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A comparison of methods for detection of phosphate limitation in microalgae

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ABSTRACT

This paper presents the results of studies into the use of the emerging techniques of nutrient induced fluorescence transients (NIFTs) and Fourier Transform InfraRed (FTIR) spectroscopy to determine the nutrient status of microalgae. Four species of microalgae were grown under conditions where growth rate was P-limited or P-replete, and NIFT responses and FTIR spectra in response to the re-supply of P (as PO_4^{3-}) measured. These responses were compared to more conventional measures of algal nutrient status such as P-uptake rates, P quotas and transient effects of PO_4^{3-} on oxygen exchange. The NIFT technique and FTIR spectroscopy gave results that were consistent with those obtained by the other techniques. Furthermore, we were able to demonstrate NIFT responses in phytoplankton samples taken from Lake Lucerne (total ambient $P \leq 0.13$ μM) but not from Lake Zürich (total P 0.55 μM). The potential and limitations of the various techniques are discussed.

Introduction

Aquatic primary productivity is frequently rate-limited by the availability of nutrients. Principally this involves limitations in the supply of nitrogen and phosphate (Birch et al., 1981; Lean and Pick, 1981; Schindler, 1977; Wynne and Berman, 1980) in marine and freshwater ecosystems respectively (Hecky and Kilham, 1988; but see

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Elser et al., 1990 and Dodds et al., 1993). However more recent investigations have shown that some marine systems such as the North Pacific Sub-tropical Gyre (Karl, 1999) and regions of the Mediterranean Sea (Krom et al., 1991) are, in fact, P limited. Furthermore, phytoplankton productivity is iron limited in "high nutrient, low chlorophyll" areas such as the equatorial Pacific (e.g. Behrenfeld et al., 1996) and Southern Ocean (Timmermanns et al., 1998) and even micro-nutrients such as zinc may be the limiting factor in some situations (Bruland, 1989; Morel et al., 1994). The ability to identify limiting nutrients is of considerable importance to our understanding of the ecology of aquatic plants and for guiding water management practices.

A number of techniques have been employed in the past in order to determine factors limiting growth and production of algae. All of these suffer in one way or another from deficiencies and/or potential artifacts. Most methods presently available entail destruction of the sample, are complicated and time consuming. The problems, associated with the various ways of assessing nutrient limitation, have been reviewed by Hecky and Kilham (1988), Wood and Oliver (1994) and Beardall et al. (2001, this issue). However, recently developed techniques based on fluorescence (Wood and Oliver, 1994; Beardall et al., 1996; Beardall et al., 2001 – this issue) or Fourier Transform Infrared (FTIR) spectroscopy (Giordano et al., 2001) offer the potential to determine nutrient limitation rapidly and non-destructively in natural populations of phytoplankton.

In this paper we report on the use of nutrient-induced fluorescence transients (NIFT) and FTIR spectroscopy to detect phosphate limitation in freshwater microalgae. These techniques are compared to more conventional assays for P-limitation such as comparison of rates of P uptake and observation of P-induced perturbations in rates of O_2 evolution.

Methods

Organisms and growth conditions

Axenic cultures of *Phormidium luridum* var *olivace* Boresch (EAWAG culture 426), *Nitzchia* sp. (ex University of Minnesota culture collection), *Scenedesmus quadricauda* (Turp.) Breb (University of Texas 1648) and *Sphaerocystis schroeteri* Choda (University of Minnesota culture LB 562) were grown in the WC medium of Guillard (1975). Cultures (1000 mL) were grown in semi-continuous mode with dilutions (made with medium containing 5 µM orthophosphate) made every second day to provide a growth rate of 0.25 d^{-1}. Where appropriate, phosphate-limited cultures were re-supplied with P to determine changes in various physiological parameters. For the phosphorus-replete cultures, the added medium contained 100 µM orthophosphate.

Basic cell parameters for the cultures used in this study are given in Table 1. Cell densities for all eucaryotic cultures were maintained below 10^5 cells mL^{-1}. Cell numbers were not estimated for the cyanobacterium *Phormidium*. In this case, cell densities were equivalent to ~ 160 µg chl L^{-1}.

For some experiments, surface water from Lake Zurich or Lake Lucerne was used. These samples were dominated by the cyanobacterium *Planktothrix.* Chlorophyll content and P content of these samples are given in Table 1.

