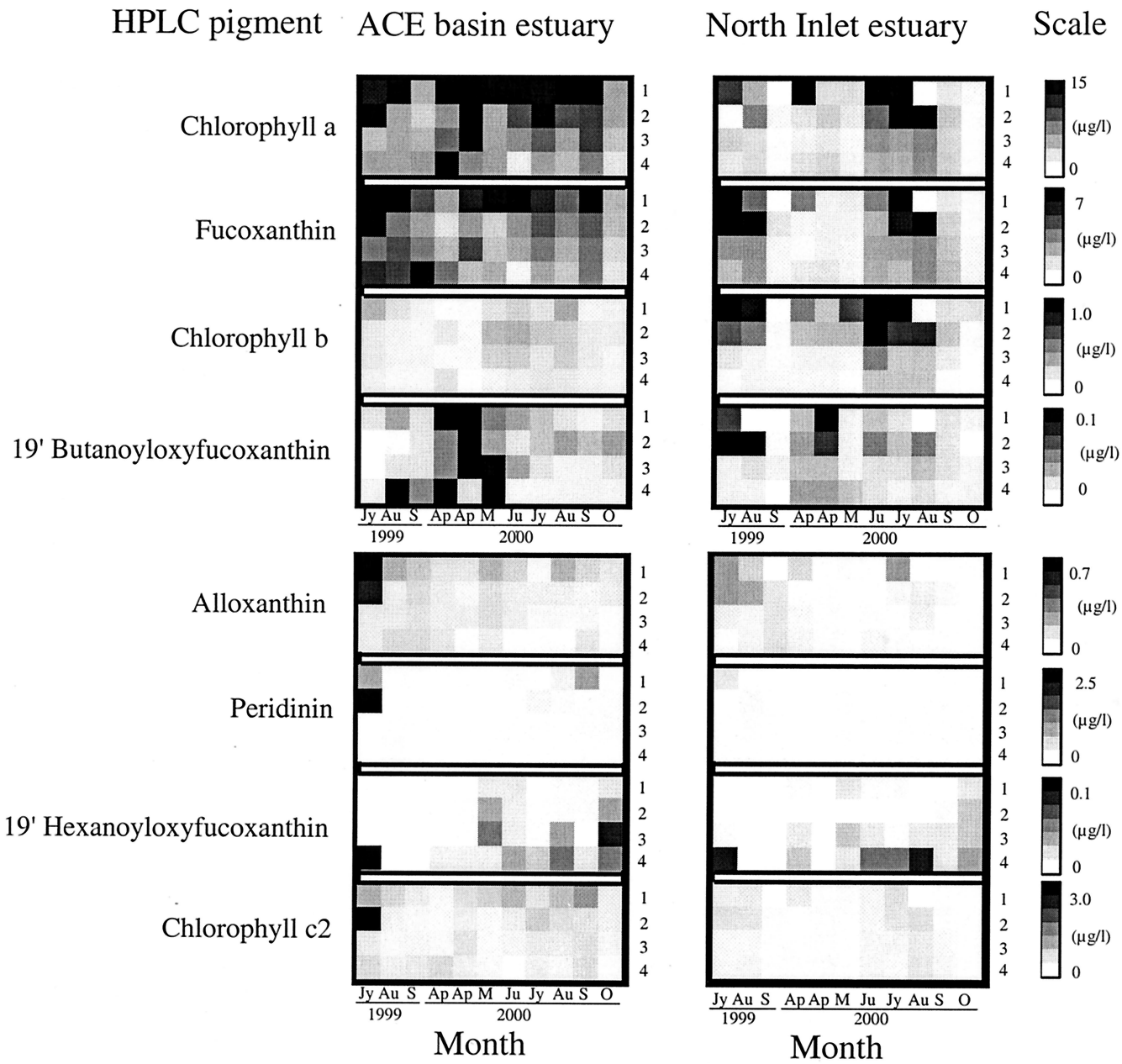

FIG. 2. Spatial and temporal variations in HPLC mean pigment concentrations. *y* axis, stations along the salinity gradient, including stations 1 (salt marsh) to 4 (coastal ocean); *x* axis, sequential sample collection times (note that samples were collected in April in the first and third weeks). Jy, July; Au, August; S, September; Ap, April; M, May, Ju, June; O, October.

rivers in the United States (65). The average water discharge of the Edisto River is approximately 76 $m^3 s^{-1}$, with the monthly mean flows highest from January through April and lowest from June through November (7). The salinity gradient extends 32 km inland from the mouth of the river (well beyond the location of our sampling stations). The NI estuary, on the other hand, receives very little freshwater input (3% of the tidal volume [47]), and approximately 40% of the total water volume leaves the system on each ebb tide (semidiurnal) (32). The frequent tidal flushing of this estuary results in a highly variable nutrient pool, which is regulated primarily by the magnitude of the flux through the mouth, exchange between the water column and the intertidal sediments, and trophic interactions inside the estuary (47).

Sampling methods. A preliminary study was aimed at determining whether HPLC pigment profiles, nutrient contents, and other environmental parameters (listed below) varied with vertical position in the water column. The preliminary study involved collecting weekly samples at two stations (stations 1 and 2 [Fig. 1]) in the NI estuary from May to October 1998. Duplicate water samples were collected from 0.5 m below the surface and 0.5 m above the bottom by using 1-liter plastic bottles at each sample station. The temperature was measured with a thermometer on site, the salinity was measured with a refractometer, the inorganic nutrient contents (nitrite and nitrate, ammonium, orthophosphate, silicate) were measured by automated colorimetric analyses by using Technicon autoanalyzers, and the dissolved organic carbon (DOC) content was measured with a carbon analyzer (Shimazu TOC 500). The dissolved organic nitrogen (DON) and dissolved organic phosphate (DOP) contents were determined by subtracting the total inorganic N and P contents from the total dissolved N and P contents, respectively, as measured by the persulfate oxidation technique (18). The amounts of total suspended sediments (TSS) were determined by filtering samples onto preweighed GF/F glass fiber filters, drying the filters (60°C), and then reweighing the filters. The amounts of organic solids (OS) were determined based on the difference in weight after the filters were combusted (450°C). Water

