2007

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Limnol. Oceanogr., 52(6), 2007, 2679–2685

Eutrophication-induced phosphorus limitation in the Mississippi River plume: Evidence from fast repetition rate fluorometry

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Abstract

We assessed nutrient limitation in the Mississippi River plume and Louisiana continental shelf during the summer of 2002 (04–08 July). We measured nutrient concentrations, alkaline phosphatase (AP) activities, chlorophyll *a* (Chl *a*) concentrations, and four fast repetition rate fluorescence (FRRF) parameters: the maximum quantum yield of photochemistry in photosystem II (PSII), $F_v : F_m$; the functional absorption cross section for PSII, σ_{PSII}; the time for photosynthetic electron transport on the acceptor side of PSII, τ_{Qa}; and the connectivity factor, *p*, in 24-h-long nutrient addition bioassays near the Mississippi River delta. Low phosphorus (P) concentrations, elevated inorganic nitrogen-to-phosphorus ratios, high AP activities, and Chl *a* increases in response to P additions in the bioassays all indicated phosphorus limitation that was confirmed by the response of FRRF parameters. This is the first study to use FRRF to confirm results from basic oceanographic methods to demonstrate phosphorus limitation in a marine setting. $F_v : F_m$ and *p* responded positively to phosphorus addition, while σ_{PSII} and τ_{Qa} decreased in the same treatments. When nitrate alone was added, none of the measured parameters differed significantly from the control. We therefore suggest that FRRF can be used to rapidly detect phosphorus limitation in marine ecosystems.

Nutrient limitation of net primary production can be an important control on phytoplankton growth in aquatic environments, and understanding it can help to limit eutrophication (Howarth and Marino 2006). Determining the extent of nutrient limitation has been a fundamentally important question of aquatic scientists for decades. Many methods, both direct and indirect, are available for addressing this problem, including nutrient concentrations and ratios, enzyme assays, fluorescence parameters, and nutrient addition bioassays (Beardall et al. 2001*b*). Fast repetition rate fluorescence (FRRF) allows quick, non-invasive assessment of phytoplankton in vivo fluorescence signatures that provides the user with photosynthetic parameters including $F_v : F_m$, σ_{PSII}, τ_{Qa}, and *p* (Kolber et al. 1998). $F_v : F_m$ is an indicator of the photosynthetic efficiency of a cell or community when measured in a dark-acclimated state. Healthy algae can have an $F_v : F_m$ as high as 0.65 (Kolber et al. 1998). The absorption cross section of PSII (σ_{PSII}) changes in response to cellular pigment concentrations and the efficiency of energy transfer from pigments to PSII reaction centers, thus making it subject to both nutrient and light availability (Kolber et al. 1988; Moore et al. 2006). σ_{PSII} is typically lower in nutrient-replete cells relative to unhealthy cells (Kolber et al. 1988). The time constant for photosynthetic electron transfer on the acceptor side of PSII (τ_{Qa}) reflects the minimum turnover time for electron transport (Kolber et al. 1988). *p* is the probability of energy transfer between PSII reaction centers (Kolber et al. 1998). Higher *p* values indicate higher probabilities of electron transfer and have been implicated in recovery from iron limitation (Vassiliev et al. 1995). Collectively, these FRRF parameters can be used to assess the physiological response of phytoplankton cells and/or communities to nutrient stress, such as P limitation.

The Mississippi River plume (MRP), herein defined as the area near the Mississippi Delta, especially Southwest Pass, and directly to the west of it (Fig. 1A), is a dynamic system for studying nutrient limitation. The widespread cultivation of maize and soybeans in the Mississippi River watershed has resulted in high nitrate loads in runoff to the Mississippi River that in turn have been implicated as a cause of the eutrophication in the river delta. The eutrophication here is thought to be the primary cause of the large hypoxic zone seen each summer off the Louisiana coast (Rabalais et al. 2002). Production in the MRP is affected by the annual river flow pattern and the water column light regime. While N is thought to control phytoplankton biomass on the Louisiana shelf because of its surplus in the system, a recent study convincingly documented spring and early summer P limitation in 2001 followed by a switch to N limitation in the fall (Sylvan et al. 2006). This is important because the P limitation occurred

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Acknowledgments

We thank Frank Natale for running the fast repetition rate fluorometer during the cruise, Zbignew Kolber for comments on the FRR fluorometry data, Paul Falkowski for the use of his FRR fluorometer, Ronald Lauck for running the nutrient samples, and the crew of the *R/V* Pelican. We also thank Maxim Gorbunov for comments on an early version of the manuscript and three anonymous reviewers for their input, which significantly improved the final manuscript.

