Effects of Nitrogen and Phosphorus Additions on Phytoplankton Productivity and Chlorophyll *a* in a Subtropical Estuary, Charlotte Harbor, Florida

By Ralph T. Montgomery, Environmental Quality Laboratory, Inc.,
Port Charlotte, Florida;
Benjamin F. McPherson, U.S. Geological Survey; and
Edward E. Emmons, Environmental Quality Laboratory, Inc.,
Port Charlotte, Florida

U.S. GEOLOGICAL SURVEY
Water-Resources Investigations Report 91–4077

Prepared in cooperation with the
FLORIDA DEPARTMENT OF ENVIRONMENTAL REGULATION

Tallahassee, Florida
1991

U.S. DEPARTMENT OF THE INTERIOR
MANUEL LUJAN, JR., Secretary

U.S. GEOLOGICAL SURVEY
Dallas L. Peck, Director

For additional information,
write to:

District Chief
U.S. Geological Survey
Suite 3015
227 North Bronough Street
Tallahassee, Florida 32301

Copies of this report may be
purchased from:

U.S. Geological Survey
Books and Open-File Reports
Federal Center
Box 25425
Denver, Colorado 80225

Contents

Figures

Tables

CONVERSION FACTORS AND ABBREVIATIONS

Multiply	By	To obtain
millimeter (mm)	0.0394	inch
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot
kilometer (km)	0.6214	mile
square kilometer (km^2)	0.3861	square mile
cubic meter per second (m^3/s)	35.31	cubic foot per second
kilogram per day (kg/d)	2.205	pound per day

Additional abbreviations used in report:

microcuries (μCi)
micrometer (μm)
milligram per liter (mg/L)
milliliter (mL)
parts per thousand (‰)

Effects of Nitrogen and Phosphorus Additions on Phytoplankton Productivity and Chlorophyll *a* in a Subtropical Estuary, Charlotte Harbor, Florida

By Ralph T. Montgomery, Benjamin F. McPherson, and Edward E. Emmons

ABSTRACT

The response of natural phytoplankton assemblages in a subtropical coastal plain estuary, Charlotte Harbor, Florida, to inorganic nitrogen and phosphorus additions was determined from measurements of relative changes in both the uptake of carbon-14 and concentrations of chlorophyll *a*. The effects of nitrate plus nitrite nitrogen, ammonia nitrogen, and orthophosphorus additions over a series of concentrations were evaluated through *in situ* experiments conducted during periods of seasonally low and high river inflows. The responses to nutrient additions were evaluated for three different size fractions of phytoplankton. Relative changes of phytoplankton carbon uptake and chlorophyll-*a* concentrations were highly variable with regard to season, location, nutrient, and size fractions.

Within areas of the estuary characterized by lower salinities, phytoplankton exhibited a distinct seasonal pattern to additions of inorganic nitrogen. Under seasonally high freshwater inflow, phytoplankton showed little response to inorganic nitrogen additions, whereas under seasonally low inflow, phytoplankton responded to the inorganic nitrogen additions. The seasonally high freshwater inflow increased ambient inorganic nitrogen concentrations and water color. The high water color greatly reduced light penetration in the water column and limited phytoplankton productivity. The effect of nutrient additions in the higher salinity reaches of the estuary indicates that, under normal conditions, these areas are continually nitrogen limited. During periods of high freshwater inflow during the summer months, the estuary can be divided conceptually into a low-salinity zone where phytoplankton production is mediated by light availability, as determined by high water color, and a high-salinity zone where phytoplankton production is nitrogen limited. Seasonal nutrient concentration data and comparisons among inorganic nitrogen inputs and observed phytoplankton productivity support the experimental bioassay results. Each of these lines of evidence indicates that, exclusive of seasonal riverine influences that affect light penetration of the water column, nitrogen availability normally limits phytoplankton production within the Charlotte Harbor estuarine system.

INTRODUCTION

Background

Redfield (1958) suggested that phosphorus might ultimately be responsible for limiting net organic production in both freshwater and marine systems. Evidence that has become available since then, however, has led to a widely accepted generalization that marine environments are, for the most part, nitrogen limited (Ryther and Dunstan, 1971), whereas phosphorus predominantly limits production in freshwater systems (Jones and Lee, 1982; Sakshaug and Olsen, 1986). Common exceptions to this would include those aquatic systems influenced by either phosphate-rich deposits or nutrient pollution, although other exceptions have been reported. Myers and Iverson (1981) found evidence of phosphorus-limiting primary production in an estuary in the northeastern Gulf of Mexico, whereas McComb and others (1981) found evidence of seasonal shifts between nitrogen and phosphorus limitation in an estuary in western Australia. Davies and Sleep (1981) reported that phytoplankton production in the English Channel at times could be simultaneously and additively stimulated by additions of ammonia, nitrate, and phosphate, whereas Agins and Jaccarini (1982) found phosphorus to be the limiting nutrient in parts of the Mediterranean Sea. Wurtsbaugh and others (1985) observed nitrogen stimulation of phytoplankton carbon-14 uptake in a tropical alpine lake.

Although light, silica, iron, trace elements, and grazing have all been observed under certain conditions to limit phytoplankton production, macronutrient availability, especially nitrogen and phosphorus, has generally been demonstrated to be the key factor regulating primary productivity in estuarine and coastal waters (Ketchum, 1967; Boynton and others, 1982). Nitrogen and phosphorus exist in a variety of dissolved and particulate forms that may be directly or indirectly used for phytoplankton production. The distribution and availability of these various nutrient forms are dependent on the complex interactions of physical, chemical, and biological processes. It has been suggested that, in estuaries, nutrient recycling sustains much of the shorter term seasonal phytoplankton productivity (Nixon, 1981), whereas the input of new nutrients increases total availability, thus supporting longer term production. Anthropogenic development in coastal basins has often resulted in increased nutrient loadings and has been implicated in estuarine enrichment and increased phytoplankton productivity (Jaworski, 1981).

Measurements of a wide variety of growth or photosynthetic characteristics have been utilized to evaluate the response of phytoplankton communities to potential nutrient limiting conditions. Some of the more commonly used measurements of phytoplankton responses include changes in biomass (Graneli and others, 1986); assays of physiological and enzymatic properties (Smith and Kalff, 1981; Kana and others, 1985; Elser and Kimmel, 1986); measurements of ambient-nutrient ratios (Sklar and Turner, 1981) and use of these ratios in kinetic models (Kunikane and Kaneko, 1984); culture and chemostat studies of nutrient uptake rate and growth (Caperon and Myer, 1972a, 1972b; Collos, 1984; Kunikane and others, 1984; Sommer, 1985; Sanders and others, 1987); and field measurements of nutrient uptake or carbon fixation (Goldman, 1960; Davies and Sleep, 1981; Nalewajko and Garside, 1983; Wurtsbaugh and others, 1985).

This report is one of several published by the U.S. Geological Survey, in cooperation with the Florida Department of Environmental Regulation, as part of the Survey's environmental assessment of Charlotte Harbor. The report discusses the effects of nutrient additions on phytoplankton populations in the harbor. Phytoplankton constitute a major primary producer in the harbor. Increased population growth in the basin is projected to increase nutrient loading to the harbor (Hammett, 1988) and could stimulate phytoplankton growth and cause changes in water quality.

In this study, changes in the incorporation of carbon-14 and chlorophyll-*a* concentrations were used as measures of the response of phytoplankton assemblages to a series of added nutrient concentrations. Changes in the phytoplankton in response to nutrient additions were evaluated for the whole and each of three different size fractions: net- (>20 μm), nano- (between 5 and 20 μm), and pico-phytoplankton <5 μm). The importance of segmenting phytoplankton production and biomass measurements, such as chlorophyll *a*, by size fractions has been emphasized in freshwater (Munawar and others, 1978; Lane and Goldman, 1984), estuarine (Bruno and others, 1983; Furnas, 1983; Furuya and Marumo, 1983; Sellner, 1983), and marine (Malone, 1971; Marshall, 1985; Probyn, 1985) habitats. Although a variety of terms and size ranges have been applied (Sicko-Goad and Stoermer, 1984), the value of partitioning phytoplankton into functional groups based on size has been shown in assessing food-chain value (Janicki and DeCosta, 1984; Stockner, 1988) and in determining phytoplankton community responses to environmental variables (Malone, 1980). Differential responses of size-based assemblages to nutrient levels and ratios (Lane and Goldman, 1984; Probyn, 1985), as well as pollutants (Munawar and others, 1983), have been demonstrated.

Microscopic surveys of the taxonomic composition of the phytoplankton assemblages within each of the three measured size fractions have indicated the following generalized patterns with regard to salinity ranges within Charlotte Harbor. The smallest size fraction (<5 μm), at both intermediate and higher salinities, is characteristically dominated by Cryptophyceae (*Chroomonas* spp. and *Cryptomonas* spp.), with small Bacillariphyceae (*Thalossiosira* spp., *Nitzschia* spp. *Naviculla* spp.) also common nano-plankton components. In high salinity waters, the net-plankton size fraction is often dominated by chain-forming and larger diatoms. *Skeletonema costatum, Asterionella glacialis, Odentella sinensis, Corethron criphilum, Coscinodiscus centralis,* and *C. eccentricus,* as well as species of *Chaetoceros* and *Rizosolenia,* are seasonally important diatoms. Dinophyceae (*Ceratium* spp. and *Peridinum* spp.) are seasonally common in the largest size fraction during the summer months.

At intermediate salinities, blooms of *Skeletonema costatum* are commonly associated with relative increases in carbon uptake and chlorophyll-*a* concentration within the largest size fraction. Occasionally, however, dinoflagellates (*Prorocentrum micans, P. minimum, Gymnodinium* spp., and *Gyrodinium* spp.) comprise major components of the largest size fractions. Specifically, blooms of *Gyrodinium resplendens* seasonally dominate the larger size fractions at the mouth of the Peace River.

The pico-plankton size fraction at the low salinities often contained significant numbers of nonflagellated, smooth, circular to ovoid, green cells. Taxonomically, such cells probably include both Cyanophyceae (*Synechoccus* spp., *Chroococcus* spp., and *Anacystis* spp.) as well as Chlorophyceae (*Nannochloris* spp. and *Chlorella* spp.). Small phytoflagellates (*Chlamydomans* spp., *Carteria* spp., *Chroomonas* spp., and *Cryptomonas* spp.) are also common components of the pico-plankton at the lowest harbor salinities. The larger size fractions in the riverine areas of the estuary generally are characterized by mixtures of both Chlorophyceae (*Ankistrodesmus* spp., *Coelastrum* spp.,

Crucigenia spp., *Pediastrum* spp., *Scenedesmus* spp., and *Tetraedron* spp.), *Bacillariophyceae* (*Cyclotella* spp., *Nitschia* spp., *Navicula* spp., and *Fragillaria* spp.), and Cyanophyceae (*Anabaena* spp., *Anacystis* spp.).

Purpose and Scope

This report presents the results of a study to evaluate the effects of inorganic nitrogen and phosphorus additions on phytoplankton productivity and chlorophyll-*a* concentrations in a subtropical Florida coastal plain estuary and assesses the potential effects of increased nutrient concentrations on future phytoplankton production within the Charlotte Harbor system. The results of this estuarine phytoplankton productivity study are discussed in terms of the seasonal and areal variations in ambient-nutrient concentrations and hydrologic conditions. The potential implications that increased anthropogenic-nutrient inputs may have on primary productivity within specific hydrologically defined areas of the harbor also are examined.

DESCRIPTION OF STUDY AREA

Charlotte Harbor in southwest Florida (fig. 1) is a large, relatively shallow, subtropical, coastal plain estuary enclosed by a series of barrier islands. Water exchange with the Gulf of Mexico occurs primarily through Boca Grande Pass at the southern end of the harbor. The estuarine system covers an area of approximately 300 km^2 and has an average depth of only about 3 m. The estuary is vertically well mixed because of tidal flow, wind, and the shallow water depth. However, strong vertical-density stratification does occur in the northern parts of the harbor, predominantly during periods of high freshwater inflow (Fraser and Wilcox, 1981; Fraser, 1986).

Two rivers flow into the upper estuary: the Peace River, which has an average, total gaged, long-term inflow of approximately 49.5 m^3/s; and the Myakka River, which has an average inflow of about 18 m^3/s. Seasonal variations of inflow to the estuary are reflected in the hydrograph for the Peace River (fig. 2).

Charlotte Harbor is one of the least disturbed estuarine complexes in Florida (Taylor, 1974; Fraser and Wilcox, 1981; Froelich and others, 1985). During the past decade, however, there has been concern over the long-term effects of rapid population growth in the area and the effects of nonpoint sources of runoff on the water quality of the estuary. Parts of the northern Charlotte Harbor drainage basin contain rich phosphate deposits in the surficial and intermediate aquifers. Phosphate mining and processing have been active in the upper Peace River basin since the late 1800's, first beginning with direct dredging of river sediments. These deposits are natural sources of phosphorus to the Peace and Myakka Rivers and, ultimately, to Charlotte Harbor.

METHODS

The effects of nutrient additions were evaluated through a series of *in situ* experiments conducted during seasonally low and high periods of river inflows. In each experiment, natural phytoplankton populations were exposed to increasing concentrations of added nitrate plus nitrite (NO_3-N), ammonia (NH_4-N), and orthophosphorus (PO_4-P). The patterns of the responses of the natural phytoplankton assemblages to increased concentrations of each of these nutrients were determined from measurements of relative changes in the uptake of carbon-14 and chlorophyll-*a* concentrations. Estimates of phytoplankton production and biomass were made using the procedures described below.

In Situ C-14 Incubations

To reduce the chances of trace inhibitory or stimulatory contamination (Marra and Heinemann, 1984), all incubation bottles and lined caps were washed in 6 normal hydrochloric acid (6N HCL) prior to use, rinsed, triple rinsed with double-distilled water, and rinsed again with sample water immediately prior to filling. At each station, 450-mL subsurface-water samples were filtered through 500-micron cloth screening to remove particulate material and larger zooplankton. All *in situ* incubations were conducted using 473-mL, clear, flint glass, narrow mouth, round bottles. A predetermined number of replicate treatment bottles were then prepared by the addition of 0.5 mL of stock nutrient solutions to obtain the desired final added concentrations. An identical volume of the deionized, double distilled water used to prepare the stock nutrient solutions was added to an equal number of replicate controls. Dark bottles were used to determine blank background uptake. All incubation bottles were then inoculated with 10 µCi of trace-metal-free carbon-14 (Fitzwater and others, 1982) and quickly suspended at the predetermined 50-percent light depth and incubated *in situ*. To reduce potential diel variability (Harding and others, 1982a, 1982b; Malone, 1982; Brown and Field, 1985; Kana and others, 1985), samples at all stations were incubated for a predetermined period of time around apparent noon. After incubation, the samples were stored in the dark on ice and quickly returned to the laboratory where they were filtered.

Determination of Photosynthetic C-14 Uptake

To determine the total carbon-14 uptake of the whole sample and that attributable to the net-, nano- and pico-plankton size fractions, three separate 50-mL subsamples (Arthur and Rigler, 1967) were taken from each of the replicate-sample bottles. The initial subsample was filtered directly onto a 2.4-cm glass fiber filter using a low vacuum (<120 µm) of mercury (Hg). Two subsequent subsamples

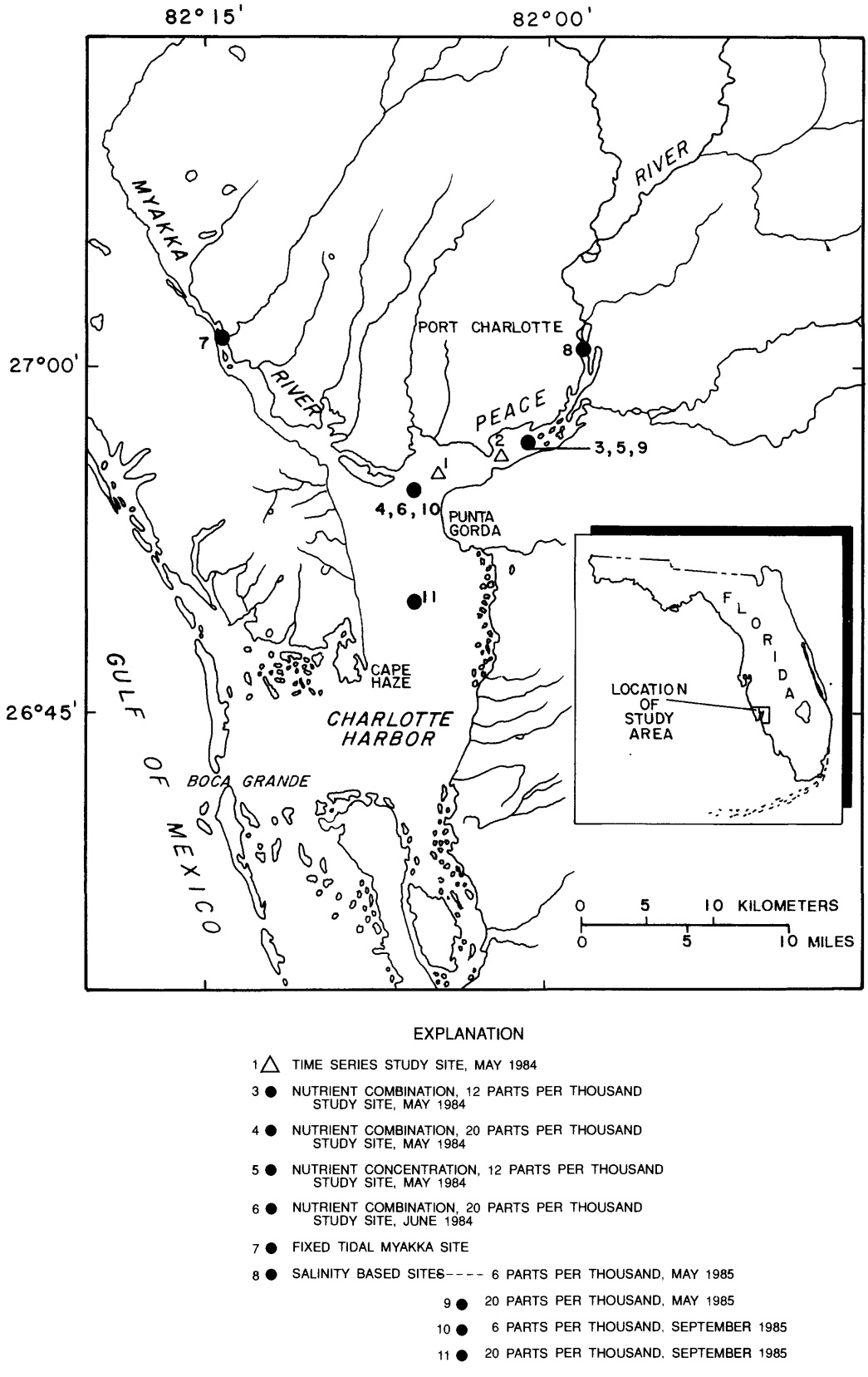

Figure 1. Location of Charlotte Harbor study area and *in situ* sites, 1984-85.

