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CHLOROPHYLL *A* AND ITS USE AS AN INDICATOR OF EUTROPHICATION IN THE CALOOSAHATCHEE ESTUARY, FLORIDA

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ABSTRACT: *The use of phytoplankton chlorophyll* a *to indicate eutrophication in the Caloosahatchee Estuary and San Carlos Bay was evaluated. Responses of chlorophyll* a *to nutrient loading and freshwater discharge at the Franklin Lock and Dam located at the head of the estuary were examined. Relationships between chlorophyll* a *and dissolved oxygen and light attenuation in the downstream estuary and bay were also investigated. Statistically significant positive correlations with nutrient loading in the lower estuary and San Carlos Bay, significant association between increasing chlorophyll* a *and decreasing dissolved oxygen in bottom waters in the estuary, and positive correlation between light attenuation and chlorophyll* a *in San Carlos Bay argue for the use of chlorophyll* a *to indicate eutrophication. Relationships between chlorophyll* a *and freshwater discharge indicated a flushing or 'wash out' effect. Review of the literature suggested that discharge of dark, colored water enhanced light attenuation. Both effects of discharge would suppress the accumulation of chlorophyll biomass. While chlorophyll* a *might be used to indicate eutrophication in the Caloosahatchee, useful interpretation of the response of this indicator to future reductions in nutrient loading must account for the modulating effects of freshwater discharge exerted through flushing and reductions in light availability.*

Key Words: Eutrophication, indicator, chlorophyll *a*

EXCESSIVE fertilization or eutrophication of coastal waters with nitrogen and phosphorus is a continuing world-wide problem (Palmer et al., 2004; Smith et al., 2003; Cloern, 2001; Eyre, 2000). Conceptual understanding of the responses of coastal ecosystems to eutrophication has changed. In a recent review, Cloern (2001) describes three phases in the evolution of this concept. The first emphasized the link between nutrient input, enhanced production of phytoplankton biomass, and the subsequent depletion of dissolved oxygen (e.g. Ryther and Dunstan, 1971). Observation over the past several decades has shown that estuarine systems do not respond generically to enhanced nutrient input. For example, while phytoplankton may bloom in some systems, macroalgae may be favored in others (Harlan, 1995). The Phase II model attempts to explain this diversity of estuarine response. It recognizes a variety of direct responses that can lead to a variety of indirect responses. A good example is the decline of seagrass associated with eutrophication. Increased nutrient supplies lead to increased chlorophyll biomass in the water column (a direct response) that shades out submerged aquatic vegetation (an indirect response, Twilley et al., 1985). Diversity of response is also explained in part by system specific physical and biological attributes or "filters" such as tidal range
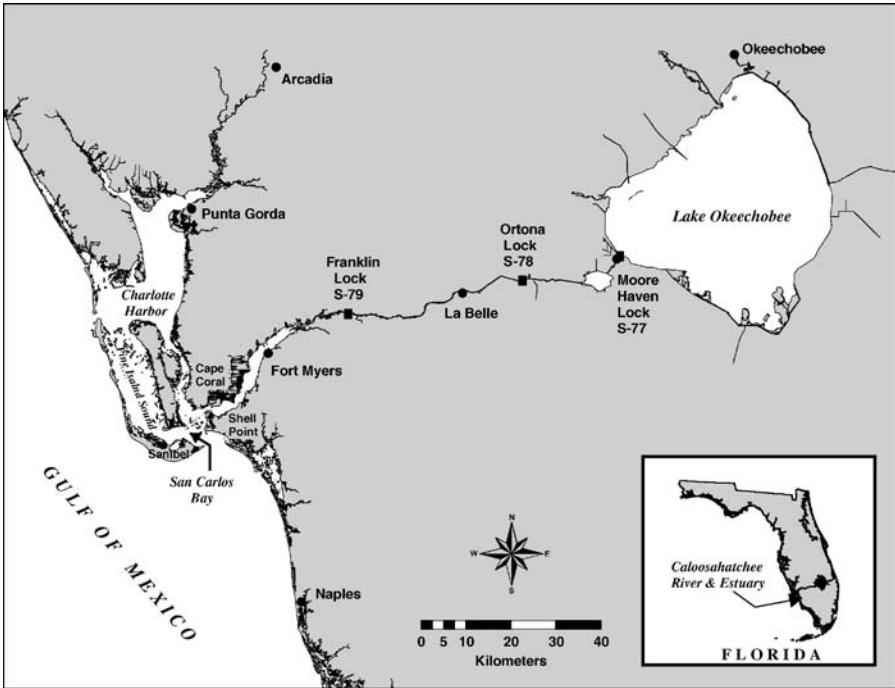
FIG. 1.   Location of the Caloosahatchee River and Estuary.

(Monbet, 1992), residence time (Nixon et al., 1996; Welch et al., 1972), and dense populations of filter feeders (Officer et al., 1982; Meeuwig et al., 1998). These attributes can enhance or mask the expression of eutrophication (Cloern, 2001). Understanding how these filters work and how they interact with other stressors is central to the development of the next (Phase III) conceptual model of eutrophication.

The response of phytoplankton biomass to increased nutrient input comprises a major pathway in many conceptual models of eutrophication (Gray, 1992; Harlan, 1995; Smith et al., 1999; Cloern, 2001). Chlorophyll *a*, a measure of phytoplankton biomass, is commonly employed as an indicator of eutrophication (Bricker et al., 1999). Yet, variation in chlorophyll *a* within and between estuarine systems does not always reflect differences in nutrient loading (Tomasko et al., 1996; Cloern, 2001). Use of chlorophyll *a* as an indicator should be considered within the context of our more complex understanding of eutrophication (Phase II, Cloern, 2001).

The Caloosahatchee River and Estuary, located on the southwest coast of Florida, are part of the larger Charlotte Harbor system (FIG. 1). The Caloosahatchee River runs 67 km from Lake Okeechobee to the Franklin Lock and Dam (S-79). S-79 separates the freshwater river from the estuary that terminates 40 km downstream at Shell Point (FIG. 1). The system has been modified. The River has been straightened, deepened and three water control structures have been added. The last, S-79, was completed in 1966 to act in part as a salinity barrier (Flaig and Capece,

1998). The River has also been artificially connected to Lake Okeechobee to convey regulatory releases of water to tide. The estuarine portion of the system has also been modified. Seven automobile bridges and one railroad bridge connect the north and south shores of the estuary. A navigation channel has been dredged and in the 1960's a causeway was built across the mouth of San Carlos Bay. Historic oyster bars upstream of Shell Point have been mined and removed for road construction.

Water quality has been a concern in the Caloosahatchee since the late 1970s and early 1980s. A waste-load allocation study in the Caloosahatchee conducted by the Florida Department of Environmental Regulation concluded that the estuary had reached its nutrient loading limits as indicated by elevated chlorophyll *a* and depressed dissolved oxygen concentrations (DeGrove, 1981). The purpose of this report is to (1) characterize nutrient loading at S-79 and quantify its relationship with chlorophyll *a* in the downstream estuary; (2) evaluate potential nutrient limitation and (3) to evaluate the use of chlorophyll *a* as an indicator of eutrophication in this system.

In keeping with the conceptual evolution of eutrophication described by Cloern (2001), evaluation of chlorophyll *a* as an indicator of eutrophication focused on component relationships of the Phase II model. First, the direct response of chlorophyll *a* to nutrient loading was evaluated. Two indirect responses were also examined: the relationship between chlorophyll *a* concentrations in surface water with oxygen concentrations in bottom water and the relationship between light extinction and chlorophyll *a*. The latter analysis also quantified the contribution of color and total suspended solids to light attenuation in the Caloosahatchee. Finally, the effects of freshwater discharge at S-79 on the downstream distribution of chlorophyll *a* are examined to determine if this parameter may be an important "filter" *sensu* Cloern (2001).

METHODS—*Data sets*—The water quality data evaluated here came from six (6) monitoring programs either conducted or supported by the South Florida Water Management District. All programs monitored the quality of surface waters with samples being taken within the top 0.5 m of the water column using a van Dorn, Kimmerer or similar bottle.

