Temporal and spatial variations of nutrients in the Ten Mile Creek of South Florida, USA and effects on phytoplankton biomass

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Water quality throughout south Florida has been a major concern for many years. Nutrient enrichment in the Indian River Lagoon (IRL) is a major surface water issue and is suggested as a possible cause of symptoms of ecological degradation. In 2005–06, water samples were collected weekly from seven sites along Ten Mile Creek (TMC), which drains into the Indian River Lagoon, to investigate and analyze spatial and temporal fluctuations of nutrients nitrogen (N) and phosphorus (P). The objective of this study was to understand the relationships among chlorophyll *a* concentration, nutrient enrichment and hydrological parameters in the surface water body.
High median concentrations of total P (TP, 0.272 mg L^{-1}), PO_4-P (0.122 mg L^{-1}), and dissolved total P (DTP, 0.179 mg L^{-1}); and total N (TN, 0.988 mg L^{-1}), NO_3^--N (0.104 mg L^{-1}), NH_4^+-N (0.103 mg L^{-1}), and total Kjeldahl N (TKN, 0.829 mg L^{-1}), were measured in TMC. The concentrations of TP, PO_4-P, DTP, TN, NO_3^--N, NH_4^+-N, and TKN were higher in summer and fall than in winter and spring. However, chlorophyll *a* and pheophytin concentrations during this period in TMC varied in the range of 0.000–60.7 and 0.000–17.4 μg L^{-1}, with their median values of 3.54 and 3.02 μg L^{-1}, respectively. The greatest mean chlorophyll *a* (10.3 μg L^{-1}) and pheophytin (5.71 μg L^{-1}) concentrations occurred in spring, while the lowest chlorophyll *a* (1.49 μg L^{-1}) and pheophytin (1.97 μg L^{-1}) in fall. High concentrations of PO_4-P (>0.16 mg L^{-1}), DTP (>0.24 mg L^{-1}), NO_3^--N (>0.15 mg L^{-1}), NH_4^+-N (>0.12 mg L^{-1}), and TKN (>0.96 mg L^{-1}), occurred in the upstream of TMC, while high concentrations of chlorophyll *a* (>6.8 μg L^{-1}) and pheophytin (>3.9 μg L^{-1}) were detected in the downstream of TMC. The highest chlorophyll *a* (11.8 μg L^{-1}) and pheophytin (6.06 μg L^{-1}) concentrations, however, were associated with static and open water conditions. Hydrological parameters (total dissolved solid, electrical conductivity, salinity, pH, and water temperature) were positively correlated with chlorophyll *a* and pheophytin concentrations ($P < 0.01$) and these factors overshadowed the relationships between N and P concentrations and chlorophyll *a* under field conditions. Principal component analysis and the ratios of DIN/DP and TN/TP in the water suggest that N is the limiting nutrient factor for phytoplankton growth in the TMC and elevated N relative to P is beneficial to the growth of phytoplankton, which is supported by laboratory culture experiments under controlled conditions.

Introduction

Eutrophication of surface waters is a major environmental issue worldwide.[1,2] In freshwater ecosystems, phosphorus (especially biologically available P) is commonly considered to be the major driving factor for eutrophication.[3,4] Nitrogen can also be an important limiting factor for phytoplankton growth, depending on its bioavailability and the composition of the community.[5–9] Phytoplankton blooms are often associated with advanced stages of eutrophication[10,11] and anthropogenic activities can accelerate the rate of eutrophication.[12,13] Discharge of waste water and widespread use of chemical fertilizers in aquatic watersheds can lead to highly elevated levels of nitrogen and phosphorus transport into water ecosystems, resulting in alterations to their structure and function.[14–16] The specific impacts of nutrient loads vary according to the structure of individual ecosystems and other factors, such as meteorological and hydrological conditions.[17,18] For example, variability of phytoplankton biomass can be associated with inter-annual variability of river flow that is linked to changes in rainfall amounts.[19–21] Furthermore, physico-chemical parameters, such as salinity, dissolved oxygen (DO) concentration, water temperature, pH, light availability and water residence time can affect phytoplankton growth and biomass accumulation.[13,20–24]

In Florida, nutrient enrichment of the Indian River Lagoon (IRL) is a major surface water quality issue, as it relates to observed degradation in the ecology of the lagoon. One of the most impacted regions of the lagoon is the St. Lucie estuary, located in the

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southern-most part of the lagoon. Three drainage canals (C-23, C-24, and C-44) and Ten Mile Creek deliver at least 8.6×10^5 kg of nitrogen (N), 9.1×10^5 kg of phosphorus (P), and 3.6×10^8 kg of suspended solids (SS) to the estuary each year.[25] By the year 2010, the total N load to the estuary is projected to increase by 32%.[26] Restoration targets of 30 and 70% reductions of total N and P loading have been proposed for the St. Lucie Estuary.[27]

This study focused on the contribution of the Ten Mile Creek (TMC), a major source of nutrient-enriched drainage, to the St. Lucie River Estuary. The TMC watershed includes substantial inputs from agricultural, commercial, and residential land-uses.[28] Rainfall runoff in the region transports substantial amounts of fine soil particles and dissolved nutrients N and P into the TMC, and ultimately into the St. Lucie Estuary. In recent years, phytoplankton blooms have become a regular feature of the estuary, with water-column chlorophyll *a* (Chla) concentrations often exceeding 50 μg L^{-1} (maximum of 120 μg L^{-1}).[22] Some of these blooms involve potentially toxic species of algae, including the cyanobacterium *Microcystis aeruginosa* (Phlips *et al.* unpublished data). However, few studies have examined the role of the TMC as a supplier of nutrients and phytoplankton for eutrophication of the estuary. The objectives of this study were: (1) to determine temporal and spatial variations of nutrient concentrations and phytoplankton standing crops (Chla and Phea) in the TMC; and (2) to define the correlations between physical and chemical characteristics of the TMC and Chla.