This work was supported by National Science Foundation grant OCE 0196175 and National Oceanographic and Atmospheric Administration grant NA03NOS4780041 to J.W.A.

This paper is contribution no. 2829 of the Virginia Institute of Marine Science, The College of William and Mary.

during the time and in the location of highest phytoplankton primary production that is responsible for much of the formation of summer bottom-water hypoxia on the Louisiana shelf (Rabalais et al. 2002). Other investigations in this area provide corroborating evidence of P limitation of phytoplankton during the late spring and early summer (Smith 1994; Lohrenz et al. 1999; Ammerman and Glover 2000).

Until now, FRRF has not been employed to address the issue of nutrient limitation on the Louisiana shelf. During a cruise from 04 to 08 July 2002, we used FRRF to confirm results from chlorophyll *a* (Chl *a*) response to added nutrients in nutrient addition bioassays conducted in the MRP to examine nutrient limitation in the region. Dissolved inorganic nitrogen (DIN = NO_3^- + NO_2^- + NH_4^+) and dissolved inorganic phosphate (P_i) concentrations, DIN : P_i ratios, and alkaline phosphatase (AP) activities were also measured in the initial samples from those bioassays. Our findings support the hypothesis that P was the limiting nutrient during early July in this ecosystem.

Materials and methods

Sample collection—Seawater samples were collected aboard the R/V *Pelican* at the surface (1 m) in 5-L Niskin bottles and subsampled for nutrient addition incubation experiments, AP assays, nutrient and Chl *a* concentrations, and FRRF analysis during 04–08 July 2002. Fourteen samples in total were collected within the MRP (Fig. 1A).

Chl a *and nutrient concentrations*—Seawater was filtered onto GF/F filters and frozen immediately in liquid nitrogen. After returning to the lab (≤6 d later), filters were thawed on ice and placed in DMSO/90% acetone (40 : 60) in the dark at room temperature for 2–6 h. Chl *a* fluorescence was measured using a calibrated Turner 10AU fluorometer. Concentrations of Chl *a* (corrected for phaeopigments) were determined according to Lohrenz et al. (1990).

Nutrient samples were filtered through prerinsed 0.45-μm cellulose ester filters (Millipore) to remove particulates. Filtrate was placed in acid-washed (10% HCl) 250-mL polyethylene Nalgene bottles rinsed twice with filtrate from the sample and then frozen until analysis on a Lachat QuikChem AE autoanalyzer for P_i and DIN.

AP assays—AP is an enzyme that cleaves inorganic phosphate from organic phosphate esters when environmental inorganic phosphate is low, but AP activity is very low or absent when phosphate concentrations are replete, >0.30 μmol L^{-1} P_i (Hoppe 2003). It is therefore used as an indicator of microbial community P stress. AP activity was measured according to Ammerman and Glover (2000) using a Tecan Genios fluorescence microplate reader, the substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DifMUP) and its respective standard, 6,8-difluoro-7-hydroxy-4-methylcoumarin (DifMU), both from Molecular Probes. Substrate was added directly to seawater in quadruplicate microplate wells at a saturating substrate