The Caloosahatchee River (CR) program sampled just upstream of the Franklin Lock and Dam (S-79). The program began in January, 1981 and continues to the present. Data from 1981 through June 2003 were analyzed. The frequency of sampling varied throughout the period of record generally being 6–8 times per year but ranging from 3 to 12 (monthly) times per year.

The Caloosahatchee Estuary (CAL) program sampled water quality at 17 stations in the estuary (Shell Point to S-79), San Carlos Bay, Matlacha Pass, and Pine Island Sound. The stations, sampled monthly from December 1985 to May 1989, were all located downstream of S-79. At each station, vertical profiles (0.5 m intervals) of temperature, salinity and dissolved oxygen were obtained electronically using Hydrolab or YSI sonde units.

The Caloosahatchee Estuary High Flow (CALHF) effort sampled monthly at 8 stations from October 1994 to August 1996. Seven stations were located in the estuary and San Carlos Bay, while one was located in freshwater upstream of S-79.

The Center for Environmental Studies (CES) program sampled 7 stations in the estuary (S-79 to Shell Point) and one (1) station upstream of S-79 on a monthly basis from April 1999 to March 2002. Temperature, salinity and dissolved oxygen were measured at 0.5 m below the surface and 0.5 m from the bottom. As of May 2002, the number of stations was reduced to 4, with one upstream of S-79 and the rest in the downstream estuary. This reduced sampling effort continues to the present. Data through June 2003 were used in the analysis.

The Southeastern Environmental Research Center (SERC) program sampled 8 stations in San Carlos Bay, Pine Island Sound, Matlacha Pass and the Gulf of Mexico on a monthly basis beginning in January 1999. The project continues to the present. Data through March 2003 were used in the analysis.

The Environmental Research and Design Program (ERD) sampled 15 sites in the Caloosahatchee Estuary and San Carlos Bay (ERD, 2003). This program was not designed to detect long term trends and therefore was not used in the analysis of water quality or loading. Stations were sampled for two month-long periods in each of three years (2000, 2001, and 2002). Each year one wet season month and one dry season month was sampled. During each sampling month, estuarine stations were occupied 4 times, once every ten days. In addition to vertical profiles of temperature, salinity and dissolved oxygen, vertical profiles of photosynthetically active radiation (PAR) were obtained using a Li-COR PAR Meter with 2 pi deck and submerged collectors.

Water quality—In the field, samples for dissolved inorganic nutrients (NH_4 = ammonia, NOx = Nitrate + Nitrate, DIN = NH_4 + NO_X, DIP = dissolved inorganic phosphorus) and color were passed manually through 0.4 μm membrane filters, using a syringe. Whole water samples were retained for total Kjeldahl nitrogen (TKN), total phosphorus (TP), total suspended solids (TSS) and chlorophyll *a* (Chl *a*). Chlorophyll *a* samples were filtered and analyzed spectrophotometrically in the laboratory within 24 hrs of collection. All samples were stored on ice until their return to the laboratory.

Samples were analyzed using standard methods in the South Florida Water Management District's Water Quality Laboratory or through contracts with private sector laboratories. All laboratories were certified by the National Environmental Laboratory Accreditation Program (NELAP). All nutrient, TSS and dissolved oxygen (DO) concentrations are reported in mg/l, chlorophyll *a* concentrations, corrected for phaeophytin, in μg/l, color in Pt-Co units, salinity (SAL) in parts per thousand and Secchi Disk Depth (SDD) in meters. Total Nitrogen (TN) was calculated as TKN + NO_X.

Calculation of nutrient loads at S-79—The loads of nutrients delivered to the estuary at the Franklin Lock and Dam (S-79) were calculated by multiplying the daily average discharge of freshwater by the concentration of nutrients in the water. A daily average discharge at S-79 was available from records kept by the SFWMD dating back to the 1960s. Data taken upstream of S-79 from the CR, CALHF and CES programs were used to generate a data set of daily concentrations by linear interpolation between sampling dates. From these data daily, 30-day and annual loads were calculated.

Analysis of loads at S-79 concentrated on temporal trends and sources of variation in the load (concentration or discharge). Temporal trends in annual discharge and loads of total nitrogen and phosphorus from 1981 to 2002 were evaluated using Kendall's Tau b correlation coefficient (SAS, 1989). Trends in daily loads were evaluated as follows. Only loads calculated for days upon which a concentration at S-79 was actually measured were considered. Daily load and concentration data were averaged by year and month to avoid undue influence of any time period. This procedure yielded a daily average load for each month in which S-79 was sampled. Temporal trends were evaluated both with Kendall's Tau b and Spearman's Rank correlation coefficients (SAS, 1989).

Multiple regression was employed to evaluate the contribution of daily discharge and concentration to variation in daily load. Only loads calculated for days upon which a concentration at S-79 was actually measured were considered.

Water quality in the estuary—Water quality varies in both time and space. In order to account for spatial variation, the Caloosahatchee system was divided into 4 areas (FIG. 2) each encompassing stations from the various sampling programs summarized below (Table 1). Only data from the CAL, CALHF, CES and SERC programs were used to evaluate trends in water quality. To account for potential differences in detection limits, the detection limits for the CAL program were applied to all data. Values less than the CAL detection limits were set to one half the detection limit.

In each region data were sorted by year and month and then averaged across stations. This produced a set of monthly observations in each region. The data were discontinuous, falling into 3 time periods: December 1985–May 1989 (CAL), November 1994–August 1996 (CALHF) and April 1999–June 2003 (CES, SERC).
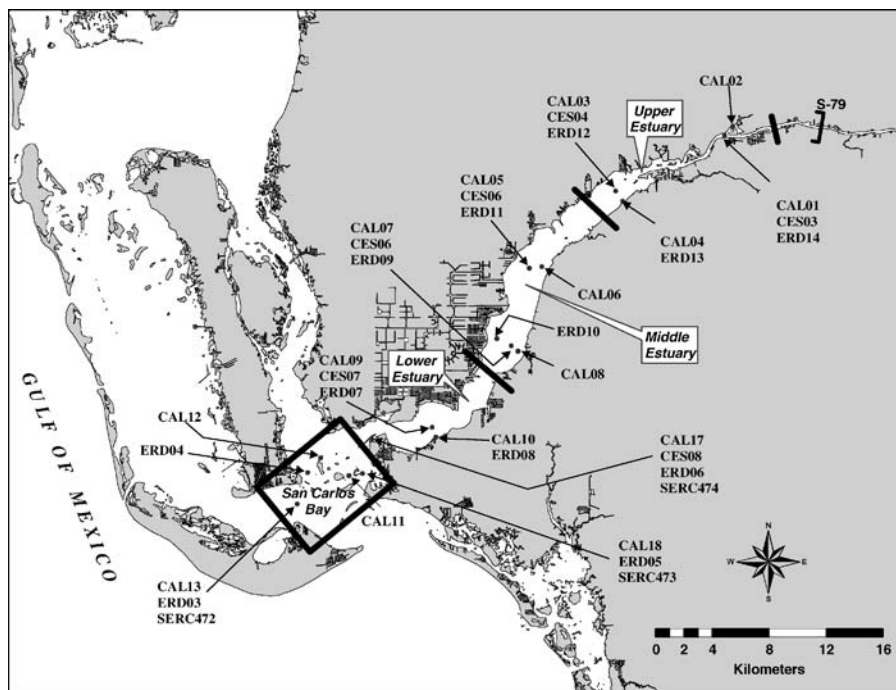
Fɪɢ. 2. Location of water quality sampling stations in the Caloosahatchee Estuary and San Carlos Bay.

Statistically, regional differences in water quality parameters were evaluated with the Kruskal-Wallis H test (NPAR1WAY, SAS 1989). This test amounts to a non-parametric one-way analysis of variance on ranked data with region being the treatment factor. If the H test was statistically significant, data were ranked and differences between regions were evaluated using Proc Multtest, with a bootstrap correction of the p-value to account for making multiple t-tests on ranks (equivalent to a Wilcoxon rank-sum test).