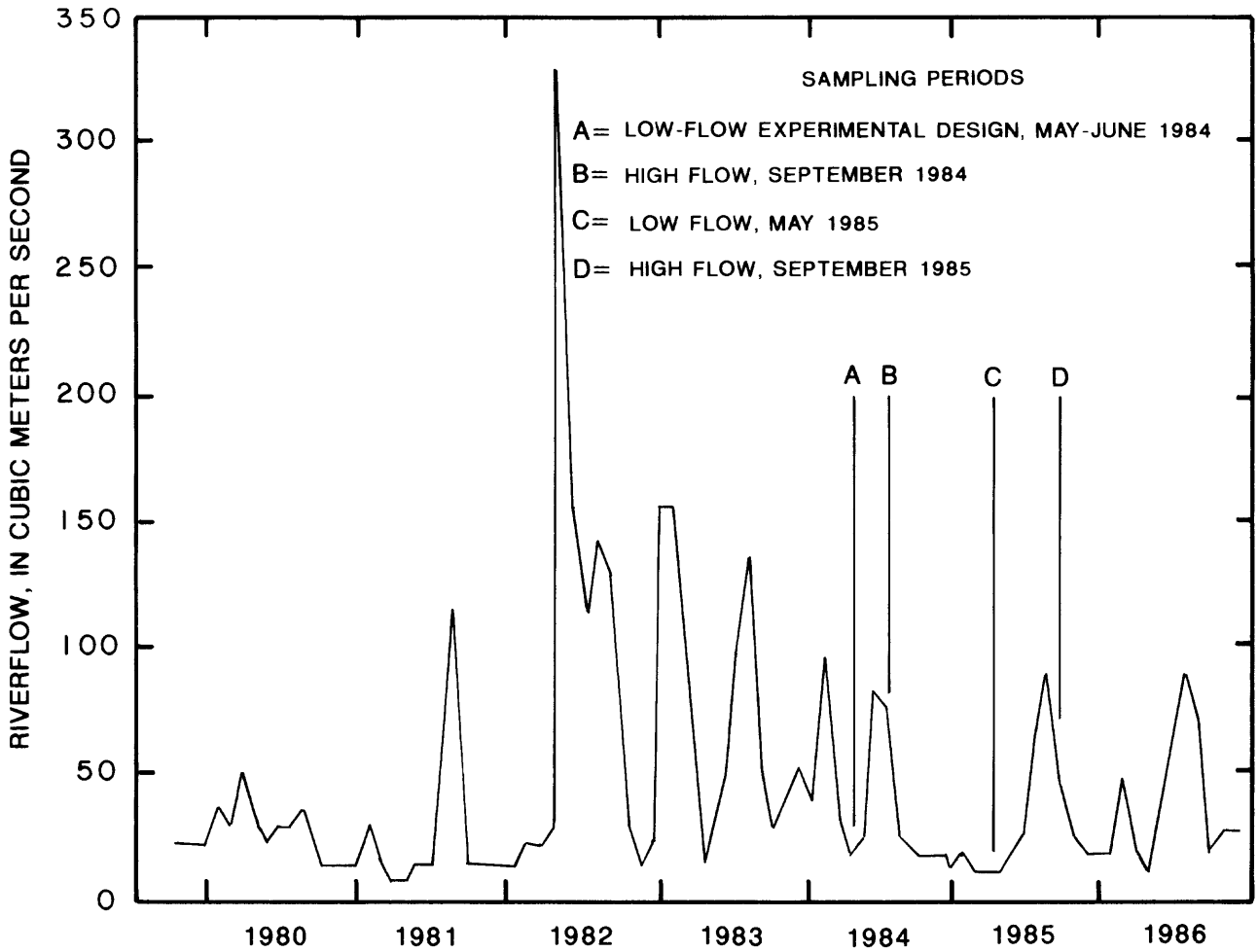

Figure 2. Total gaged flow of the Peace River, 1980-86, and dates of *in situ* flow-related studies.

were likewise filtered after having been respectively filtered through 20- and 5-micron screening. Three parallel filter blanks for each station were prepared by adding 10 µCi of carbon-14 to a corresponding 450-mL ice-stored water sample, taking 50-mL subsamples, and filtering and treating the blanks in the same manner as the initial subsample described above. The wet filters were then placed in scintillation vials. Any residual inorganic carbon-14 was removed by adding 0.5 mL of 0.1 N HCL (Lean and Burnison, 1979) and by allowing the vials to stand for 3 hours before the addition of 10.0 mL of scintillation fluor. The scintillation vials were stored in the dark for at least 24 hours before being counted. Each sample was counted for 30 minutes using a liquid scintillation system to provide counts per minute (CPM) in spectral region A with standard deviations of less than 0.2 percent. CPM were then converted to disintegrations per minute (DPM) using the channels ratio method. The counting efficiency was determined using a series of filters quenched by increasing amounts of phytoplankton onto which a known amount of carbon-14 labeled sucrose (Pugh, 1970) had been dried. The specific activity of the stock carbon-14 bicarbonate added to the samples was checked (Iverson and others, 1976) after each experiment. DPM were converted to milligrams carbon per cubic meter fixed, based on equations of Strickland and Parsons (1972).

Determination of Chlorophyll *a*

Chlorophyll-*a* concentrations for the >20 µm, 5 to 20 µm, and <5 µm size phytoplankton fractions were determined fluorimetrically (Strickland and Parsons, 1972) using 90-percent acetone extracted from unground filters prepared using the same filtering procedures used for the determination of carbon-14 incorporation. All reported chlorophyll-*a* concentrations represent single determinations obtained by compositing equal volumes from each of the replicate incubation bottles.

Chemical Analyses

Corresponding water samples at each station were collected in new, clean polyethylene containers. The containers were rinsed with sample water, filled, and immediately placed on ice in the dark. Analyses were conducted within recommended holding times for unpreserved samples using either U.S. Environmental Protection Agency (1979) or American Public Health Association (1989) standard methods (table 1).

Table 1. Methods used for chemical analyses

[EPA, U.S. Environmental Protection Agency, 1979; APHA, American Public Health Association, 1989]

Constituent or property	Method and reference	
Chloride	4500B	(APHA)
Color	110.2	(EPA)
NO_2+NO_3 nitrogen	353.2	(EPA)
NH_4 nitrogen	350.1	(EPA)
Silica	370.1	(EPA)
Inorganic carbon	415.1	(EPA)
Orthophosphorus	365.1	(EPA)
Total kjeldahl nitrogen	351.3	(EPA)
Total phosphorus	365.2	(EPA)
Chlorophyll *a* (fluorometric)	10200H,3	(APHA)

Data Analyses

Graphical and statistical analyses of results were conducted utilizing procedures within PC SAS (Statistical Analysis System Institute, Inc., 1987). In a number of experiments, carbon uptake and chlorophyll-*a* responses to nutrient additions were tested over a series of nitrogen and phosphorus concentrations. In these instances, general linear model procedures (polynomial regression or curve-fitting analysis) were employed to determine if the coefficients of the resulting phytoplankton responses over the range of nutrient concentrations tested were significantly different from zero and to describe the shape of the response curves. This methodology explicitly differs from that of the more often applied multiple-comparative procedures, such as Duncan's Multiple Range of Analysis of Variance (Duncan, 1957; Waller and Duncan, 1969). Multiple-comparative analysis would treat the series of nutrient concentrations as qualitatively different variables and test and contrast means for individual concentrations.

In testing responses to treatments with increasing concentrations of a single quantitative variable, such as a nutrient, polynomial regression or curve-fitting analysis provides the most effective and statistically valid method of estimating the relation of the observed response with the applied treatment (Chew, 1971; 1980; Peterson, 1977; Little, 1981). In utilizing polynomial regression to estimate the relation between the dependent response (phytoplankton carbon or chlorophyll *a*) and independent treatment variables (nitrogen forms and phosphorus), it is usually sufficient to partition the resultant treatment sum of squares into its linear, cubic, and quadratic components (table 2) while testing the significance of each (Chew, 1971). The advantages of this methodology over standard analysis of variance procedures are evident when applied to actual field data where progressive patterns in the data may be obscured by the variability between discrete-measurement intervals.

Nutrient concentrations were log transformed before applying polynomial regression procedures because phytoplankton responses were tested over nutrient concentration ranges of up to three orders of magnitude. The relative shapes of the resulting response curves were further analyzed using nonlinear, least squares, and fitting procedures to solve and plot the estimated resultant curves.

DESIGN OF NUTRIENT ADDITION EXPERIMENTS

A series of experiments was initially conducted to develop an overall experimental design. The purpose of the design was to evaluate adequately the potential phytoplankton-community responses to nutrient additions under high and low freshwater inflow conditions.

Time Series

Collos and Slawyk (1984) noted the importance of conducting an incubation time series experiment as part of any interpretation of the response of C-14 uptake to nitrogen additions. Their results indicated that, during the early periods of a time-course experiment, the addition of nitrogen could result in inhibition of C-14 uptake, whereas longer incubations resulted in marked stimulation. In addition, experimental incubation periods should be of sufficient length to observe a response, if present, while at the same time be short enough to reduce the possible "bottle-induced" effects often associated with extended incubations.

To define an adequate incubation period, two independent time-series experiments were conducted at estuarine salinities of approximately 12‰ during a period when ambient NO_3-N and NH_4-N concentrations were at detection limits (table 3). Each time-series experiment was conducted by incubating sets of control and treated bottles for periods ranging from 2 to 12 hours centered around apparent noon (eastern standard time). The longest incubation period samples were collected first. A drogue was used to mark the initial body of water sampled and sequential samplings were conducted hourly up to the 2-hour incubation period, which was collected 1 hour prior to apparent noon.

Table 2. Summaries by experiment of linear, quadratic, and cubic determinations from general linear model procedures for changes in carbon uptake and chlorophyll *a* in response to nutrient additions

[Student t value for testing null hypothesis; significance level, probability, underlined equals statistical significance; mg/m^3, milligrams per cubic meter]

Experiment	Parameter estimate			Student T			Significance level			Standard error		
	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
Carbon uptake (mg/m^3)												
Nutrient concentration												
12‰												
NO_3-N	167.279	11.450	-1.479	2.61	6.51	-1.67	0.0139	0.0001	0.1056	64.168	1.759	0.888
NH_4-N	-89.478	-6.848	1.464	1.50	-5.26	1.62	.1421	.0001	.1124	59.743	1.301	.901
20‰												
NO_3-N	297.327	9.159	-3.367	3.85	4.91	-2.70	.0005	.0001	.0105	77.191	1.865	1.248
NH_4-N	318.211	9.050	-3.575	3.39	3.91	-2.35	.0018	.0004	.0249	93.782	2.314	1.522
Flow-related studies												
Myakka fixed station												
September 1984												
PO_4-P	- 2.774	.598	- .066	- .24	1.23	- .36	.8135	.2309	.7226	11.638	.487	.185
NO_3-N	76.137	-1.682	-1.072	2.62	-1.38	-2.31	.0148	.1796	.0291	29.086	1.218	.463
NH_4-N	14.071	-1.583	- .237	.53	-1.42	- .56	.6007	.1662	.5787	26.554	1.111	.422
May 1985												
PO_4-P	-38.526	- .402	.439	-3.20	- .80	2.29	.0036	.4329	.0302	12.047	.504	.191
NO_3-N	55.627	2.020	- .366	3.22	2.79	-1.33	.0035	.0099	.1955	17.272	.724	.275
NH_4-N	3.906	4.693	.619	.21	6.02	2.10	.8355	.0001	.0460	18.622	.779	.296
September 1985												
PO_4-P	- 1.459	.092	.024	-1.52	2.29	1.57	.1401	.0304	.1275	.958	.040	.015
NO_3-N	- .059	- .022	- .003	- .05	- .46	- .17	.9593	.6474	.8664	1.135	.047	.018
NH_4-N	.906	- .046	- .019	.89	-1.07	-1.16	.3827	.2939	.2574	1.021	.043	.016
Peace River salinity zones												
May 1985												
6‰												
PO_4-P	-36.438	.931	.516	-2.29	1.40	2.04	.0302	.1734	.0131	15.895	.665	.252
NO_3-N	-17.823	.255	.304	-1.63	.56	1.75	.1145	.5813	.0912	10.913	.457	.173
NH_4-N	20.202	.429	- .197	2.11	1.07	-1.30	.0445	.2937	.2054	9.569	.400	.152
20‰												
PO_4-P	13.916	- .243	- .246	1.92	- .80	-2.14	.0660	.4306	.0421	7.252	.031	.115
NO_3-N	82.569	2.041	- .631	2.96	1.75	-1.42	.0065	.0921	.1661	27.886	1.167	.443
NH_4-N	82.122	8.590	- .058	3.96	9.90	- .18	.0005	.0001	.8618	20.738	.868	.329
September 1985												
6‰												
PO_4-P	2.485	- .074	- .037	.93	- .67	- .89	.3589	.5098	.3842	2.661	.111	.042
NO_3-N	3.031	.262	- .019	1.18	2.45	- .48	.2471	.0212	.6375	2.560	.107	.041
NH_4-N	3.411	.177	- .037	1.06	1.32	- .74	.2970	.1976	.4687	3.206	.134	.051
20‰												
PO_4-P	-16.847	.414	.358	- .65	.38	.87	.5208	.7056	.3912	25.880	1.083	.411
NO_3-N	230.491	2.134	-2.682	2.48	.55	-1.82	.0198	.5876	.0806	92.866	3.886	1.475
NH_4-N	309.663	-5.945	-4.477	4.16	-1.91	-3.79	.0003	.0672	.0008	74.370	3.112	1.181
Chlorophyll *a* (mg/m^3)												
Nutrient concentration												
12‰												
NO_3-N	14.565	.278	- .185	1.88	1.31	-1.72	.1569	.2824	.1831	7.753	.213	.107
NH_4-N	10.002	.349	- .114	1.60	2.60	-1.21	.1705	.0485	.2793	6.252	.135	.094
20‰												
NO_3-N	-8.7419	.897	.217	-18.88	80.16	29.01	.0001	.0001	.0001	.463	.011	.007
NH_4-N	-7.6802	.746	.192	- 2.79	11.20	4.30	.0495	.0004	.0127	2.757	.066	.045

Table 2. Summaries by experiment of linear, quadratic, and cubic determinations from general linear model procedures for changes in carbon uptake and chlorophyll *a* in response to nutrient additions—Continued

[Student t value for testing null hypothesis; significance level, probability, underlined equals statistical significance; mg/m^3, milligrams per cubic meter]

Experiment	Parameter estimate			Student T			Significance level			Standard error		
	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic	Linear	Quadratic	Cubic
					Chlorophyll *a* (mg/m^3)—Continued							
Flow-related studies												
Myakka fixed station												
September 1984												
2‰												
PO_4-P	.298	- .20	- .007	1.08	-1.72	-1.69	.3216	.1364	.1423	.276	.012	.004
NO_3-N	2.508	.015	- .027	2.08	.30	-1.44	.0827	.7758	.2005	1.206	.050	.019
NH_4-N	1.610	.073	- .011	2.35	2.55	- .97	.0572	.0438	.3694	.686	.029	.011
May 1985												
23‰												
PO_4-P	- .810	.008	.011	-1.92	.46	1.57	.1033	.6643	.1679	.422	.018	.007
NO_3-N	.961	.152	.004	1.09	4.13	.30	.3182	.0062	.7714	.883	.037	.014
NH_4-N	- .472	.296	.037	- .37	3.68	1.22	.8142	.0103	.2697	1.920	.080	.031
September 1985												
0‰												
PO_4-P	- .054	- .004	.001	- .62	- .97	.58	.5611	.3718	.5857	.087	.004	.001
NO_3-N	.218	- .013	.004	3.14	4.40	-3.49	.0201	.0045	.0130	.069	.003	.001
NH_4-N	- .025	- .003	.001	- .48	-1.19	.35	.6453	.2785	.7398	.051	.002	.001
Peace River salinity zones												
May 1985												
6‰												
PO_4-P	- .304	.007	.011	- .51	.30	1.20	.6251	.7767	.2739	.591	.025	.009
NO_3-N	1.072	.038	- .007	1.80	1.55	- .78	.1212	.1728	.4631	.594	.249	.009
NH_4-N	.598	.750	.005	1.88	5.64	1.07	.1086	.0013	.3247	.315	.013	.005
20‰												
PO_4-P	- .673	.045	.012	-1.56	2.49	1.76	.1706	.0472	.1295	.432	.018	.007
NO_3-N	1.573	.311	.016	.47	2.20	.30	.6578	.0897	.7763	3.376	.141	.054
NH_4-N	.193	.496	.048	.09	5.53	1.42	.9312	.0015	.2059	2.143	.090	.034
September 1985												
6‰												
PO_4-P	.082	- .001	- .001	.62	- .26	- .61	.5606	.8033	.5627	.134	.006	.002
NO_3-N	.247	.013	- .003	1.24	1.62	-1.00	.2608	.1556	.3556	.199	.008	.003
NH_4-N	.244	.017	- .003	2.10	3.43	-1.68	.0801	.0139	.1430	.116	.005	.002
20‰												
PO_4-P	.061	.017	- .002	.16	1.05	- .37	.8791	.3354	.7212	.385	.016	.006
NO_3-N	- .920	.889	.102	- .18	4.18	1.26	.8622	.0058	.2552	5.081	.212	.081
NH_4-N	2.490	.297	- .001	.55	1.57	- .01	.6024	.1679	.9897	4.530	.190	.071

The first time-series experiment investigated responses to the addition of 0.5 mg/L of NO_3-N. The results indicated a lag of 8 to 10 hours was required before a significant increase in the rate of carbon uptake was observed (fig. 3). A slightly shorter period of time was needed to observe increases in concentrations of chlorophyll *a* above control levels (fig. 4). No significant differences were observed among the measured phytoplankton size fractions in their responses to nitrogen addition.

A second experiment was conducted to further investigate phytoplankton reactions to nitrogen additions with respect to incubation length. Additions of 0.5 mg/L of NO_3-N and NH_4-N were compared with controls (figs. 5 and 6). Stimulation patterns of carbon uptake and chlorophyll *a* were similar for both nitrogen forms. The incubation period required to observe an increase in concentrations of chlorophyll *a* was again slightly less than that required for a corresponding significant increase in carbon uptake.

Time lags, as observed in these experiments between nutrient additions and measurable stimulation, should be expected to vary both with phytoplankton community composition and physiological condition. In each of the preceding experiments, incubation periods of 6 to 8 hours were sufficient to observe increases in phytoplankton carbon uptake and concentrations of chlorophyll *a*. However, to assure adequate detection of phytoplankton responses to experimental nutrient additions, standardized 10-hour incubations, centered at 1200 hours eastern standard time, were utilized in all subsequent investigations.