Table 1. Cell biomass and phosphorus content of the medium in P-limited and P-resupplied cultures and natural lake waters at the time of sampling for physiological parameters. Biomass in P-resupplied cultures is lower in some cases because of the dilution effect in running semi-continuous cultures

Species	Cell density (cells mL^{-1}) or *chlorophyll content ($\mu g\ L^{-1}$)		Orthophosphate concentration (μM)	
	limited	Re-supplied	limited	Re-supplied
Sphaerocystis	20 ± 3	22.7 ± 1.2	<0.1	0.14
Scenedesmus	9 ± 1	3 ± 1	0.1	12.1
Nitzchia	22 ± 2	18.6 ± 3.4	0.15	9.95
*Phormidium**	~148–168 $\mu g\ L^{-1}$	~148–168 $\mu g\ L^{-1}$	>0.1	21.7
*Lake Lucerne**	1.08 $\mu g\ L^{-1}$	–	0–0.13[a]	–
*Lake Zurich**	2.09 $\mu g\ L^{-1}$	–	0.55[a]	–

[a] Values for Lake Lucerne and L. Zurich are given as total P.

Oxygen exchange. Oxygen exchange rates in the light and dark were measured with Hansatech DW1 oxygen electrodes. Electrodes were calibrated by bubbling distilled water in the chambers with oxygen-free N_2 (zero) and air (272 µM). An AD converter card linked to a laptop computer monitored the output from the electrodes. Culture (2 mL) was placed in each of two electrode chambers. One chamber was darkened for estimates of dark respiration and the other was illuminated at 300 µmol photons $m^{-2}\ s^{-1}$, a photon flux which preliminary experiments had shown to be saturating for photosynthetic oxygen evolution. After a pre-incubation of ~10 min, phosphate was added to 10 µM and rates of oxygen evolution or consumption followed for an additional 10 min. Care was taken to avoid elevated oxygen concentrations in the electrode chambers. If necessary, suspensions were bubbled with N_2 to reduce oxygen to 50% air equilibrium.

Phosphate uptake rates. Phosphate uptake rates were determined by the disappearance of orthophosphate from the medium following addition of K_2HPO_4 to 20 µM. Phosphate, in filtered (0.45 µm) samples of medium, was determined by the method of Stevens (1962). P quotas were determined from uptake of phosphate from the medium and concurrent changes in cell density.

Fluorescence analysis

All NIFT assays were performed with a pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Waltz, Germany). Sample aliquots (2 mL) of algal material were placed in disposable plastic cuvettes and dark-adapted for 15 min. Each cuvette was then placed in a purpose-built holder positioned to enable magnetic stirring from below. The fibre optic of the PAM fluorometer, used for both sample illumination and fluorescence collection, was positioned in the cuvette and the fluorescence parameters initial fluorescence (F_o), maximal fluorescence following a saturating pulse (F_m), steady state fluorescence (F) and maximal fluorescence under actinic light (F'_m) determined. Actinic light was set at 117 µmol $m^{-2}\ s^{-1}$.

At the start of each experiment, using dark-adapted suspensions, initial fluorescence (F_o) and maximal fluorescence following a saturating pulse (F_m) were used to calculate F_v/F_m with the formula:

$$F_v/F_m = (F_m - F_o)/F_m$$

where the variable fluorescence, F_v, is the difference between maximal and minimal fluorescence.

Using the Diving-PAM's chart mode function, following determination of F_v/F_m the actinic light was switched on and steady state fluorescence (F) and maximal fluorescence under actinic light (F'_m) were logged and plotted in real time.

NIFT experiments were typically run for 30 minutes with a 30 μL addition PO_4^{3-} (1 mM) injected into the cuvette after the first 10 minutes. Controls with similar volumes of distilled water, ammonia or urea (10 mM) provoked no response.

A NIFT response is seen as a transitory change in chlorophyll fluorescence output. Changes in either or both of the parameters F and F'_m have been observed (Beardall et al., 1996; Wood and Oliver, 1995; Beardall et al., 2001).

In one set of experiments, the photon flux of the actinic light was varied and the Diving PAM used to determine electron transport rates (ETR) before and during a NIFT response in cells. Relative ETR (rETR) is given by

$$rETR = [(F'_m - F_o')/F'_m] \times \text{photon flux} \times 0.5$$

Values of ETR estimated in this way assume that the light absorption properties of the cells are unchanged prior to and during the NIFT response.