The discontinuity of data sets precluded traditional analysis for temporal trends using, for example, Kendall's Tau b. Interpreting differences between periods as temporal trends, even those that increase or decrease progressively from one period to the next, should be done with great caution. It is beyond the scope of this effort to evaluate the data for temporal trends or attempt to explain differences between periods. However, we have compared the three periods for a selected set of water quality parameters relevant to this study (Salinity, TN, DIN, DIP, DIN:DIP, Chl *a*). Differences in water quality between the three periods were evaluated for each region using the same approach as just described with period being the treatment factor. Rain fall and discharge from Lake Okeechobee and to the estuary at S-79 also were summarized for each period. In order to compare periods of different lengths, monthly values during each

TABLE 1. Sampling stations in the four regions of the Caloosahatchee Estuarine System.

Region of Estuary	Program				Km from S-79
	CAL, CALHF	CES	SERC	ERD	
Upper	01,02,03,04	03,04	—	12,13	6–14
Mid	05,06,07,08	05,06	—	9,10,11	14–28
Lower	09,10,17	07,08	474	6,7,8	28–41
San Carlos Bay	18,11,12,13	—	472,473	3,4,5	41–49

period were summed, divided by the number of months in the period and multiplied by 12 to produce a 12 month, periodic average for each time period.

Nutrient loading and chlorophyll—The dependence of chlorophyll *a* concentrations in the estuary on loading at S-79 was established by simple linear correlation. Data from stations within each region were averaged by sampling date to produce one observation per region per date. Correlations between concentration in the estuary and the loading that had occurred over the 30 days prior to sampling were calculated.

Other standard correlation and regression techniques applied to the data are described in the results section. All statistical analyses were performed using SAS Version 8 software (SAS, 1989).

Potential nutrient limitation—Although providing only a first approximation, comparison of nutrient concentrations with literature values for the half-saturation constant of nutrient uptake by phytoplankton furnishes a measure of nutrient limitation (Fisher et al., 1988). Half-saturation constants range between 0.014 and 0.028 mg/l for DIN and 3.1 and 15.5 μg/l for DIP. Concentrations below these ranges indicate a potential for nutrient limitation (Fisher et al., 1988). As a measure of potential limitation in each region, the proportion of concentration measurement above and below the lower limit of theses ranges were calculated.

Chlorophyll and dissolved oxygen—The correlation between chlorophyll *a* concentration in surface waters and dissolved oxygen in bottom waters was examined using data from the CES sampling program. Visual analysis of graphed data was employed to demonstrate dependence over short time scales (weeks). On monthly time scales, linear correlation coefficients were calculated with lags up to 2 months.

Chlorophyll and light extinction—Photosynthetically Active Radiation (PAR) data required for the calculation of the light extinction coefficient (K_d) was consistently collected only during the ERD study (ERD, 2003). On each sampling date, data were averaged across stations in a region. Stepwise multiple regressions relating variation in the extinction coefficient to chlorophyll *a*, color and total suspended solids were calculated for each region. Statistically, the regression approach identified the water quality parameters that most influence change or variation in light extinction. Following McPherson and Miller (1994), the approach also was used to partition the light extinction coefficient and quantify the contribution of individual water quality parameters to the total light extinction using individual regression coefficients. The concentration of an individual water quality constituent on each sampling date was multiplied by its regression coefficient from the multiple regression equation. The result was divided by the corresponding light extinction coefficient on the same day.

Chlorophyll and freshwater discharge—The potential for freshwater discharge at S-79 to influence the distribution of chlorophyll *a* was examined regionally in the same way as the relationship with nutrient loading described above. The effect of discharge on the longitudinal position of maximum chlorophyll *a* in the study area (S-79 to San Carlos Bay) was examined following Doering and co-workers (1994) and Doering and Chamberlain (1999). On each sampling date, the station with the highest chlorophyll *a* concentration was identified along with its distance from S-79. The correlation between the position of the chlorophyll maximum and discharge at S-79 was determined. In addition, the position data were classified into several flow ranges increasing from low to high and analyzed by one-way ANOVA. The treatment was flow range. The flow ranges were based on the salinities they produce in the estuary and the tolerances of estuarine organisms (Chamberlain and Doering, 1998; Doering et al., 2002). Flows less than about 14 m³/sec (500 cfs) do not maintain the full (0–35 ppt) salinity gradient in the estuary. At flows greater than about 79 m³/sec (2800 cfs) salinity declines in the lower estuary impacting marine seagrasses typical of this area. Flows greater than about 127 m³/sec (4500 cfs) lower salinity sufficiently in San Carlos Bay to impact seagrasses there (Chamberlain and Doering, 1998).

Not all the data could be used for this analysis. The CAL and CALHF program sampled the entire study area. The CES and SERC programs sampled the Caloosahatchee Estuary and San Carlos Bay
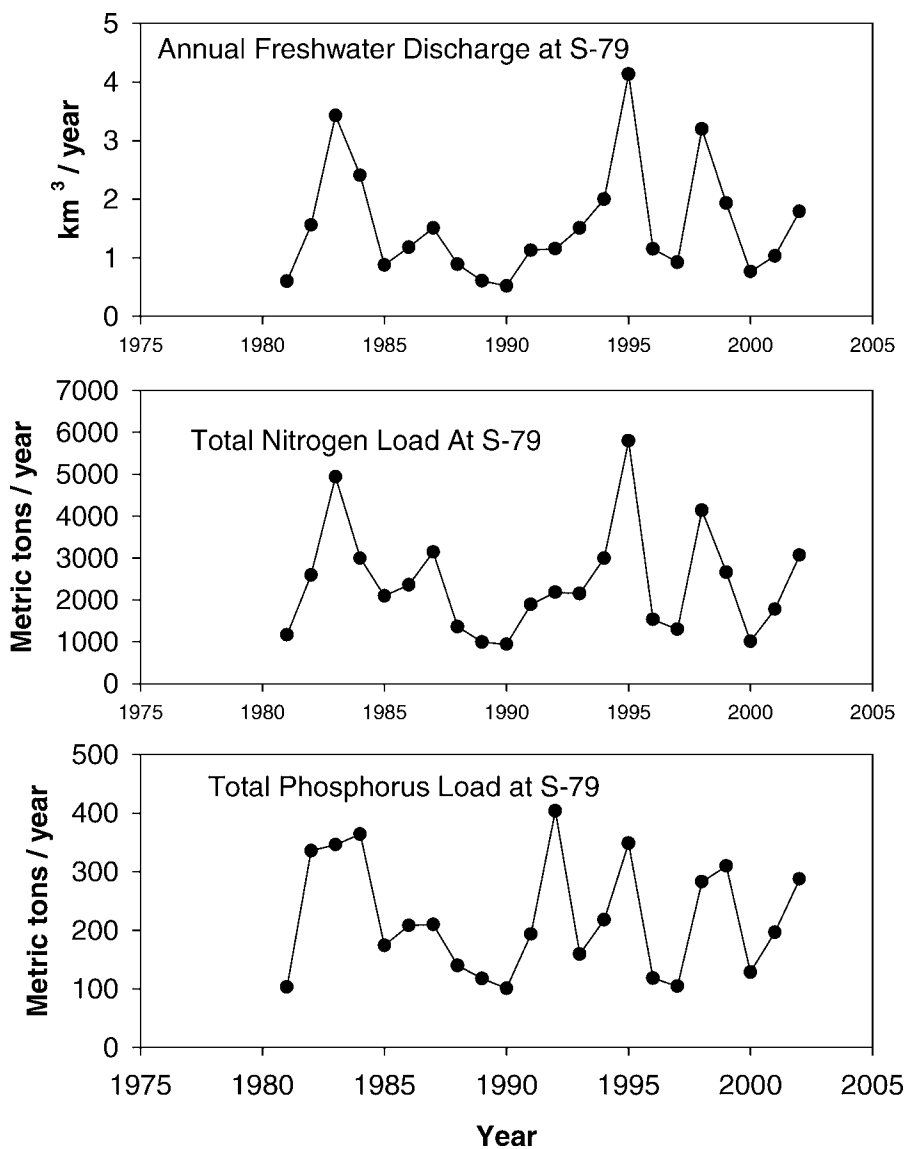
FIG. 3.   Annual discharge and annual loading of total nitrogen and phosphorus at S-79.

respectively. In most instances, the two programs sampled their respective areas within a day or two of each other and these were considered as one event. At other times sampling was not so coincidental. Events occurring more than a week apart were eliminated from the analysis.