Materials and methods

Study site descriptions

The watershed for the Indian River Lagoon (IRL) is a major vegetable and citrus production area in Florida, and an area of rapid urban development. Vegetable crops and citrus are grown on flatwoods soils, which are Spodosol (sandy, siliceous, hyperthermic, Alfic Alaquods) and Alfisol (sandy, siliceous, hyperthermic, Arenic Glossaqualf), respectively, and both are characterized by low water and nutrient holding capacity. A shallow hardpan layer in the sediments restricts downward movement of water soluble compounds, and increases the lateral movement of nutrients *via* surface drainage. During heavy irrigation or abundant rainfall, nutrient-enriched water drains into creeks and canals and eventually into the IRL. Ten Mile Creek is the largest subbasin draining water to the North Fork of the St. Lucie Estuary, which has been designated as an Outstanding Florida Water body (OFW). The St. Lucie Estuary is part of the southern IRL, which has been characterized as the most biologically diverse estuary in North America.[29] As such, nutrient and phytoplankton concentrations in the TMC affect water quality negatively throughout a critically important region of Florida.

Field procedures

Water samples were collected weekly from seven sampling sites along the TMC, and the location and environmental conditions of each site are provided in Table 1 and Fig. 1. The sampling period covered June 28th, 2005 to June 27th, 2006. Water temperature, dissolved oxygen (DO), salinity, and electrical conductivity (EC) were directly measured in the field using a portable environmental multi-probe (YSI Model EC300, YSI Environmental, Yellow Springs, Ohio, USA) and a handheld dissolved oxygen instrument (YSI Model 550A-25, YSI Environmental, Yellow Springs, Ohio, USA). Water samples were collected using grab sampling devices, and aliquots of water were placed into pre-washed sample bottles. Chlorophyll *a* samples were collected on site by filtering 300 mL of the water sample through a GF/F glass fiber filter (0.7 μm) and wrapping the filter paper with aluminium foil.[21] All the samples were placed inside an ice chest immediately after collection and transported to the lab for physical, chemical, and biological analyses.

Table 1 Site description of water sample collection along the Ten Mile Creek

Location ID	GPS location	Description
SL1	27°24′4.20″N 80°25′11.34″W	Most upstream site in this study, surrounded by citrus ground. Water way is narrow (12.8 m). Sampling was carried out in the center of the water current under a crossing-river bridge
SL2	27°24′10.8″N 80°23′56.88″W	River water level was maintained by a water dam (28.0 m). Sampling was carried out in the center of the water current under a crossing-river bridge
SL3	27°24′15.66″N 80°21′59.58″W	Water way is narrow (11.9 m). Sampling was carried out in the center of the water current under a crossing-river bridge
SL4	27°24′31.62″N 80°21′29.22″W	A tributary stream (Five Mile Creek) with a narrow water way (7.62 m), passing by residential district, discharges into Ten Mile Creek.
SL5	27°22′26.88″N 80°20′33.42 W	Ten Mile Creek flows through White city park. TMC has a broad water way (31.1 m) at this site. Sampling was carried out in the center of the water current under a crossing-river bridge
SL5B	27°19′23.76″N 80°19′59.23″W	Ten Mile Creek flows through river park Mariana. River water has a broad water way (45.1 m). Sampling was carried out in the water under a dock inside the park, where water dynamic is relatively static and open, and was seasonally influenced by tide.
SL6	27°16′26.98″N 80°19′15.54″W	Ten Mile Creek flows through River gate park, where the river water is frequently influenced by Tide. TMC at this site has the broadest water way (62.5 m) in the seven studied sites. Sampling was in the water beside a walking dock in the park.

Chemical analyses of water samples

Water pH and turbidity were measured immediately after water samples were transported into the lab using a pH/ion/conductivity meter (pH/Conductivity Meter, Model 220, Denver Instrument, Denver, CO, USA) and a Turbidity meter (DRT 100B, HF Scientific, inc. USA), respectively. Total dissolved solid (TDS) concentrations of unfiltered water samples were measured using a gravimetric method. Concentrations of NH_4^+-N were determined using an N/P discrete autoanalyzer (Easychem Plus, Systea Scientific, LLC, Illinois, USA), after the samples were filtered through a 0.45 μm membrane filter. Concentrations of TKN were determined using the same instrument as NH_4^+-N, after the water samples were digested with acidified cupric sulfate

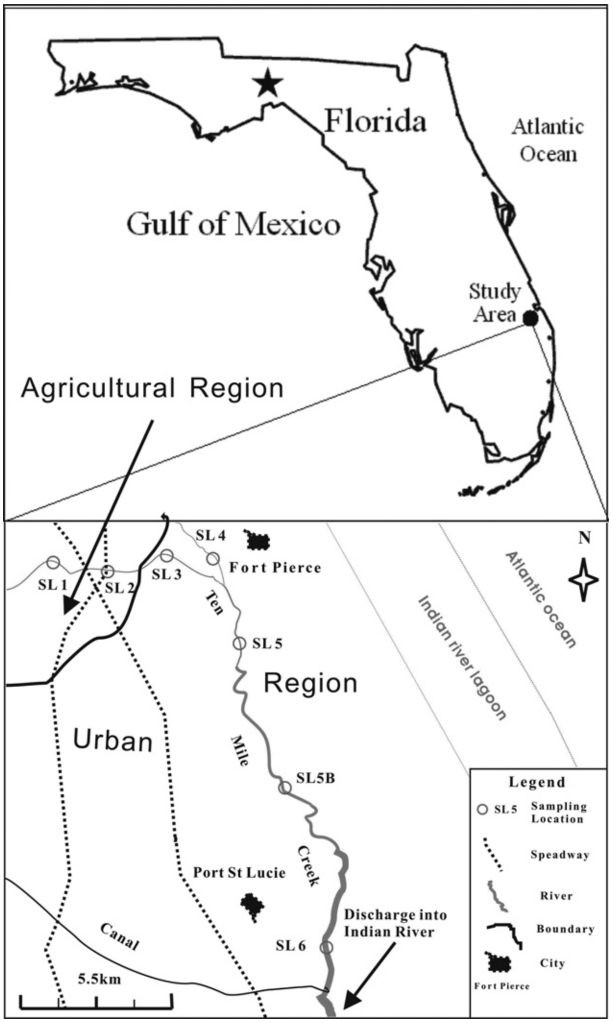

Fig. 1 Sampling locations in the Ten Mile Creek and land use types in the watershed.