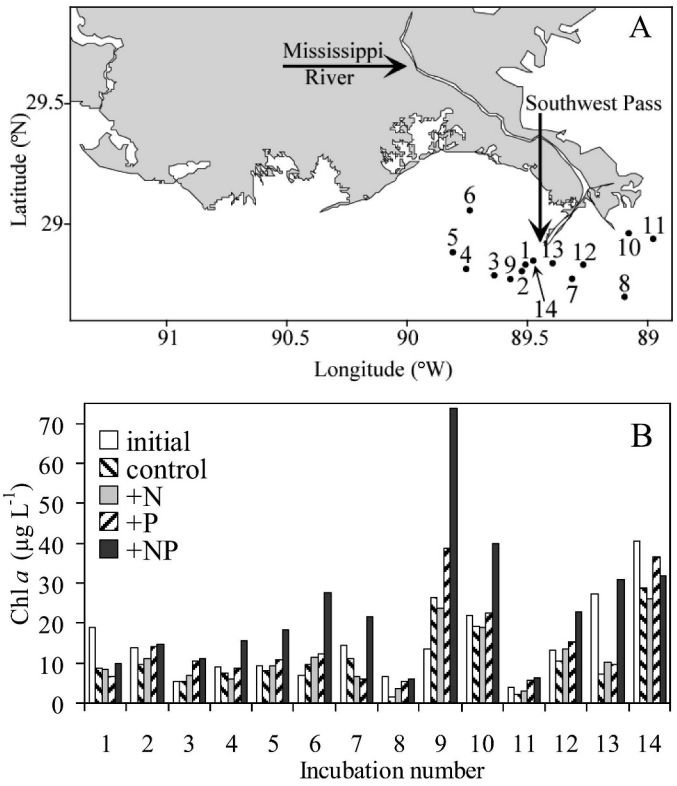

Fig. 1. (A) Location of stations used for nutrient addition bioassays during the 04–08 July 2002 cruise. Large arrows point to the locations of the Mississippi River and Southwest Pass. Small arrow indicates the location of station 14. (B) Chl *a* biomass (μg L^{-1}) for each treatment and all incubations. Initial Chl *a* was taken at t = 0 h, and control, +N, +P, and +NP were taken at t = 24 h.

concentration of 10 μmol L^{-1} based on prior kinetics data (Ammerman and Glover 2000). Fresh blanks and standard curves were included with each run. Killed controls were run periodically and indicated no significant autohydrolysis of the substrate.

FRRF data acquisition—A Chelsea Instruments first-generation commercial FAST^{tracka} FRR fluorometer was used in benchtop mode with only the dark chamber active. The instrument was configured to generate single turnover flashes. The single turnover consisted of a sequence of 100 excitation flashlets, each 1 μs duration, separated by a 1-μs interval, and a series of 20 relaxation flashlets. This protocol allowed for 3–4 quanta to be absorbed per reaction center in PSII (Kolber et al. 1998) and has been used successfully before in field experiments (Suggett et al. 2001). The same excitation protocol was maintained throughout the cruise. Samples taken during the day and after dusk were low-light acclimated in opaque polyethylene bottles and kept in a cooler with surface water for 30 min prior to assessment with the FRRF. This may not be enough time to allow for complete repair of photodamaged PSII, but it is assumed to be sufficient to eliminate photoinhibition by allowing for all PSII reaction centers to relax, making it possible to measure the maximum potential quantum yield. We assume no photo-

damage because there is no correlation between photosynthetically active radiation (PAR) and mapped underway $F_v : F_m$ in surface water samples during this cruise when plotted versus each other (data not shown). On the same plot are high values of $F_v : F_m$ at high PAR levels, but one would expect to see depressed $F_v : F_m$ correlate with elevated PAR levels if photodamage had occurred. Depressed quantum yields were therefore correlated to nutrient limitation rather than to photoinhibition (Kolber et al. 1998). Further details can be found in Kolber et al. (1998) and Suggett et al. (2001).

FRRF data processing—Data were acquired and processed with a program coded in TurboPascal as described by Kolber et al. (1998) to determine the parameters $F_v : F_m$, σ_{PSII}, τ_{Qa}, and p. This program is not related to the FRS.EXE program distributed with the Chelsea FAST^{tracka} unit. We present only data derived from single turnover analysis, and τ_{Qa} was calculated with a monophasic function. A baseline correction for all gain levels was applied for scattering correction based on measurements using ultrapure deionized water. Comparisons with filtered seawater and ultrapure deionized water yielded similar results, as found in other studies (Behrenfeld et al. 2006), indicating no significant background fluorescence from our blanks.

Nutrient addition bioassays—Seawater collected from Niskin bottles was analyzed before the addition of any nutrients at $t = 0$ h for Chl *a*, nutrient concentrations, AP activity, and FRRF parameters. This sample is hereafter referred to as the "initial" sample. Aliquots were subsequently placed into acid washed 250-mL Nalgene polyethylene bottles and received one of four additions: control (no additions), +N (30 μmol L^{-1} NO_3^-), +P (2 μmol L^{-1} PO_4^{3-}) or +NP (30 μmol L^{-1} NO_3^- + 2 μmol L^{-1} PO_4^{3-}). While an addition of 2 μmol L^{-1} PO_4^{3-} reagent could introduce trace amounts of Fe or Zn, Fe limitation is unlikely in the MRP because of dissolved Fe concentrations of 7–30 nmol L^{-1} in the river plume, even during the spring of 2000 when the high river flow was only half of normal (Powell and Wilson-Finelli 2003). Bottles were incubated in a clear incubator on deck with continuous surface water flowing through for 24 h before they were sampled again for Chl *a* and FRRF. Samples taken at 24 h will be referred to as control, +N, +P, and +NP, respectively. The average photoperiod during the cruise was 13:11 light:dark with a PAR of 824 μmol quanta m^{-2} s^{-1}, measured by an on-deck PAR meter. Samples in the incubator received approximately 55 ± 12% (354–552 μmol quanta m^{-2} s^{-1}) of ambient light. One-way analysis of variance (ANOVA) was used to assess statistical relationships between the different treatments. Post hoc comparisons were performed using the Tukey test. Statistical analyses were performed using JMP 5.1 (SAS Institute, Inc.).