FTIR-MS analysis of cellular composition

Fourier-transform infrared (FTIR) spectroscopy uses mid-infrared absorbance spectra from fixed and dried microalgal samples. This can be combined with a suitable microscope and microspectrometer (FTIR-MS) to obtain spectral information from small fields of view or even single cells. When FTIR spectroscopy is applied to intact cells, the resulting spectra reflect the total biochemical composition of the cells (Naumann et al., 1991). Furthermore, FT-IR spectra can be used to determine the relative concentration of macromolecules such as proteins, lipids and carbohydrates in cells (Heise, 1997).

Cell samples used for FT-IR MS analysis were first concentrated by centrifugation at 500–600 g for 5 to 10 min. Cell pellets were then resuspended in small volumes of supernatant, such that final cell concentrations were approximately 10^8 cells mL^{-1}. Although spectra can be obtained, using a suitable microscope and microspectrometer, from single cells, high cell densities were used here to provide average population data and improve signal to noise ratios. Sub-samples (10 μL) of cell suspension were then deposited on IR-transparent substrates that included KRS-5, ZnSe, CaF_2, and a gold-coated silicon wafer for transmission/reflection spectra, then dried under vacuum (1 atm).

Spectra were generated using a Bruker IRscope II, fitted with a liquid nitrogen cooled mercury-cadmium-telluride detector and coupled to IFS-55 Equinox spectrometer, with a spectral range from 7000–600 cm^{-1} at a resolution of 8 cm^{-1}. For each deposit a sub-region of 50 × 50 $(\mu m)^2$ was encapsulated with a glass-edge aperture and one spectrum recorded at a resolution of 8 cm^{-1} with 512 scans co-added in the 7000–600 cm^{-1} range. All spectra presented are raw in form i.e. they have not been mathematically processed in any way (Wood et al., 1996).

Results and Discussion

A large number of techniques have been used in the past to determine nutrient status of phytoplankton. These include measurements of alkaline phosphatase activity as a measure of P-limitation, effects of nutrient re-supply on dark carbon fixation as an index of N-limitation, elemental composition of algae and bioassays. For a review of the approaches used, see Beardall et al., 2001. In the present study we have concentrated on comparing several conventional indices of P-limitation (P-cell quotas, effects of nutrient re-supply on oxygen evolution and P-uptake capacity) with the emerging techniques of NIFTs and FTIR spectroscopy.

Biomass (as chlorophyll) and phosphate concentrations in the cultures before and after phosphate was added are given in Table 1, together with data for samples from Lakes Zürich and Lucerne. Ambient phosphate concentrations in the culture medium of P-limited cultures were always < 1 μM whereas after re-supply concentrations were higher by an order of magnitude or more. Lake Zürich water contained relatively high concentrations of P at the time of sampling, whereas that of Lake Lucerne was comparatively P-poor (Table 1).

Oxygen exchange and P uptake

Phosphate uptake rates (measured by disappearance of phosphate from the external medium) were high in P-limited cultures but considerably lower or undetectable in cultures after P had been re-supplied (Table 2). This is a well-established phenomenon in microalgae and "luxury" uptake of P as a response to P limitation has been reported in a range of species (Gotham and Rhee, 1981; Turpin and Harrison, 1979; Graziano et al., 1996). However, the absolute rates of P uptake in P limited cultures reported in the present study varied more than 5 fold between algal species (Table 2) and such species specificity would make this a poor index of P status in natural populations.

The rates of net oxygen evolution ranged from ~1 to 96 μmol O_2 min^{-1} $(10^6$ cells$)^{-1}$ depending on species (Table 2). In all cases photosynthetic rates increased markedly after 24 hours when P was re-supplied to P-limited cultures. Note, however, that in these P-limited cultures, oxygen evolution was strongly inhibited immediately following the addition of 10 μM orthophosphate. After approximately 5–10 min, rates recovered to values equivalent to those before the P-spike (Fig. 1). Similar results have been reported by Healy (1979) for P-limited *Scenedesmus* and for NH_4^+ re-supply to N-limited *Selenastrum* (Elrifi and Turpin, 1986). Twenty four

Table 2. Phosphorus quota, maximal P-uptake rates and maximal rates of photosynthesis (measured by oxygen evolution) in the 4 algal species prior to and 24 h after re-supply with P