Single and Multiple Nutrient Additions

An experiment was conducted to investigate potential differences in the responses of phytoplankton communities at two salinity zones within the estuary to different concentrations and combinations of potentially limiting nutrients. Phytoplankton were collected from two salinity zones (approximately 12 and 20‰) at a time when both areas of the estuary were characterized by low ambient-nitrogen levels (table 3). Figures 7 and 8 depict the effects of nutrient additions. Differences in carbon uptake were analyzed using analysis of variance and contrast methods (alpha = 0.05) to compare mean uptake rates for various treatments.

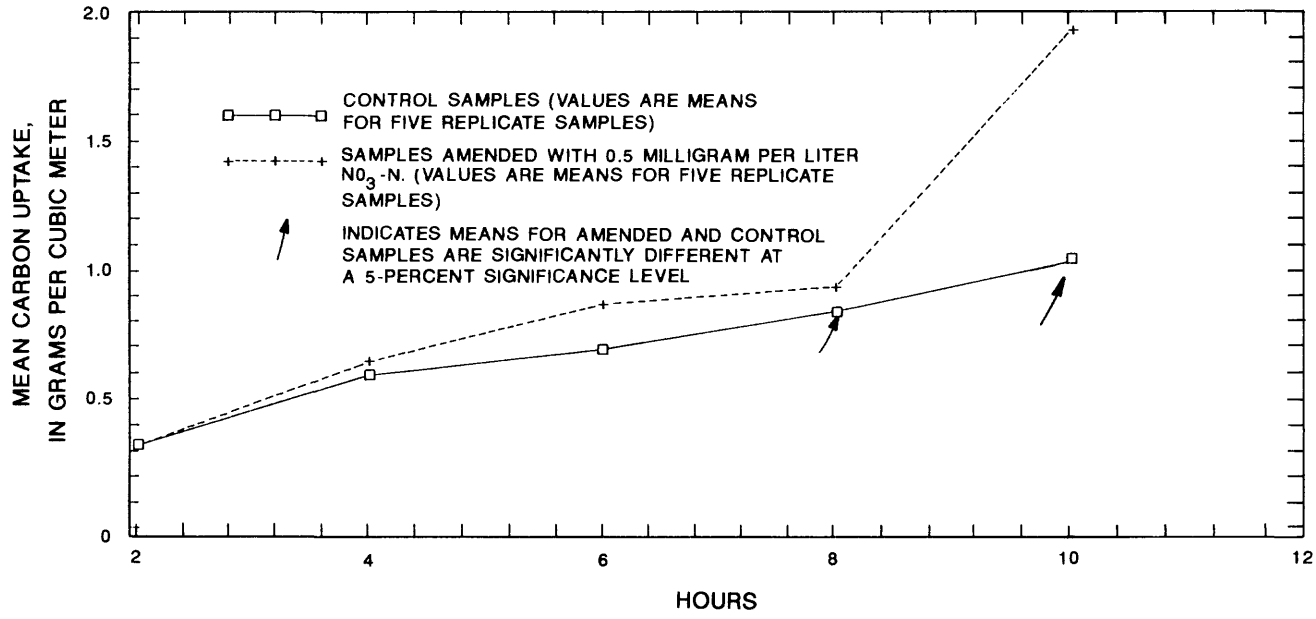

Figure 3. Relation between carbon uptake and period of *in situ* incubation at site 1 (approximately 12-parts-per-thousand salinity), May 10, 1984.

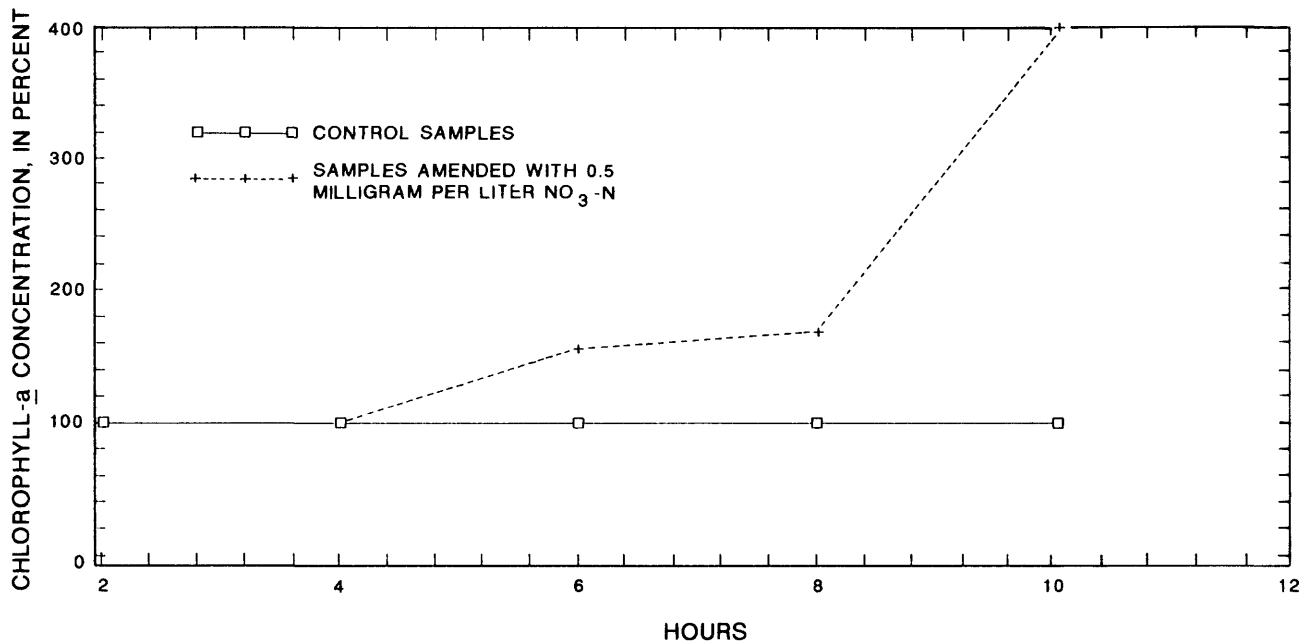

Figure 4. Relation between chlorophyll-*a* concentration of amended samples as percent of chlorophyll *a* in control samples and period of *in situ* incubation at site 1, May 10, 1984.

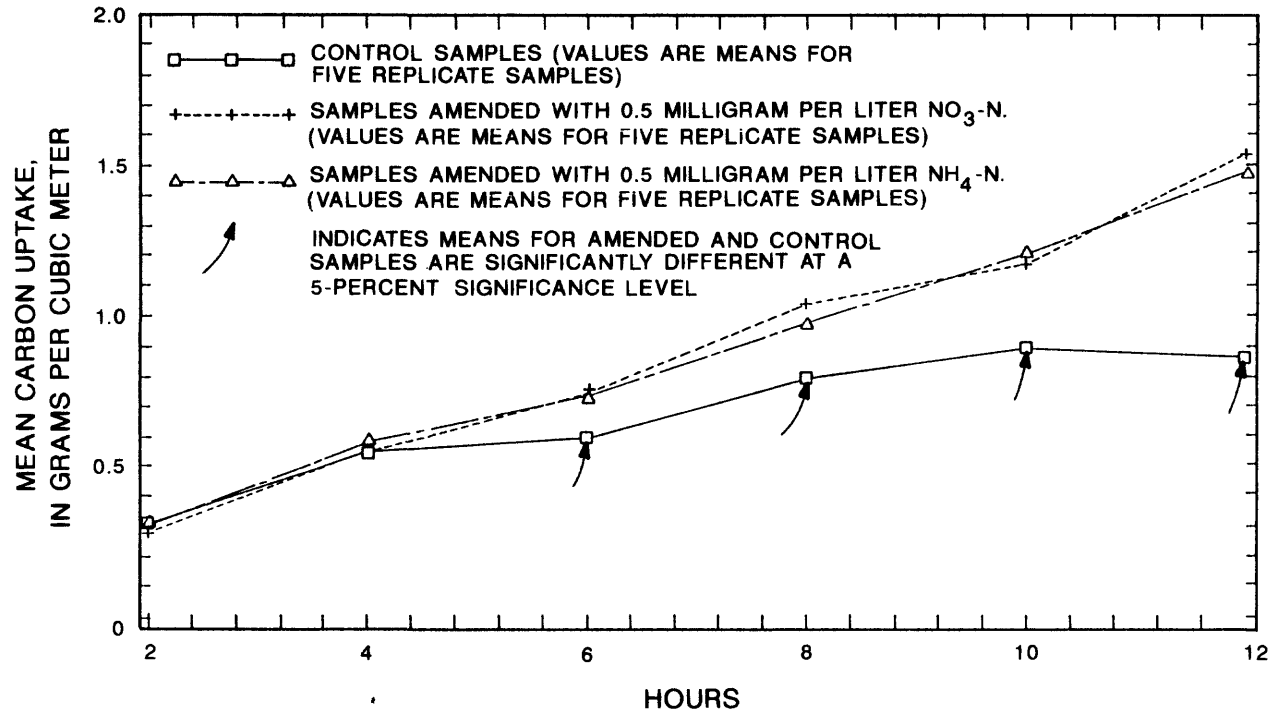

Figure 5. Relation between carbon uptake and period of *in situ* incubation at site 2 at a salinity of approximately 12 parts per thousand on May 17, 1984. (No significant differences between means of amended samples.)

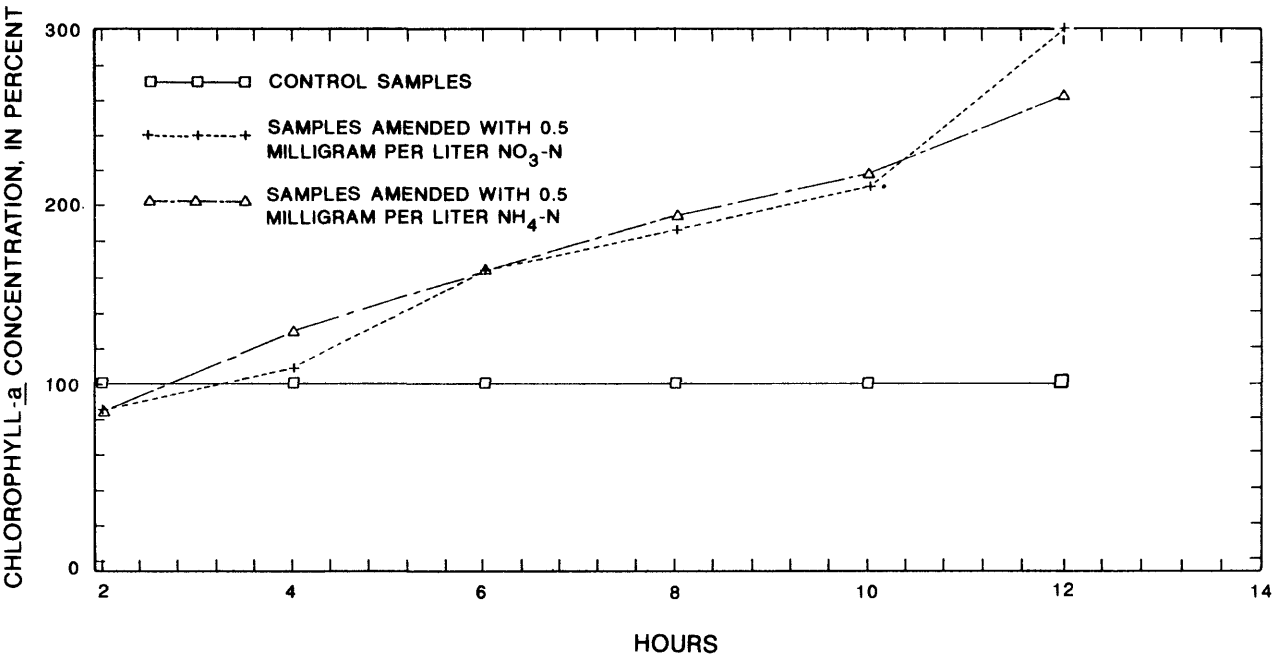

Figure 6. Relation between chlorophyll-*a* concentration of amended samples as percent of chlorophyll *a* in control samples and period of *in situ* incubation at site 2, May 17, 1984.

Table 3. Ambient water-quality data and preceding Peace River flows for each experiment

[NO_3 = nitrite plus nitrate; NH_4 = annomia nitrogen; OP = orthophosphate; IOC = inorganic carbon; TKN = total kjeldahl nitrogen; TP = total phosphorus; < = less than; ‰ = parts per thousand. All values are in milligrams per liter except color, which is in platinum-cobalt units]

Experiment	Date	Chloride	Color	NO_3	NH_4	OP	Silica	IOC	TKN	TP	Average total gaged Peace River flow, in meters per second for the preceding indicated number of days		
											7 days	14 days	28 days
Preliminary design experiments													
Time series no. 1													
2 hours	5/10/84	7,110	49.0	<0.001	<0.013	0.363	1.34	18.1	1.01	0.393			
4 hours	5/10/84	7,260	50.0	< .001	< .013	.360	1.37	18.1	.96	.395			
6 hours	5/10/84	7,270	50.5	< .001	< .013	.363	1.41	18.6	.99	.398	5.7	12.5	26.8
8 hours	5/10/84	7,130	52.0	< .001	< .013	.378	1.44	18.2	1.26	.403			
10 hours	5/10/84	7,270	54.0	< .001	< .013	.368	1.47	18.3	.99	.395			
Time series no. 2													
2 hours	5/17/84	6,480	50.0	.002	< .004	.396	1.40	17.8	1.14	.689			
4 hours	5/17/84	6,480	50.0	.001	< .004	.404	1.40	17.4	1.02	.486			
6 hours	5/17/84	6,610	51.0	.001	< .004	.380	1.40	18.1	.95	.423			
8 hours	5/17/84	6,280	51.5	.001	< .004	.397	1.39	17.8	1.02	.469	16.1	5.7	18.3
10 hours	5/17/84	6,160	53.0	.002	< .004	.401	1.40	17.9	1.02	.441			
12 hours	5/17/84	6,460	51.0	.002	< .004	.389	1.38	17.9	1.02	.442			
14 hours	5/17/84	6,470	52.0	.004	.004	.400	1.40	18.2	1.10	.469			
Nutrient combinations													
12‰	5/22/84	7,500	50.0	< .003	< .018	.399	1.16	19.2	1.15	.454	5.3	4.8	15.1
20‰	5/22/84	10,800	33.0	< .003	< .018	.247	.78	22.1	.78	.272			
Nutrient concentrations													
12‰	5/31/84	6,670	50.0	.003	< .010	.471	1.17	17.2	.93	.543	9.3	4.1	5.7
20‰	6/01/84	10,900	28.0	< .001	< .001	.251	.42	19.9	.78	.280	9.1	3.6	5.2
Flow-related studies													
Myakka River fixed station													
September 1984													
2‰	9/27/84	1,300	122	.017	.019	.227	2.25	16.8	.90	.267	23.8	38.8	64.9
May 1985													
23‰	5/22/85	12,500	33.0	< .001	< .001	.397	.32	19.6	1.35	.412	2.4	1.3	1.8
September 1985													
0‰	9/16/85	190	390	.483	.185	.518	3.18	6.72	1.47	.559	122.2	68.2	43.9
Peace River salinity zones													
May 1985													
6‰	5/08/85	4,220	52	.077	.052	.830	1.29	2.5	.96	.886	.9	1.8	2.4
20‰	5/10/85	11,200	43	< .001	< .001	.313	.04	25.4	1.10	.330	1.9	1.7	2.3
September 1985													
6‰	9/17/85	3,720	275	.003	.221	.631	3.68	12.6	1.39	.674	111.8	77.0	40.5
20‰	9/24/85	10,700	70	.001	< .001	.183	1.08	18.5	.76	.204	36.4	111.8	39.6

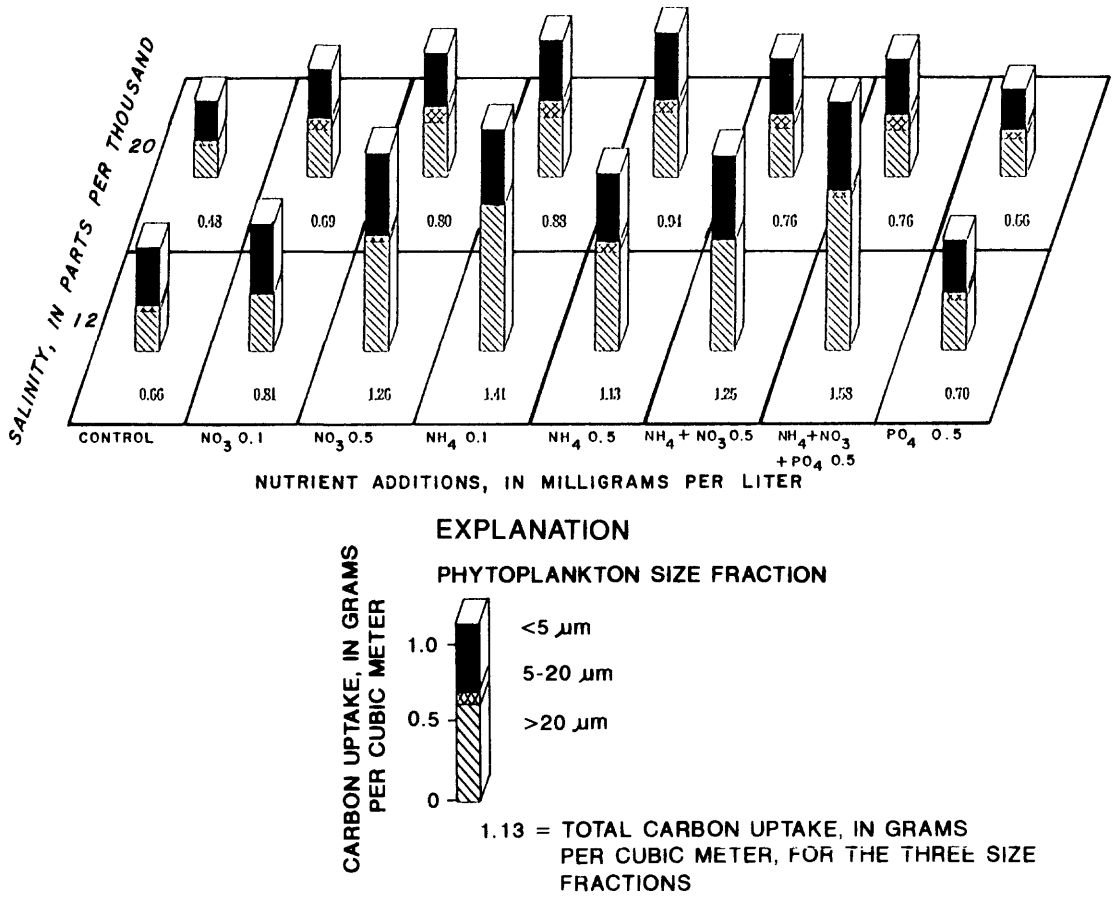

Figure 7. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at salinities of approximately 12 (site 3) and 20 (site 4) parts per thousand, May 22, 1984. (Each bar represents mean of five replicates.)