RESULTS—*Nutrient loading*—Annual discharge of freshwater at S-79 averaged 1.57 km^3/yr (1.27 million acre-ft), with a minimum of 0.52 km^3 (424 thousand ac-ft) in 1990, a drought year, and a maximum of 4.17 km^3 (3.38 million ac-ft) in 1995,

TABLE 2. Fraction of variation in daily loading at S-79 explained by fluctuations in discharge and nutrient concentration. All fractions are statistically significant at p<0.0001.

	Discharge	Concentration	Total Variation
Total Nitrogen	0.908	0.023	0.931
Dissolved Inorganic Nitrogen	0.692	0.082	0.774
Total Phosphorus	0.716	0.117	0.833
Dissolved Inorganic Phosphorus	0.503	0.260	0.763

a very wet year (FIG. 3). No long term trend in discharge was detected (Kendall Tau b, p>0.60). Annual loading of total nitrogen at S-79 averaged 2412 metric tons/year with a minimum of 938 metric tons in 1990 and a maximum of 5801 metric tons in 1995. Over the period 1981 through 2002 there was no general increase or decrease in the annual total nitrogen load (p>0.8). Loading of total phosphorus averaged 220 metric tons/year with a minimum of 101 metric tons in 1990 and a maximum of 403 metric tons in 1992. No long term trends were detected (p>0.8). The molar ratio of the total N load to the total P load averaged 24.4 and ranged from 12 to 37.

Variation in daily nutrient loads at S-79 was primarily a function of freshwater discharge (Table 2). In multiple regressions, this variable explained between 50 and 90% of the variation in nutrient loads. Concentration explained a significant but substantially smaller proportion of the total variation (range 2–26%).

No long term trends in the daily loads (Table 3) of total nitrogen, total phosphorus, dissolved inorganic nitrogen or dissolved inorganic phosphorus at S-79 were detected. The molar N to P ratio of the daily total nutrient load averaged nearly 30 and the median was 26. The molar ratio of the daily inorganic load averaged about 9.5 over the 22 year period of record with a median of 7.5.

Differences between periods—Hydrologic conditions varied between the three sampling periods. Period 2 was the wettest with a 12 month periodic average rainfall of 1.67 m, compared to Period 1 with 1.17 m and Period 3 with 1.45 m. This result is expected given that 1995 was a very wet year (FIG. 3). Period 2 also had the highest 12 month periodic average discharge at S-79 (3.22 km^3) with 60% accounted for by discharge from Lake Okeechobee. Discharge at S-79 for Period 1 averaged 1.09 km^3/12 months with only 11% being released from Lake Okeechobee. For Period 3, discharge at S-79 averaged 1.55 km^3/12 months with 30% being released from Lake Okeechobee.

Salinity reflected these hydrologic conditions in all regions being lower during

TABLE 3. Summary of daily loads at S-79.

	Daily Load (kg/day)			
	Mean	Median	Minimum	Maximum
Total Nitrogen	7,018	2,618	0	46,885
Dissolved Inorganic Nitrogen	1,385	567	0	12,874
Total Phosphorus	657	253	0	5,141
Dissolved Inorganic Phosphorus	426	157	0	3,352

TABLE 4. Median values for selected water quality parameters during three time periods in four regions of the Caloosahatchee estuarine system. Letters indicate statistical differences between periods at p<0.05. Medians with the same letter are not statistically different. DIN:P is the molar ratio of DIN to DIP.

Region	Period	Water Quality Parameter					
		SAL	TN	DIP	CHL *a*	DIN	DIN:P
Upper Estuary	1985–1989	4.1 a	1.43 a	0.08 a	10.3	0.10	2.7 b
	1994–1996	0.3 b	1.31 a	0.04 b	3.5	0.17	8.6 a
	1999–2003	1.0 a	1.13 b	0.06 b	8.6	0.19	5.5 a
Mid Estuary	1985–1989	13.9 a	1.30 a	0.06 a	8.1	0.01 b	0.4 b
	1994–1996	1.0 b	1.29 a	0.04 b	7.3	0.09 a	7.9 a
	1999–2003	8.8 a	0.91 b	0.04 b	10.5	0.04 a	3.4 a
Lower Estuary	1985–1989	25.3 a	0.95 a	0.04 a	4.7	0.01 c	0.95 c
	1994–1996	15.3 b	0.99 a	0.03 ab	5.5	0.13 a	11.7 a
	1999–2003	26.8 a	0.33 b	0.02 b	3.6	0.03 b	3.5 b
San Carlos Bay	1985–1989	30.7	0.83 a	0.015 a	3.1	0.01 b	1.9 c
	1994–1996	27.9	0.83 a	0.014 ab	3.4	0.15 a	19.9 a
	1999–2003	31.8	0.25 b	0.008 b	3.4	0.01 b	5.2 b

Period 2 than at other times (Table 4). The concentration of TN was lower in all regions during Period 3 than at other times. In general, DIP concentrations were highest and DIN concentrations were lowest in Period 1. Chlorophyll *a* did not change. The molar ratio of DIN:DIP was generally lowest during Period 1, highest during Period 2 and intermediate during Period 3 (Table 4).

Spatial trends in water quality and potential nutrient limitation—Evaluation of the overall spatial variation in water quality indicated several patterns (Table 5). As expected, median salinity increased from the upper estuary to San Carlos Bay. Many

TABLE 5. Median values of water quality parameters by estuarine region. Letters indicate statistical differences between regions at p<0.05. Medians with the same letter are not statistically different. Medians calculated for all three sampling periods combined except for SDD, Color, and TSS. These parameters were measured in San Carlos Bay only during the 1985–1989 and 1994–1996 sampling periods.

Parameter	Region			
	Upper Estuary	Mid Estuary	Lower Estuary	San Carlos Bay
SAL	4.1 d	10.1 c	22.6 b	29.4 a
TN	1.26 a	1.05 b	0.67 c	0.55 d
DIN	0.18 a	0.10 b	0.07 b	0.05 c
NH4	0.038 a	0.027 b	0.025 b	0.026 b
NOx	0.15 a	0.08 b	0.04 c	0.02 d
Chl *a*	10.7 a	12.7 a	5.3 b	4.2 b
TP	0.14 a	0.13 a	0.09 b	0.05 c
DIP	0.07 a	0.06 b	0.03 c	0.02 d
TSS	9.8 b	15.0 b	24.8 a	21.0 a
Color	93 a	73 a	42 b	20 c
SDD	1.05 c	1.13 bc	1.33 ab	1.37 a

TABLE 6. Potential nutrient limitation. Percentage and (n = number) of measured nutrient concentrations falling below (limiting) and above (not limiting) half-saturation constants for the uptake of DIN (0.014 mg/l) and DIP (3.1 μg/l).

Region	Nutrient Status	DIN Percent (n)	DIP Percent (n)
Upper Estuary	Limiting	24% (73)	2% (7)
	Not Limiting	76% (236)	98% (302)
Mid Estuary	Limiting	45% (125)	3% (9)
	Not limiting	55% (152)	97% (268)
Lower Estuary	Limiting	40% (107)	4% (10)
	Not Limiting	60% (163)	96% (260)
San Carlos Bay	Limiting	63% (164)	17% (45)
	Not Limiting	37% (96)	83% (215)

water quality parameters showed an inverse pattern, decreasing from the upper estuary to San Carlos Bay: TN, DIN, NO_X, DIP, Color. Others such as TSS and SDD followed the same pattern as salinity, increasing towards the Gulf of Mexico. Concentrations of some parameters (Chl *a*, TP, Color) suggested two regions of differing water quality: an upper and mid estuarine region with higher concentrations and a lower estuary- San Carlos Bay region with lower concentrations.

Potential nutrient limitation as judged by measured DIN and DIP concentrations relative to half-saturation constants for nutrient uptake indicated nitrogen limitation more often than phosphorus limitation (Table 6). Furthermore, the percentage of measurements indicating nitrogen limitation increased progressively from 24% in the upper estuary to 40–45% in the lower and mid-estuary to 63% in San Carlos Bay.