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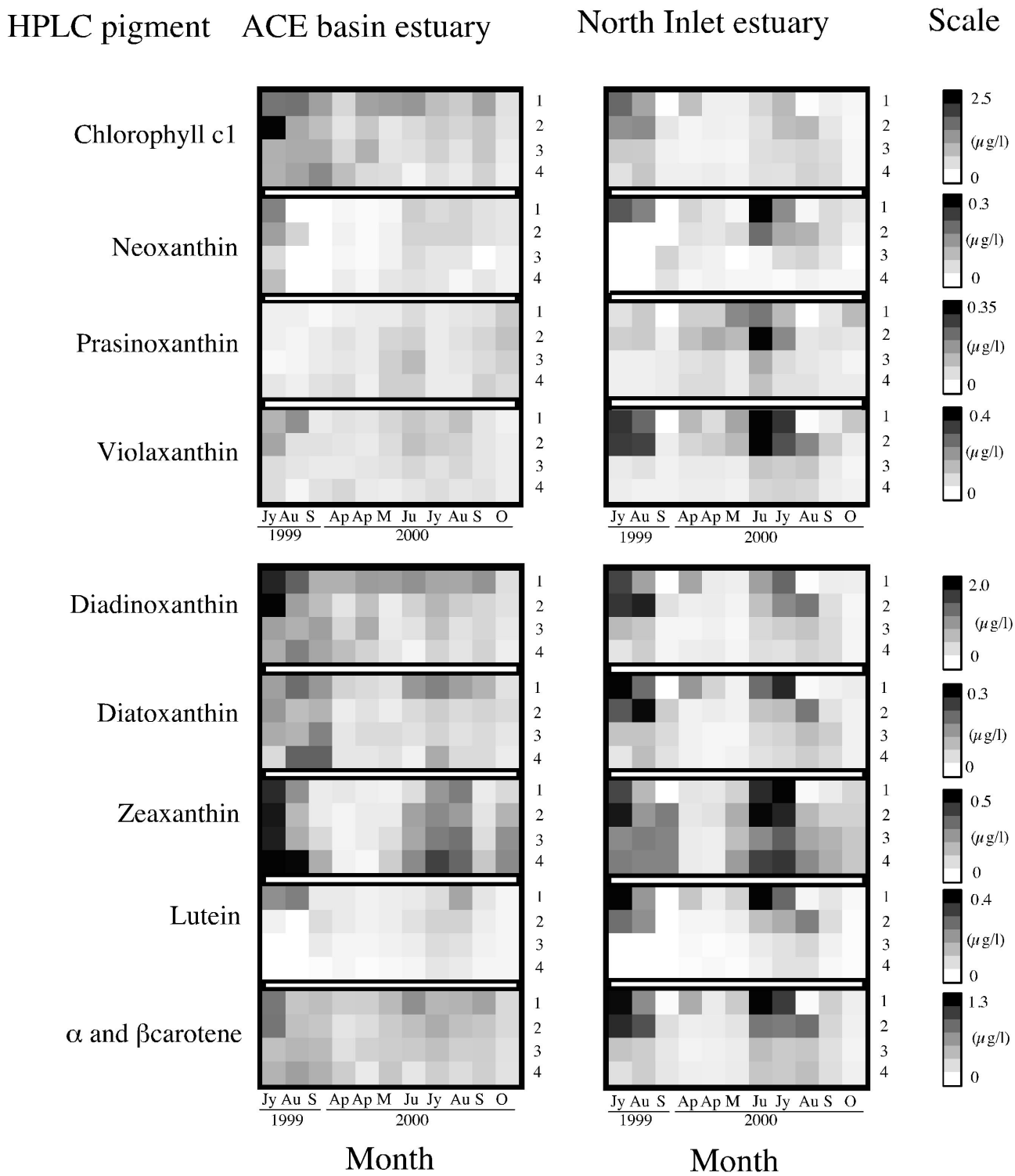

FIG. 2—*Continued.*

column irradiance profiles were determined by using a Li-Cor spherical quantum sensor (LI-193SA). Light attenuation coefficients were calculated by using the K_d equation (31).

Information gained from the preliminary study was used to guide the main study, which involved collecting bimonthly samples from July to September 1999 and from April to October 2000. Near-surface samples (i.e., 0.5 m below the surface) were collected at mid-ebb to low tide at five stations in each estuary (Fig. 1). The environmental conditions at mesohaline station 5 were distinct from those at the other sites in each estuary, and therefore this station was used as an internal reference station for quality assurance and control of sample processing and analysis.

Analyses of the plankton community by HPLC. HPLC was used to detect 18 pigments that have known chemotaxonomic importance for phytoplankton identification. Pigments were extracted in 100% acetone by using a tissue grinder. The slurry was then filtered through a Teflon HPLC syringe filter (pore size, 0.45 μm) containing a glass fiber prefilter. The pigment analysis was conducted by

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using a Beckman System Gold HPLC with an external column heater, a 125 solvent module dual pump, and a photodiode array detector with a deuterium lamp (monitoring 450 nm). The protocol was a modification of the protocol of Van Heukelem and Thomas (66), in which temperature control and a polymeric column were used. Pigment standards were obtained from L. Van Heukelem (Horn Point Laboratory, University of Maryland). Each water sample was processed in triplicate. The mean of the triplicate samples was used for statistical analysis.

Data for statistical analysis. The complete preliminary data set for 1998 consisted of HPLC profiles of 87 water samples (i.e., profiles based on the means for triplicate subsamples), and each profile represented 17 pigments collected at a specific station and time.

The complete data set for the main study (i.e., 1999 and 2000 samples) consisted of data for 108 water samples (i.e., data based on the means for triplicate samples; 11 sampling dates × five stations × two estuaries, minus two samples from station 5 that were not used because of quality assurance problems). Each profile consisted of 18 pigments (the average for triplicate subsamples) and represented a unique operational community unit (OCU) (i.e., sampling station and time). The complete data set included all the pigments used in the preliminary study (except that α- and β-carotenes were combined to represent a single HPLC pigment, carotene) plus chlorophyll c_2 and prasinoxanthin. For principal-component and cluster analyses, the concentration of each pigment in a profile was normalized to the chlorophyll *a* concentration of the profile. Each HPLC profile had a corresponding suite of data for environmental variables, which included temperature, salinity, tidal height, and ammonium (NH_4), nitrate-nitrite (NO_3-NO_2), DON, orthophosphate (PO_4), DOC, OS, TSS, and DOP contents.

Statistical analysis. Pearson product-moment correlation was used to determine the degree of association between variables. Linear regressions were used to estimate the relationship of one variable to another (62). An analysis of variance (ANOVA) was used to determine the source of variability in the experimental data. The Student-Newman-Keuls (SNK) test was employed to determine whether the difference between any two means in a set of means was significant (41). ANOVA and SNK tests were conducted by using the GLM (General Linear Model) procedure in the SAS program (release 6.11; SAS Inc., Cary, N.C.).

RESULTS

Water characteristics of the ACE Basin and NI estuaries. The environmental measurements obtained during the study are shown in Table 1. The nutrient concentrations tended to decrease along the salinity gradient toward the mouth of both estuaries (e.g., stations 1 to 4). A Student's *t* test indicated that there was no statistically significant difference in the average salinity values for station 1 or 2. However, the water at ACE Basin stations 3 and 4 had lower salinities than the water at the corresponding NI stations, reflecting the effects of freshwater drainage at the confluence of the ACE Basin estuary (Table 1).

There were no statistically significant differences in average temperature or NH_4 concentration at the sites. However, the water in the ACE Basin estuary had much higher concentrations of PO_4 and NO_3-NO_2 than the water in the NI estuary (Table 1) ($P < 0.01$), reflecting the effects of nutrient loading due to riverine drainage.

The significantly higher light attenuation coefficients ($P < 0.01$, as determined by the Student *t* test; df = 38) (Table 2), TSS contents ($P < 0.01$; df = 77), and OS contents ($P < 0.01$; df = 47) in the ACE Basin estuary suggested that riverine drainage affected the optical properties of the water column. We were not able to precisely determine which factors were responsible for the increased turbidity at stations in the ACE Basin estuary; however, the TSS content was substantially higher in the ACE Basin estuary (34.3 ± 27.8 mg liter^{-1}; n = 40) than in the NI estuary (19.0 ± 19.5 mg liter^{-1}; n = 39) (Table 2). Furthermore, the ratios of OS content to TSS content in ACE Basin samples were considerably lower (range, 0.23 to 0.25; Table 2) than the ratios in NI station samples (range, 0.28 to 0.33), suggesting that flocculent material and/or riverine debris (and not microbially derived debris) made up a larger proportion of the TSS at ACE Basin sampling stations than at NI sampling stations.

Pigment distribution and abundance. (i) Preliminary study of surface and bottom samples. In the preliminary study (i.e., 1998 samples) we examined the effects of sample depth on pigment distribution at NI stations 1 and 2. We found that with the exception of chlorophyll *b*, there was no statistically significant difference in the pigment concentrations in samples collected near the surface (0.5 m below the surface) and samples collected near the bottom (0.5 m above the bottom) of the water column. The chlorophyll *b* concentrations were higher in surface samples (mean ± standard deviation, 0.09 ± 0.06 μg liter^{-1}; n = 63) than in samples collected near the bottom (0.06 ± 0.05 μg liter^{-1}; $P < 0.01$, as determined by Student *t* test; n = 55). The higher chlorophyll *b* concentrations in surface samples were associated with elevated nitrogen (NO_3-NO_2, total dissolved nitrogen, and DON), phosphate (DOP and total dissolved phosphate), and/or DOC levels (data not shown). For example, the NO_3-NO_2 concentration near the surface was 1.1 ± 0.7 μM (average ± standard deviation; n = 55), while the concentration near the bottom was 0.6 ± 0.6 μM (n = 55). The DOP concentrations were 0.3 ± 0.3 μM (average ± standard deviation; n = 63) near the surface and 0.1 ± 0.3 μM (n = 55) near the bottom, while the DOC concentrations were 687 ± 245 mg liter^{-1} (n = 63) near the surface and 605 ± 167 mg liter^{-1} (n = 55) near the bottom. Nonetheless, because the concentration of only one measured pigment varied significantly with water column depth, we chose to collect samples only from near the surface (i.e., 0.5 m below the surface) for subsequent sampling in 1999 and 2000.