Comparisons of phytoplankton carbon uptake at 12‰ indicated:

1. No significant difference between controls and replicates treated with PO_4-P;
2. Both forms of nitrogen stimulated production, with the effect of NH_4-N at 0.1 mg/L being significantly greater;
3. Increasing the concentration of NO_3-N resulted in increased uptake, whereas increasing NH_4-N from 0.1 to 0.5 mg/L had the opposite effect;
4. NO_3-N + NH_4-N had no greater effect than either of the two nutrients individually, whereas the addition of PO_4-P in combination with the two forms of nitrogen resulted in a significantly higher level of carbon uptake; and
5. NO_3-N at 0.5 mg/L and both concentrations of NH_4-N significantly increased the relative amount of carbon taken up by the largest phytoplankton size fraction (>20 µm).

The effects on carbon uptake to similar nutrient additions at 20‰ salinity showed slightly different patterns:

1. No significant difference was noted between controls and replicates treated with PO_4-P;
2. Both forms of nitrogen significantly increased carbon uptake. Stimulation by NH_4-N was significantly greater than that for NO_3-N;
3. Increasing the concentration of either of the forms of nitrogen did not significantly increase carbon uptake;
4. Neither the combination of nitrogen forms nor the further addition of PO_4-P increased carbon uptake;
5. Both forms of nitrogen at 0.5 mg/L resulted in a significant increase in relative percent of production by the 5- to 20-µm size fraction.

Within each salinity zone, chlorophyll-*a* concentrations generally exhibited stimulation patterns similar to those observed for carbon uptake.

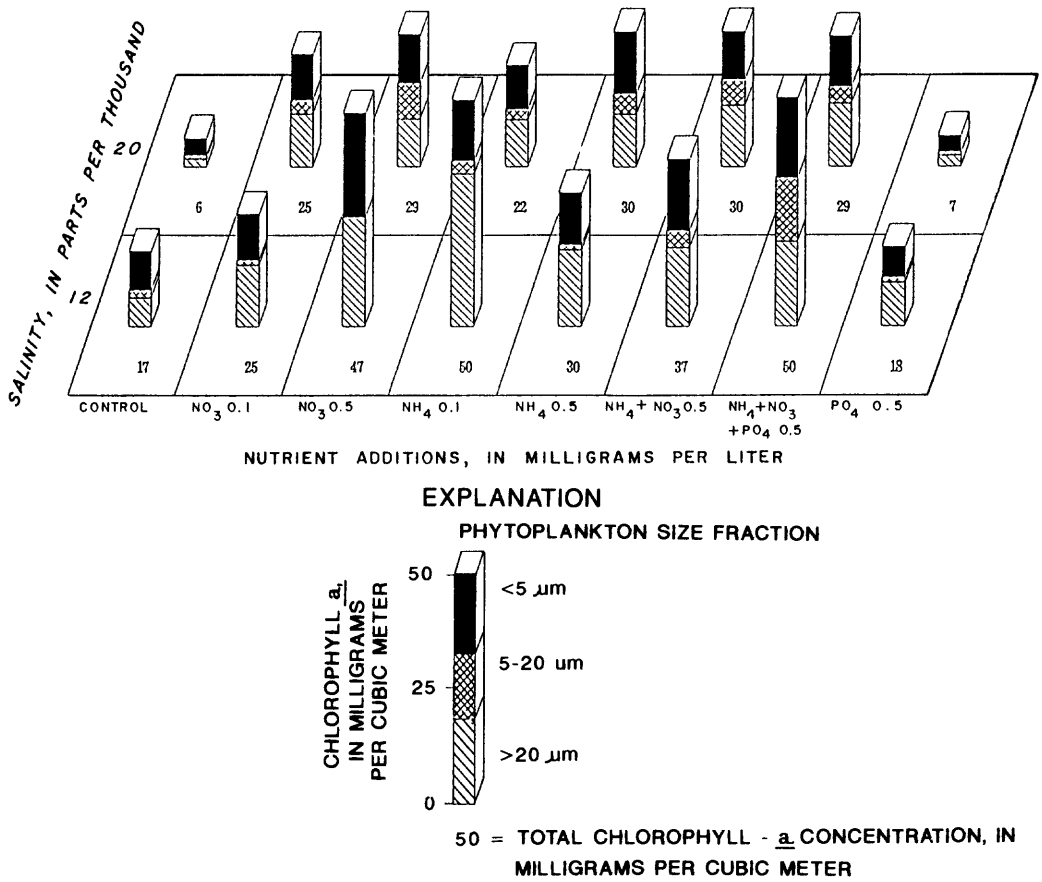

Figure 8. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at salinities of approximately 12 (site 3) and 20 (site 4) parts per thousand, May 22, 1984.

Concentration Response

Based on the results of the preceding experiments, the effects of nutrient additions over a wider range of nitrogen concentrations were measured at the same two salinities. The purpose of this experiment was to determine a standardized range of added-nutrient concentrations adequate to statistically evaluate phytoplankton responses within different estuarine salinity regimes. At 12‰, the effects of nitrogen on phytoplankton carbon uptake and concentrations of chlorophyll *a* were investigated using nutrient additions that resulted in final concentrations for NO_3-N ranging from 0.0 to 1.0 mg/L and from 0.0 to 0.8 mg/L for NH_4-N. At 20‰ salinity, phytoplankton responses to nutrient additions were tested over final concentrations ranging from 0.0 to 0.4 mg/L for both NO_3-N and NH_4-N.

Observed changes in phytoplankton carbon uptake and chlorophyll *a* over ranges of nitrogen additions at the 12‰ salinity zone are shown in figures 9 and 10. Concentrations of the additions are presented in ascending order and are not linear. Changes in carbon uptake and chlorophyll *a* are indicated for the whole (total block height) and each of the three measured size fractions. In this (and following concentration experiments), polynomial regression procedures were used to test for changes in the carbon uptake and chlorophyll-*a* concentrations. Total phytoplankton responses were tested for significant linear, cubic, and quadratic treatment sums for models with coefficients significantly different from zero. Additions of NO_3-N significantly increased carbon uptake, which steadily increased and then flattened at the highest concentrations. Analysis indicated that the overall carbon uptake response to NO_3-N could best be described by linear and quadratic functions (table 2). Carbon uptake also increased with NH_4-N additions. The pattern of the treatment response, however, was significantly different from that for NO_3-N. Carbon uptake was greatest at the lowest levels of NH_4-N addition and then slowly declined with increasing concentrations. Analysis of this pattern indicated a significant quadratic component (table 2). Corresponding changes in chlorophyll-*a* concentrations that resulted from increasing additions of the two nitrogen forms (fig. 10) were similar to one another and characteristically different from the patterns of increase in carbon uptake. Maximum stimulation of chlorophyll *a* took place at much lower NO_3-N concentrations

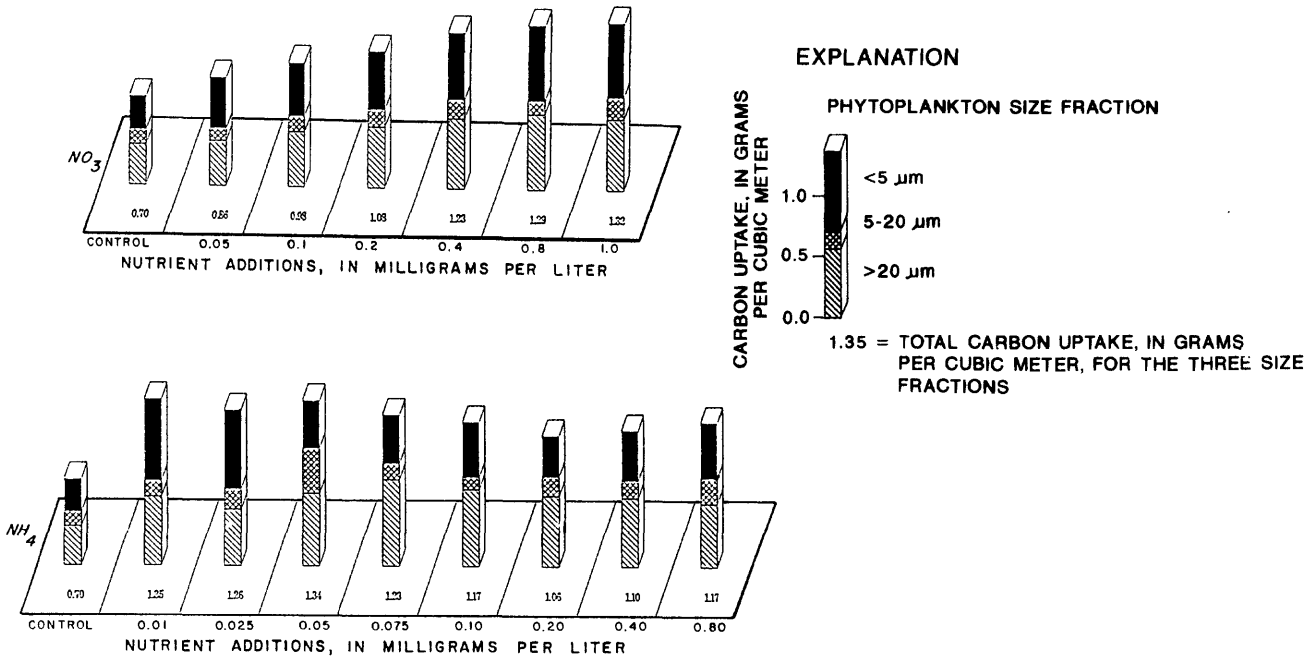

Figure 9. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at site 5, May 31, 1984. (Each bar represents mean of five replicates.)

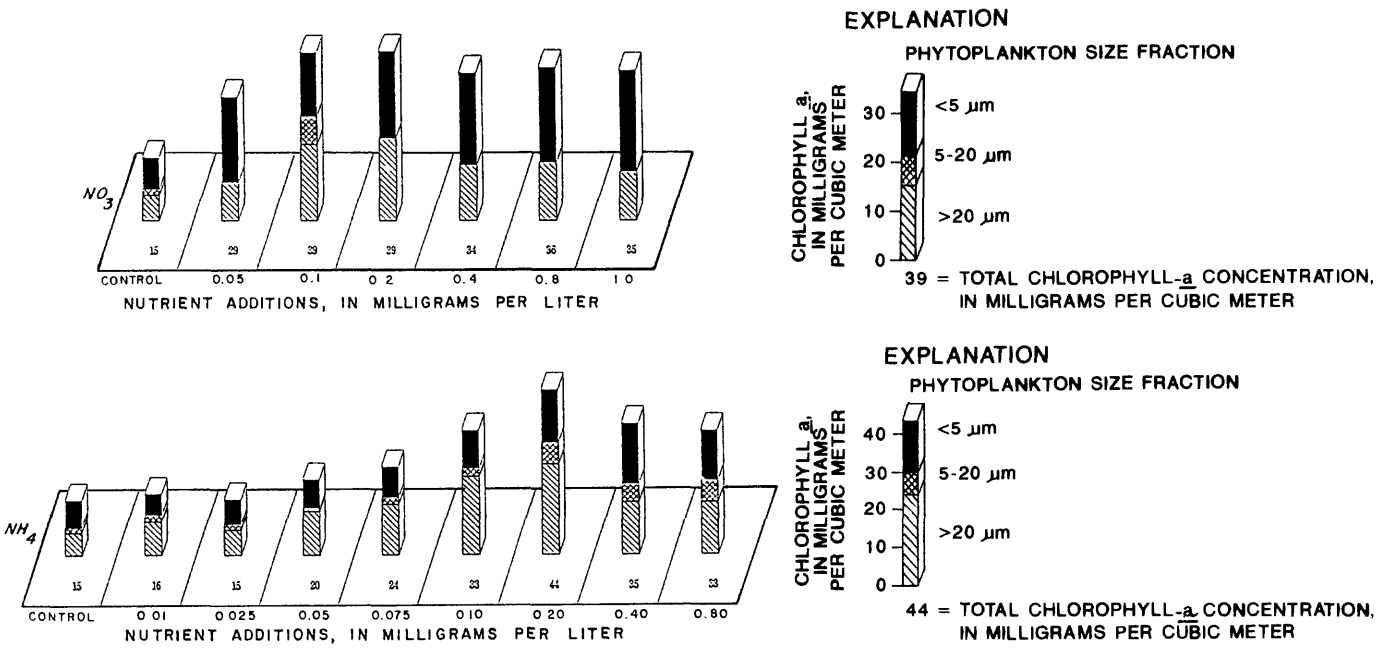

Figure 10. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at site 5, May 31, 1984.

than that for carbon uptake. By comparison, the maximum stimulation of chlorophyll a occurred at high levels of NH_4-N, unlike the carbon uptake response.

At 20‰ salinity, both carbon uptake and chlorophyll-*a* concentrations (figs. 11 and 12) significantly increased with additions of NO_3-N and NH_4-N (table 2). Solution of carbon uptake models, using nonlinear least squares fitting procedures, indicated that the general shape of the resulting curves were similar, with the asymptote of the response curve being slightly higher for NH_4-N than for NO_3-N. Corresponding analysis of chlorophyll-*a* increases in response to increasing NO_3-N and NH_4-N additions indicated the resulting curves were similar.

Analysis of relative changes in carbon uptake and chlorophyll-*a* concentrations within the three measured phytoplankton size fractions in response to nutrient additions at 12 and 20‰ salinity generally failed to indicate any systematic or progressive differences. An exception, however, was the relative changes in chlorophyll-*a* concentrations among size fractions in response to increasing NO_3-N levels at 20‰. Although chlorophyll-*a* concentrations within all size fractions rose with increasing NO_3-N concentrations, there was a progressive increase in the relative percent of chlorophyll *a* in the >20-µm size fraction and a corresponding decrease in the <5-µm fraction.

RESULTS OF NUTRIENT ADDITION EXPERIMENTS

Based on the preceding series of experimental findings, a methodology was developed to investigate the response of phytoplankton communities within the Charlotte Harbor estuarine system to nutrient additions under differing conditions of freshwater inflow. Changes in carbon uptake and chlorophyll-*a* concentrations that resulted from additions of NO_3-N, NH_4-N, and PO_4-P, each over a series of 10 final concentrations (0.0, 0.001, 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/L), were investigated. Nutrient addition experiments were conducted at a fixed location in the tidal part of the Myakka River (fig. 1) during two periods of high and one period of low freshwater inflow and at two salinity zones (6 and 20‰) within the lower tidal Peace River-upper Charlotte Harbor part of the estuary during corresponding periods of low and high Peace River inflow.

In each experiment polynomial models of the form

$$\text{carbon uptake} = b_0 + b_1(\text{Conc.}) + b_2(\text{Conc.})^2 + b_3(\text{Conc.})^3$$

were constructed for each nutrient. Similar models also were constructed using chlorophyll-*a* concentrations as a response variable.

Significance determinations of the models were done by testing the coefficient for each term of the regression (b_1, b_2, b_3 not equal to 0). Significance of these coefficients dictated shape of the response curve. A significant linear (b_1) response was characterized by monotonic changes in carbon uptake with increasing nutrient concentrations; significant quadratic (b_2) responses by polynomial curves with one inflection point; and significant cubic responses by polynomial curves with two inflection points. Different response curves were generated for each experiment (location, date), nutrient, and response variable (carbon uptake, chlorophyll-*a* concentrations) and are summarized in table 2.

Tidal Myakka River Fixed Station

High Flow

The first experiment at the fixed Myakka River site was conducted in September 1984 during a period of relatively high river flow (fig. 2). Ambient water conditions were characterized by low salinity, high color, and relatively high macronutrient concentrations (table 3). Phytoplankton carbon uptake and chlorophyll-*a* concentrations were highest in the smallest size fraction (<5 µm), as indicated by the controls (figs. 13 and 14).

Carbon uptake and chlorophyll-*a* concentrations significantly increased in response to nitrogen additions (table 2). Uptake and concentrations initially increased and then reached a point where the effects of stimulation from increasing NO_3-N additions remained relatively constant (figs. 13 and 14). The observed changes in carbon uptake in response to increasing NH_4-N additions, by comparison, were greatest at lower concentrations and then declined steadily with increasing NH_4-N concentrations. Changes in chlorophyll-*a* concentrations in response to increasing NH_4-N and NO_3-N additions initially increased and then remained relatively constant with further additions. There were no systematic changes or differences apparent in the responses of the three measured phytoplankton size fractions resulting from stimulation by nitrogen additions.

Graphical analysis appeared to indicate that both carbon uptake and chlorophyll-*a* concentrations declined slightly with increasing concentrations of PO_4-P (figs. 13 and 14). Statistical analysis, however, failed to identify any significant decline (table 2).

Low Flow

A second experiment was conducted at the Myakka River site in May 1985 during a period of low river flow. Ambient water conditions were characterized by high salinity, reduced water color, and inorganic nitrogen concentrations at detection limits. Phytoplankton carbon uptake and chlorophyll-*a* concentrations were divided nearly equally between the largest (>20 µm) and smallest (<5 µm) size fractions, as indicated by the untreated controls.

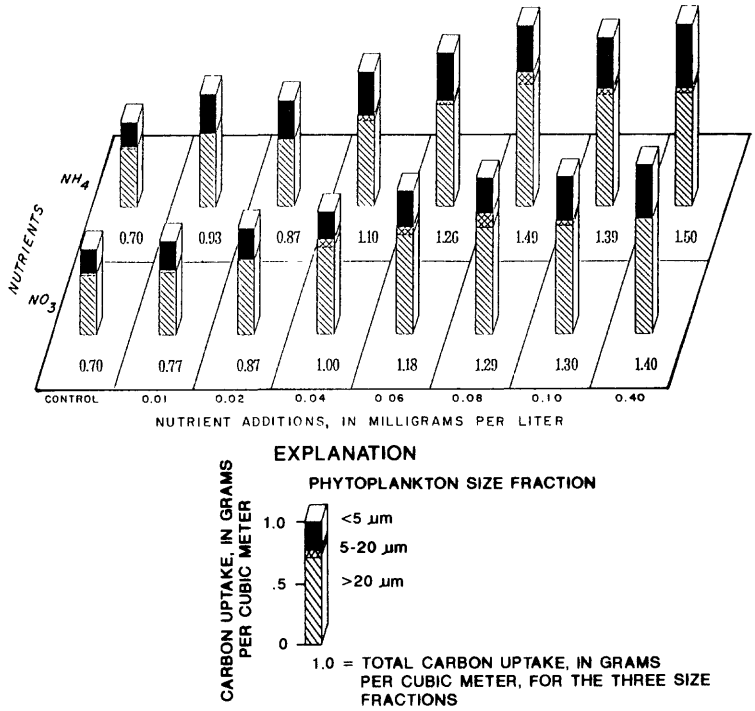

Figure 11. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 20 parts per thousand at site 6, June 1, 1984. (Each bar represents mean of

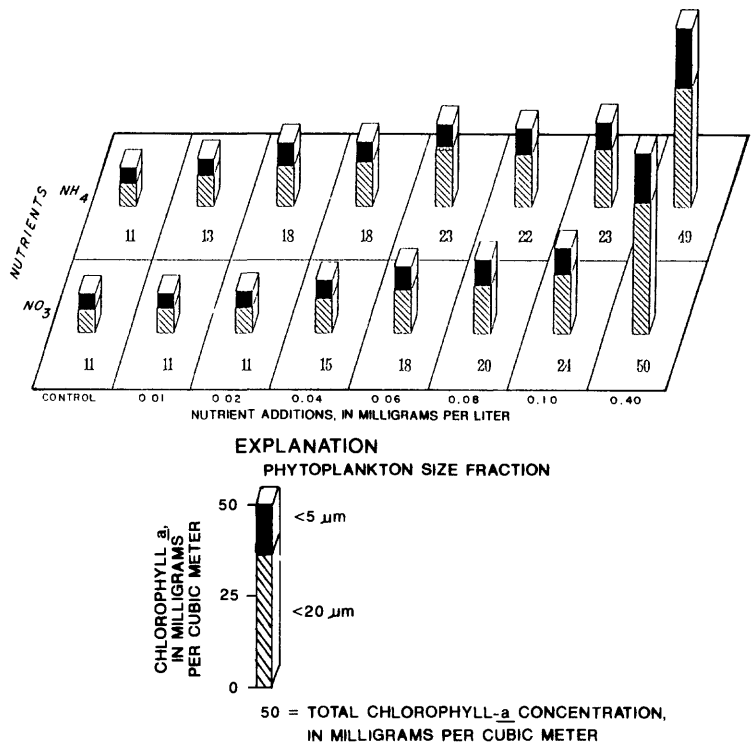

Figure 12. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 20 parts per thousand at site 6, June 1, 1984.