Nutrient loading and chlorophyll—The correlation between chlorophyll *a* concentration in the Calooshatchee estuary and the loading of total nitrogen during the 30 days prior to sampling varied spatially (FIG. 4). In San Carlos Bay and the lower estuary, increased loading corresponded to increased chlorophyll *a*. In the mid-estuary the correlation was not significant. In the upper estuary, the relationship was negative with increased loading associated with a reduction in the concentration of chlorophyll *a*. It is worth noting that while TN loading is featured in Figure 4, this does not mean that TN limits the growth of phytoplankton in the Caloosahatchee. Chlorophyll *a* concentrations showed the same regional relationships with DIN loading, DIP loading and TP loading: positive in the lower estuary and San Carlos Bay, not significant in the mid-estuary and negative in the upper estuary (Table 7).

Except in the mid-estuary, these relationships were seasonally robust. In the upper estuary, lower estuary and San Carlos Bay correlations for the wet (November–April) and dry (May–October) seasons showed the same patterns as when all data were considered together. The negative relationship in the upper estuary during the wet season was significant only at the $p < 0.10$ level. By contrast, the relationship between loading and chlorophyll *a* in the mid-estuary was positive in the dry season (r = 0.229, $p < 0.05$) and not significant in the wet season.

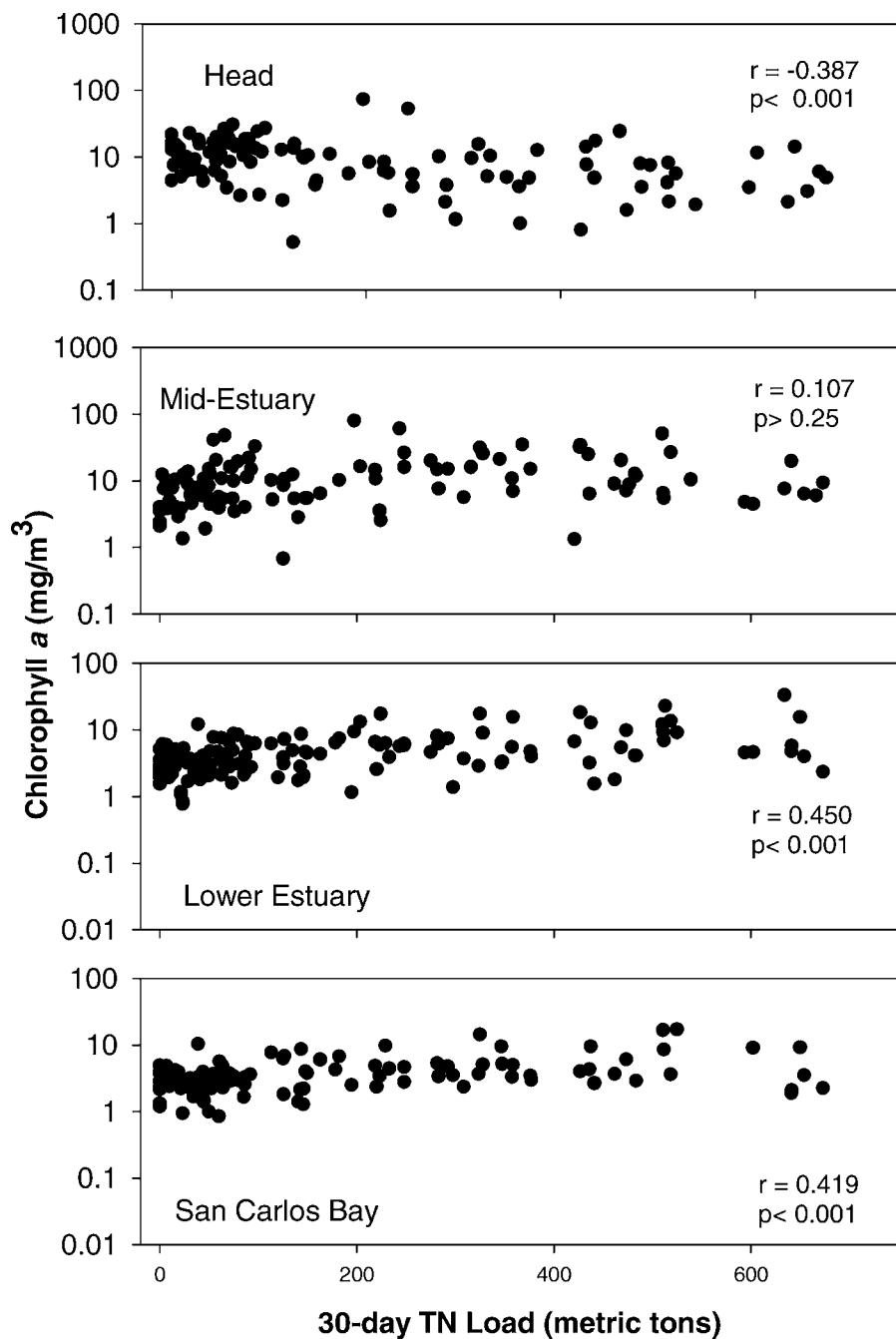*Chlorophyll and dissolved oxygen*—High concentrations of chlorophyll *a* in

Fig. 4.   Concentration of chlorophyll *a* as a function of total nitrogen loading at S-79 for the 30-days prior to sampling. r = Pearson correlation coefficient.

TABLE 7. Correlation between nutrient loading (kg per 30 days prior to sampling) at S-79 and chlorophyll *a* (log10 transformed) in 4 regions of the Caloosahatchee Estuary. * = Pearson correlation coefficients (r) statistically significant at p<0.05. n = 114–146 observations.

Region	DIN Load	TP Load	DIP Load
Upper Estuary	−0.457*	−0.452*	−0.404*
Mid Estuary	0.141	0.123	0.035
Lower Estuary	0.492*	0.508*	0.528*
San Carlos Bay	0.569*	0.559*	0.588*

surface waters can be associated with low concentrations of dissolved oxygen in bottom waters (0.5 m above bottom) in the Caloosahatchee on short time scales of weeks (FIG. 5). During the month of June 2000, the crash of a chlorophyll *a* bloom coincided with a rapid decline in oxygen in bottom waters. On longer time scales, the high concentrations of chlorophyll *a* may be associated with lower oxygen concentrations one or two months in the future (Table 8).

Chlorophyll and light extinction—Photosynthetically Active Radiation (PAR) data required for the calculation of the light extinction coefficient was consistently collected only during the ERD (2003) study. The results of stepwise multiple regressions relating variation in the extinction coefficient to chlorophyll *a*, color and total suspended solids are given in Table 9. Color explained most of the variation in light extinction in the upper, mid and lower estuary. In San Carlos Bay, chlorophyll
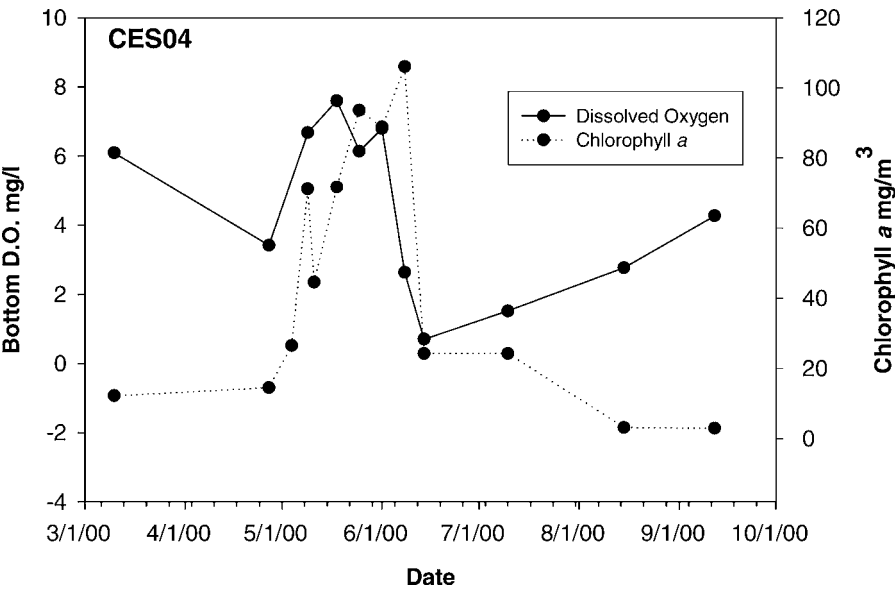

FIG. 5. Time series of chlorophyll *a* and dissolved oxygen in bottom water at station CES04 in the upper estuary (see Figure 2 for location). Note the marked decline in dissolved oxygen following a phytoplankton bloom.