(ii) Comparison of phytoplankton pigments by estuary. In general, chlorophyll *a* concentrations tended to be highest at stations that were most affected by freshwater input (e.g., station 1) (Fig. 2). The maximum chlorophyll *a* concentrations in the ACE Basin and NI estuaries were 19.7 and 16.8 μg liter^{-1}, respectively. The Student *t* test revealed that there were statistically significant differences in the mean chlorophyll *a* concentrations between the estuaries ($P < 0.01$; n = 44), with the highest concentrations occurring in the ACE Basin estuary from April to September and in the NI estuary in April, June, and July (Fig. 2).

The following pigments occurred at significantly higher concentrations in the ACE Basin estuary than in the NI estuary (as determined by the Student *t* test; n = 44): fucoxanthin ($P < 0.01$), alloxanthin ($P < 0.01$), peridinin ($P < 0.01$), chlorophyll c_1 ($P < 0.01$), chlorophyll c_2 ($P < 0.01$), and diadinoxanthin ($P < 0.02$). For each pigment, the concentration, the time of the maximum concentration (peak time), and the station locations were compared to determine relevant trends by site. For most pigments, the maximum concentration occurred at station 1 or 2 in July or August (Fig. 2). However, in most cases, the peak times for the two estuaries were different (i.e., they varied by month and/or year), indicating that there was interestuary variability in phytoplankton community dynamics.

The following pigments occurred at higher concentrations in the NI estuary than in the ACE Basin estuary: chlorophyll *b*

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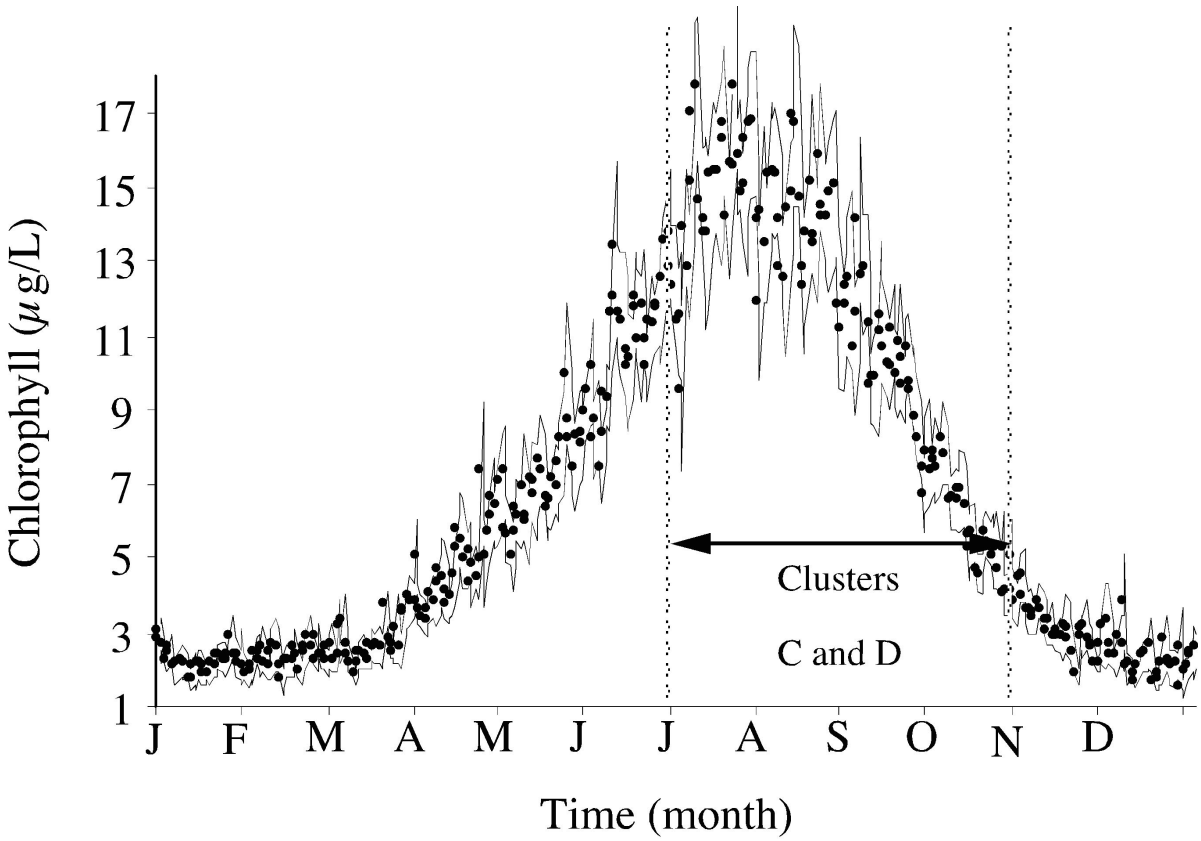

FIG. 3. Daily concentrations of chlorophyll *a* (averages ± standard errors) for site 2 in the NI estuary based on 10 years of samples collected from 1981 to 1991 by using the NSF Long Term Ecological Research data set available at http://inlet.geol.sc.edu. From left to right the months are January, February, March, April, May, June, July, August, September, October, November, and December.

($P < 0.02$), prasinoxanthin ($P < 0.03$), and violaxanthin ($P < 0.03$). With exception of prasinoxanthin in the ACE Basin (station 3), the maximum concentrations of these pigments occurred at station 1 or 2. The concentrations of chlorophyll *b* and violaxanthin reached their maximum values in August in the ACE Basin estuary and in June in the NI estuary, while the prasinoxanthin concentration peaked in June, 2000 in both estuaries.

The average concentrations of 19′-butanoyloxyfucoxanthin (19′-But), 19′-hexanoyloxyfucoxanthin (19′-Hex), neoxanthin, diatoxanthin, lutein, total carotenes, and zeaxanthin were not statistically significantly different in the two estuaries. However, there were definitive patterns in the spatial and temporal distributions of some of these pigments, which often varied substantially in the two estuaries (Fig. 2). For example, in both estuaries, high concentrations of 19′-Hex were associated with samples collected near the ocean margin (i.e., station 4). In the NI estuary, increases in neoxanthin, diatoxanthin, lutein, and total carotene concentrations closely followed the summer phytoplankton bloom (as shown in Fig. 3), with the highest concentrations occurring at stations 1 and 2 (Fig. 2). A similar trend was not observed in the ACE Basin estuary.

(iii) Shannon index of phytoplankton pigments by estuary. The Shannon index was used to evaluate how pigment diversity differed spatially and temporally in the two estuaries. In general, pigment diversity for the ACE Basin phytoplankton and pigment diversity for the NI phytoplankton were quite similar, and changes in diversity followed the same temporal (Fig. 4) and spatial (data not shown) trends, indicating that seasonal changes rather than spatial changes along the estuaries (i.e., differences between stations) affected phytoplankton diversity. Substantial decreases in pigment diversity occurred in both estuaries in April and May 2000 and appeared to be primarily associated with increases in 19′-But concentrations at all ACE Basin stations (Fig. 2) and at some NI stations. Comparisons of the 19′-But concentrations with environmental parameters re-

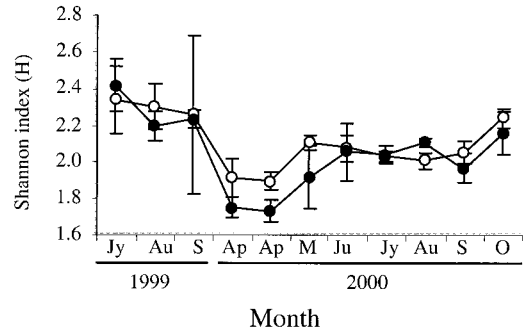

FIG. 4. Variation in phytoplankton pigment diversity over time in the NI (○) and ACE Basin (●) estuaries. *y* axis, sequential sample collection times. Note that in April samples were collected in the first and third weeks. Jy, July; Au, August; S, September; Ap, April; M, May, Ju, June; O, October.