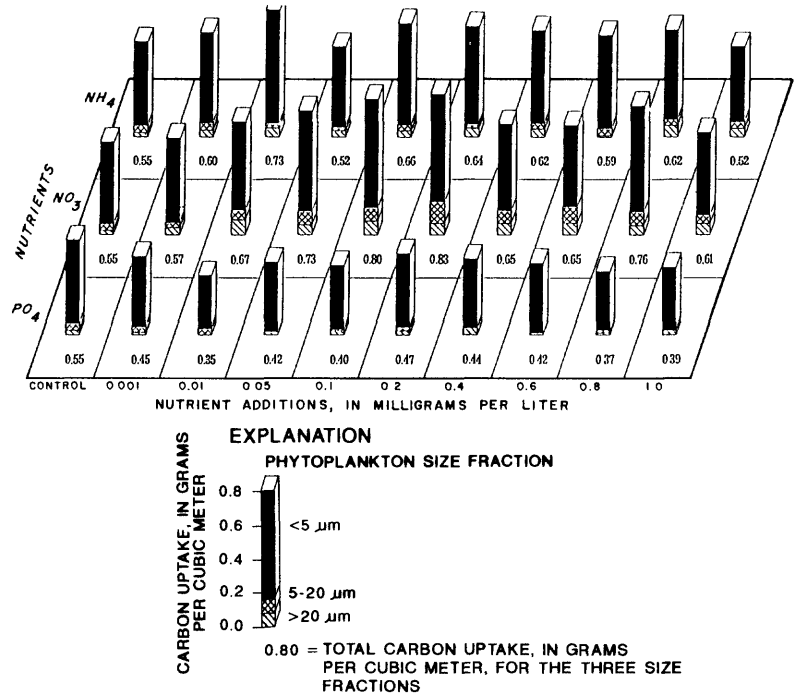

Figure 13. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at site 7 on the tidal Myakka River, September 27, 1984. (Each bar represents mean of three replicates.)

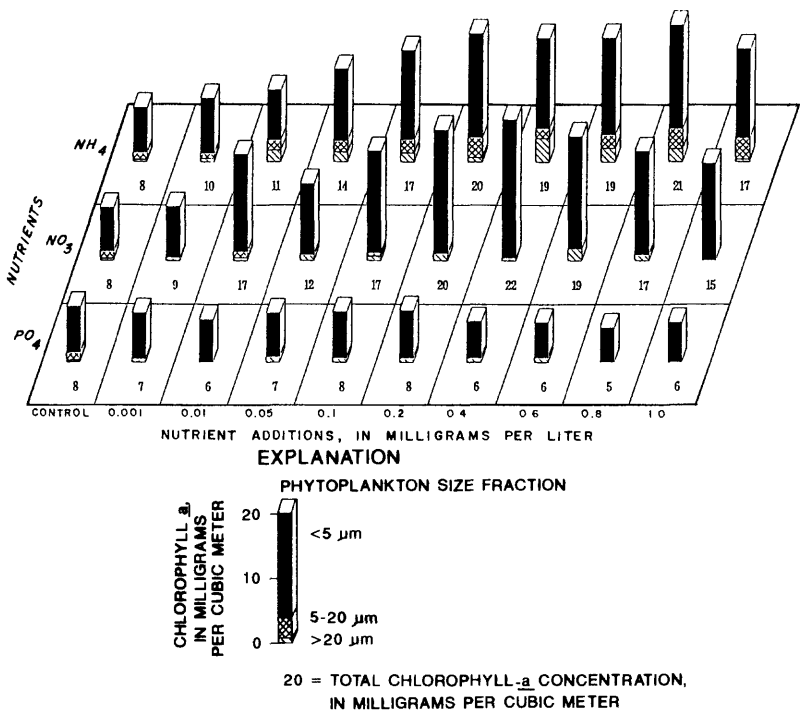

Figure 14. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at site 7 on the tidal Myakka River, September 27, 1984.

The additions of both forms of nitrogen significantly (table 2) increased carbon uptake and chlorophyll *a* (figs. 15 and 16). The patterns of changes in carbon uptake in response to increasing additions of NO_3-N and NH_4-N were similar. In each instance, carbon uptake increased with increasing nitrogen additions and then remained relatively constant at concentrations greater than 0.2 mg/L. Solutions of these models using nonlinear-fitting procedures indicated that the response curve for NO_3-N initially increased at a slightly greater rate than that for NH_4-N. The asymptote of the NH_4-N response curve, however, was higher than that for NO_3-N. Analysis of relative changes among the size fractions indicated the smallest phytoplankton size fraction (<5 µm) accounted for most of the observed increase in carbon uptake and chlorophyll-*a* concentration in response to NO_3-N and NH_4-N additions.

In contrast to the stimulation by nitrogen, carbon uptake significantly (table 2) decreased with increasing PO_4-P additions. Graphically, chlorophyll-*a* concentrations also seemed to decrease slightly with increasing PO_4-P additions. This conclusion was supported by the results of the general linear-models analysis. Further examination indicated that the largest phytoplankton size fraction (>20 µm) was responsible for the observed decrease in carbon uptake and chlorophyll-*a* concentration.

Second High Flow

The final experiment at the Myakka River site was conducted in September 1985, again during a period of high river flow (fig. 2). Ambient water conditions were characterized by low salinity, extremely high water color, and elevated concentrations of macronutrients (table 3). The controls indicated that, although the phytoplankton chlorophyll-*a* concentration was slightly less than that of the preceding experiments at this location, the relative rate of carbon uptake was extremely low (less than 15 percent) when compared to previous observed rates (figs. 17 and 18). Neither phytoplankton carbon uptake nor chlorophyll-*a* concentrations indicated any biologically significant response to the additions of nutrients.

Peace River and Charlotte Harbor Salinity Stations

Low Flow

Experiments were conducted at 6‰ salinity in the lower Peace River and at approximately 20‰ in upper Charlotte Harbor in May 1985, a period of extremely low river inflow (fig. 2). Ambient water conditions at the lower salinity zone were characterized by low color and relatively high concentrations of NO_3-N, NH_4-N, and PO_4-P (table 3). The controls indicated that approximately 75 percent of phytoplankton chlorophyll *a* and carbon uptake within the phytoplankton community occurred in the <5-µm size fraction (figs. 19 and 20). Ambient conditions in the higher salinity zone, by comparison, were characterized by low color and NO_3-N and NH_4-N concentrations at or below detection limits. Phytoplankton carbon uptake and chlorophyll-*a* concentrations within the controls at 20‰ were divided approximately equally between the largest and smallest size fractions (figs. 21 and 22).

Carbon uptake at 6‰ (fig. 19) salinity was slightly depressed by the addition of PO_4-P, slightly stimulated by NH_4-N, and showed no significant response to additions of NO_3-N (table 2). Graphical analysis (fig. 20) indicated that the concentration of chlorophyll *a* at 6‰ salinity was initially relatively high and moderately (approximately 30-50 percent) stimulated by additions of each of the three macronutrients (table 2). None of the observed changes in carbon uptake or chlorophyll *a* in response to increasing nutrient additions resulted from systematic differences in stimulation among the three measured phytoplankton size fractions.

At 20‰ salinity, additions of PO_4-P caused no biologically meaningful changes in either phytoplankton carbon uptake or chlorophyll-*a* concentrations (figs. 21 and 22). Additions of both nitrogen forms, by comparison, resulted in highly significant increases. In general, the patterns of the responses to NO_3-N and NH_4-N were similar. Solutions of these curves using nonlinear fitting procedures indicated that the curve for NO_3-N initially increased at a rate greater than that of NH_4-N, whereas the asymptote of the NH_4-N response curve was significantly higher. The responses of chlorophyll *a* followed patterns similar to those for carbon uptake (table 2). Both nitrogen forms caused threefold increases in phytoplankton chlorophyll-*a* concentration.

Analysis by size fraction of changes in carbon uptake in response to nitrogen additions indicated that, as concentrations of both forms increased, there were significant small increases in the relative percentages attributable to the two smallest (<5 µm and <20 and >5 µm) size fractions. A similar pattern occurred with respect to changes in concentration of chlorophyll *a* with increasing NH_4-N additions.

High Flow

A second series of experiments was conducted in the Charlotte Harbor Estuary at approximately 6 and 20‰ salinity in September 1985, a period of relatively high river inflow (fig. 2). Water color and the ambient concentration of NH_4-N at the more riverine-influenced lower salinity were extremely high, whereas the ambient level of NO_3-N was relatively low (table 3). Phytoplankton chlorophyll-*a* concentrations and carbon uptake at 6‰ salinity were both quite low (figs. 23 and 24 controls). Ambient water-quality conditions at 20‰ salinity, by comparison, were

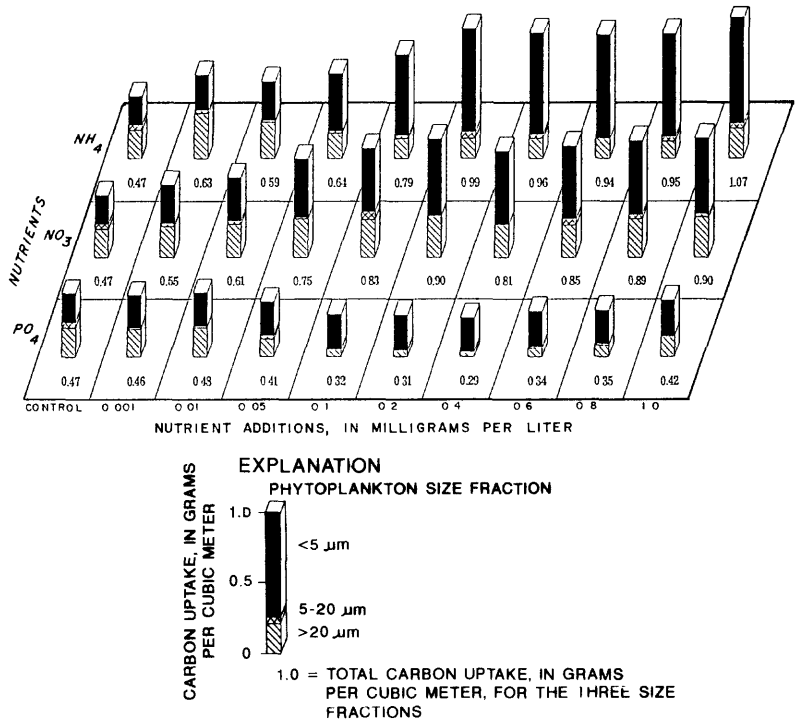

Figure 15. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at site 7 on the tidal Myakka River, May 23, 1985. (Each bar represents mean of three replicates.)

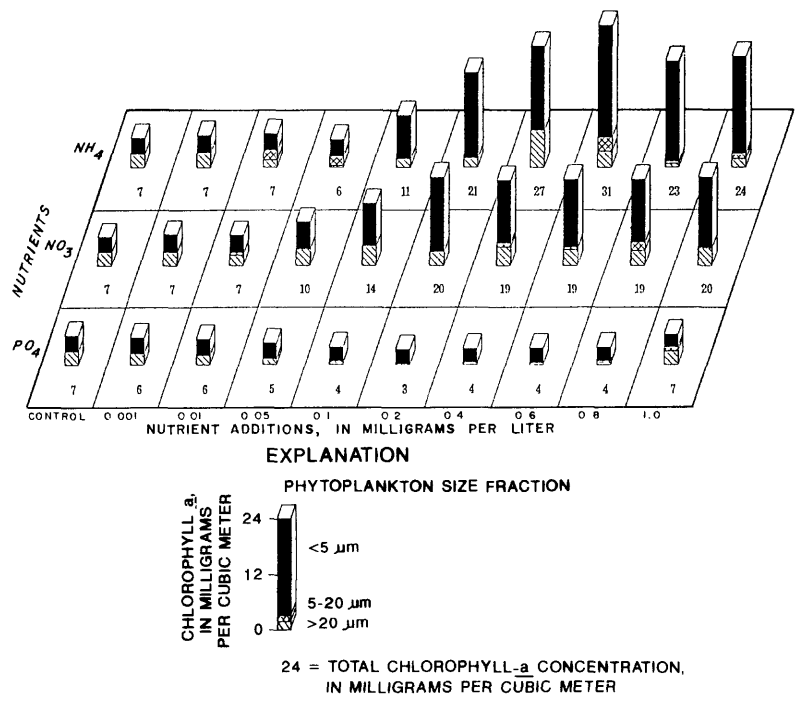

Figure 16. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at site 7 on the tidal Myakka River, May 23, 1985.

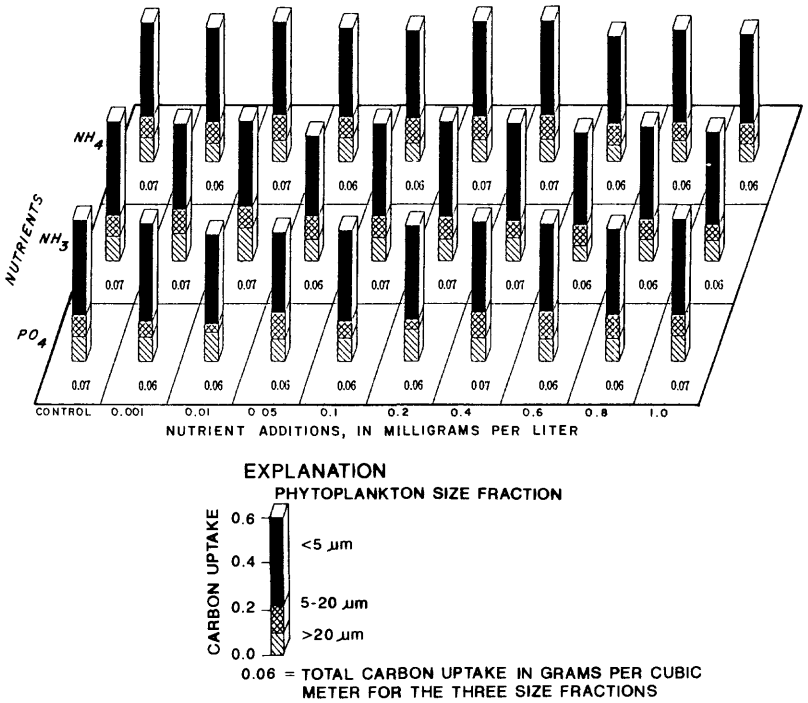

Figure 17. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at site 7 on the tidal Myakka River, September 16, 1985. (Each bar represents mean of three replicates.)

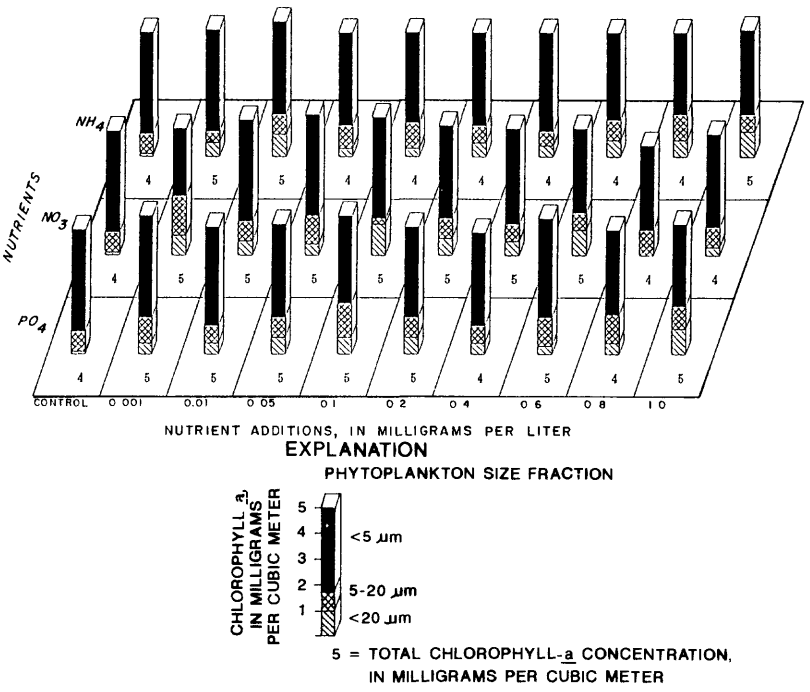

Figure 18. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at site 7 on the tidal Myakka River, September 16, 1985.

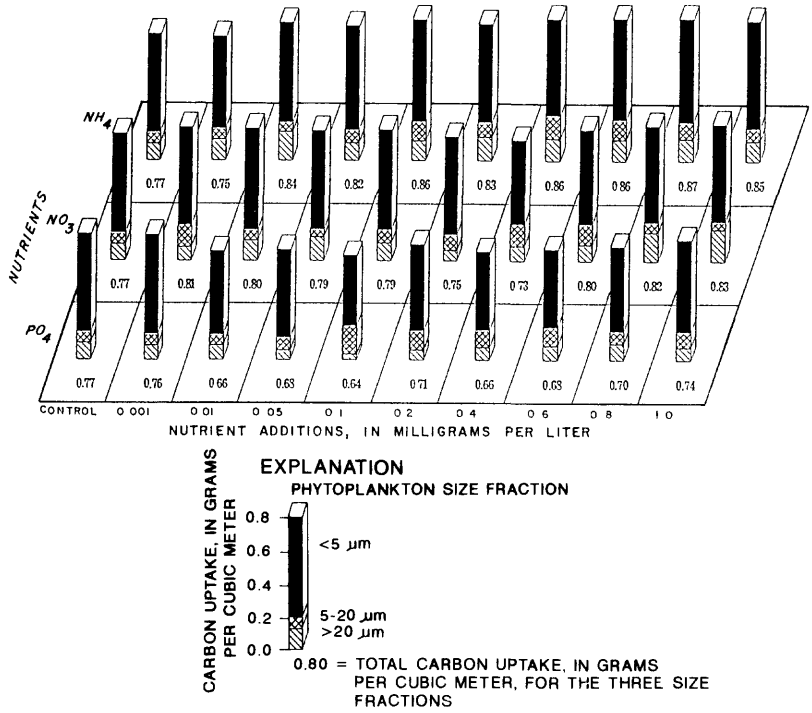

Figure 19. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 6 parts per thousand at site 8, May 8, 1985. (Each bar represents mean of three replicates.)