and potassium sulfate (EPA method 351.2).[30] Total P (TP) was determined using the molybdenum-blue method,[31] after the unfiltered water samples were digested with acidified ammonium persulfate.[32] After the samples were filtered through a 0.45 μm membrane filter, dissolved total P (DTP) was determined following EPA method 200.7[33] using inductively coupled plasma atomic emission spectrometry (ICP-AES, Ultima, JY Horiba, Edison, NJ, USA), and ortho-phosphate (PO_4-P) and NO_3^--N were measured within 48 h after sample collection using an ion chromatography (DX 500; Dionex Corporation Sunnyvale, CA, USA).

Chlorophyll *a* and pheophytin concentrations were determined according to Phlips *et al.*[7] Chlorophyll was extracted from filtered samples using 95% ethanol in a 78 °C water bath for 5 min. After 24 h of subsequent extraction in the dark, filters were removed and centrifuged to exclude particulate debris. Chlorophyll *a* and pheophytin concentrations were colorimetrically determined using a dual beam spectrophotometer (Hitachi U2000, Japan).

If not specified, all the physico-chemical and biological parameters were measured in 28 days after samples were collected. Nutrient forms of soluble P (PO_4-P and DTP) and N (NH_4^+-N, NO_3^--N, and TKN) were investigate in this study due to their being readily available to phytoplankton growth, while TP and TN were analyzed to survey the general levels of these nutrients in river water of the TMC.

Results

Phosphorus and nitrogen concentrations

Total phosphorus (TP) concentrations in the 371 water samples, collected from the 7 sites over the study period, ranged from 0.110–15.4 mg L^{-1}, with a median concentration of 0.272 mg L^{-1} (Fig. 2). Three TP concentration peaks (15.3, 14.0, and 11.4 mg L^{-1}, respectively) were detected during the period from June 28th, 2005 to October 10th, 2005 (Fig. 3). After then, TP concentrations were low, with a grand average of 0.260 mg L^{-1} (Fig. 3).

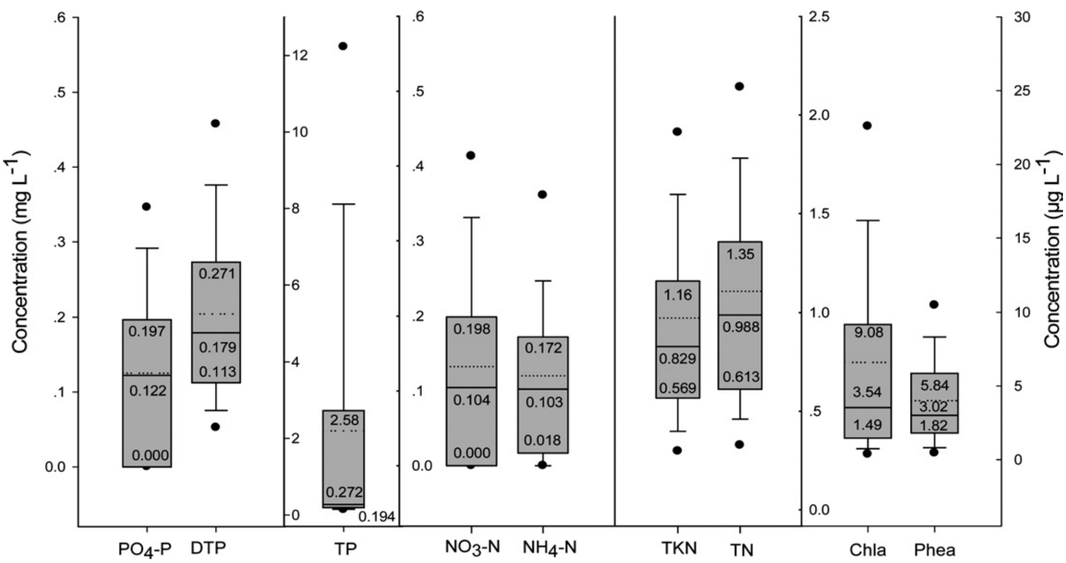

Fig. 2 The concentrations of orthophosphate (PO_4-P), dissolved total P (DTP), total P (TP), NO_3^--N, NH_4^+-N, total kjeldahl N (TKN), total N (TN) (in mg L^{-1}), and chlorophyll *a* and pheophytin (in μg L^{-1}) in the Ten Mile Creek water samples, 2005–06 (n = 371). The circles represent 5 and 95 percentile of the data and the error bars represent the 10 and 90 percentile of the data. The middle line is the median value of the data range. The upper value of the box is the 75 percentile and the lower value of the box is the 25 percentile. Dotted lines represent mean values.