Results

Fourteen nutrient addition incubations were performed in the MRP area near Southwest Pass over a 5-d period. This area is known to be P limited in July based on elevated $DIN : P_i$ ratios and high AP activities found in previous studies (Ammerman and Glover 2000; Sylvan et al. 2006). Station locations are shown in Fig. 1A, and seawater characteristics from each station are given in Table 1. The area in July 2002 was characterized by low P_i (mean = 0.18 μmol L^{-1}), high $DIN : P_i$ ratios (mean = 53), and very high AP activities (mean = 347 nmol L^{-1} h^{-1}).

The +P incubations yielded higher Chl *a* concentrations than the +N incubations in 11 of the 14 nutrient addition experiments (Fig. 1B). The +NP incubations had higher Chl *a* biomass after 24 h than the +N incubation for all 14 nutrient addition experiments and higher biomass than the +P incubation for 13 of 14 experiments. To summarize all 14 incubations, we calculated mean parameters for each treatment using the values from all the incubations (Fig. 2). The average Chl *a* concentration in the initial samples was 14.6 μg L^{-1}. This was greater than the mean concentration of Chl *a* in both the control (11.1 μg L^{-1}) and the +N (11.3 μg L^{-1}) treatment after 24 h. The mean Chl *a* concentration in the +P treatment was 14.5 μg L^{-1} at 24 h, similar to that in the initial sample. Only the +NP treatment yielded an increase of Chl *a* over the initial sample (62%; 23.6 μg L^{-1}) after 24 h. The control and +N treatments were significantly lower than all the others ($p = 0.033$, one-way ANOVA).

FRRF parameters give a snapshot of the overall status of the phytoplankton community, enhancing our understanding of the effects of the added nutrients. For this reason, the $F_v : F_m$ ratio is commonly used to rapidly assess the status of phytoplankton cultures (Kolber et al. 1998) and field populations (Suggett et al. 2001). The mean $F_v : F_m$ value in the initial samples was 0.42 ± 0.004 (standard error; Fig. 2). In both the control and the +N treatment, mean $F_v : F_m$ decreased after 24 h to 0.32 ± 0.004 and 0.32 ± 0.006, respectively. $F_v : F_m$ in the +P treatment was 0.41 ± 0.004 after 24 h and was 0.41 ± 0.003 in the +NP treatment. Both were higher than the corresponding control and close to the initial ratio. The control and +N treatments were again significantly lower from all the others ($p < 0.001$; Fig. 2).

The mean σ_{PSII} in all the initial samples was 238 ± 3.19 Å^2 quanta^{-1}. The control treatment yielded a mean σ_{PSII} of 228 ± 7 Å^2 quanta^{-1} after 24 h, and the +N treatment had a mean σ_{PSII} of 237 ± 6 Å^2 quanta^{-1} (Fig. 2). Mean σ_{PSII} was significantly lower in the +P and +NP treatments after 24 h, 207 ± 3 and 194 ± 3 Å^2 quanta^{-1}, respectively, than the initial and +N treatments ($p < 0.001$). The control treatment was significantly higher than the +NP treatment but was not significantly different from either the initial and +N or the +P.

Mean τ_{Qa} was 421 ± 17 μs in the initial samples and increased dramatically for both the control (535 ± 43 μs) and the +N (519 ± 42 μs) incubation after 24 h (Fig. 2), while the +P treatment showed a very slight increase over the initial sample to 425 ± 15 μs, and the +NP treatment decreased from the initial sample to 412 ± 12 μs. None of the treatments were significantly different from each other ($p > 0.05$).