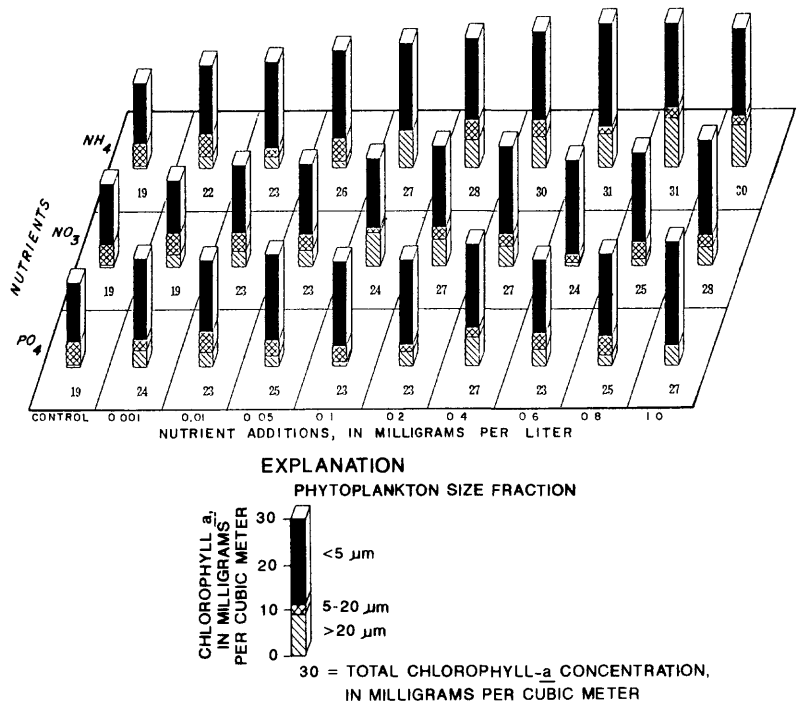

Figure 20. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 6 parts per thousand at site 8, May 8, 1985.

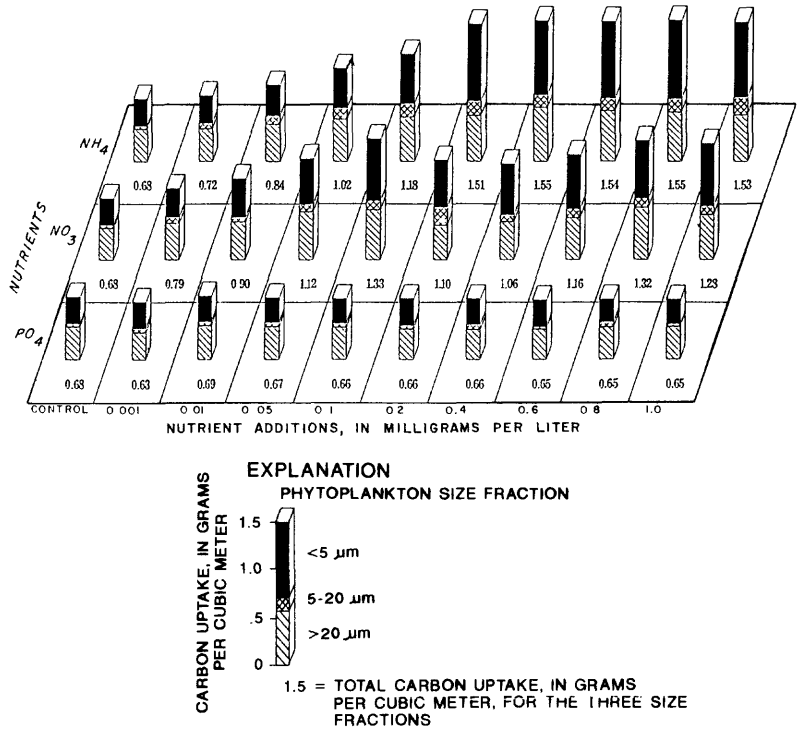

Figure 21. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 20 parts per thousand at site 9, May 10, 1985. (Each bar represents mean of three replicates.)

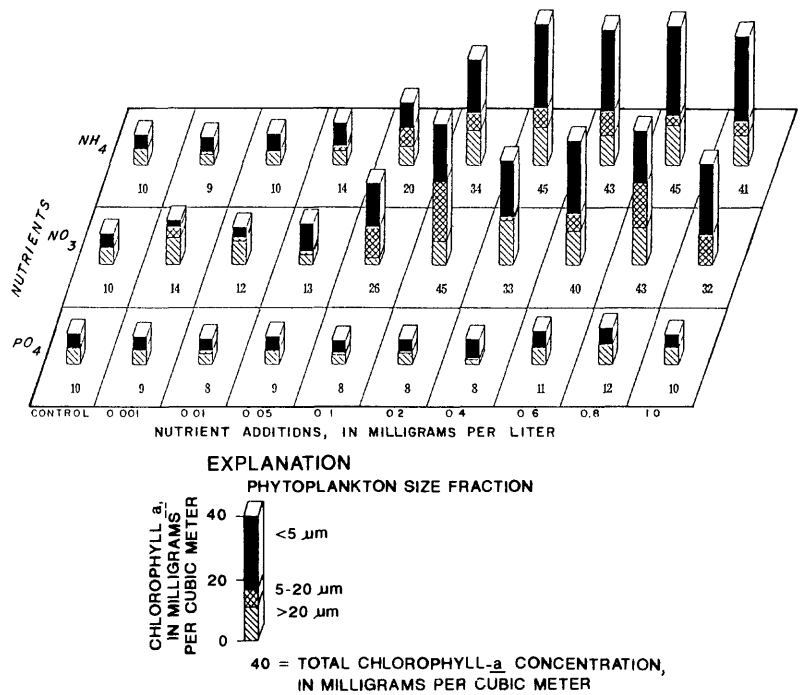

Figure 22. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 20 parts per thousand at site 9, May 10, 1985.

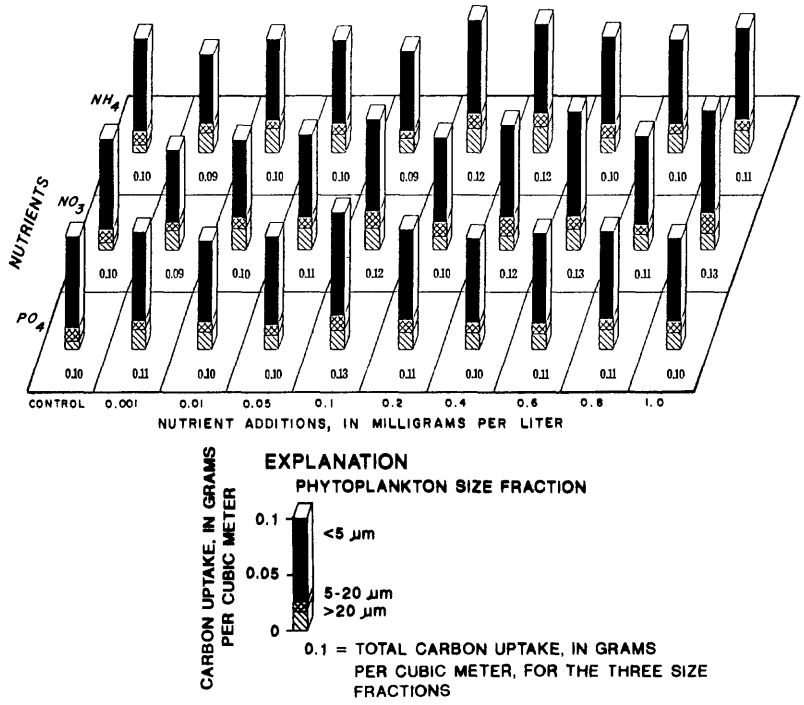

Figure 23. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 6 parts per thousand at site 10, September 17, 1985. (Each bar represents mean of five replicates.)

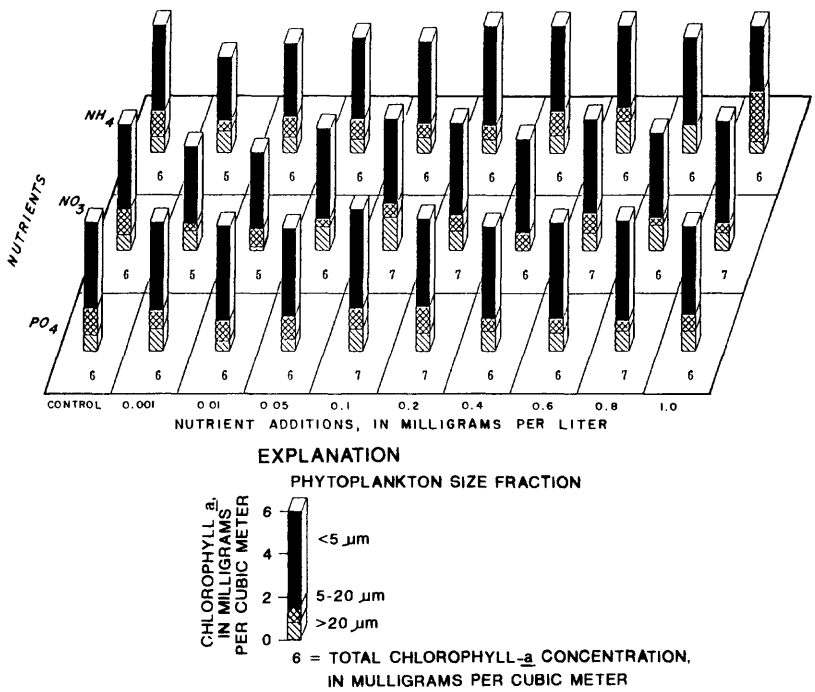

Figure 24. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 6 parts per thousand at site 10, September 17, 1985.

characterized by moderate color and by NO_3-N and NH_4-N concentrations near detection limits (table 3). Initially, chlorophyll-*a* concentrations and carbon uptake rates at 20‰ salinity (figs. 25 and 26 controls) were higher than those at 6‰ salinity.

Experimental nutrient additions at 6‰ salinity had no biologically significant effects on either carbon uptake or chlorophyll-*a* concentration (figs. 23 and 24). This was in sharp contrast to the effects of corresponding additions at the higher salinity.

Additions of NO_3-N and NH_4-N at 20‰ salinity significantly increased (table 2) carbon uptake by more than 100 percent (fig. 25). The carbon uptake responses for nitrogen initially increased with increasing concentrations, reached maximum levels, and then significantly declined with further additions. Solutions of the response curves, using nonlinear fitting procedures, indicated no significant differences between the asymptotes of the two nitrogen response curves. The shape of the NH_4-N curve, however, indicated greater stimulation at lower concentrations.

Chlorophyll-*a* concentrations significantly increased (table 2) as much as fivefold in response to experimental nitrogen additions at 20‰ salinity (fig. 26). The shapes of the responses of phytoplankton chlorophyll-*a* concentration to the two forms of nitrogen (fig. 26) increased to maximum levels and then declined slightly at the highest additions. The magnitude of chlorophyll-*a* stimulation was greater than that of carbon uptake, whereas the decline of chlorophyll-*a* concentrations at the highest nitrogen concentrations was less dramatic.

Analysis by size fraction indicated that, over the ranges of NO_3-N additions, where carbon uptake and chlorophyll *a* were stimulated, the relative percentage attributable to the 5- to 20-µm size fraction increased with increasing concentration of NO_3-N. By comparison, the relative percentage of chlorophyll *a* within the largest size fraction (>20 µm) increased in response to NH_4-N stimulation.

EFFECTS OF NITROGEN AND PHOSPHORUS ADDITIONS ON PHYTOPLANKTON PRODUCTIVITY AND CHLOROPHYLL *a*

The experiments conducted within the 6‰ salinity part of the Charlotte Harbor Estuary showed distinct seasonal changes in phytoplankton carbon uptake and chlorophyll *a* that resulted from inorganic nitrogen additions. Other investigators, such as Myers and Iverson (1981) working in the fresh and brackish estuarine waters of Apalachicola Bay in northwest Florida and D'Elia and others (1986) working in Chesapeake Bay, have also observed seasonal differences in the response of phytoplankton assemblages to nitrogen and phosphorus additions. The seasonal changes in phytoplankton responses in Charlotte Harbor were directly attributable to seasonal periods of low and high freshwater inflow. Nutrient additions at the end of the wet season, when water color and ambient macronutrient concentrations were high (September 1985), did not stimulate phytoplankton carbon uptake or chlorophyll-*a* production at either the riverine Myakka River (0‰ salinity) or Peace River (6‰ salinity) locations. The experimental results, by comparison, from the Myakka River site in May 1985 (23‰ salinity) and the 20‰ salinity zone in upper Charlotte Harbor in May and September 1985 indicate that phytoplankton communities within the more saline parts of the estuary do not exhibit seasonality in their response to nitrogen additions and are continually nitrogen limited, except possibly under very high freshwater inflow conditions.

The results from the nutrient addition experiments indicate that, during low freshwater inflow, the availability of nitrogen may limit phytoplankton productivity over a wide range of salinities throughout much of the Charlotte Harbor system. During seasonal high freshwater inflow, however, nitrogen availability probably does not limit phytoplankton production in these lower salinity areas of the estuary. Freshwater runoff from the Peace and Myakka River basins contains high concentrations of dissolved humic compounds that color the water and greatly reduce light penetration (McPherson and Miller, 1987; Fraser, 1991; McPherson and others, in press). In such highly colored waters, light availability probably is the dominant factor controlling phytoplankton production. In regions of the estuary characterized by higher salinities, dilution and photooxidation of humic substances reduce water color and increase light penetration. Thus, during periods of high freshwater inflow, the estuary conceptually can be divided into two regions based on salinity. In the lower salinity region, phytoplankton production is limited by light availability mediated by elevated water color, whereas in the higher salinity regions, phytoplankton communities are nutrient (nitrogen) limited.

Ambient conditions are often useful in determining potential limiting factors controlling phytoplankton productivity within estuarine systems. The results from Charlotte Harbor indicate a general pattern of increasing phytoplankton response to nitrogen additions with decreasing ambient nutrient concentrations. Other investigators (Davies and Sleep, 1981; Myers and Iverson, 1981; D'Elia and others, 1986) have reported similar patterns.

Mean monthly ambient values for selected macronutrients, color, phytoplankton production, and chlorophyll-*a* concentrations in the Charlotte Harbor Estuary from June 1983 through June 1988 at 6 and 20‰ salinity are shown in figure 27 (Montgomery and Emmons, 1989). Several of these measurements exhibit distinct patterns related to seasonal river inflow. Basin rainfall and runoff are characterized by a spring dry season and a summer wet period. Orthophosphorus concentrations within the more riverine-influenced upper harbor increase significantly in the spring dry season as the concentration of phosphorus in the rivers increases with declining river flow and then declines through

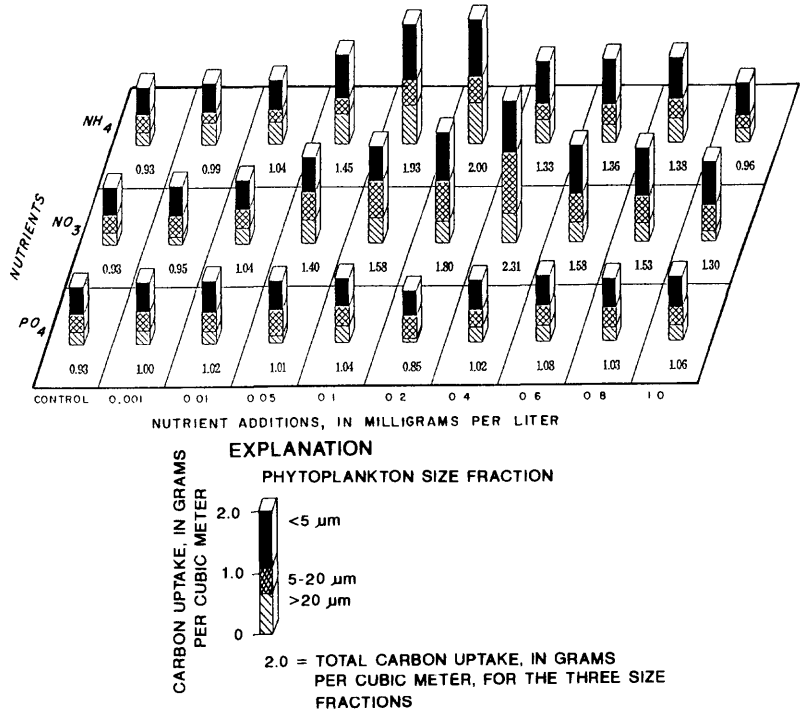

Figure 25. Phytoplankton carbon uptake for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 20 parts per thousand at site 11, September 24, 1985. (Each bar represents a mean of three replicates.)

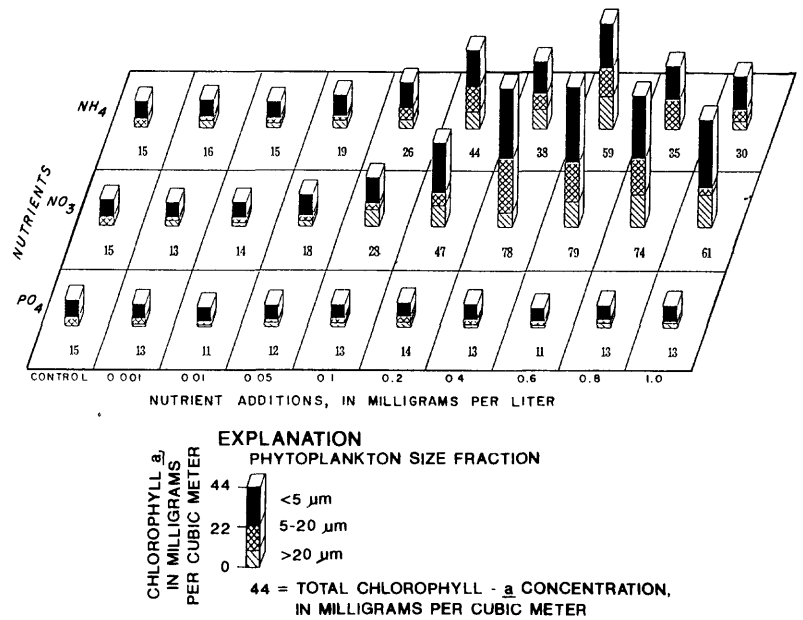

Figure 26. Phytoplankton chlorophyll-*a* concentrations for control and nutrient-amended samples for 10-hour *in situ* incubations at a salinity of approximately 20 parts per thousand at site 11, September 24, 1985.