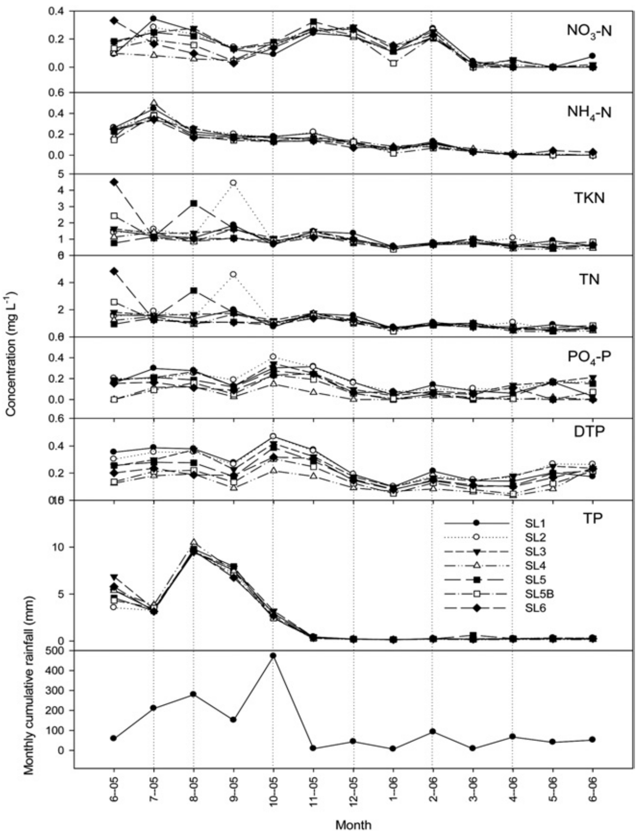

Fig. 3 Temporal variations of orthophosphate (PO_4-P), dissolved total P (DTP), total P (TP), NO_3^--N, NH_4^+-N, total kjeldahl N (TKN), total N (TN) in the Ten Mile Creek and their relationships with regional cumulative rainfall.

Ortho-phosphate (PO_4-P) and total dissolved phosphorus (DTP) ranged in 0.000–0.546 and 0.019–0.611 mg L^{-1}, with median values of 0.122 and 0.179 mg L^{-1} (Fig. 2), respectively. The two dissolved forms of phosphorus (PO_4-P and DTP) had similar monthly variation of concentrations, with peaks occurring in October, 2005, and other pulses scattered through July and August in 2005, and February and May of 2006 (Fig.3). Concentrations of TP, PO_4-P, and DTP were higher in the summer and fall than in the winter or spring (Fig. 3).

Spatially, dissolved total phosphorus (DTP) concentrations were higher at the upstream sites (Sites SL1, SL2 and SL3) than downstream (Table 2). Site SL4, where Five Mile Creek (FMC) discharges into the TMC, had the lowest mean values for the two soluble phosphorus parameters among the seven sites (Table 2). By contrast, all the seven monitoring sites had similar TP concentrations.

Total nitrogen (TN) concentrations ranged from 0.054–12.4 mg L^{-1}, with a median value of 0.988 mg L^{-1} (Fig. 2). Excluding two exceptional peaks, 12.4 mg L^{-1}on September 21st, 2005, and 11.3 mg L^{-1} on August 26th, 2005, at Sites SL2 and SL5, respectively, TN concentrations in all water samples were below 3 mg L^{-1}, with a mean value of 1.03 mg L^{-1} (Fig. 3).

The concentrations of NO_3^--N, NH_4^+-N, and total Kjedahl N (TKN) ranged from 0.000–0.820, 0.000–0.810, and 0.054–12.4 mg L^{-1}, with median values of 0.104, 0.103, and 0.829 mg L^{-1}, respectively (Fig. 2). NH_4^+-N concentrations peaked in July,

Table 2 Mean concentrations of measured P and N forms, and phytoplankton biomass through June 28th, 2005 to June 27th, 2006 in different sampling locations in the Ten Mile Creek[a]

	PO_4-P mg L^{-1}	DTP	Total P	NO_3^--N	NH_4^+-N	TKN	TN	Chla μg L^{-1}	Phea	DIN/DIP	TN/TP
SL1	0.170 ± 0.130	0.257 ± 0.140	2.20 ± 3.71	0.147 ± 0.160	0.134 ± 0.146	0.995 ± 0.517	1.14 ± 0.590	4.43 ± 7.78	2.82 ± 2.11	2.77 ±2.18	6.85 ± 5.89
SL2	0.186 ± 0.124	0.262 ± 0.128	2.17 ± 3.68	0.148 ± 0.152	0.128 ± 0.135	1.18 ± 1.62	1.33 ± 1.64	5.99 ± 9.65	3.14 ± 3.25	3.03 ± 2.77	6.15 ± 4.79
SL3	0.168 ± 0.113	0.242 ± 0.112	2.29 ± 3.75	0.153 ± 0.146	0.118 ± 0.114	0.958 ± 0.536	1.11 ± 0.609	6.47 ± 9.21	3.64 ± 3.49	3.16 ± 2.56	5.65 ± 4.33
SL4[b]	0.045 ± 0.087	0.126 ± 0.097	2.25 ± 3.93	0.102 ± 0.124	0.132 ± 0.142	0.861 ± 0.486	0.963 ± 0.530	1.98 ± 1.70	2.71 ± 1.53	6.33 ± 5.98	7.42 ± 5.83
SL5	0.128 ± 0.111	0.202 ± 0.103	2.24 ± 3.68	0.147 ± 0.135	0.119 ± 0.121	1.05 ± 1.48	1.20 ± 1.50	8.65 ± 8.29	3.91 ± 3.24	3.68 ± 3.03	5.22 ± 3.91
SL5B	0.078 ± 0.102	0.157 ± 0.097	2.10 ± 3.68	0.110 ± 0.128	0.101 ± 0.122	0.872 ± 0.418	0.982 ± 0.474	11.9 ± 7.71	6.06 ± 3.48	4.27 ± 5.19	6.92 ± 5.64
SL6	0.102 ± 0.097	0.185 ± 0.085	2.12 ± 3.78	0.121 ± 0.141	0.110 ± 0.101	0.897 ± 0.625	1.02 ± 0.678	6.84 ± 4.48	5.81 ± 2.82	4.16 ± 5.05	6.78 ± 5.39
$LSD_{0.05}$	0.0419	0.0421	1.4300	0.0543	0.0486	0.3602	0.3739	2.866	1.125	1.564	1.981
Gordy Road Dam[c]	0.355[d]			0.157	0.078	1.003					

[a] Data are presented as Mean±1 STDEV. PO_4-P: ortho-phosphate; DTP: Dissolved total P. DIN: dissolved inorganic N. DIP: dissolved inorganic P. TP: total P. DIN/DIP and TN/TP are molar ratios. [b] A tributary stream, Five Mile Creek (FMC), discharges into the Ten Mile Creek. [c] Mean of Environmental Monitoring Station data from Gordy Road Dam in 2005. [d] Total P.