Species	Phosphorus quota (pg cell^{-1})			Maximum phosphorus uptake rate (pg P cell^{-1} h^{-1})			Maximum photosynthetic rate (nmol min^{-1} (10^6 cells)$^{-1}$		
	limited	re-supplied	% change[b]	limited	re-supplied	% change[a]	limited	re-supplied	% change[b]
Sphaerocystis	0.5	7.4	1480	3.7	0.5	–86	2.3	8.1	352
Scenedesmus	1.7	46.1	2711	10.3	0	–100	17.4	96.9	554
Nitzchia	0.7	7.7	1100	1.7	0	–100	1.1	2.5	227
*Phormidium**	N/m	N/m	N/m	0.92*	0	–100	N/m	N/m	N/m

* Biomass based data for *Phormidium* are unavailable due to a lack of cell density or chlorophyll data to form a basis for comparison of P- quota or rates of oxygen exchange. Rates of P uptake in this instance are given as µmol min^{-1} L^{-1}.

[a] Determined as (limited-resupplied)/limited × 100.

[b] Given by (resupplied/limited) × 100.

N/m = not measured.

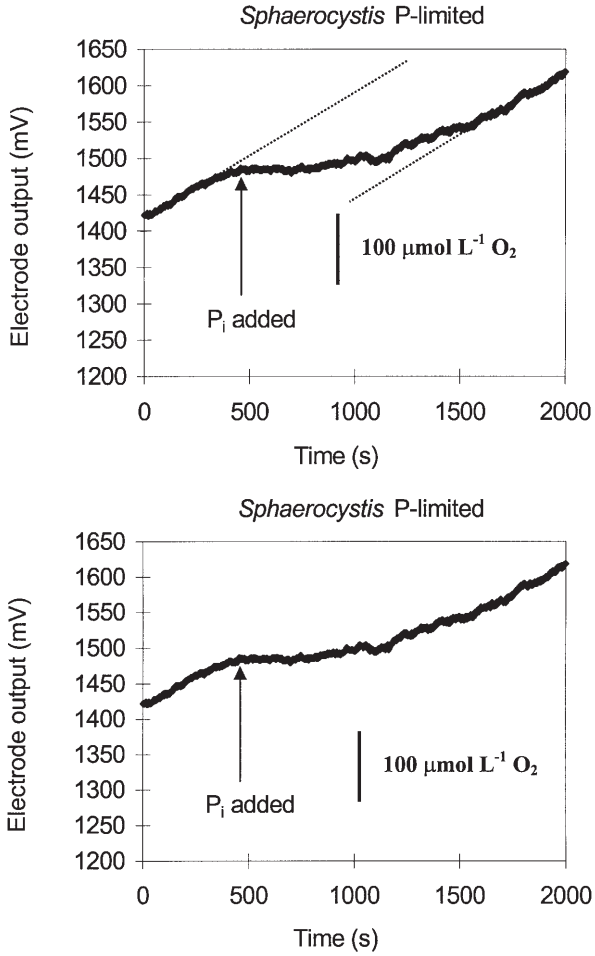


Figure 1. Oxygen evolution by suspensions of *Sphaerocystis* prior to (upper panel) and 24 h after (lower panel) re-supply of orthophosphate to a P-limited culture. Addition of P causes a transient suppression of oxygen evolution in P-limited, but not in P-resupplied, cultures

hours after re-supply of P, maximal photosynthetic rates had increased by up to 6 fold. Further P addition to these P-replete cells caused only slight or no perturbation of oxygen exchange in the light (Fig. 1).

Ratios of rates of phosphate uptake to maximal rates of photosynthesis were high in P-limited cells, but considerably lower (zero in most instances) in cells re-supplied with P (Table 3). The combination of enhanced nutrient uptake by nutrient limited cells and diminished oxygen evolution and carbon assimilation rates has been reported elsewhere (Lean and Pick, 1981; Turpin, 1983; Birch et al., 1986; Istvanovics et al., 1992). Lean and Pick (1981) proposed using the molecular ratio of P uptake to C fixation (or oxygen evolution) as an index of limitation with ratios <30 being indicative of nutrient sufficiency and higher ratios suggesting different

Table 3. Ratios of P uptake to photosynthetic rate for the four algal cultures prior to, and 24 h after, re-supply with P

Species	Ratio of maximum P uptake to maximum photosynthetic rate (mol P/mol O_2 evolved)	
	P-limited	After P re-supply
Sphaerocystis	463	33.5
Scenedesmus	187	0
Nitzchia	873	0
Phormidium	245	0

degrees of limitation. Clearly the data presented here confirm the strongly P-limited nature of cultures before P was re-supplied. In most cases the ratio of P uptake to photosynthesis following P re-supply was zero although *Sphaerocystis* still exhibited some capacity for P uptake under these conditions.