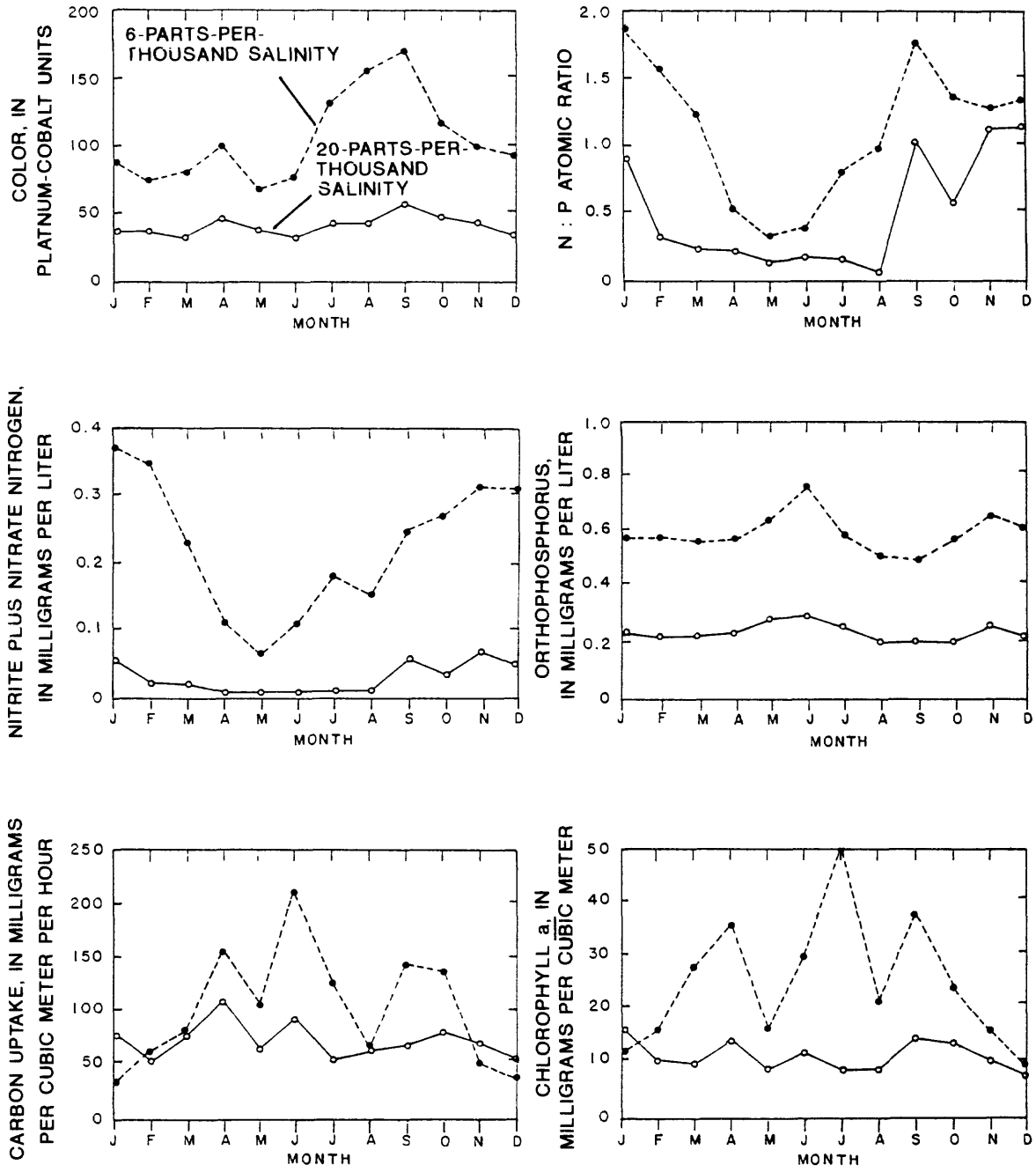

Figure 27. Mean values for selected macronutrients and measurements of phytoplankton production collected monthly from June 1983 through June 1988 at 6 and 20 parts per thousand along salinity transects in the lower Peace River and upper Charlotte Harbor. (From Montgomery and Emmons, 1989.)

the summer as PO_4-P concentrations in river inflow decrease. Ambient nitrite plus nitrate concentrations in the highly riverine-influenced areas of the estuary (6‰ salinity) are highest in winter and then decline sharply throughout the spring as phytoplankton production increases. Nitrate concentrations in this salinity zone increase again during the wet season as river inflow increases and high water color results in decreased phytoplankton production. Concentrations of NO_3-N in the more saline parts of the harbor (20‰ salinity) remain near detection limits through all but the winter months. These generalized macronutrient patterns are similar to those reported by Fraser (1986; 1991) from long-term seasonal studies of water-quality characteristics of the lower Peace River.

Low ambient nutrient levels by themselves, however, cannot be taken as definitive indications of potential nutrient limitation. Smith (1984) proposed further evaluation of potential limiting nutrient conditions in estuarine systems by

analyzing inorganic atomic N:P ratios and comparisons of concentrations of nutrients and conservative constituents along natural gradients. The N:P ratios of natural phytoplankton assemblages generally average near the Redfield (1958) value of 16. Waters with ambient N:P ratios of less than 10 usually indicate potential conditions in which phytoplankton production is nitrogen limited, whereas values of greater than 21 are commonly indicative of phosphorus limitation (Ram and Plotkin, 1983; Sakshaug and Olsen, 1986). Determinations of limiting nutrient conditions based on ambient inorganic N:P ratios and corresponding results from bioassay determinations generally have been found to be in good agreement (Schanz and Juon, 1983). Extensive natural-phosphate deposits within the Charlotte Harbor drainage basin result in unusually high ambient-orthophosphorus concentrations and unusually low inorganic N:P ratios throughout the entire estuarine system. The nitrogen and phosphorus ratios in 6‰ and 20‰ salinity zones are shown in figure 27.

Another method commonly used to evaluate potential limiting nutrient conditions for phytoplankton growth is the comparison of concentrations of nutrients and conservative constituents along natural gradients (Smith, 1984). Fraser and Wilcox (1981), Froelich and others (1985), and McPherson and Miller (1990) evaluated nutrient sources and distributions in Charlotte Harbor using nutrient dilution curve models constructed by plotting nutrient concentrations against salinity. Nitrite-nitrate concentrations always deviated from conservative behavior, declining rapidly with increasing salinity, whereas orthophosphorus levels exhibited conservative behavior reflecting simply straight dilution. Such nutrient dilution curve observations, combined with determinations of atomic N:P ratios and studies of seasonal ambient-nutrient concentration patterns (fig. 27), support the overall findings of the nutrient-addition experiments. The results of these experiments stress the importance of nitrogen in limiting phytoplankton production, except in those estuarine areas that are seasonally influenced by high water color.

Population growth in the Charlotte Harbor basin is expected to increase nitrogen loading to the estuary by more than 2,700 kg/d by the year 2020 (Hammett, 1988). Such additional nitrogen loading would be equivalent to 18 percent of the current gaged-basin nitrogen input (McPherson and Miller, 1990). New inorganic nitrogen would be immediately available for phytoplankton uptake, whereas mineralization by bacteria and other microorganisms would make other forms available over time. Additional nitrogen loadings could be expected to result in increased seasonal phytoplankton production in those areas of the harbor where water color is low enough to allow for sufficient light availability.

Total freshwater inorganic nitrogen inputs in Charlotte Harbor (Hammett, 1988; Fraser, 1991) are more than an order of magnitude below that required to support observed levels of phytoplankton production (Montgomery and Emmons, 1989; McPherson and others, in press). Therefore, some mechanism, such as the rapid recycling of nitrogen by processes such as remineralization by microheterotrophs (Furnas and others, 1986), is required to support observed photosynthesis rates. Pennock (1987) suggested that, in the Delaware Estuary system, more than 80 percent of the annual nitrogen demand is supplied by recycling of NH_4-N, even though NO_3-N is the dominant ambient-nitrogen form in estuarine freshwater inflow (Fisher and others, 1982). Other than during ephemeral events associated with extremely large inputs of freshwater (as may occur during tropical depressions), the inorganic nitrogen entering the estuary is rapidly taken up by phytoplankton and joins a larger organic pool of fixed nitrogen. Such a concept is consistent with a number of findings from other estuarine systems.

Phytoplankton responses to stimulation by nitrogen in the presented bioassay experiments exhibited a number of well-defined patterns. Distinct differences existed in the response time and the absolute magnitudes of the two productivity measurements, carbon uptake and chlorophyll *a*. Other investigators (Vince and Valiel, 1973; Davies and Sleep, 1981; Wurtsbaugh and others, 1985; Graneli and others, 1986) have noted that nitrogen stimulation of carbon uptake is normally associated with corresponding increases in chlorophyll-*a* concentrations. Results from the presented time-series experiments indicated that chlorophyll *a* responded more quickly to nitrogen stimulation than carbon uptake. More apparent, however, were the differences observed in the absolute magnitudes of the maximum responses of carbon uptake and chlorophyll *a*. Nitrogen additions resulted in maximum increases in carbon uptake ranging from 40 to 150 percent, whereas corresponding increases in chlorophyll *a* were always far greater, increasing from 150 to 400 percent.

Another characteristic distinction of the response of the phytoplankton assemblages to nitrogen additions was the observed difference in carbon uptake stimulation between the two forms of nitrogen, NO_3-N and NH_4-N. Nitrogen uptake, under limiting conditions, generally has been found to be both extremely rapid and nearly light independent (Goldman and others, 1981; Fisher and others, 1982; Whalen and Alexander, 1984; Morel, 1987; Pennock, 1987; Priscu, 1987). Researchers have almost universally reported that phytoplankton preferentially take up more reduced forms of nitrogen, with uptake rates generally being greater for the smaller phytoplankton size fractions (Malone, 1971; Malone and Neale, 1981). Carpenter and Dunham (1985) and Pennock (1987), working on the seasonal estuarine uptake of nitrogen, found strong preferences for NH_4-N over NO_3-N. They, and others (Takahashi and Saijo, 1981), also observed that NH_4-N inhibited NO_3-N uptake. Such repressive inhibition was not apparent at high concentrations of NO_3-N or low concentrations of NH_4-N. Dortch and others (1982) found that, under nitrogen limitation, the ability to take up NO_3-N may actually decrease, whereas the potential to take up NH_4-N

Table 4. Dates and locations where there were significant changes in relative percentage of either carbon uptake or chlorophyll *a* for three measured size fractions of phytoplankton in response to nutrient additions

[A (+) denotes a significant increase ($\alpha = 0.05$) in the percent contribution of a size fraction over the controls, and a (—) indicates a corresponding significant decline. < = less than; > = greater than; µm = micrometer]

	Carbon uptake for indicated size fraction			Chlorophyll *a* for indicated size fraction		
	<5 µm	5-20 µm	>20 µm	<5 µm	5-20 µm	<20 µm
Myakka River						
May 1985						
23‰						
PO_4-N	+	—	—	+		—
NO_3-N	+	—	—	+	—	—
NH_4-N	+	—	—	+	—	—
Peace River						
June 1984						
20‰						
NO_3-N						
NH_4-N				—		+
May 1985						
20‰						
PO_4-N						
NO_3-N	+	+	—			
NH_4-N	+	+	—	+	+	—
September 1985						
20‰						
PO_4-N	—	+	—	—	+	—
NH_4-N				—	—	+

increases. Under such conditions, phytoplankton, even at low ambient NH_4-N levels, may be able to satisfy cellular demands quickly. Such observations support the findings of the current study in which the maximum response of carbon uptake was often greater for NH_4-N than for NO_3-N.

Nitrogen stimulation during several of the experiments also caused relative changes in the distribution of carbon uptake and chlorophyll-*a* concentrations among phytoplankton size fractions. The patterns of such responses often differed between nitrogen forms. Within an estuary, such as Charlotte Harbor, natural phytoplankton communities are exposed continuously to both short-term patchy and long-term seasonally ephemeral physical and chemical gradients that may differentially limit the growth of various taxonomic groups. Differences in nutrient ratios also may be an important factor in determining the relative dominance of specific taxonomic groups. Sommer (1985) found that neither pulsed nutrient additions, such as those that occur naturally during periods of high river input, nor steady-state nutrient conditions uniformly provided a competitive advantage to any particular size class of phytoplankton; rather, stimulation was taxa specific.

Sanders and others (1987) investigated the effects of seasonal additions of ammonium, nitrate, and phosphorus to large cultures of natural phytoplankton assemblages from the Pautuxent River Estuary. Their results indicated that nitrogen enrichment during the summer and fall quickly changed the species composition of the phytoplankton community from a predominance of flagellates to one dominated by small centric diatoms. Such changes in dominance were observed to result from increased growth rates of specific taxa. Malone (1971; 1982) observed that smaller phytoplankton size fractions are often stimulated at far lower concentrations than larger size fractions. He proposed that smaller size fractions might be expected to respond more quickly because such cells, with their greater surface to volume ratio, generally have greater uptake rates and lower half-saturation constants for macronutrients.

Findings of those experiments where nutrient addition over a range of concentrations not only stimulated either phytoplankton carbon uptake or chlorophyll-*a* concentrations but resulted in significant changes in the relative percentages among the three measured size fractions are summarized in table 4. As indicated, nutrient additions at

lower salinities either did not stimulate phytoplankton carbon uptake or chlorophyll *a* (high-flow conditions), or resulted in an equal increase among all three size fractions. Similar nutrient additions at higher salinities, by comparison, often resulted in significant differences in percentages of carbon uptake and chlorophyll *a* among size fractions of the phytoplankton. Although the overall results of this study tend to support the view that smaller size fractions generally exhibit a greater response to nutrient additions, the data indicated that the relative responses of the size fractions were highly variable with regard to season, location, and nitrogen form.

Additions of orthophosphorus over a series of concentrations did not stimulate carbon uptake during any of the bioassay experiments. However, such additions did inhibit carbon uptake slightly in May 1985 at both the Myakka River site and at 6‰ salinity along the Peace River. Ambient conditions at these sites included high orthophosphorus concentrations (0.397 and 0.830 mg/L, respectively) and low atomic N:P ratios (<0.5).

A possible explanation for the inhibition of carbon uptake might be the differences in availability between the measured ambient and added forms of orthophosphorus, combined with the widely observed phenomenon of luxury-phosphorus consumption by phytoplankton. Procedures for determining ambient-orthophosphorus concentrations measure "reactive forms" of the nutrient, large percentages of which can exist as "lightly bound" forms. In much of the Charlotte Harbor basin, large percentages of the measured orthophosphorus may be in the form of trimetaphosphate associated with calcium ions. Although this form is chemically measurable as "reactive" orthophosphorus, its direct availability to phytoplankton across cellular membranes could be more limited (Kuhl, 1974). The orthophosphorus experimentally added, by comparison, was in a chemically "pure" form (NaH_2PO_4), readily available for phytoplankton uptake. Orthophosphorus uptake, unlike that of nitrogen, generally has been found to be stimulated by light and dependent on adenosine triphosphate (ATP) (Nalewajko and Lee, 1983). Thus, luxury-phosphorus consumption has the potential of competing with other metabolic-intensive processes, such as photosynthesis, for ATP and may explain the observed slight depression of carbon uptake at the two sites in May 1985.

SUMMARY AND CONCLUSIONS

1. In regions of Charlotte Harbor characterized by low salinities, phytoplankton carbon uptake and chlorophyll-*a* concentrations were stimulated by additions of inorganic nitrogen during seasonal periods of low freshwater inflow. Neither phytoplankton measurement, by comparison, responded to nitrogen additions at low salinities during periods of high freshwater inflows. This could be directly attributed to the effects of increased water color, reduced light availability, and high ambient nitrogen concentrations.
2. Under normal conditions, in the more saline reaches of the estuary, phytoplankton carbon uptake and chlorophyll *a* may be continually nitrogen limited.
3. During periods of high summer freshwater inflow, the estuary can be divided conceptually into a low salinity zone where phytoplankton production is mediated by light availability, as determined by high water color, and a high salinity zone, where production is nitrogen limited.
4. Results from seasonal and dilution curve studies of ambient-nutrient concentration patterns, as well as comparative calculations of inorganic nitrogen inputs and observed phytoplankton productivity, support the observations of the bioassay investigations. Each of these lines of evidence indicates that, exclusive of seasonal riverine influences affecting light penetration of the water column, nitrogen availability normally limits phytoplankton production within the Charlotte Harbor estuarine system.
5. Although the overall results of this study tend to support the view that smaller size fractions generally exhibit a greater response to nitrogen additions, the data indicated that the relative responses of the size fractions were highly variable with regard to season, location, and nutrient form.

The reported experimental results indicate that seasonal freshwater inflows influence the occurrence and distribution of the nutrient limitation of phytoplankton production in the Charlotte Harbor estuarine system. Care must be taken, however, in directly transferring the results of such short-term macronutrient-bioassay determinations to the ecosystem level. The scope of the present study was limited to specific salinities and flows and may have neglected other nutrients and biological and physical factors that could have significantly influenced the potential responses of the phytoplankton assemblages. Factors that limit bottle-held phytoplankton growth and productivity or both may differ from those that limit production over an entire system. Understanding the components controlling phytoplankton production in a shallow, subtropical estuarine system such as Charlotte Harbor is further confounded by the complex interactions of physical factors such as salinity, temperature, light (as influenced by both annual cycles and seasonal increases in water color), and nutrient additions through inputs and regeneration.

Conceptually, phytoplankton populations can seldom be classified as either strictly light or nutrient limited. First, the potential exists for distinct spatial and temporal differences among the factors controlling phytoplankton production. Further, various algal taxonomic groups, influenced by distinctive uptake and growth rates, can exhibit marked variations in their responses to limiting conditions.

The view that potentially limiting nutrients are available for phytoplankton uptake at homogeneous low concentrations is, furthermore, restrictive. Phytoplankton assemblages within estuarine systems encounter a wide variety of ambient nutrient concentrations, including discrete nutrient pulses, deterministic of a patchy environment, as well as ephemeral and seasonal influences.

Population growth in the Charlotte Harbor basin is expected to cause an increase in nitrogen loading to the estuary by more than 2,700 kg/d by the year 2020. Such additional nitrogen loading would be equivalent to 18 percent of the current gaged-basin nitrogen input. New inorganic nitrogen would be immediately available for phytoplankton uptake, whereas mineralization by bacteria and other microorganisms would make other forms available over time. Additional nitrogen loadings could be expected to result in increased seasonal phytoplankton production in those areas of the harbor where water color is low enough to allow for sufficient light availability.

Incorporation of the results of nutrient-limiting studies, such as presented here, as well as seasonal patterns of phytoplankton production into generalized hydrodynamic models would aid the development of predictive management tools. The present study indicates that management strategies developed to maintain existing water quality need to emphasize point and nonpoint loadings of nitrogen into the Charlotte Harbor system, seasonal and locational factors, and rates of nitrogen recycling.