Mean initial p was 0.32 ± 0.02 (unitless) and decreased to 0.24 ± 0.02 and 0.22 ± 0.01 in the control and +N

Table 1. Station data for incubation experiments. All water was collected at 1 m with a Niskin bottle. DIN and P_i are units of μmol L^{-1}; DIN : P_i, $F_v : F_m$, and p are unitless; Chl a is in μg L^{-1}; AP activity is in nmol L^{-1} h^{-1}; σ_{PSII} is in $Å^2$ $quanta^{-1}$; and τ_{Qa} is in μs. Date and time are local. N.D. = no data.

Station	Date and time (July 2002)	Latitude (°N)	Longitude (°W)	Salinity	Temperature (°C)	DIN	P_i	DIN : P_i	Chl a	AP activity	Initial $F_v : F_m$	Initial σ_{PSII}	Initial p	Initial τ_{Qa}
1	04, 22:53	28.804	89.523	12.9	31.3	14.30	0.21	68	19.0	240	0.426	245	0.287	460
2	05, 10:58	28.832	89.508	14.7	30.5	5.12	0.21	24	13.9	315	0.351	255	0.281	558
3	05, 12:27	28.787	89.639	16.5	30.9	8.33	0.21	40	5.3	81	0.437	258	0.274	425
4	05, 14:12	28.814	89.755	17.5	31.1	N.D.	N.D.	N.D.	8.9	151	0.407	254	0.299	486
5	05, 15:43	28.883	89.811	16.9	31.1	7.48	0.20	37	9.2	143	0.518	178	0.438	403
6	05, 18:11	29.057	89.740	15.7	30.7	7.75	0.14	55	7.0	218	0.355	270	0.327	430
7	06, 11:37	28.773	89.315	24.4	28.8	10.88	0.21	52	14.3	65	0.372	238	0.271	407
8	06, 14:53	28.698	89.096	29.3	30.2	3.27	0.06	55	6.6	2190	0.404	244	0.356	446
9	07, 22:57	28.772	89.571	16.2	30.2	23.71	0.21	113	13.4	193	0.413	256	0.315	404
10	07, 14:00	28.961	89.079	21.2	30.9	9.56	0.23	42	22.0	418	0.507	207	0.407	386
11	07, 15:34	28.939	88.974	30.5	30.1	3.79	0.10	38	3.9	74	0.398	252	0.223	501
12	07, 19:49	28.831	89.268	27.8	29.9	6.38	0.15	43	13.1	140	0.311	270	0.386	311
13	07, 20:39	28.838	89.396	22.3	30.2	10.48	0.18	58	27.2	340	0.457	238	0.332	364
14	07, 21:42	28.848	89.476	19.1	30.3	14.87	0.22	68	40.4	291	0.504	219	0.303	374
Mean				20.4	30.4	9.7	0.18	53	14.6	347	0.42	242	0.32	425
Median				18.3	30.4	8.33	0.21	52	13.3	206	0.41	249	0.31	416

treatments, respectively, after 24 h (Fig. 2). However, p remained the same in the +P treatment (0.32 ± 0.01) after 24 h and increased in the +NP treatment to 0.36 ± 0.01. The initial sample and +P and +NP treatments were found to be significantly higher than the control and +N treatments ($p < 0.001$).

Discussion

In this work, multiple lines of evidence demonstrated P limitation of the phytoplankton community in the MRP along the Louisiana continental shelf during early summer 2002. In combination, the FRRF parameters $F_v : F_m$, σ_{PSII}, τ_{Qa}, and p supported findings based on Chl a, AP activities, and nutrient concentrations and ratios, thereby providing a mechanistic understanding of how the community responded to changes in nutrient availability. This study confirms and extends temporally the seasonal pattern of P limitation on the Louisiana shelf, recently shown by Sylvan et al. (2006).

On the Louisiana continental shelf, DIN concentrations and primary productivity are typically highest in the late spring and early summer, shortly after the annual peak discharge of the Mississippi River (Lohrenz et al. 1997, 1999). The high levels of DIN carried in the river stimulate phytoplankton growth and result in high DIN : P_i ratios and high AP activities. Chl a concentrations >30 μg L^{-1} were observed in separate cruises during July–August 1990 (Lohrenz et al. 1999). In July 2001, mean Chl a concentration for the entire Louisiana shelf was >17.0 μg L^{-1}, with concentrations >24 μg L^{-1} closer to the MRP (Sylvan et al. 2006). Although we focused on a smaller area in the current study, mean Chl a concentration was still high, 14.6 μg L^{-1} (Table 1). AP activities during this study were very high (Table 1) and in combination with those seen in this area in the past (Ammerman and Glover 2000; Sylvan et al. 2006) are among the highest reported for marine and estuarine ecosystems (Hoppe 2003).