Response of fluorescence parameters to nutrient limitation

In Table 4 we summarize the results of measurements made on two fluorometric parameters that have been suggested to be indicative of physiological state or nutrient status in algae; the ratio of variable to maximal fluorescence (F_v/F_m) (Graziano et al., 1996) and the magnitude of the NIFT response (see Beardall et al., 2001 for a review). In the cases of *Nitzchia* and *Phormidium,* values for F_v/F_m prior to re-supply of P were below 0.5, suggesting considerable impairment of physiological 'health' of the cultures. In *Sphaerocystis* and *Scenedesmus*, F_v/F_m values were considerably higher (0.66–0.67), suggesting a lower degree of stress. In all cases, however, values of F_v/F_m increased 24 h after P was re-supplied (by 21–114%). Again the lower end of this range was found in the two green algae, while *Nitzchia* and *Phormidium* showed a greater increase in F_v/F_m (Table 4).

Table 4. Fluorescence characteristics of the 4 algal species prior to, and 24 h after, re-supply with P. Also shown are the NIFT responses from L. Lucerne and L. Zurich

Species	F_v/F_m		NIFT response (% drop in F'_m)	
	P-limited	After P re-supply	P-limited	After P re-supply
Sphaerocystis	0.676	0.821	19.6	0
Scenedesmus	0.664	0.864	34.3	6.9
Nitzchia	0.465	0.857	ud	ud
Phormidium	0.333	0.714	16.9	6.5
L. Lucerne	–	–	43	–
L. Zurich	–	–	0	–

ud = undetectable.

Nutrient induced fluorescence transients (NIFTs) have been observed in P- and N-limited algae in response to re-supply of the limiting nutrient (see Beardall et al., 2001 – this issue – for a review). Transient decreases in F'_m were found when P limited cultures of *Scenedesmus*, *Sphaerocystis* and *Phormidium* were spiked with 15 µM. orthophosphate (see Fig. 2 for examples). These NIFT responses were less apparent or absent after cultures had been re-supplied with P (Table 4, Fig. 2). No NIFT response was found with *Nitzchia*. We are unsure why this is so, but we have also been unable to detect NIFTs in another diatom *Chaetoceros muelleri* (Beardall and Roberts, unpublished). Whether this is a characteristic of diatoms in general requires further study. Samples of Lake Lucerne water, dominated by the cyanobacterium *Planktothrix*, showed a very marked NIFT response. In contrast, Lake Zurich waters, which also had a preponderance of *Planktothrix* but higher P concentrations, showed no response (Table 4). In some cases (e.g. *Phormidium*), both F and F'_m showed a NIFT response while in others (e.g. *Sphaerocystis*) changes were only apparent in F'_m (Fig. 2). No NIFT response was detectable with additions of N (as urea, nitrate or ammonia) or distilled water (data not shown). The duration of the NIFT response was consistent with the perturbations in oxygen evolution and the time required for P to be depleted from the medium at the rates cited in Table 2.

Measurements of electron transport rate (ETR) as a function of photon flux (Fig. 3) show that addition of orthophosphate to P-limited suspensions of *Scenedesmus* caused a marked decrease in light saturated rates of ETR but that the effect at sub-saturating photon flux was less apparent. Similar effects of P-addition on photosynthesis vs light relationships have been described for rates of oxygen evolution in Gauthier and Turpin (1997) and imply that a major consequence of the NIFT response is an impairment in rates of carbon assimilation rather than in rates of light harvesting, consistent with the notion that P-acquisition competes with C-assimilation for photochemically generated ATP (Roberts and Beardall, unpublished; Beardall et al., 2001). It is also well known from work on higher plant chloroplasts that elevated cytosolic concentrations of phosphate cause perturbations of photosynthesis and chlorophyll fluorescence quenching by enhancing the depletion of triose-phosphates from the chloroplasts into the cytosol via the triose-phosphate translocator in the chloroplast envelope (Cerovic et al., 1991).