TABLE 8. Correlation between chlorophyll *a* and the concentration of dissolved oxygen (log 10) in bottom waters. Monthly Data from CES Data Set POR: 3/99–4/2002. * $p<0.05$, ** $p<0.01$, $n = 33$–35.

	Chlorophyll *a*		
	Lag in Months		
Region	0	1	2
Upper Estuary	−0.041	−0.534**	−0.633**
Mid-Estuary	0.009	−0.170	−0.359*
Lower Estuary	−0.286	−0.458**	−0.266

a explained the majority of variation. The contribution of color to K_d ranged from 20–30% depending on location and chlorophyll *a* from 10–25%. TSS accounted for 17% of the total light extinction in the upper estuary.

Chlorophyll and freshwater discharge—Freshwater discharge at S-79 also explained variation in the concentration of chlorophyll *a* in the downstream estuary (FIG. 6). The regional relationships were the same as those for loading: positive in the lower estuary and San Carlos Bay, not significant in the mid-estuary and negative in the upper estuary. In contrast to loading, there was apparent curvature in the relationships with discharge. In the mid and lower estuary and San Carlos Bay the concentration of chlorophyll *a* increased with increasing discharge up to a maximum and then began to decrease. In the mid-estuary this inflection point occurred at a 30-day average discharge of about 85 m^3/sec (3000 cfs). To the right of the inflection point, chlorophyll *a* concentration was positively correlated with discharge ($r = 0.384$, $p<0.001$, $n = 90$) and to the left negatively correlated ($r = −0.463$, $p<0.02$, $n = 25$). In the lower estuary ($r = 0.326$, $p<0.01$, $n = 131$) and San Carlos Bay ($r = 0.390$, $p<0.01$, $n = 109$) the concentration of chlorophyll *a* was positively correlated at discharges of less than 127–141 m^3/sec (4500–5000 cfs). At higher flows linear correlation coefficients were negative but not statistically significant (lower estuary $r = −0.400$, $p<0.15$, $n = 10$; San Carlos Bay $r = −0.533$, $p<0.12$, $n = 10$).

Analyzing the relationships on a seasonal basis yielded results similar to those for nutrient loading. In the upper estuary, lower estuary and San Carlos Bay both wet

TABLE 9. Mean ($\pm$ SD) light attenuation coefficient (K_d) and percentage of total variation in the light extinction coefficient explained by variation (Var) in color, chlorophyll *a* and total suspended solids (TSS) in stepwise multiple regressions. Also given is the mean ($\pm$ SD) percentage of light extinction attributable to each parameter calculated from the regression equations. Significance level for entry in the model was $p<0.05$ in all cases except for the Upper Estuary where $p<0.10$.

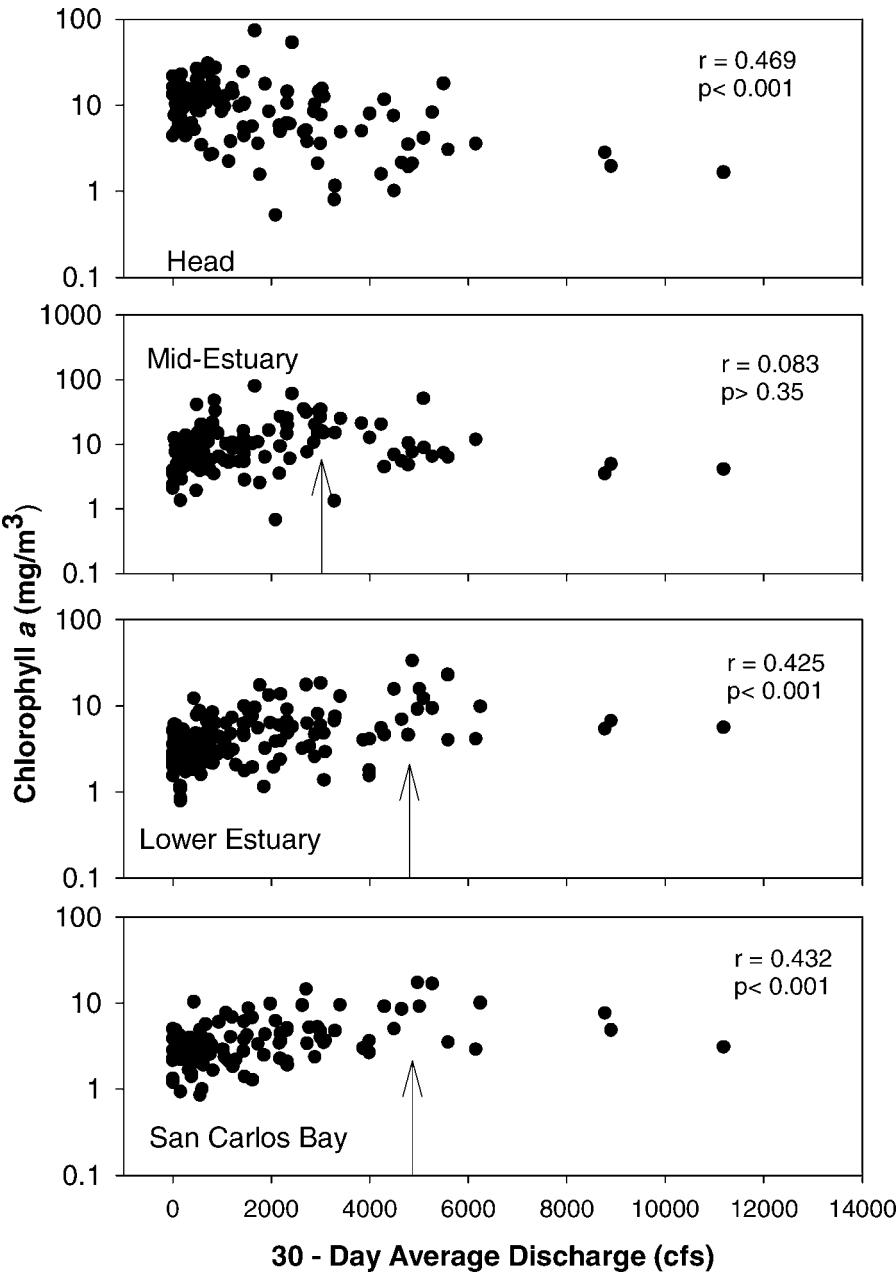|	Color		Chlorophyll *a*		TSS		
Percentage	Var	K_d	Var	K_d	Var	K_d	Mean K_d
Upper Estuary	13	20 ± 13	0	0	11	17 ± 12	2.91 ± 0.73
Mid-Estuary	72	30 ± 19	12	12 ± 10	0	0	2.25 ± 0.94
Lower Estuary	78	23 ± 18	11	10 ± 10	0	0	1.55 ± 0.75
San Carlos Bay	0	0	68	25 ± 17	0	0	1.21 ± 0.39