2005, and then gradually decreased. Concentrations of NO_3^--N were characterized by three main peaks in July and November, 2005, and February, 2006 (Fig. 3). Except for SL6 in June, 2005, SL5 in August, 2005, and SL2 in September, 2005), no common peaks of TKN were evident (Fig. 3). Concentrations of TN, NO_3^--N, NH_4^+-N, and TKN were higher in the summer and fall than in the winter or spring (Fig. 3).

Spatially, N concentrations showed less variability than P (Table 2). Overall the sites upstream of SL4 tended to have higher median N values. Downstream sites usually had low concentrations of NO_3^--N, NH_4^+-N, and TKN. However, some trends of increasing NO_3^--N, NH_4^+-N, and TKN were visible at Site SL6. The lowest NO_3^--N, TKN, TN, but the highest NH_4^+-N, were observed at Site SL4 (Table 2).

Mean ratios of DIN/DIP and TN/TP were less than 10 in the seven sampling locations, with their highest ratio found at location SL4 (Table 2). Of the four seasons, river water in spring and summer had the lowest DIN/DIP (0.376) and TN/TP (1.82) ratios, respectively.

Chlorophyll *a* and pheophytin concentrations

Chlorophyll *a* and pheophytin concentrations during this period varied in the range of 0.000–60.7 and 0.000–17.5 μg L^{-1}, with a median value of 3.54 and 3.02 μg L^{-1}, respectively (Fig. 2). River water samples in spring had the highest chlorophyll *a* (10.3 μg L^{-1}) and pheophytin (5.71 μg L^{-1}) concentrations, and the lowest chlorophyll *a* (1.49 μg L^{-1}) and pheophytin (1.97 μg L^{-1}) concentrations in fall (Fig. 4).

During the period from July, 2005–June, 2006, greatest chlorophyll *a* and pheophytin concentrations were detected at Site SL5B, followed by Site SL5 for chlorophyll *a*, and followed by Site SL6 for pheophytin. The lowest chlorophyll *a* and pheophytin concentrations were measured at Site SL4 (Table 2).

Minimal variance of chlorophyll *a* concentrations was observed before April 10th, 2006. Mostly, they were less than 20 μg L^{-1} with a mean value of 6.60 μg L^{-1}. However, a peak of chlorophyll *a* concentration occurred on April 25th, 2006 (Fig. 4). Similar to chlorophyll *a*, pheophytin concentrations varied minimally before April 10th, 2006, and mostly were below 10 μg L^{-1}, with a grand average of 4.01 μg L^{-1}, though a peak occurred on April 25th, 2006 (Fig. 4). The highest chlorophyll *a* concentrations were measured in spring, while the lowest occurred in fall and SL4 had the lowest chlorophyll *a* levels among all the seven sites (Fig. 4). Similar trends were observed for pheophytin (Fig. 4).

Correlations among water physical, chemical properties and biological characteristics

Physical properties of water samples in the TMC during this study period are presented in Table 3. Except for DO and