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TABLE 3. Pearson correlation coefficient matrix for pigment data obtained from the ACE Basin estuary ($n = 44$)

HPLC pigment	Pearson correlation coefficient															
	Chlorophyll[a]	Fucoxanthin	Chlorophyll *b*	19′-But	Alloxanthin	Peridinin	19′-Hex	Chlorophyll c_2	Chlorophyll c_1	Neoxanthin	Prasinoxanthin	Violaxanthin	Diadinoxanthin	Diatoxanthin	Zeaxanthin	Lutein
Fucoxanthin	0.63[a]															
Chlorophyll *b*	0.11	0.12														
19′-But	0.40	−0.01	−0.19													
Alloxanthin	0.33	0.57[a]	0.32	−0.02												
Peridinin	0.24	0.43	0.13	−0.30	0.71[a]											
19′-Hex	−0.34	−0.46	0.10	−0.13	−0.40	−0.20										
Chlorophyll c_2	0.47	0.63[a]	0.15	−0.25	0.63[a]	0.91[a]	−0.24									
Chlorophyll c_1	0.43	0.83[a]	−0.03	−0.12	0.73[a]	0.72[a]	−0.50[a]	0.82								
Neoxanthin	0.13	0.22	0.51[a]	−0.36	0.45	0.58[a]	0.12	0.55[a]	0.28							
Prasinoxanthin	−0.17	−0.42	0.22	0.11	−0.27	−0.21	0.51[a]	−0.25	−0.54[a]	0.08						
Violaxanthin	0.40	0.60[a]	0.65[a]	−0.21	0.62[a]	0.50[a]	−0.20	0.59[a]	0.54[a]	0.55[a]	−0.18					
Diadinoxanthin	0.47	0.79[a]	0.05	−0.12	0.80[a]	0.76[a]	−0.50[a]	0.81[a]	0.95[a]	0.39	−0.60[a]	0.61[a]				
Diatoxanthin	0.09	0.70[a]	0.06	−0.18	0.43	0.33	−0.50	0.35	0.64[a]	0.02	−0.51[a]	0.36	0.66[a]			
Zeaxanthin	−0.30	0.16	0.30	−0.46	0.18	0.35	0.27	0.30	0.23	0.53[a]	−0.20	0.33	0.34	0.33		
Lutein	0.27	0.35	0.55[a]	−0.07	0.49[a]	0.25	−0.25	0.21	0.18	0.26	−0.13	0.71[a]	0.33	0.32	0.12	
Carotenes	0.39	0.78[a]	0.36	−0.16	0.74[a]	0.69[a]	−0.36	0.72[a]	0.78[a]	0.54[a]	−0.34	0.57[a]	0.81[a]	0.66[a]	0.41	0.32

[a] $P < 0.001$.

vealed that these concentrations were positively correlated with turbidity (e.g., TSS; $r = 0.73$; $n = 44$) and the light attenuation coefficient ($r = 0.66$; $n = 24$), indicating that these parameters may promote the growth of 19′-But-containing phytoplankton relative to the growth of other taxa or that 19′-But is associated with benthic resuspension. A similar effect was not as obvious in the NI estuary, which may reflect interestuary differences in riverine discharge.

In September 1999, the Shannon index for the NI samples was highly variable (as shown by the standard deviation in Fig. 4). The large standard deviation was due to a single low diversity value that was obtained for station 1. Of the 18 pigments examined, only chlorophyll *a*, fucoxanthin, and chlorophyll *b* were detected in samples obtained at this station. Moreover, the concentrations of the pigments were very low compared to the concentrations in samples collected from other stations at the same time. With the exception indicated above, the Shannon index did not change substantially between stations within an estuary, indicating that pigment diversity was more or less consistent for all stations from which samples were collected on the same date regardless of the estuary.

(iv) Correlations between pigments by estuary. Pearson correlation coefficients of pigment concentrations were used to establish the relationships between major and minor accessory pigments and to obtain an understanding of how pigment concentrations change (relative to one another) at the two sites (Tables 3 and 4). In general, correlations were higher between pigments collected from NI samples (Table 3) than between pigments collected from ACE Basin samples (Table 4). Only 45 pigment combinations (of the 136 possible combinations) for samples collected from the ACE Basin estuary yielded statistically significant correlations at a P level of <0.001, compared to 76 pigment combinations for samples collected from the NI estuary.

Cluster and principal-component analyses of phytoplankton pigment profiles. A data matrix of 108 pigment profiles was constructed to examine the similarities among OCU, with each OCU representing a specific sample location and time in the ACE Basin and NI estuaries. The concentrations of all pigments used for these analyses were normalized to the chlorophyll *a* concentration. Canthaxanthin was excluded from these analyses because it was not detected in most OCU. By using

TABLE 4. Pearson correlation coefficient matrix for pigment data obtained from the NI estuary ($n = 44$)

HPLC pigment	Pearson correlation coefficient															
	Chlorophyll *a*	Fucoxanthin	Chlorophyll *b*	19′-But	Alloxanthin	Peridinin	19′-Hex	Chlorophyll c_2	Chlorophyll c_1	Neoxanthin	Prasinoxanthin	Violaxanthin	Diadinoxanthin	Diatoxanthin	Zeaxanthin	Lutein
Fucoxanthin	0.72[a]															
Chlorophyll *b*	0.73[a]	0.61[a]														
19′-But	0.36	0.44	0.37													
Alloxanthin	0.40	0.72[a]	0.36	0.38												
Peridinin	0.22	0.48[a]	0.15	0.33	0.51[a]											
19′-Hex	−0.09	−0.12	−0.26	−0.27	−0.33	−0.12										
Chlorophyll c_2	0.73[a]	0.92[a]	0.49[a]	0.35	0.59[a]	0.29	0.13									
Chlorophyll c_1	0.61[a]	0.96[a]	0.53[a]	0.44	0.78[a]	0.66[a]	−0.20	0.82[a]								
Neoxanthin	0.65[a]	0.41	0.83[a]	0.17	0.25	0.18	−0.33	0.27	0.37							
Prasinoxanthin	0.47	0.24	0.75[a]	0.32	0.05	−0.15	−0.16	0.21	0.14	0.55[a]						
Violaxanthin	0.68[a]	0.71[a]	0.94[a]	0.45	0.51[a]	0.29	−0.30	0.56[a]	0.66[a]	0.76[a]	0.72[a]					
Diadinoxanthin	0.66[a]	0.95[a]	0.64[a]	0.54[a]	0.79[a]	0.56[a]	−0.27	0.81[a]	0.95[a]	0.43	0.26	0.78[a]				
Diatoxanthin	0.65[a]	0.88[a]	0.66[a]	0.46	0.82[a]	0.66[a]	−0.28	0.71[a]	0.92[a]	0.54[a]	0.21	0.77[a]	0.94[a]			
Zeaxanthin	0.47	0.67[a]	0.55[a]	0.10	0.47	0.20	0.07	0.62[a]	0.58[a]	0.41	0.42	0.63[a]	0.59[a]	0.58[a]		
Lutein	0.68[a]	0.73[a]	0.89[a]	0.42	0.54[a]	0.42	−0.25	0.56[a]	0.70[a]	0.79[a]	0.43	0.90[a]	0.79[a]	0.83[a]	0.52[a]	
Carotenes	0.71[a]	0.88[a]	0.85[a]	0.43	0.66[a]	0.46	−0.23	0.72[a]	0.84[a]	0.67[a]	0.45	0.91[a]	0.91[a]	0.91[a]	0.71[a]	0.93[a]

[a] $P < 0.001$.