FIG. 6.   Concentration of chlorophyll *a* as a function of discharge of freshwater at S-79 for the 30-days prior to sampling. Arrows indicate inflection point. r = Pearson correlation coefficient.
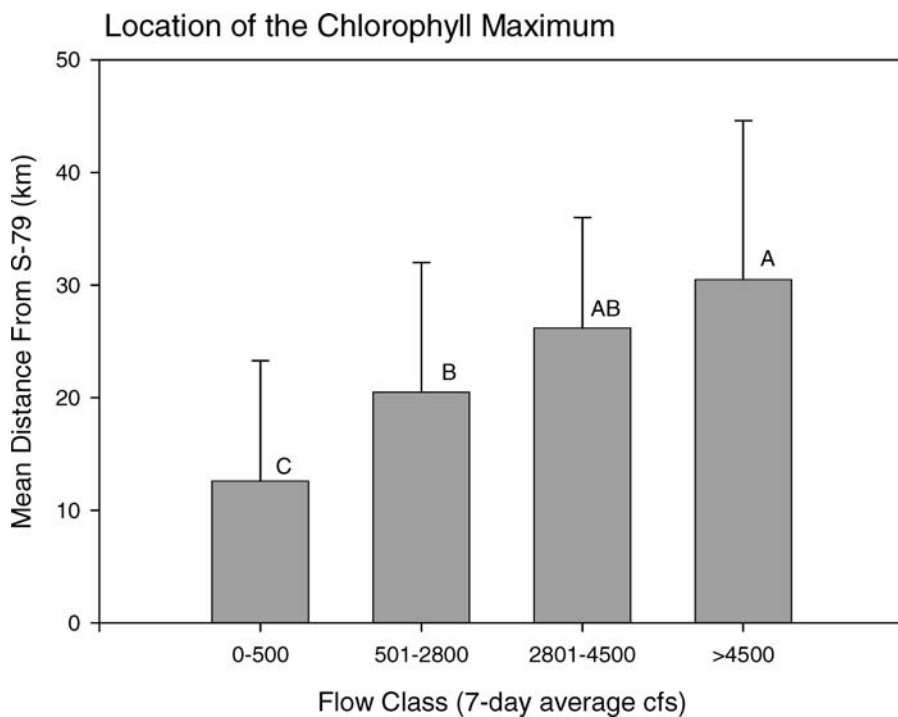
FIG. 7.   Mean distance of the chlorophyll *a* maximum downstream of S-79 as a function of freshwater discharge at S-79.

and dry season relationships were the same as the overall. In the mid-estuary, the relationship was weakly positive in the dry season (r = 0.210, p<0.08) and not significant in the wet season.

The rate of discharge at S-79 also influenced the position of maximum chlorophyll *a* found on a sampling date in the downstream estuary. The correlation between position of the maximum (in km from S-79) and discharge averaged over the 7 days prior to sampling was r = 0.526 (p<0.001, n = 91 sampling events). This pattern is illustrated in Figure 7 where the location data have been classified into several ecologically based flow classes. The mean distance of the chlorophyll maximum from S-79 increased as flow increased.

TABLE 10. Comparison of average daily nutrient loads (kg/day) at S-79. Annualized estimates from the ERD study are the average of the wet and dry season mean daily loads.

	ERD Study			This Study
	Wet Season	Dry Season	Annual	Annual
TN	11,051	2,408	6,730	7,018
DIN	2,476	608	1,542	1,385
TP	1,040	355	698	657
DIP	474	211	343	426

DISCUSSION—*Nutrient loading*—Annual loads of total nitrogen delivered to the Caloosahatchee at S-79 calculated in this study agree well with those estimated previously by Janicki Environmental (2003). Although the period of record examined here was longer than the Janicki study, agreement is remarkable for the period of overlap (1990–2002). Discharge at S-79 explained most of the variance in loading and the good agreement between studies most likely stems from the use of similar discharge data and similar methods for calculating loads.

Environmental Research and Design (2003) measured nutrient loads at S-79 intermittently during 2000–2002 and derived mean daily estimates for the wet and dry seasons. The ERD study shows that most of the annual nutrient load is delivered during the wet season (Table 9). The annually averaged daily loads reported here fall within the range of seasonal loads reported by ERD (2003). When an annualized daily load is derived from the ERD data, means compare well with this study (Table 9).

The present study examined nutrient loads at S-79 only. There are other prominent nutrient inputs to the Caloosahatchee including waste water treatment facilities (WWTF). In the 1980s and early 1990s, five WWTFs discharged directly into the Caloosahatchee Estuary (Baker 1990). By 2000, the effluent from the Cape Coral plant had been reclaimed and under ordinary circumstances discharges to the Caloosahatchee had ceased.

The ERD (2003) study compared nutrient loading at S-79 with that from the remaining four plants. In general, average daily nutrient loads at S-79 exceeded those from all 4 plants combined by an order of magnitude in both the wet and dry seasons. This is not to say that loading from WWTFs is never important. During drought conditions when no flow and hence no loading occurs at S-79, WWTFs can dominate nutrient loading (ERD 2002).

Differences between periods—Hydrologically, the three periods ranged from relatively dry in Period 1 (1986–1989) to relatively wet in Period 2 (1994–1996) with Period 3 (1999–2003) being intermediate and may be viewed as capturing a range of natural variation. There were statistical differences in water quality between periods (Table 4). Whether these differences reflect natural variation or temporal changes caused by other factors remains unknown in the absence of a time series of appropriate length. The question of whether the differences obviated other analyses presented here deserves consideration. Certainly, relationships between nutrient loading or freshwater discharge at S-79 and chlorophyll *a* in the downstream estuary were not affected since all three exhibited no trend over the period of record. Other analyses (dissolved oxygen, light attenuation) relied on smaller data sets taken recently, within Period 3 (1999–2003). The spatial analyses of water quality may have been influenced but results agree with all previous investigations of the system (see below).

Water quality and nutrient limitation—The distribution of nutrients and other water quality parameters reported here (Table 5) is similar to those described previously (McPherson and Miller, 1990; Doering and Chamberlain, 1998; Doering

and Chamberlain, 1999). The spatial distribution of nutrients and color largely reflects freshwater input at S-79: concentrations are high near the structure and decrease as proximity to clearer ocean water increases. Water clarity as measured by secchi disk showed the same pattern. TSS shows the opposite pattern suggesting a major input of suspended sediment from the Gulf of Mexico (Doering and Chamberlain, 1999; McPherson and Miller, 1990). This pattern also could arise from greater resuspension of sediments in saltier more open regions of the system such as San Carlos Bay and Pine Island Sound.

Understanding nutrient limitation of primary productivity can be considered a keystone of the study of eutrophication (Smith et al., 1999). Restricting the loading of the limiting nutrient(s) should control eutrophication. Eutrophication of the Caloosahatchee has been a concern since the late 1970s and early 1980s. A waste load allocation study conducted by the Florida Department of Environmental Regulation concluded that the estuary had reached its nutrient loading limits as indicated by elevated chlorophyll *a* and depressed dissolved oxygen concentrations (DeGrove, 1981). Nutrient addition experiments conducted in October during high flow conditions indicated nitrogen limitation of phytoplankton growth in the upper reaches and phosphorus limitation in the lower reaches of the Caloosahatchee Estuary. McPherson and Miller (1990) and McPherson and coworkers (1990) concluded that nitrogen was likely the most limiting nutrient in the Charlotte Harbor estuarine system because concentrations of inorganic nitrogen frequently fell below detection limits and atomic ratios (N:P) were generally less than 3:1 and well below the traditionally accepted ratio for balanced uptake by phytoplankton of 16:1 (Day et al., 1989). While the analysis of inorganic nutrient concentrations presented here indicates nitrogen is most likely to limit phytoplankton productivity, potential limitation by phosphorus may also occur, especially in San Carlos Bay (Table 6). Median nutrient ratios (Table 4) were consistent with these conclusions. These ratios were less than 16:1 in all regions during all periods except in San Carlos Bay during Period 2 (DIN:DIP = 19.9). Since the Caloosahatchee River at S-79 is a major source of nutrients to the estuary there is a general increase in the potential for nutrient limitation by either N or P as distance from S-79 increases (Table 6).