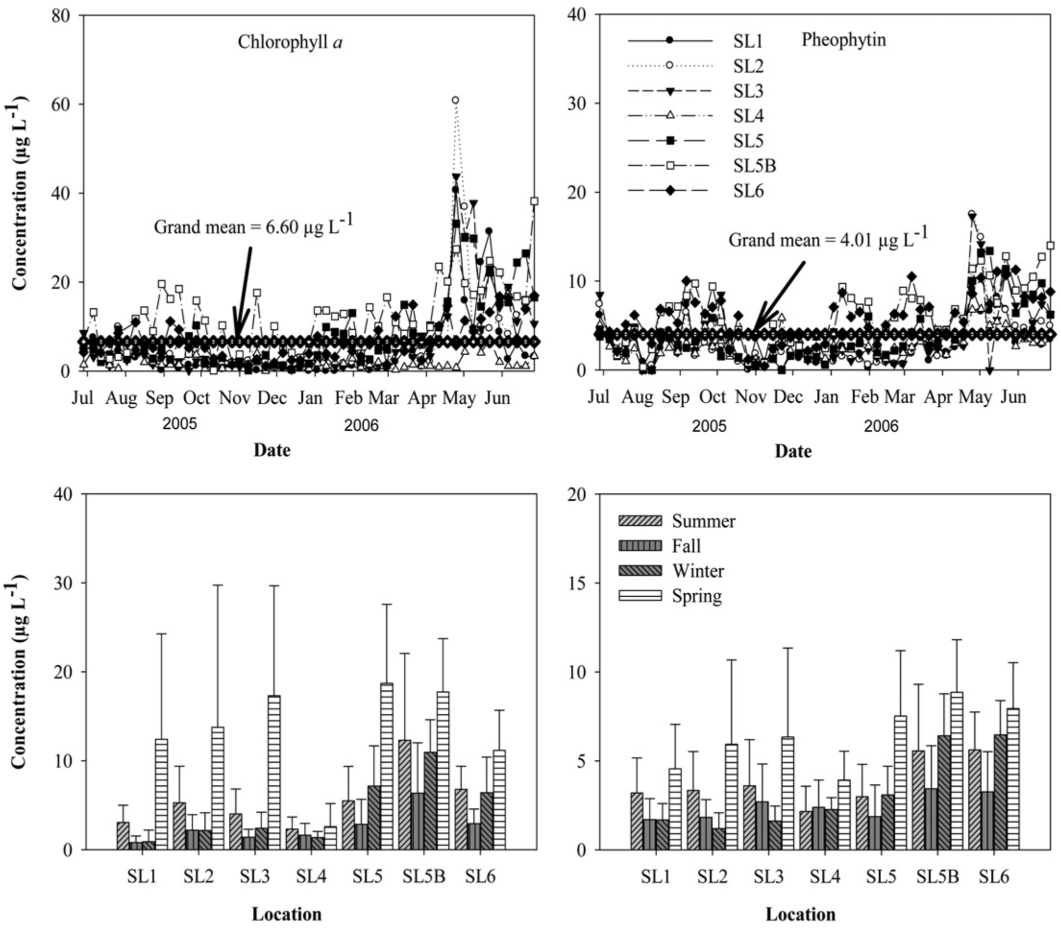

Fig. 4 Temporal variations of chlorophyll *a* and pheophytin concentrations in the Ten Mile Creek.

Table 3 Physical properties of water samples through June 28th, 2005 to June 27th, 2006 in different sampling locations in the Ten Mile Creek[a]

	WT (°C)	pH	EC (μS cm^{-1})	Salinity (PSS)	DO (mg L^{-1})	Turbidity (NTU)	TDS (g L^{-1})
SL1	23.7 (14.3–29.5)	7.20 (6.58–7.60)	1477 (195.3–2419)	0.7(0.1–1.2)	4.37(1.77–7.43)	5.19(1.68–21.5)	0.95(0.085–1.98)
SL2	23.9 (14.6–30.0)	7.24 (6.55–7.59)	1477 (202.6–2303)	0.7 (0.1–1.2)	4.07(1.99–6.47)	5.72(1.27–28.2)	0.91(0.040–2.00)
SL3	23.9 (14.6–29.9)	7.30 (6.50–7.67)	1342 (213.1–2016)	0.6(0.1–1.0)	5.10(1.44–8.30)	9.31(3.52–28.8)	0.85(0.080–1.89)
SL4	23.5 (13.6–29.9)	7.42 (6.54–7.80)	1023 (7.3–1406)	0.5(0.1–0.7)	5.21(0.26–8.64)	15.9(4.32–53.9)	0.66(0.075–1.13)
SL5	24.2 (14.3–29.9)	7.33 (6.61–7.88)	1227 (232.1–1980)	0.6(0.1–1.0)	5.10(2.74–7.90)	10.6(4.75–26.3)	0.76(0.095–1.59)
SL5B	25.1 (15.3–31.8)	7.38 (6.69–7.78)	2127 (45.0–10620)	1.1(0.1–6.0)	5.07(2.75–8.93)	10.1(4.01–107)	1.24(0.050–6.99)
SL6	25.2 (16.5–32.0)	7.43 (6.70–8.75)	6546(253.9–25420)	4.2 (0.1–15.4)	5.20(2.05–8.80)	7.21(3.47–15.9)	4.10(0.065–16.6)
$LSD_{0.05}$	1.61	0.231	1240.3	0.77	0.568	2.754	0.806

[a] Data are presented as mean (min.~max.). WT = water temperature; EC = electric conductivity; DO = dissolved oxygen; TDS = total dissolved solid.

Table 4 Correlations among microbiological index and the physical and chemical properties of water samples collected during June 28th, 2005 to June 27th, 2006 in the Ten Mile Creek[a]

	WT	pH	EC	TDS	Salinity	DO	Turbidity
Chlorophyll *a*	0.289**	0.171**	0.243**	0.195**	0.213**	0.114	−0.131
Pheophytin	0.353**	0.206**	0.413**	0.361**	0.390**	0.098	−0.094

	NO_3^--N	NH_4^+-N	TN	PO_4-P	DTP	TP	DIN/DIP	TN/TP
Chlorophyll *a*	−0.369**	−0.356**	−0.199**	−0.169**	−0.168**	−0.121*	−0.261**	−0.123*
Pheophytin	−0.461**	−0.330**	−0.174**	−0.239**	−0.183**	0.005	−0.241**	−0.173**

[a] WT = water temperature; EC = electric conductivity; DO = dissolved oxygen; TDS = total dissolved solid; TN = total N, calculated by NO_3^--N + total Kjeldahl N; DTP = dissolved total P; TP = total P; PO_4-P = ortho-phosphate; DIN = dissolved inorganic N; DIP = dissolved inorganic P. *and ** mean correlation at significant levels of $P < 0.05$ and $P < 0.01$ (N = 371), respectively.

turbidity, physical properties were positively correlated with chlorophyll *a* and pheophytin concentrations at significant levels of $P < 0.01$. Water temperature had the most influence on chlorophyll *a* concentration, while EC was correlated to pheophytin concentration (Table 4). None of the nutrients were positively correlated to chlorophyll *a* or pheophytin, and high TP concentrations were not accompanied by elevated phytoplankton growth in the TMC (Table 4). However, DTP, NH_4^+-N, NO_3^--N and TN concentrations had negative correlations with both chlorophyll *a* and pheophytin at significant levels of $P < 0.01$ (Table 4). This suggests that growth of phytoplankton was accompanied by the consumption of N and P.

(NH_4 + NO_3)/DTP ratios were both negatively logarithmically correlated with (chla + phea)/TP ($r = -0.318$, $P < 0.01$, $N = 371$) and (chl *a* + phe)/TN ($r= -0.354$, $P < 0.01$, $N = 371$) ratios (Fig. 5). Therefore, growth of phytoplankton (chla + phea) in the TMC may be more sensitive to N than P.