Nutrient addition experiments were conducted in the high-Chl a, midsalinity area adjacent to the Mississippi River mouth (Table 1), where phytoplankton production is typically highest (Lohrenz et al. 1997). Chl a biomass in the control bottles decreased, on average, after 24 h of incubation. While the mean biomass in the control and +N additions decreased by 22% and 24%, that of the +P addition maintained the original biomass (Fig. 2). Biomass in the +NP addition increased by 62%. This type of result for nutrient addition bioassays is not uncommon, especially in short incubations (<48 h), and appears to reflect the physiological lag between nutrient uptake and conversion to biomass (Downing et al. 1999). Grazers were not examined during this study, so it is possible that they were responsible for the decrease in Chl a in the control and +N incubations. However, given the short duration of the incubations, this was unlikely. The decrease in Chl a biomass was likely a result of isolation of an already P-depleted water mass with no new sources of P available, making new growth difficult. The higher Chl a biomass in the +NP treatment than the +P treatment likely resulted from additional growth fueled by the added N above what

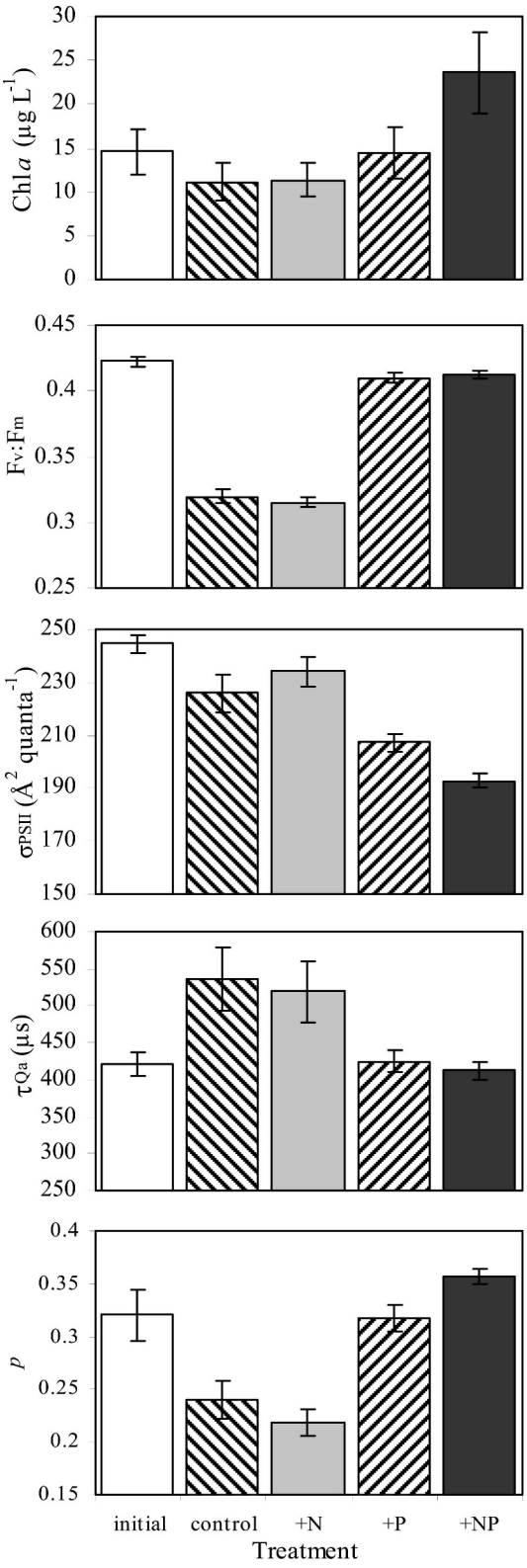

Fig. 2. Mean responses in incubations of Chl *a*, $F_v:F_m$, σ_{PSII}, τ_{QA}, and *p* after 24 h (± standard error) for all incubations. Sampling and notation are the same as in Fig. 1. For FRRF parameters, data from the +P addition of incubation 3 and all of incubation 13 were lost and so were not included.

could be achieved with the added P alone. Cells in the +P addition could only use the excess P in combination with the N already present in the initial sample, resulting in N limitation following drawdown of the initial N, while additional growth was possible in the +NP treatment. Additionally, it may take more than 24 h to see an increase in Chl *a* concentrations above the initial in the +P treatments. However, the response in the +NP treatments indicates that the phytoplankton were indeed nutrient limited because if their nutrient uptake systems were saturated, Chl *a* in the +NP treatment would not have increased above the initial. Therefore, the higher Chl *a* biomass in the P additions (compared with the control) is important evidence for P limitation in the region.