The magnitude of the NIFT responses observed in the present study is less than those previously reported in the literature (Wood and Oliver, 1995; Beardall et al., 1996). Several factors could be responsible for this. The NIFT response is dependent on the photon flux of actinic light used (Fig. 3). In our experiments, the actinic light source of the Diving PAM was set at an arbitrary 117 µmol quanta m^{-2} s^{-1} which might have been too low to get full expression of the NIFT response. Alternatively, the NIFT response is also dependent on the extent of nutrient limitation. In cultures that are extremely nutrient limited or in cultures where limitation is only just setting in, NIFT responses are small (Roberts and Beardall, unpublished).

FT-IR MS

Fourier-transform infrared microspectroscopic (FT-IR MS) analysis revealed dramatic spectral differences between P-limited and P-replete states for all four algae

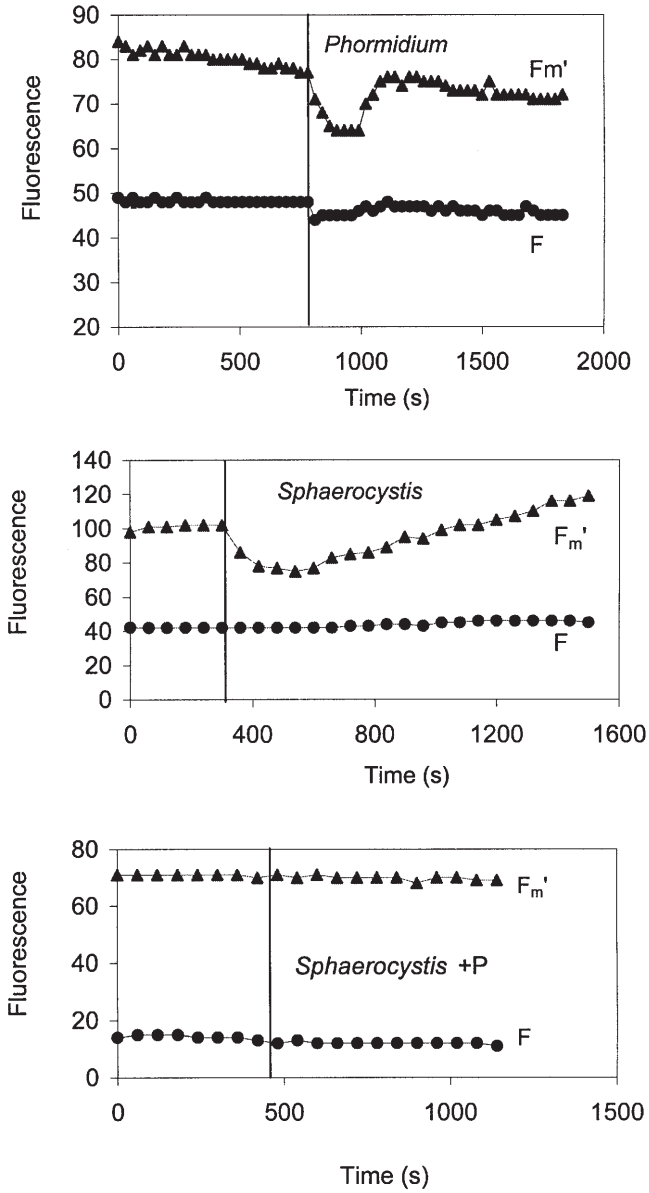


Figure 2. Nutrient induced fluorescence transient (NIFT) responses to the addition of P (indicated by the vertical bar) in P-limited *Phormidium* (upper panel), P-limited *Sphaerocystis* (middle panel) and *Sphaerocystis* after the re-supply of orthophosphate (bottom panel). Note that in *Phormidium* NIFT responses were exhibited by F'_m and F whereas in *Sphaerocystis,* only F showed a detectable transient under the experimental conditions. When orthophosphate was re-supplied to the *Sphaerocystis* culture, the NIFT response in F'_m disappeared