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Euclidean distance with complete linkage, a dendrogram revealed that there were four major clusters at a distance of 0.3 and several minor clusters at a distance of 0.2 (Fig. 5).

Characteristics of major and minor clusters are shown in Table 5. Cluster A was composed mostly of OCU collected from the ACE Basin estuary, cluster B was composed of OCU collected from both estuaries, and clusters C and D were composed of OCU collected from the NI estuary. ANOVAs revealed that the following pigments varied between clusters: chlorophyll c_2, chlorophyll c_1, peridinin, fucoxanthin, neoxanthin, 19′-Hex, alloxanthin, diadinoxanthin, diatoxanthin, zeaxanthin, and total carotenes. Chlorophyll c_1, fucoxanthin, diadinoxanthin, alloxanthin, diatoxanthin, zeaxanthin, and total carotenes occurred at significantly higher concentrations in clusters C and D than in clusters A and B (Table 5), while higher concentrations of chlorophyll c_1 and fucoxanthin and lower concentrations of total carotenes were found in cluster D than in cluster C. Higher concentrations of chlorophyll c_1 and c_2 and fucoxanthin distinguished cluster B from cluster A. These results were consistent with our spatial-temporal analysis of pigment concentrations in the two estuaries (Fig. 2).

SNK tests provided information on within-cluster differences (i.e., differences within clusters B and C) (Table 5). Subcluster B1 was distinguished from other cluster B subclusters by having low fucoxanthin concentrations, perhaps indicating a lower abundance of diatoms. Subcluster B3 was distinguished from the other cluster B subclusters by having high concentrations of chlorophyll c_2 and peridinin, presumably indicating a higher level of dinoflagellates. Finally, subcluster C1 was distinguished from subcluster C2 by having lower concentrations of chlorophyll c_2, diatoxanthin, zeaxanthin, and alloxanthin and higher concentrations of fucoxanthin and diadinoxanthin.

ANOVA and SNK tests provided information on the linkages between clusters and physical and chemical characteristics of the water samples. The following variables were significantly different in different clusters: oxygen saturation, PO_4 concentration, TSS content, OS content, and DOC content. Oxygen saturation was lowest in clusters representing OCU collected during the annual NI phytoplankton bloom (subcluster C1 and cluster D). PO_4 concentrations were lowest in some of the clusters representing the NI phytoplankton bloom (subcluster C2 and cluster D). SNK tests did not reveal a clear trend for TSS and OS contents, even though they appeared to be lower for NI samples than for ACE Basin samples. The DOC content was substantially higher for some of the NI samples collected during the annual phytoplankton bloom.

The same data matrix employed for cluster analysis was used to construct an ordination plot to examine the distribution of samples relative to pigments explaining most of the variance in the matrix and to provide support for the cluster analysis results. Figure 6 shows an ordination plot that accounts for 51.1% of the total matrix variance. Two large groups distributed along the principal component 1 (PC1) axis had considerable coherence (Fig. 5). This coherence was examined further by labeling points in the plots with the corresponding cluster designations from the dendrogram. The PC1 axis explained 32.2% of the total variance and was correlated strongly with the following pigments (correlation coefficients are indicated in parentheses): chlorophyll c_1 (0.91), diadinoxanthin (0.87), fucoxanthin (0.81), diatoxanthin (0.81), chlorophyll c_2 (0.70), and alloxanthin (0.70). OCU associated with clusters C and D were clearly distinct from OCU associated with clusters A and B. The PC2 axis explained 18.9% of the total variance and was correlated with the following pigments (correlation coefficients are indicated in parentheses): chlorophyll b (0.91), prasinoxanthin (0.72), and neoxanthin (0.71). The pigments strongly correlated with the PC1 and PC2 axes are the same pigments identified in Table 5 which can be used to discriminate the major and minor clusters in the dendrogram (Fig. 5 and Table 5), indicating that there is general agreement in the two approaches. However, there was considerable overlap in the distribution of OCU within major groups in the ordination plot, indicating that subtle distinctions between clusters A and B or between clusters C and D, as well as the minor clusters (at a distance of 0.2), might be too fine to make any generalizations. However, we did not alter the cluster designations since the ordination plot explained only slightly more than one-half of the total variance of the data.

To summarize, the ordination plot and dendrogram results showed that pigment concentrations varied by estuary and with time, and the OCU collected during the summer (July to October) phytoplankton bloom in the NI estuary were different from the OCU collected at other times in the NI estuary and at all times in the ACE Basin estuary.

DISCUSSION

The effects of nutrient loading on phytoplankton have been extensively examined in estuarine ecosystems. For example, it has been well established that nutrient loading increases phytoplankton biomass, production and decomposition of phytoplankton-derived organic matter, and ultimately depletion of oxygen from bottom waters (10, 27, 64). However, the effects of nutrient loading on estuarine ecosystems are highly dependent on ecosystem-specific attributes, such as the salinity gradient, the extent of tidal mixing, the amount of riverine drainage, and the optical properties of the water column which affect light exposure to phytoplankton (as well as other light-dependent organisms). These attributes modulate the responses of estuaries to nutrients well before there are substantial increases in phytoplankton biomass, production of excessive organic matter, and/or oxygen depletion (10). We reasoned that analysis of HPLC pigment profiles might provide a valuable tool for monitoring the effects of nutrient loading on estuarine function because changes in pigment concentrations and composition can reflect the responses of phytoplankton communities to environmental conditions (2, 26, 37, 51, 52, 55, 57, 71, 72).

Effects of riverine discharge. We selected the ACE Basin and NI estuaries as study sites for this project because they belong to the same biogeographic province, have similar salinities, water temperatures, and mean tidal ranges, and are surrounded by intertidal marshes and forested wetlands with minimal anthropogenic development. Moreover, these estuaries are relatively close to each other (within 100 km). The major differences between these estuaries include the amount of riverine drainage, which can have substantial effects on water column nutrient quantity and quality, the DOC concentration, the turbidity (e.g., TSS and OSS), and light attenuation and in turn can significantly affect phytoplankton community struc-

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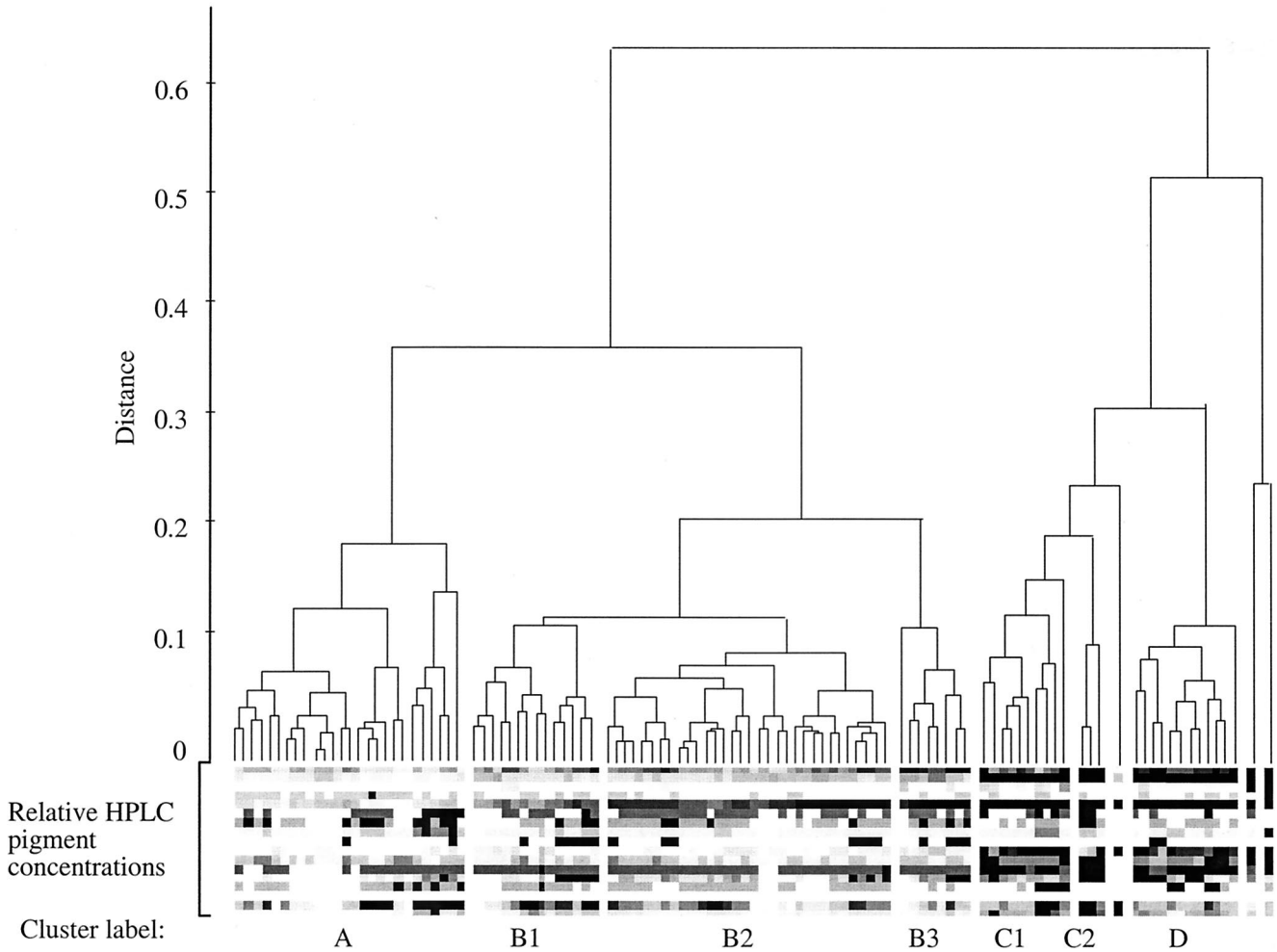