Further supporting evidence was revealed by careful examination of the physiological parameters measured using FRRF. Mean $F_v:F_m$ values for the +P and +NP treatments were nearly equal to the initial, untreated sample, while the treatments lacking added P suffered a decline in $F_v:F_m$. These results indicated either that the P additions maintained the phytoplankton at their pretreatment physiological status or that the added P enhanced photosynthetic efficiency of cells after an initial decline, thereby allowing them to maintain a relatively higher $F_v:F_m$. The lack of increase of $F_v:F_m$ over the initial in the +P and +NP treatments indicates that perhaps there was some additional stressor in the incubations preventing these treatments from approaching the theoretical maximum $F_v:F_m$. Perhaps, as seems to be the case with the Chl *a* concentration in the +P treatments, 24 h was not enough time for slowly adapting assemblages to attain their maximum $F_v:F_m$. Alternatively, a lack of additional P may have caused the already P-limited cells to crash in the control and +N treatments, while the additional P in the +P and +NP treatments allowed the cells in these treatments to maintain their $F_v:F_m$. It may have required more P to see a further increase in $F_v:F_m$ over the initial. While photodamage was not evident in the initial samples, it is possible that cells experienced a low level of photodamage in our incubators during the experiment. However, in combination with the increase in Chl *a*, especially that of +NP over the initial, our results indicate that P limitation was the ultimate cause for the changes in Chl *a* biomass and $F_v:F_m$. Cells in the +P and +NP treatments experienced a higher growth rate because of the added P, resulting in higher overall Chl *a* and elevated $F_v:F_m$ values compared to the control and +N treatments.

σ_{PSII} decreased below the control in the +P and +NP treatments and was lowest in the +NP treatment. Both the +P and +NP treatments were significantly lower than the initial. Previous work has shown an increase in σ_{PSII} under N limitation (Kolber et al. 1988), but this is the first evidence of a decrease under release from nutrient limitation, illustrating that σ_{PSII} is responsive to nutrient conditions. The decrease in σ_{PSII} in the +P and +NP additions was correlated with an increase in $F_v:F_m$ in those treatments.

The time for the reoxidation of Q_a is given by τ_{Qa}. Q_a reoxidation occurred slightly faster in treatments with P added than those without, supporting the improvement in

the physiological status of the cells, consistent with an increased $F_v : F_m$ in the +P treatments (Fig. 2). Because of high background noise inherent to the measurement of τ_{Qa} (M. Gorbunov pers. comm.), it was not possible to obtain statistically significant results, but mean τ_{Qa} was shorter in treatments with added P than those without.

Lastly, an increase in *p* indicates a higher probability that energy will be passed between the reaction centers in PSII. Decreased *p* is associated with N limitation in laboratory-grown cultures (Kolber et al. 1988) but has not been examined in the field. Mean *p* was greatest in the +NP treatment and greater in the +P treatment (which was equal to the initial sample) than in the control and +N treatment (Fig. 2). Overall, the FRRF results for these bioassays confirmed the nutrient data and enzyme assays from the initial samples and the Chl *a* responses to added nutrients in our bioassays, indicating that phytoplankton were P stressed near the MRP in July 2002. The increase in $F_v : F_m$ and *p* and coincident decrease in σ_{PSII} and τ_{Qa} provide a signature for release from P limitation by the phytoplankton in this area, supporting the value of the FRRF and combined use of derived parameters to assess nutrient limitation.