SELECTED REFERENCES

Agins, C., and Jaccarini, V., 1982, The effects of nitrate and phosphate enrichment on the phytoplankton from Marsaxlokk Bay, Malta (Central Mediterranean): Hydrobiologia, v. 87, p. 89-96.

American Public Health Association, 1989, Standard methods for the examination of water and wastewater (17th ed.), Franson, M.A.H., ed.: Washington, D.C.

Arthur, C.R., and Rigler, F.H., 1967, A possible source of error in the ^{14}C method of measuring primary productivity: Limnology and Oceanography, v. 12, p. 121-124.

Boynton, W.R., Kemp, W.M., and Keefe, C.W., 1982, A comparative analysis of nutrients and other factors influencing estuarine phytoplankton production, *in* Kennedy, V.S., ed., Estuarine comparisons: New York, Academic Press, p. 69-90.

Brown, P.C., and Field, J.G., 1985, Diel variation in production rates of natural phytoplankton populations in the southern Benguela upwelling region: Botanica Marina, v. 27, p. 201-208.

Bruno, S.F., Staker, R.D., Sharma, G.M., and Turner, J.T., 1983, Primary productivity and phytoplankton size fraction dominance in a temperate North Atlantic Estuary: Estuaries, v. 6, no. 3, p. 200-211.

Caperon, J., and Myer, J., 1972a, Changes in population characteristics with steady-state growth rate, pt. I *of* Nitrogen-limited growth of marine phytoplankton: Deep-Sea Research, v. 19, p. 601-618.

——— 1972b, Uptake kinetics and their role in nutrient limited growth of phytoplankton, pt. 2 *of* Nitrogen-limited growth of marine phytoplankton: Deep-Sea Research, v. 19, p. 619-632.

Carpenter, E.J., and Dunham, S., 1985, Nitrogenous nutrient uptake, primary production, and species composition of phytoplankton in the Carmans River Estuary, Long Island, New York: Limnology and Oceanography, v. 30, p. 513-526.

Chew, V., 1971, A brief review of some multiple comparison procedures: Presented at the Meeting of the University Statisticians of the Southern Experiment Stations, College Park, Md.

——— 1976, Use and abuses of Duncan's multiple range test: Proceedings of Florida State Horticultural Society, v. 89, p. 251-253.

——— 1980, Testing differences among means: Correct interpretation and some alternatives: Horticultural Science, v. 15, p. 467-470.

Collos, Y., 1984, Interspecific variability in biomass and uptake during nitrogen starvation and resupply, pt. V *of* Transient situations in nitrate assimilation by marine diatoms: Marine Ecology Progress Series 17, p. 25-31.

Collos, Y., and Slawyk, G., 1984, Interactions in euphotic zone profiles of stratified oceanic areas, pt. III *of* ^{13}C and ^{15}N uptake by marine phytoplankton: Marine Ecology Progress Series 19, p. 223-231.

D'Elia, C.F., Sanders, J.G., and Boynton, W.R., 1986, Nutrient enrichment studies in a coastal plain estuary: Phytoplankton growth in large scale, continuous cultures: Canadian Journal of Fisheries, v. 43, p. 397-406.

Davies, A.G., and Sleep, J.A., 1981, Nutrient stimulation of carbon fixation in summertime English Channel phytoplankton assemblages: Journal of Marine Biology, v. 61, p. 551-568.

Dortch, Q., Clayton, J.R., Thoreson, S.S., Bressler, S.L., and Amred, S.I., 1982, Response of marine phytoplankton to nitrogen deficiency: Decreased nitrate uptake versus enhanced ammonium uptake: Marine Biology, v. 70, p. 13-19.

Duncan, D.B., 1957, Multiple range tests for correlated and heteroscedastic means: Biometrics, v. 13, p. 164-176.

Elser, J.J., and Kimmel, B.L., 1986, Alteration of phytoplankton phosphorus status during enrichment experiments: Implications for interpreting nutrient enrichment bioassay results: Hydrobiologia, v. 133, p. 217-222.

Fisher, T.R., Carlson, P.R., and Barber, R.T., 1982, Carbon and nitrogen primary productivity in three North Carolina estuaries: Estuarine, Coastal and Shelf Science, v. 15, p. 621-644.

Fitzwater, S.E., Knauer, G.A., and Martin, J.H., 1982, Metal contamination and its effect on primary production measurements: Limnology and Oceanography, v. 27, p. 544-552.

Fraser, T.H., 1986, Long-term water-quality characteristics of Charlotte Harbor: U.S. Geological Survey Water-Resources Investigations Report 86-4180, 43 p.

——— 1991, The lower Peace River and Horse Creek: Flow and water quality characteristics, 1976-1986, *in* Livingston, R.J., ed., The rivers of Florida: Springer-Verlag, p. 143-186.

Fraser, T.H., and Wilcox, W.H., 1981, Enrichment of a subtropical estuary with nitrogen, phosphorus and silica, *in* Neilson, B.J., and Cronin, L.E., eds., Estuaries and nutrients: New Jersey, Humana Press, p. 481-498.

Froelich, P.N., Kaul, L.W., Byrd, J.T., Andreae, M.O., and Roe, K.K., 1985, Arsenic, barium, germanium, tin, dimethylsulfide, and nutrient biogeochemistry in Charlotte Harbor, Florida, a phosphorus-enriched estuary: Estuarine, Coastal and Shelf Science, v. 20, p. 239-264.

Furnas, M.J., 1983, Nitrogen dynamics in lower Narragansett Bay. I. Uptake by size-fractionated phytoplankton populations: Journal of Plankton Research, v. 5, p. 657-676.

Furnas, M.J., Smayda, T.J., and Deason, E.A., 1986, Nitrogen dynamics in lower Narragansett Bay. II. Phytoplankton uptake, depletion rates of nitrogenous nutrient pools, and estimates of ecosystem remineralization: Journal of Plankton Research, v. 8, no. 4, p. 755-769.

Furuya, K., and Marumo, R., 1983, Size distribution of phytoplankton in the western Pacific Ocean and adjacent waters in summer: Bulletin of Plankton Society of Japan, v. 30, p. 21-32.

Goldman, C.R., 1960, Primary productivity and limiting factors in three lakes of the Alaska peninsula: Ecological Monograph, v. 30, p. 207-230.

Goldman, J.C., Dennett, M.R., and Riley, C.B., 1981, Marine phytoplankton photosynthesis and transient ammonium availability: Marine Biology Letters, v. 2, p. 323-331.

Graneli, E., 1987, Nutrient limitation of phytoplankton biomass in a brackish water bay highly influenced by river discharge: Estuarine, Coastal and Shelf Science, v. 25, p. 555-565.

Graneli, E., Graneli, W., and Rydberg, L., 1986, Nutrient limitation at the ecosystem and the phytoplankton community level in the Laholm Bay, South-East Kattegat: Ophelia, v. 26, p. 181-194.

Hammett, K.M., 1988, Land use, water use, streamflow, and water-quality characteristics of the Charlotte Harbor inflow area, Florida: U.S. Geological Survey Open-File Report 87-472, 104 p.

Harding, L.W., Prezelin, B.B., Sweeney, B.M., and Cos, J.L., 1982a, Diel oscillations of the photosynthesis-irradiance (P-I) relationship in natural assemblages of phytoplankton: Marine Biology, v. 67, p. 167-178.

——— 1982b, Primary production as influenced by diel periodicity of phytoplankton photosynthesis: Marine Biology, v. 67, p. 179-186.

Hutchinson, G.E., 1961, The paradox of the plankton: American Naturalist, v. 95, p. 137-145.

Iverson, R.L., Bittaker, H.F., and Myers, V.B., 1976, Loss of radiocarbon in direct use of Aquasol for liquid scintillation counting of solutions containing ^{14}C-$NaHCO_3$: Limnology and Oceanography, v. 21, p. 756-758.

Janicki, A., and DeCosta, J., 1984, The filtering rates of four herbivorous crustaceans on nannoplankton and netplankton fractions in an acid lake in West Virginia: International Revue ges. Hydrobiology, v. 69, p. 643-652.

Jaworski, N.A., 1981, Sources of nutrients and the scale of eutrophication problems in estuaries, *in* Neilson, B.J., and Cronin, L.E., eds., Estuaries and nutrients: New Jersey, Humana Press, p. 329-385.

Jones, R.A., and Lee, G.F., 1982, Recent advances in assessing impact of phosphorus loads on eutrophication-related water quality: Water Research, v. 16, p. 503-515.

Kana, T.M., Watts, J.L., and Gilbert, P.M., 1985, Diel periodicity in the photosynthetic capacity of coastal and offshore phytoplankton assemblages: Marine Ecology Progress Series 25, p. 131-139.

Ketchum, B.H., 1967, Phytoplankton nutrients in estuaries, *in* Lauff, G.H., ed., Estuaries: Washington, D.C., American Association for the Advancement of Science, p. 329-385.

Kuhl, A., 1974, Phosphorus, *in* Stewart, W.D.P., ed., Algal physiology and biochemistry: London, Blackwell Scientific, Botanical Monographs, v. 10, p. 636-654.

Kunikane, S., and Kaneko, M., 1984, Growth and nutrient uptake of green alga, Scenedesmus dimorphus, under a range of nitrogen/phosphorus ratio–II: Water Research, v. 18, p. 1313-1326.

Kunikane, S., Kaneko, M., and Maehara, R., 1984, Growth and nutrient uptake of green alga, Scenedesmus dimorphus, under a wide range of nitrogen-phosphorus ratio–I: Water Research, v. 18, p. 1299-1311.

Lane, J.L., and Goldman, C.R., 1984, Size-fraction of natural phytoplankton communities in nutrient bioassay studies: Hydrobiologia, v. 118, p. 219-223.

Lean, D.R.S., and Burnison, B.K., 1979, An evaluation of errors in the ^{14}C method of primary production measurement: Limnology and Oceanography, v. 24, p. 917-928.

Little, T.M., 1981, Interpretation and presentation of results: Horticultural Science, v. 16, p. 637-640.

Malone, T.C., 1971, Diurnal rhythms in netplankton and nannoplankton assimilation ratios: Marine Biology, v. 10, p. 285-289.

——— 1980, Algal size, *in* Morris, I., ed., The physiology of phytoplankton: London, Blackwell Scientific, p. 433-464.

——— 1982, Phytoplankton photosynthesis and carbon specific growth: Light saturated rates in a nutrient-rich environment: Limnology and Oceanography, v. 27, p. 226-235.

Malone, T.C., and Neale, P.J., 1981, Parameters of light-dependent photosynthesis for phytoplankton size fractions in temperate estuative and coastal environments: Marine Biology, v. 61, p. 289-297.

Marra, J., and Heinemann, K., 1984, A comparison between noncontaminating and conventional incubation procedures in primary production measurements: Limnology and Oceanography, v. 29, p. 389-392.

Marshall, H.G., 1985, Comparison of phytoplankton concentrations and cell volume measurements from the continental shelf off Cape Cod, Massachusetts, U.S.A.: Hydrobiologia, v. 120, p. 171-179.

McComb, A.J., Atkins, R.P., Birch, P.B., Gordon, D.M., and Lukatelich, R.J., 1981, Eutrophication in the Peel-Harvey estuarine system, western Australia, *in* Neilson, B.J., and Cronin, L.E., eds., Estuaries and nutrients: New Jersey, Humana Press, p. 323-342.

McPherson, B.F., and Miller, R.L., 1987, The vertical attenuation of light in Charlotte Harbor, a shallow, subtropical estuary, southwest Florida: Estuarine, Coastal and Shelf Science, v. 25, p. 721-737.

——— 1990, Nutrient distribution and variability in the Charlotte Harbor estuarine system, Florida: Water Resources Bulletin, v. 26, no. 1, p. 67-80.

McPherson, B.F., Montgomery, R.T., and Emmons, E.E., in press, Temporal and spatial variability of phytoplankton productivity in the Charlotte Harbor estuarine system, Florida: Journal review.

Montgomery, R.T., and Emmons, E.E., 1989, Production and structure of phytoplankton communities within the lower Peace River and upper Charlotte Harbor estuarine system, chapter 3 *in* Hydrobiological monitoring program report, March 1987-February 1988, for the lower Peace River and Charlotte Harbor: Submitted to the Southwest Florida Water Management District by General Development Utilities, Inc.

Morel, F.M.M., 1987, Kinetics of nutrient uptake and growth in phytoplankton: Journal of Phycology, v. 23, p. 137-150.

Munawar, M., Munawar, I.F., Culp, L.R., and Dupuis, G., 1978, Relative importance of nanoplankton in Lake Superior phytoplankton biomass and community metabolism: Journal of Great Lakes Research, v. 4, p. 462-480.

Munawar, M., Murdoch, A., Munawar, I.F., and Thomas, R.L., 1983, The impact of sediment-associated contaminants from the Niagara River mouth on various size assemblages of phytoplankton: Journal of Great Lakes Research, v. 9, p. 303-313.

Myers, V., and Iverson, R.L., 1981, Aspects of nutrient limitation of the phytoplankton productivity in the Apalachicola Bay system: Florida Marine Research Publications, v. 26, p. 68-74.

Nalewajko, C., and Garside, C., 1983, Methodological problems in the simultaneous assessment of photosynthesis and nutrient uptake in phytoplankton as functions of light intensity and cell size: Limnology and Oceanography, v. 28, no. 3, p. 591-597.

Nalewajko, C., and Lee, K., 1983, Light stimulation of phosphate uptake in marine phytoplankton: Marine Biology, v. 74, p. 9-15.

Nixon, S.W., 1981, Remineralization of nutrient cycling in coastal marine ecosystems, *in* Neilson, B.J., and Cronin, L.E., eds., Estuaries and nutrients: New Jersey, Humana Press, p. 111-138.

Pennock, J.R., 1987, Temporal and spatial variability in phytoplankton ammonium and nitrate uptake in the Delaware Estuary: Estuarine, Coastal and Shelf Science, v. 24, p. 841-857.

Peterson, R.G., 1977, Use and misuse of multiple comparison procedures: Journal of Agronomy, v. 69, p. 205-208.

Priscu, J.C., 1987, Time-course of inorganic nitrogen uptake and incorporation by natural populations of freshwater phytoplankton: Freshwater Biology, v. 17, p. 331-339.

Probyn, T.A., 1985, Nitrogen uptake by size-fractionated phytoplankton populations in the southern Benguela upwelling system: Marine Ecology Progress Series, v. 22, p. 249-258.

Pugh, P.R., 1970, Liquid scintillation counting of ^{14}C-diatom material on filter papers for use in productivity: Limnology and Oceanography, v. 15, p. 652-655.

Ram, N.M., and Plotkin, S., 1983, Assessing aquatic productivity in the Housatonic River using the algal assay bottle test: Water Research, v. 17, no. 9, p. 1095-1106.

Redfield, A.C., 1958, The biological control of chemical factors in the environment: American Scientist, v. 46, p. 205-222.

Ryther, J.H., and Dunstan, W.M., 1971, Nitrogen, phosphorus, and eutrophication in the coastal marine environment: Science, v. 171, p. 1008-1013.

Sakshaug, E., and Olsen, Y., 1986, Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition: Canadian Journal of Fisheries, v. 43, p. 389-396.

Sanders, J.G., Cibik, S.J., D'Elia, C.F., and Boynton, W.R., 1987, Nutrient enrichment studies in a coastal plain estuary: Changes in phytoplankton species composition: Canadian Journal of Fisheries, v. 44, no. 1, p. 83-90.

Schanz, F., and Juon, H., 1983, Two different methods of evaluating nutrient limitations of periphyton bioassays, using water from the River Rhine and eight of its tributaries: Hydrobiologia, v. 102, p. 187-195.

Sellner, K.G., 1983, Importance of size fractionated phytoplankton productivity biomass and species composition in carbon export, pt. I *of* Plankton productivity and biomass in a tributary of the upper Chesapeake Bay: Estuarine, Coastal and Shelf Science, v. 17, p. 187-206.

Sicko-Goad, L., and Stoermer, E.F., 1984, The need for uniform terminology concerning phytoplankton cell size fractions and examples of picoplankton from the laurentian Great Lakes: Journal of Great Lakes Research, v. 10, p. 90-93.

Sklar, F.H., and Turner, R.E., 1981, Characteristics of phytoplankton production off Barataria Bay in an area influenced by the Mississippi River: Continental Marine Science, v. 24, p. 93-106.

Smith, R.E.H., and Kalff, J., 1981, The effect of phosphorus limitation on algal growth rates: Evidence from alkaline phosphatase: Canadian Journal of Fisheries, v. 38, p. 1421-1427.

Smith, S.V., 1984, Phosphorus versus nitrogen limitation in the marine environment: Limnology and Oceanography, v. 29, p. 1149-1160.

Sommer, U., 1985, Comparison between steady state and non-steady state competition: Experiments with natural phytoplankton: Limnology and Oceanography, v. 30, p. 335-346.

Statistical Analysis System Institute, Inc., 1987, SAS/STAT guide for personal computers (6th ed.): Cary, N.C., Statistical Analysis System Institute, Inc., 1,028 p.

Stockner, J.G., 1988, Phototrophic picoplankton: An overview from marine and freshwater ecosystems: Limnology and Oceanography, v. 33, p. 765-775.

Strickland, J.D.H., and Parsons, T.R., 1972, A practical handbook of seawater analysis: Ottawa, Fisheries Research Board of Canada, 310 p.

Takahashi, M., and Saijo, Y., 1981, Ammonium and nitrate uptake by phytoplankton, pt. I *of* Nitrogen metabolism in Lake Kizaki, Japan: Hydrobiologia, v. 91, p. 393-407.

Taylor, J.L., 1974, The Charlotte Harbor estuarine system: Florida Scientist, v. 37, p. 205-216.

Tilman, D., Kilham, S.S., and Kilham, P., 1982, Phytoplankton community ecology: The role of limiting nutrients: Annual Review Ecological Systems, v. 13, p. 349-372.

U.S. Environmental Protection Agency, 1979, Methods for chemical analysis of water and wastes: Office of Research and Development, EPA-600/4-79-020.

Vince, S., and Valiel, I., 1973, The effects of ammonium and phosphate enrichment on chlorophyll-*a* pigment ratio and species composition of phytoplankton of Vineyard Sound: Marine Biology, v. 19, p. 69-73.

Waller, R.A., and Duncan, D.B., 1969, A Bayes rule for the symmetric multiple comparisons problem: Journal of the American Statistical Association, v. 64, p. 1484-1503.

Whalen, S.C., and Alexander, V, 1984, Diel variations in inorganic carbon and nitrogen uptake by phytoplankton in an artic lake: Journal of Plankton Research, v. 6, p. 571-590.

Wurtsbaugh, W.A., Vincent, W.F., Tapia, R.A., Vincent, C.L., and Richerson, P.J., 1985, Nutrient limitation of algal growth and nitrogen fixation in a tropical alpine lake, Lake Titicaca (Peru/Bolivia): Freshwater Biology, v. 15, p. 185-195.

*U.S. Government Printing Office: 1991 — 631-117/40008