FIG. 5. Dendrogram showing the distribution of OCU in clusters at a dissimilarity level of 2.0 and the corresponding concentrations of HPLC pigments. The order of the HPLC pigments in each column (from top to bottom) is as follows: chlorophyll c_2, chlorophyll c_1, peridinin, 19′-But, fucoxanthin, neoxanthin, prasinoxanthin, violaxanthin, 19′-Hex, diadinoxanthin, alloxanthin, diatoxanthin, zeaxanthin, lutein, canthaxanthin, chlorophyll *b*, and carotenes. The intensities of the boxes indicate the relative concentrations of the pigments in samples, with white representing a low concentration and black representing a high concentration.

ture. In the ACE Basin estuary, relatively high nutrient levels (e.g., the levels of PO_4 and NO_3-NO_2 [Table 1]) were associated with higher levels of phytoplankton biomass (i.e., chlorophyll *a* concentrations [Fig. 2]) than the levels occurring in the NI estuary. This is consistent with a stimulatory effect of increased nutrient loading on phytoplankton population growth in the ACE Basin.

Riverine drainage might also be responsible for differences in DOC content between estuaries. Multiple sources of estuarine DOC are possible, including physicochemical processing (1, 5, 11, 17, 20, 21, 28, 45, 46, 49, 60, 63) and/or biological processing, such as phytoplankton exudates (3), sloppy feeding by higher trophic levels (23, 35, 40, 54, 56), and the effects of viroplankton (25, 70). Note, for example, the association of a high DOC content with the NI phytoplankton bloom period (Table 5). Comparative analyses of DOC composition and flux are needed to determine the relationship between riverine drainage and DOC concentrations in the estuaries.

The following factors presumably contributed to the high turbidity values observed in the ACE Basin estuary: riverine debris (e.g., terrestrially derived organic carbon), suspended and resuspended sediments, bioturbation (in the sense of Davis [14] and DeDeckere et al. [15]), flocculation, and/or abundant phytoplankton biomass near the surface of the water column. Previous studies have demonstrated that there is a well-developed estuarine turbidity maximum (ETM) in the ACE Basin (7, 44) but not in the NI. ETM are typically created by interactions between river flow and tidal forcing and are located at the boundary of the salt wedge (i.e., approximately station 3) (38). They are characterized as regions where there are elevated concentrations of flocculent material and suspended solids (7, 44). Our findings that the TSS content was substantially higher and the ratio of OS content to TSS content was lower in the ACE Basin estuary (Table 2) than in the NI estuary suggested that flocculent material and/or riverine debris, and not microbially derived debris, was primarily responsible for most of the observed turbidity in the ACE Basin estuary. High turbidity was associated with relatively high light

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TABLE 5. Properties possessed by OCU in clusters shown in Fig. 5