Previously, $F_v : F_m$ has been measured in N- and Fe-stressed or -limited laboratory and field phytoplankton, but the only field study to measure $F_v : F_m$ in conjunction with observations of P limitation used pulse amplitude modulated (PAM) fluorometry in an estuary in the Netherlands (Kromkamp and Peene 1999), where decreased $F_v : F_m$ correlated with low DIN and P_i. There are no published field studies of P limitation using FRRF. However, a few laboratory studies of P limitation using fluorometrically derived parameters also exist. Cultured *Microcystis aeruginosa* displayed an increase in $F_v : F_m$ when PO_4^{3-} was added to P-limited cells (Wood and Oliver 1995). *Dunaliella tertiolecta* grown in a chemostat exhibited lower $F_v : F_m$ when grown under P-starved conditions than when P replete (Graziano et al. 1996), and lab-grown *Sphaerocystis*, *Scenedesmus*, *Nitzchia*, and *Phormidium* exhibited an increase in $F_v : F_m$ within 24 h of P addition to P-limited cells (Beardall et al. 2001*a*). P limitation caused a decrease in $F_v : F_m$ and $\Delta F' : F_m'$ compared to P-replete cells, and spiking the P-limited cells with P caused a recovery of $\Delta F' : F_m'$ to the same level as P-replete cells in *Alexandrium minimum* (Lippemeier et al. 2003). While these studies used either a spectrofluorometer (Wood and Oliver 1995) or a PAM fluorometer (Graziano et al. 1996; Beardall et al. 2001*a*; Lippemeier et al. 2003) with laboratory-grown phytoplankton, their results supported our finding that $F_v : F_m$ increases with the relief of P limitation and that $F_v : F_m$ is decreased in nutrient-stressed phytoplankton.

One potential drawback to the FRRF is that it cannot be effectively used to study species whose effective absorption bands of the light harvesting pigments in PSII do not overlap with those of the FRR fluorometer, such as the filamentous cyanobacteria *Nodularia spumigena* and *Aphanizomenon sp.* (Raateoja et al. 2004), which contain phycoerythrocyanin instead of phycoerythrin. While we did not perform species-level identifications of the phytoplankton taxa in this study, past work in this area indicates that filamentous cyanobacteria are not present at high concentrations. In April and October 2000, the single-celled *Synechococcus* dominated coastal waters, while *Prochlorococcus* was abundant at high-salinity stations (Liu et al. 2004) along the Louisiana coast. These same distributions were seen in May 2000 (Jochem 2003).

Some lab studies suggest that $F_v : F_m$ may not be an accurate method for assessing nutrient limitation in the field (Parkhill et al. 2001; Kruskopf and Flynn 2006). We found that $F_v : F_m$, as well as σ_{PSII} and *p*, changed significantly in response to nutrient additions. One excellent study assessed the usefulness of variable fluorescence in a number of different phytoplankton in laboratory cultures and found they took variable amounts of time to show decreases in $F_v : F_m$ resulting from nutrient stress (Yentsch et al. 2004). These authors also found $F_v : F_m$ to vary directly with nutrient input at multiple field sites and indicated that the ratio can be used to measure potential nutrient stress, as it has in many other studies. It is likely that a limited response of $F_v : F_m$ to nutrient changes in lab cultures is a result of the variable stress tolerances of different species to ranges of nutrient conditions. Future FRRF and other fluorometric studies should continue to assess and then validate the usefulness of this method for evaluating nutrient limitation.

This work used a combination of traditional measurements and FRRF to demonstrate P limitation in coastal Louisiana, influenced by the Mississippi River runoff. The high concentrations of DIN and high $DIN : P_i$ ratios measured during this study indicate that the P limitation was driven by surplus DIN in addition to low P. Our findings are consistent with past studies that also measured P limitation of phytoplankton on the Louisiana continental shelf using direct (Smith and Hitchcock 1994; Sylvan et al. 2006) and indirect methods (Lohrenz et al. 1999). Here we have provided the first field measurements using FRRF to study P-limitation in marine phytoplankton. In a set of nutrient addition bioassays near the MRP, low P_i concentrations and high $DIN : P_i$ ratios and AP activities in the initial samples coupled with Chl *a* response over a 24-h period in nutrient addition bioassays were indicative of P limitation. FRRF parameters measured in these bioassays showed statistically significant responses that corresponded to the traditional data and outlined a fluorescence signature for release from P limitation consisting of increased $F_v : F_m$ and *p* coupled with decreased σ_{PSII} and τ_{Qa}. This study suggests that the use of FRRF coupled with short-term incubations can provide more rapid results than traditional bioassays of nutrient limitation requiring multiple days, and more detailed information about the physiological state of the community in the region being assessed, all without a significant increase in effort.

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Received: 28 July 2006
Accepted: 21 June 2007
Amended: 29 June 2007