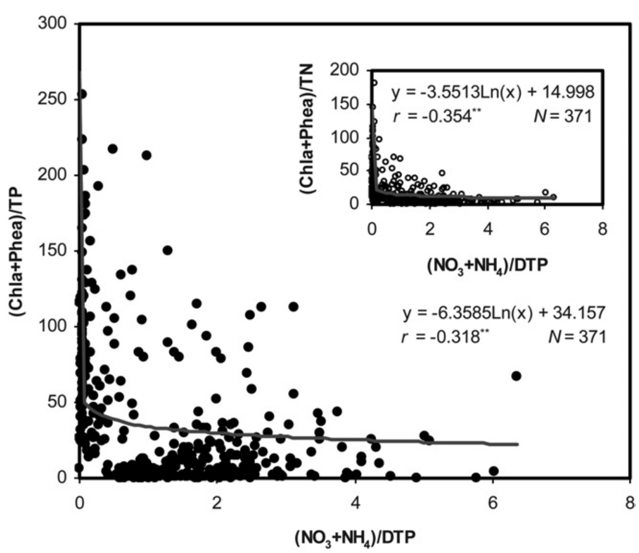

Fig. 5 Relationships of phytoplankton biomass with inorganic N (NO_3 + NH_4), DTP, and total P (TP) and total N (TN) concentrations. ** indicates a significant level of $P < 0.01$.

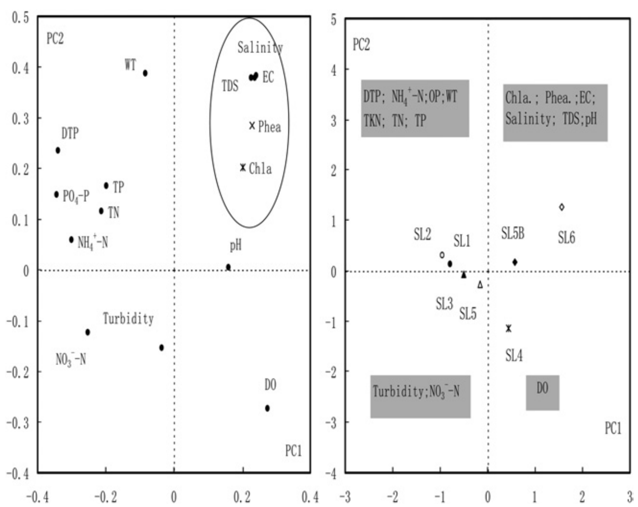

Fig. 6 Principal Component Score of field observation data in the Ten Mile Creek. Phea: pheophytin; Chla: chlorophyll *a*; WT: water temperature; DTP: dissolved total P; TP: total P; PO4-P: ortho-phosphate; TKN: total Kjeldahl N; TN: total N; DO: dissolved oxygen; TDS: total dissolved solid. EC: electricity conductivity.

A PCA analysis was carried out to understand the factors most closely related to chlorophyll *a* and pheophytin levels, where PC1 and PC2 explained 30.07% and 18.61% of the variance, respectively (Fig. 6). For all sampling sites in the study, chlorophyll *a* and pheophytin clustered most closely with salinity, TDS and EC (Fig. 6), reflecting the general downstream increase in all of these parameters. Individual sampling sites were distinguished sets of related parameters: Sites SL5B and SL6 differed from other sites because of high chlorophyll *a*, pheophytin, EC, salinity, TDS and pH values. Site SL4 was featured by its high DO value. Upstream Sites SL1 and SL2 were characterized by high nutrient levels (PO_4-P, DTP, TP, NH_4^+-N, TKN and TN), while Sites SL3 and SL5 were most closely linked by turbidity and NO_3^--N (Fig. 6).

Discussions

The results of this study demonstrate considerable differences in water nutrient levels and phytoplankton growth among upstream and downstream sampling locations in the TMC (Table 2, Fig. 2–4). The upper portion of the TMC receives surface runoff from agricultural fields high in N and P, as reflected in the concentrations observed at Sites SL1–2 (Table 2). Nutrient load to the upper TMC results in total P levels up to 15.4 mg L^{-1}. Median concentrations of TP for the seven sampling sites during the monitored period, which range from 2.1 to 2.3 mg L^{-1}, are much higher than the USEPA nutrient concentration guideline for the region of 0.04 mg L^{-1}. Total P concentrations in the TMC (Fig. 2) are much greater than TP concentrations in the southern IRL, which range from 0.03–0.14 mg L^{-1}.[34] In the case of total nitrogen concentrations, the range of median values (Table 2) was just above the USEPA nutrient guideline of 0.90 mg L^{-1}. Soluble inorganic forms of P and N were also present at high concentrations, including orthophosphate levels (PO_4-P) up to 0.55 mg L^{-1} and nitrate levels (NO_3^--N) up to 0.82 mg L^{-1}.

Downstream sites are also influenced by saline water from the Indian River Lagoon (evidenced by high salinity and EC, Table 3), which enhances the settlement of suspended solids and flocculation of dissolved organic matter. This hypothesis is supported by the difference in TP and DTP concentrations among the study sites (Table 2). He *et al.*[35] reported that high total P was accompanied by high solids concentrations in the runoff water from vegetable farm runoff.

The impacts of agricultural and residential runoff on nutrient concentrations vary with climatic conditions. Seasonal rainfall events play an important role in defining surface water runoff and in turn N and P loads to the TMC. Peaks of monthly rainfall are frequently accompanied by peaks of nutrient N and P concentrations in TMC (Fig. 3), which is good evidence for climate impacting nutrient fluctuation in the TMC. For example, the late summer period of 2005 was characterized by exceptionally strong tropical storm activity in this region, especially hurricane Wilma on October 24th, 2005 which resulted in the highest rainfall in the monitoring period (Fig. 3). The impact of such a flood resulted in clearly visible peaks of PO_4-P and DTP, but a dip in total P and N concentrations, demonstrating the dilution effects of excess water runoff. However, concentrations of N quickly rebounded in the fall, in response to the normal dieoff, decomposition and mobilization of plant biomass nutrients accumulated over the summer growth period. While, reduced loss of N due to plant uptake can be accompanied by low N concentrations in river water during March–June, 2006,[36] and low or no plant growth season during November–February, 2005, can be followed by peaks of N concentrations in the TMC (Fig. 3).