Variable	Properties possessed by OCU by cluster (no. of OCU)[c]							
	A (26)	B_1 (14)	B_2 (32)	B_3 (8)	C_1 (10)	C_2 (3)	D (12)	Significance
Concn of HPLC pigments[a,b]								
Chlorophyll c_2	0.05 ± 0.01A	0.01 ± 0.02B	0.08 ± 0.02B	0.11 ± 0.02C	0.09 ± 0.02B	0.11 ± 0.01D	0.11 ± 0.01B,D	$P < 0.001$
Chlorophyll c_1	0.05 ± 0.01A	0.06 ± 0.01B	0.07 ± 0.01B	0.08 ± 0.01B	0.14 ± 0.01C	0.13 ± 0.02C	0.15 ± 0.02D	$P < 0.001$
Peridinin	0.00 ± 0.00A	0.01 ± 0.01A	0.01 ± 0.01A	0.03 ± 0.02B	0.04 ± 0.03B	0.01 ± 0.01A,B	0.02 ± 0.01B	$P < 0.001$
19′-Butanoyloxyfucoxanthin	0.01 ± 0.01	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.00	0.00 ± 0.01	0.00 ± 0.01	NS
Fucoxanthin	0.27 ± 0.03A	0.36 ± 0.02B	0.40 ± 0.02C	0.45 ± 0.03C	0.59 ± 0.04D	0.53 ± 0.03E	0.67 ± 0.03F	$P < 0.001$
Neoxanthin	0.01 ± 0.01A	0.01 ± 0.01A	0.01 ± 0.00A	0.01 ± 0.01A	0.01 ± 0.01A	0.02 ± 0.02B	0.00 ± 0.01A	$P < 0.04$
Prasinoxanthin	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	NS
Violaxanthin	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	NS
19′-Hexanoyloxyfucoxanthin	0.00 ± 0.00A	0.00 ± 0.01A	0.00 ± 0.00A	0.01 ± 0.01B	0.00 ± 0.00A	0.00 ± 0.00A	0.00 ± 0.00A	$P < 0.026$
Diadinoxanthin	0.05 ± 0.01A	0.06 ± 0.01A	0.06 ± 0.01A	0.07 ± 0.01A	0.13 ± 0.02B	0.09 ± 0.03C	0.12 ± 0.02B	NS
Alloxanthin	0.02 ± 0.01A	0.02 ± 0.01A	0.02 ± 0.01A	0.02 ± 0.01A	0.04 ± 0.01B	0.09 ± 0.02C	0.03 ± 0.01B	$P < 0.001$
Diatoxanthin	0.01 ± 0.00A	0.01 ± 0.00A	0.01 ± 0.00A	0.01 ± 0.00A	0.02 ± 0.01B	0.03 ± 0.00C	0.02 ± 0.01B	$P < 0.001$
Zeaxanthin	0.02 ± 0.01A	0.03 ± 0.03A	0.02 ± 0.01A	0.04 ± 0.02A	0.03 ± 0.01A	0.12 ± 0.02B	0.04 ± 0.02A	$P < 0.001$
Lutein	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	NS
Canthaxanthin	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	NS
Chlorophyll *b*	0.05 ± 0.04	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.03 ± 0.01	NS
Carotenes	0.04 ± 0.02A	0.05 ± 0.02A	0.04 ± 0.01A	0.05 ± 0.01A	0.08 ± 0.02B	0.10 ± 0.01B	0.07 ± 0.01A	$P < 0.001$
No. of samples from:								
NI estuary	2	3	12	8	10	3	12	
ACE estuary	24	11	20	0	0	0	0	
Sampling periods represented (months)	April to July	May to October	April to October	April to October	July to September	October	July to October	
Physical properties[a]								
Temp (°C)	25 ± 5	26 ± 4	25 ± 5	25 ± 5	28 ± 3	21 ± 1	26 ± 4	NS
Vertical attenuation coefficient	2.4 ± 1.1	2.2 ± 0.9	2.4 ± 1.1	2.4 ± 2.2	2.8 ± 2.3	2.9 ± 2.7	3.0 ± 2.7	NS
Chemical properties[a]								
pH	7.5	7.5	7.5	7.3	7.3	7.6	7.4	NS
Salinity (‰)	28 ± 7	31 ± 3	29 ± 8	29 ± 13	30 ± 8	28 ± 13	25 ± 12	NS
O_2 saturation (%)	84.9 ± 10.1A	74.4 ± 10.6A	83.4 ± 18.2A	80.6 ± 6.1A	52.5 ± 16.1B	83.3 ± 12.6A	54.3 ± 10.0B	$P < 0.001$
Silicate concn (μM)	23.3 ± 21.0	24.1 ± 16.0	20.7 ± 17.6	08.6 ± 10.1	35.6 ± 33.8	38.1 ± 53.6	18.5 ± 24.1	NS
Nutrient concn[a]								
TN (μM)	37.9 ± 27.6A	34.6 ± 23.8A	27.7 ± 28.5A	10.5 ± 19.5A	46.3 ± 23.3A	00.0 ± 0.0B	31.3 ± 31.4A	$P < 0.01$
TDN (μM)	35.9 ± 11.3	35.2 ± 7.6	34.1 ± 9.8	38.8 ± 13.1	35.3 ± 13.0	37.0 ± 12.6	32.7 ± 12.1	NS
DON (mg/liter)	35.5 ± 26.0A	32.4 ± 22.6A	24.5 ± 25.0A	10.3 ± 19.1A	41.9 ± 21.1A	00.0 ± 0.0B	29.3 ± 29.6A	$P < 0.01$
NH_4 (μM)	1.73 ± 1.2	1.95 ± 1.9	2.25 ± 2.1	1.61 ± 1.6	3.93 ± 3.4	2.64 ± 1.0	2.73 ± 1.7	NS
NO_3/NO_2 (μM)	0.91 ± 1.5	0.84 ± 0.9	1.93 ± 3.5	2.57 ± 6.6	1.33 ± 3.3	2.20 ± 3.1	0.64 ± 1.1	NS
TP (μM)	1.6 ± 1.4	1.4 ± 1.3	1.3 ± 1.7	0.2 ± 0.4	2.0 ± 1.5	0.0 ± 0.0	1.6 ± 1.9	NS
TDP (μM)	0.2 ± 0.4	0.5 ± 0.4	0.4 ± 0.4	0.0 ± 0.1	0.3 ± 0.3	0.1 ± 0.1	0.4 ± 0.4	NS
PO_4 (μM)	0.6 ± 0.3A,B	0.7 ± 0.3A,B	0.5 ± 0.4A,B	0.3 ± 0.1A,B	0.3 ± 0.1A,B	0.2 ± 0.2B,C	0.2 ± 0.2B,C	$P < 0.001$
SS (μM)	46.4 ± 24.9	24.0 ± 22.6	29.2 ± 24.8	39.3 ± 18.9	15.7 ± 19.4	28.3 ± 7.9	17.1 ± 23.7	$P < 0.002$
OS (μM)	7.9 ± 3.5	3.9 ± 3.4	5.8 ± 4.9	8.3 ± 3.8	3.5 ± 4.3	5.6 ± 1.8	3.9 ± 5.1	$P < 0.014$
DOC (mg/liter)	5.6 ± 3.1A	4.5 ± 2.2A	4.7 ± 2.7A	3.7 ± 4.3A	6.4 ± 3.6A	7.1 ± 7.0A	16.1 ± 24.3B	$P < 0.020$

[a] Values are means ± standard deviations.
[b] Relative to chlorophyll *a*.
[a] One-way ANOVA was used to assess differences in cluster properties. Clusters with the same letter in the same row are not significantly different from one another based on SNK test. NS, not significant.

attenuation in the ACE Basin estuary, which may have had an effect on phytoplankton community structure and/or pigment concentrations.

Phytoplankton pigment diversity. The pigment diversity data were quite similar at different stations and in the two estuaries, and pigment diversity changed seasonally in both estuaries (Fig. 4). We anticipated that the number of different pigments along the salinity gradient would be more or less consistent due to the extensive tidal fluxes and mixing at sampling stations. However, the decreased pigment diversity in April 2002 was difficult to explain since it occurred just before the annual phytoplankton bloom in both estuaries, indicating that a specific taxonomic group or species dominated the phytoplankton community for a short period of time. Spatial-temporal pigment analyses supported this interpretation because 19′-But occurred at relatively high concentrations in both estuaries at this time (as shown in Fig. 2), which may be indicative of the presence of prymnesiophytes (29), raphidophytes (Lewitus, unpublished data), some symbiont-bearing dinoflagellates (6), or pelagophytes (9).

Phytoplankton pigment profiles. Comparison of spatial-temporal pigment data revealed that the annual phytoplankton bloom occurred at different times in the two estuaries. In the ACE Basin estuary the bloom occurred between April and March (Fig. 2), and in the NI estuary the bloom occurred between March and August (Fig. 2 and 3). These results suggest that riverine drainage caused substantial changes in pigment concentrations and shifts in the seasonal phytoplankton bloom, with chlorophyll *a* concentrations reaching their peak levels earlier in the year in the ACE Basin estuary than in the

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NI estuary. These results may reflect the presence of a prototypical spring bloom in the ACE Basin estuary versus a summer bloom in the NI estuary, the former driven by springtime nutrient loading (freshet) and the latter driven by nutrient regenerative processes and top-down (grazing) control (35). However, correlation coefficients for pigments and nutrients, as well as other environmental variables, did not yield any statistically meaningful relationships (data not shown), which suggests that more than one factor was likely responsible for the observed shifts in peak pigment concentrations.

Cluster analysis of the pigment profiles identified potential links between the clusters and the physical, chemical, and nutrient variables (Table 5). For example, subcluster C1 and cluster D (NI samples during the summer bloom) were associated with reduced oxygen saturation levels, suggesting that OCU in these groups were associated with heterotrophic processes. Under certain conditions, biological and chemical oxygen demand can exceed primary production, resulting in reduced oxygen concentrations (16). This apparently occurred for OCU in cluster D because this cluster had high DOC and low oxygen saturation levels. High DOC values are often associated with sloppy feeding by microzooplankton, which release DOC into the surrounding water (35), or with phytoplankton exudates (3). A pronounced influence of microzooplankton grazing on phytoplankton population growth during the NI summer bloom has been demonstrated previously (12, 35, 69).