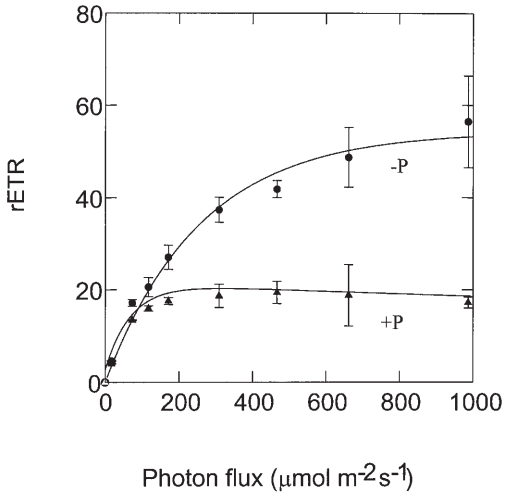
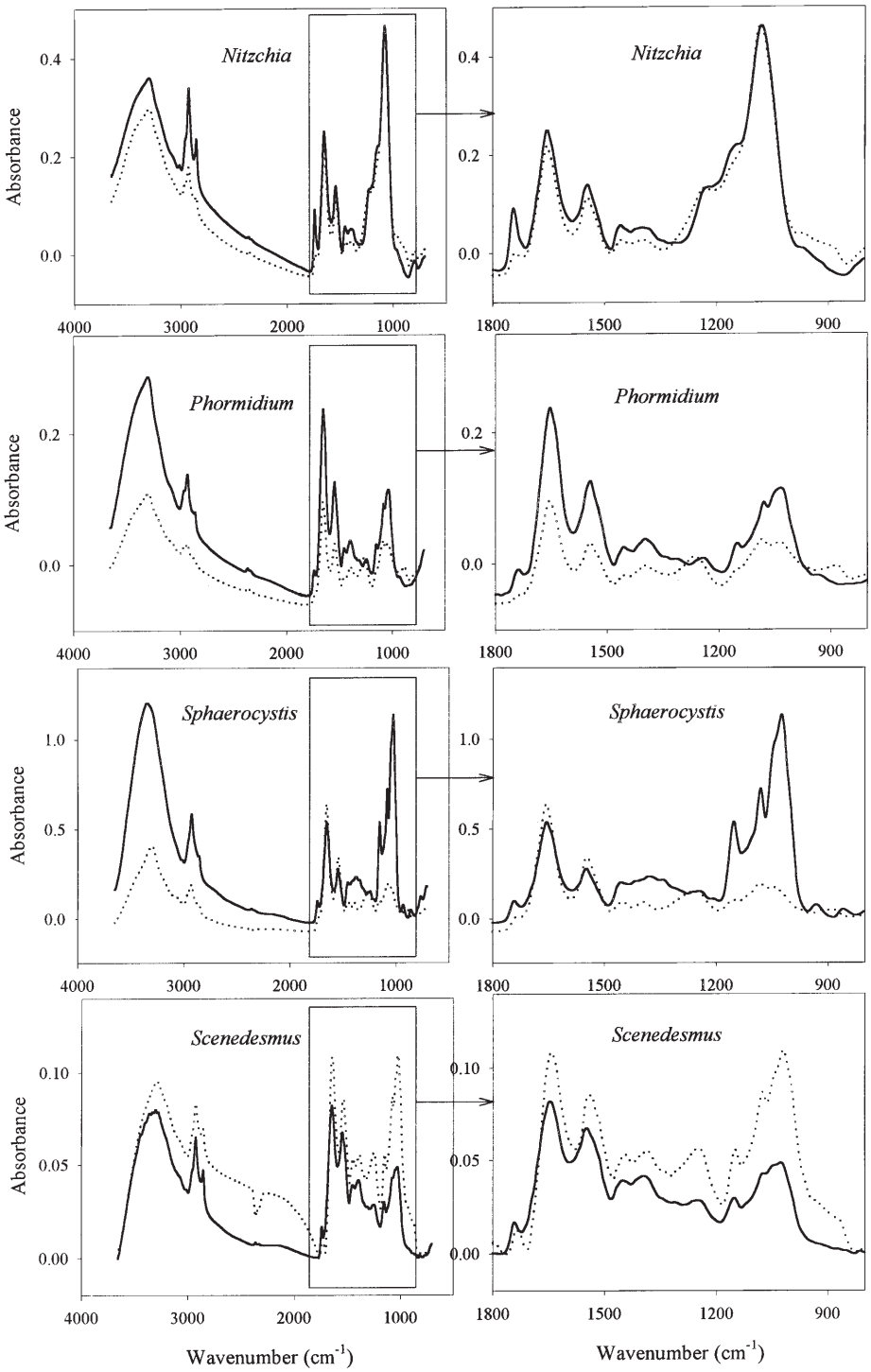