The median chlorophyll *a* concentration (3.54 μg L^{-1}, Fig. 2) in the TMC was lower than reported for IRL in 2003–04[23] and St. Johns River in 1993–96.[7] However, high chlorophyll *a* concentration didn't occur at upstream sites (SL1-3), where high N and P levels were observed (Table 2). Significantly negative correlations among chlorophyll *a* and nutrient N and P concentrations can be observed in the TMC (Table 4, Fig. 6). This may be favored by uptake and incorporation of inorganic nutrients into phytoplankton, which enhanced higher phytoplankton growth and resulted in lower nutrient concentrations at downstream sites. Furthermore, low DIN/DIP and TN/TP ratios (<10, Table 2), more negative correlations of N other than P with chlorophyll *a* (Table 4) and logarithmic relationships between N, P and chlorophyll *a* (Fig. 5) suggested that N could be the limiting nutrient in the TMC.

Another explanation for this apparent divergence is that hydrologic factors mitigate the accumulation of phytoplankton biomass.[17,18] Chlorophyll *a* reached the maximum at Site SL5B where relatively static and open water conditions persisted (Table 1). This indicates that the residence time of headwaters is important for defining phytoplankton bloom potential.[21,23,37] Peak chlorophyll *a* concentrations were observed in May (Fig. 4) when N and P concentrations were low, and rainfall was small (Fig. 3), indicating that relatively stable hydrodynamic conditions (such as warm water temperature, static water flow, etc.) are also critical for phytoplankton biomass growth when certain levels of nutrients were present.

Field observation of seasonal and spatial variations in N, P and chlorophyll *a* suggest that anthropogenic inputs govern long-term variations in nutrient concentration while climate plays an important role in phytoplankton growth.[18,38] Reasonably, acceleration of eutrophication by hydrological and meteorological factors under certain amounts of nutrient levels could have occurred, while the positive relationships between phytoplankton biomass and nutrient concentrations in water were not expected under field conditions (Fig. 6).[17,18]

Under laboratory-controlled conditions (consistent DO, WT, and light intensity, similar pH, salinity, and EC, same residence time), our culture experiments (unpublished data) found that concentrations of chlorophyll *a* and pheophytin in water were correlated positively to NO_3^--N concentration and N/P ratios and negatively to P of different forms on PC1, but were positively to N and P of different forms on PC2 (Fig. 7). Apparently, growth of phytoplankton is observed to be enhanced by the progressive consumption of N and high N/P ratio. This was also supported by field observations that the gradient decreases of N and P offshore accompanied with gradient increase of chlorophyll *a* concentrations.[39]

In this study, N is a crucial factor influencing phytoplankton growth in TMC river water, which is different from P (especially biologically available P) as the dominant in eutrophication.[3,4] This discrepancy may be better explained by high P background levels in the river water in the TMC (Fig. 2 and 3); therefore,

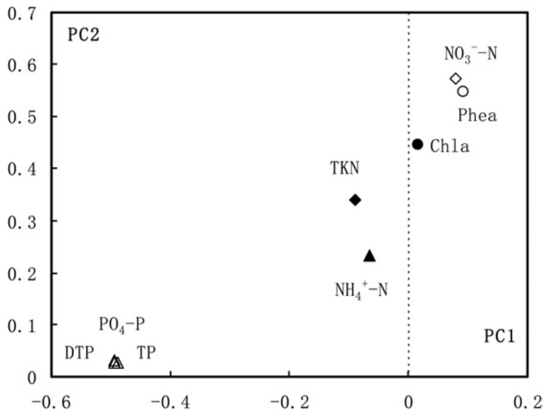

Fig. 7 Principal Component Score of laboratory incubation experiment data after 5 weeks' incubation. Phea: pheophytin; Chla: chlorophyll a; DTP: dissolved total P; TP: total P; PO4-P: ortho-phosphate; TKN: total Kjeldahl N; TN: total N; NO_3^--N: nitrate-N; NH_4^+-N: ammonia-N.

a fluctuation in N may limit phytoplankton blooming in the TMC.

Conclusions

Phosphorus and N concentrations were high upstream of the TMC, and then decreased downstream due to the precipitation of suspended solids enhanced by mixture of fresh river water from upstream with saline water from the IRL. In the most downstream water, high N and P concentrations were indicative of tidal influence. Chlorophyll *a* concentrations in water from all the monitoring sites were greater than the USEPA limitation (0.4 μg L^{-1}) of ecoregional nutrient criteria,[40] and the highest chlorophyll *a* concentrations were related to the conditions of static and open water with long residence time. Temporally high PO_4-P (0.154, 0.190 mg L^{-1}), and DTP (0.236, 0.274 mg L^{-1}) concentrations; high NO_3^--N (0.136, 0.208 mg L^{-1}), NH_4^+-N (0.212, 0.133 mg L^{-1}), and TKN (1.181, 1.056 mg L^{-1}) occurred in summer and fall, respectively. Chlorophyll *a* concentration was highest in spring, and lowest in fall. Under field conditions, Chlorophyll *a* was positively correlated to each of water TDS, EC, salinity, pH, and WT ($P < 0.01$), but negatively correlated to water DTP, NH_4^+-N, NO_3^--N or TN concentrations ($P < 0.01$). At the same phytoplankton growth level, TN was consumed more than TP, and increasing phytoplankton growth in the water of the TMC was favored by the decrease of N (NH_4^+-N + NO_3^--N) pertaining to P (DTP). The N response to phytoplankton growth was more sensitive than P. In addition, a static and open water body with long residence time is a crucial geographical condition for phytoplankton conglomeration in the TMC, although nutrient concentrations (especially NO_3^--N) and high N/P ratio are essential for phytoplankton growth.

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