Differences in the results obtained from spatial-temporal pigment analyses and those obtained from principal-component and cluster analyses were due, in part, to normalization of the data. For principal-component and cluster analyses, pigment concentrations were normalized to ambient chlorophyll *a* concentrations, while pigment data used for spatial-temporal pigment analyses were not. We normalized the data in this manner for two reasons: (i) to minimize biases due to extremely high or low chlorophyll *a* levels (i.e., extreme values for chlorophyll *a* would cause biases in the numerical analysis) and (ii) to compare our findings with those of other studies in which the authors used normalized data to calculate the best-fit pigment ratio (2, 26, 37, 51, 52, 55, 57, 71, 72). The best-fit ratio has been used by the matrix-factorization program CHEMTAX for estimating algal class abundance from HPLC pigments. CHEMTAX partitions total chlorophyll *a* into major phytoplankton groups (37, 72) and hence can provide some important insights into phytoplankton community composition. We chose not to use CHEMTAX in this study because of the paucity of information on phytoplankton pigment ratios in estuaries and because these ratios vary with growth conditions, light intensity, and nutrient quality and limitations (59). Furthermore, no comprehensive study has been conducted yet to

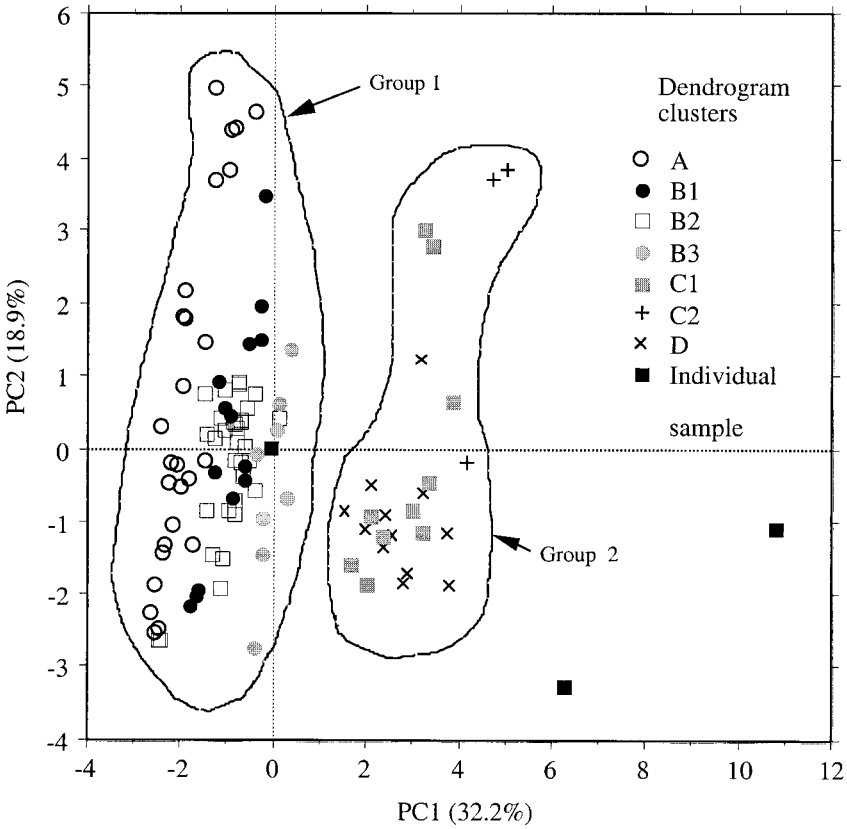

FIG. 6. Ordination plot produced from principal-component analysis of HPLC pigment concentrations normalized to the chlorophyll *a* concentration. Cluster labels refer to the dendrogram clusters (Fig. 5). Two major groups are outlined. Group 1 consists of OCU obtained from both the NI and ACE Basin estuaries, while group 2 consists of OCU obtained from NI estuary between July and October and represents phytoplankton associated with the seasonal chlorophyll *a* peak (see Fig. 3). The two outlying samples (■) are NI samples collected in July 2000.

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determine how these factors affect pigment ratios of phytoplankton associated with salt marsh estuaries.

Our observation that there are differences in the results obtained with raw and normalized pigment data highlights one of the problems associated with normalizing HPLC pigment data. Dividing HPLC pigments by the ambient chlorophyll *a* concentrations affected our ability to detect subtle changes in pigments that occurred in small concentrations relative to the chlorophyll *a* concentration (e.g., chlorophyll *b*, neoxanthin, prasinoxanthin, violaxanthin, zeaxanthin, lutein, and carotenes). However, since raw pigment concentrations most closely followed changes in mean chlorophyll *a* concentrations (based on 11 years of daily data collection by using the NI Long Term Ecological Research data set [Fig. 3]), we believe that data obtained from spatial-temporal analyses provided a more accurate reflection of pigment concentrations than normalized data (i.e., data from principal-component and cluster analyses). Nonetheless, results obtained from principal-component and cluster analyses and results obtained from spatial-temporal analysis both revealed significant differences in pigment concentrations by estuary, particularly in the period from July to October.

Pigment correlation coefficients. Correlation analyses of pigment concentrations by estuary revealed that most pigments from the ACE Basin estuary were poorly correlated with one another, indicating that the ACE Basin phytoplankton communities were changing in an inconsistent manner, presumably in response to changing local environmental conditions (e.g., riverine debris, nutrient loading, and/or ETM). Conversely, more pigments were highly correlated with one another in NI samples than in ACE Basin samples, implying that the NI phytoplankton communities were changing in a successional manner, with one dominant phytoplankton group and/or species replacing another. Such circumstances were not detectable for ACE Basin phytoplankton presumably because local environmental conditions were changing with riverine drainage and/or sample collection times were too far apart to adequately determine phytoplankton dynamics.

Contrasting phytoplankton community structure and nutrients by estuaries. Some pigments were highly correlated in both estuaries, indicating that there was substantial overlap in the phytoplankton community compositions (Table 4). This was expected because both estuaries experience frequent diurnal tidal mixing and have similar salinity and temperature regimens. However, the temporal pigment patterns differed considerably in the two estuaries. The correlations of pigment pairs for the entire time series were higher in the NI estuary than in the ACE Basin estuary, which is evidence that a greater number of phytoplankton groups persisted throughout the study in the NI estuary, although the total diversity in the NI estuary was lower. Thus, the ACE Basin phytoplankton populations appeared to be more ephemeral and diverse, while the NI populations were more predictable. This suggests that irregular or nonrepetitive changes in ACE Basin phytoplankton community composition occurred, possibly in response to irregular environmental conditions. Presumably, the ACE Basin phytoplankton communities were changing in response to changes in local environmental conditions related primarily to river drainage (e.g., suspended sediments, nutrient loading, salinity, and/or circulation).

Summary. In summary, two southeastern United States salt marsh estuaries were compared to determine their phytoplankton community spatial-temporal dynamics, as indicated by pigment composition. The estuaries differ in terms of the influence of riverine drainage, which explains the generally higher inorganic nutrient and chlorophyll *a* concentrations in the ACE Basin estuary. In fact, based on the mean chlorophyll *a* concentrations found at stations 1 to 4 in this study, 29% of the total NI samples and 7% of the ACE Basin samples should be categorized as low (≤ 5 μg liter^{-1}) according to the eutrophication classification scheme of Bricker et al. (8). Also, none of the NI chlorophyll *a* concentrations and 29% of the ACE Basin sample concentrations at these stations were more than 12.5 μg liter^{-1}, the median of the range classified as medium or moderately eutrophic (>5 to ≤ 20 μg liter^{-1}).

Our comparisons of phytoplankton community structures and regulation of these structures therefore may have revealed fundamental differences between southeastern salt marsh estuaries in different stages of eutrophication. A comparison of NI and ACE Basin pigment patterns indicated that the annual phytoplankton bloom occurred earlier in the ACE Basin estuary (spring) than in the NI estuary (summer), which may have reflected a greater influence of allochthonous environmental inputs, including NO_3 and PO_4 loading. We suggest that increased nutrient loading in tidally dominated high-salinity salt marsh estuaries may result not only in increases in phytoplankton biomass but also in a change in bloom properties from a predominance of summer blooms characterized by microbial loop dynamics (regulation by microzooplankton grazing and regenerated nutrients) to a predominance of spring blooms controlled by the availability of new nutrients (12, 13, 35). Evidence from pigment profiles also indicated that there were interestuary differences in the phytoplankton bloom community composition (although we hesitate to extrapolate to taxonomic composition until confirmatory microscopy is performed) and pigment group persistence or variability. Implied in this working hypothesis is that there are different types of estuaries, including estuaries dominated by autochthonous processes that regulate the dynamics of their biological communities, like the NI estuary, and estuaries that are regulated by allochthonous processes, like the ACE Basin estuary. The consequences of autochthonous versus allochthonous regulation may be manifested in community organizational properties. Our results are consistent with the interpretation that ecosystems that are subject to internal regulation and autochthonous processes are inherently more predictable than ecosystems that are subject to allochthonous processes.

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