Chlorophyll and eutrophication—If chlorophyll *a* is a good an indicator of nutrient enrichment or eutrophication in the Caloosahatchee Estuary then both direct and indirect effects consistent with the Phase II (Cloern 2001) model need to be established. Establishing a direct relationship between nutrient loading and chlorophyll *a* concentrations has been problematic for estuarine systems (Nixon and Pilson 1983; Monbet 1992). This may stem from a paucity of data or a weak response to loading (Nixon and Pilson 1983). Monbet (1992) argues that nutrient loadings control nutrient concentrations and the nutrient concentration actually controls phytoplankton standing crop. Statistical analysis of data from the Caloosahatchee demonstrates a direct effect of nutrient input on chlorophyll *a*: on monthly time scales, increases in nutrient loading are associated with increases in chlorophyll *a* concentration in the lower estuary and San Carlos Bay (FIG. 4). In these regions, the relationship is seasonally robust. The expected relationship does

not hold in the upper and mid estuarine regions and this is discussed below. In the lower estuary and San Carlos Bay, nutrient loading at S-79 explains only 17–35% of the variability in chlorophyll *a* concentration (FIG. 4, Table 7). While these relationships imply that reducing the nutrient load could decrease chlorophyll *a* in both wet and dry seasons, they are not of predictive significance.

The Phase II conceptual model allows for cascading secondary effects of increased chlorophyll *a* and two were investigated here: effects on dissolved oxygen and light extinction. The classic link between increases in chlorophyll *a* in surface waters and declining oxygen concentrations in bottom waters is amply demonstrated for the Caloosahatchee Estuary by the data presented in Figure 7 and Table 8.

The water quality parameters that influenced light extinction varied spatially in the estuary and San Carlos Bay (Table 9). Suspended solids acounted for 17% of the total in the upper estuary. Non-chlorophyll suspended matter (NSM) can be the major attenuator of light in the Charlotte Harbor system (McPherson and Miller 1987; 1994). We made no attempt to directly measure or calculate the contribution NSM to light extinction. We included TSS as a surrogate in the regression analysis. TSS is not a good indicator of light extinction caused by NSM (McPherson and Miller, 1987), and this may explain why TSS did not appear as a more prominent component of light extinction in the estuary or San Carlos Bay.

Water quality in the Caloosahatchee Estuary is significantly influenced by the tannin stained freshwater input at S-79 (Doering and Chamberlain, 1999). In this region, color accounted for 20–30% of the light attenuation. This estimate agrees well with those (mean 22%, range 4–93%) reported for the greater Charlotte Harbor system by McPherson and Miller (1994).

Chlorophyll *a* accounted for 25% of the light attenuation in San Carlos Bay. While this estimate also agrees with those reported for the greater Charlotte Harbor system by McPherson and Miller (1994, mean 16%, range 0–43%), it exceeds that reported by Dixon and Kirkpatrick (1999) for San Carlos Bay (3%). They found color and turbidity to be most important, respectively accounting for 60% and 37% of light attenuation.

Statistics may help explain the differing results. In our study, color was correlated with light extinction in San Carlos Bay (r = 0.740, p<0.05) but not selected in a stepwise multiple regression because the correlation with chlorophyll was stronger (r = 0.824, p<0.05). Nevertheless, based on the linear regression of K_d on color, it can be calculated that color may have accounted for 19 $\pm$ 15% of light attenuation in San Carlos Bay during the ERD study. There also appear to be concentration differences between the two studies. In the Dixon and Kirkpatrick (1999) study color concentrations ranged from 15–60 pcu, with an average of 31.8 pcu and a median of 30 pcu (n = 6). In the ERD study Bay wide averages used in the analysis ranged from 0.5–73 pcu with a mean of 14.7 pcu and a median of 11.8 pcu (n = 24). On average the concentration of color during the Dixon and Kirkpatrick (1999) study was twice that of the ERD study. Chlorophyll *a* concentrations appeared similar with a median of 5 µg/l , a mean of 6.1 µg/l and range of 2.7–13.4 µg/l for the Dixon and Kirkpatrick study, compared with a median of 4.5 µg/l a mean of 7.5 µg/l and a range of 0.5 to 25.4 µg/l for the ERD study.

The differing results of these studies have important management implications. There are extensive seagrass beds in San Carlos Bay composed primarily of *Thalassia testudinum* Banks ex Konig and *Halodule wrightii* (Acherson) (Chamberlain and Doering, 1998). A comparison of sites in the Charlotte Harbor Estuarine system, including San Carlos Bay, showed that the depth of the deep edge of bed (DDEB) depended on light attenuation (Dixon and Kirkpatrick, 1999). The DDEB decreased as light attenuation increased. The contrasting results above suggest that reductions in chlorophyll *a* attendant with reductions in nutrient loads will not always result in improved light availability in San Carlos Bay.

Freshwater discharge as a filter—In the Phase II conceptual model of eutrophication, filters act to modulate the response of an estuary to changes in nutrient loading. For example, San Francisco Bay, a highly turbid estuary, is less responsive to nutrient addition than Chesapeake Bay because light is more often limiting (Cloern, 2001). Turbidity is the filter.

In the Caloosahatchee, chlorophyll *a* responds to both nutrient loading and freshwater discharge at S-79. Because the Caloosahatchee River at S-79 is a major source of nutrients and because freshwater discharge explains much of the variability in nutrient loading at S-79 (Table 2), it is difficult to determine which of the two influence chlorophyll *a*. There is some evidence that freshwater discharge may modulate or "filter" the response of chlorophyll *a* through a 'wash out' effect (Welsh et al., 1972).

In the upper estuary the responses of chlorophyll *a* to nutrient loading and freshwater discharge are both negative. The negative relationship between nutrient loading and chlorophyll *a* in the upper estuary is counter to expectation if nutrient supply were limiting. Being closest to a major source, nutrients are least likely to limit chlorophyll in this region (Table 6). The negative relationship with freshwater discharge observed in this region is consistent with the wash out hypothesis. Finally, changes in horizontal location of the chlorophyll maximum are also consistent with this hypothesis. The maximum occurs in the upper estuary at low discharges (Fig. 7) and moves down stream as discharge increases.

In the mid and lower estuary and San Carlos Bay, chlorophyll $a -$ freshwater discharge relationships also exhibit an inflection point that suggests 'wash out'. At higher discharges (85 m^3/sec or 3000 cfs in the mid-estuary, 127–141 m^3/sec or 4500–5000 cfs in the lower estuary and San Carlos Bay), chlorophyll *a* decreases as discharge increases. This inflection point was not evident in relationships with nutrient loading.

Relationships between chlorophyll *a* and both nutrient loading and freshwater discharge in the mid-estuary varied seasonally, being slightly positive in the dry season and unrelated in the wet season. The lack of a negative relationship in the wet season was due to variability in the data. At lower flows and loadings, both relatively high and low chlorophyll *a* values occurred and these spanned the range observed at higher flows.

The tannic, dark color of freshwater discharge may modulate the response of chlorophyll *a* to enhanced nutrient supply through light limitation. McPherson and

coworkers (1990) measured phytoplankton biomass and productivity throughout the Charlotte Harbor system including a station at the near mouth of the Caloosahatchee in San Carlos Bay. They explained the responses of phytoplankton biomass and productivity to freshwater inflow as an interaction between nutrient and light availability. Increased freshwater inflow increases nutrient supply but also increases color, hence decreasing light availability. Phytoplankton biomass and productivity increase where nutrient rich colored water has been diluted enough for light to become sufficiently available. These conditions obtain in the mid-salinity regions of an estuary (McPherson et al., 1990). The present study suggests that "wash out" influences the accumulation of phytoplankton biomass. In the Caloosahatchee, the rate of freshwater inflow comprises another interacting variable. In general, the spatial distribution of chlorophyll a, with a peak in the mid-estuary suggests that it is here that conditions most often become favorable for the growth and accumulation of phytoplankton.

This interaction between color and phytoplankton productivity may also determine where and when each of these water quality constituents becomes an important contributor to light extinction. Ultimately, this interaction, moderated by freshwater inflow, may help explain why the contributions of chlorophyll a and color to light attenuation appear to vary spatially and temporally between studies conducted in different years.

In summary, correlations between chlorophyll a and nutrient loading, dissolved oxygen and light extinction recommend its use as an indicator of eutrophication in the Caloosahatchee Estuary and San Carlos Bay. However, useful interpretation of the response of this indicator to future changes in nutrient loading must account for the modulating effects of freshwater discharge exerted through flushing and reductions in light availability. These modulating effects are especially germane to changes in nutrient loads at S-79 caused by alterations in the rate of freshwater discharge.

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