Figure 3. Changes in relative electron transport rate (rETR – see text for details) in P-limited *Scenedesmus* as a function of photon flux. Curves represent results from cells before (– P) and during (+ P) a NIFT response to orthophosphate. The '+ P' curve was made using cells taken at the trough of the NIFT response (approximately 200 s after addition of PO_4^{3-}) – see Fig. 2. The addition of P affects the light saturated rates of electron transport but not the initial slope, implying that the major effect of P-re-supply is on the light-independent reactions of photosynthesis (i.e. the Calvin cycle)

species investigated. Fig. 4 displays the initial spectra (prior to P spiking), and those 24 h after P spiking, for S*phaerocystis*, *Phormidium* and *Nitzschia* species, while only the initial and a 1 h spectra were available for *Scenedesmus.*

The spectra of P-limited *Sphaerocystis* and *Phormidium* exhibit strong bands at 1024, 1080 and 1150 cm^{-1} characteristic of the C-O stretching vibrations of carbohydrates (see Wood et al., 1996 for detailed functional group assignments). These bands have virtually disappeared 24 hours after the initial P spiking. An approximation, based on the total absorbance of the 1024 cm^{-1} band, indicates that *Sphaerocystis* has undergone a 5-fold decrease in carbohydrate levels over the 24 h period. The bands associated with lipid vibrations, namely the asymmetric and symmetric CH_2 stretching vibrations at 2924 and 2854 cm^{-1} along with the band at 1737 cm^{-1} (from the C = O stretching vibration of fatty acyl chains) are significantly decreased in the spectra of all four species. This is particularly evident in *Nitzschia* where a 3-fold decrease in lipid content is observed (based on the integrated intensity of the band at 1737 cm^{-1}). The increase in intensity and broadening of the asymmetric and symmetric phosphodiester vibrations at 1244 and 1080 cm^{-1} respectively, clearly observed in the spectra of *Phormidium*, *Sphaerocystis* and *Scenedesmus,* are indicative of P uptake into the cells. Both the phosphate and carbohydrate bands are obscured in the spectra of *Nitzschia* due to the intense absorbance by the O-Si-O bands (1300–1000 cm^{-1}) from the species' characteristic silicacious frustles. That changes in FTIR spectra correspond to changes in concentrations of extracted macromolecular components has been demonstrated in the diatom *Chaetoceros muellerii* (Giordano et al., 2001).


Nitzchia
Nitzchia
Phormidium
Phormidium
Sphaerocystis
Sphaerocystis
Scenedesmus
Scenedesmus
Absorbance
Wavenumber (cm-1)
Wavenumber (cm-1)

FT-IR MS analysis indicates that *Sphaerocystis*, *Phormidium* and *Nitzschia* all showed alterations in the storage of carbon, in response to changes in nutrient availability, similar to those reported elsewhere using conventional methods (see Beardall et al., 2001). Under P limitation, carbon was stored as either lipid or carbohydrate or both, with these stores being used when the nutrient was re-supplied. However with *Scenedesmus*, spectral changes indicated that an increase in carbohydrate levels occurred within one hour of the addition of P. More investigation is needed to verify and understand the meaning of this short-term response.

FT-IR MS analysis represents a novel method to investigate macromolecular synthesis in response to changes in nutrient supply in freshwater algae. The rapidity of the technique (~20 s to acquire a spectrum) and the ability to obtain simultaneous measurements on protein, lipid, carbohydrate, nucleic acids and phosphorylated compounds on whole cell populations is clearly demonstrated by the data presented here. Although relevant data are not shown in the present paper, it is feasible, using these techniques, to obtain spectral data from single cells or single colonies of cells (as in the case of *Scenedesmus*) (Heraud et al., unpublished). Despite the fact that, at present, the method has the limitations of requiring bulky and expensive equipment, its capability to provide a comprehensive analysis of the major cellular biochemical fractions suggests that this approach has considerable future potential for studies of the physiological responses of algae to varying environmental conditions.

Conclusions

The algae examined exhibited 'conventional' responses to P-limitation i.e. reduced photosynthetic capacity, enhanced phosphate uptake capacity and P-dependent transients in oxygen evolution. However, although these parameters are easy to investigate in laboratory cultures, there are problems associated with their application to natural populations. We compared these techniques to two novel approaches, namely the use of fluorescence characteristics and FTIR spectroscopy.

We have shown that a NIFT response was evident in all P-limited algal (except *Nitzchia*) and natural phytoplankton samples. This fluorescence response is rapid and can be accurately determined with commercially available instrumentation. The fluorescent measurements are non-destructive and highly sensitive, thus requiring only small volumes and low concentration of experimental samples. These characteristics make the NIFT approach potentially very useful for exploring to nutrient status of algae and their response to environmental changes.

Figure 4. FTIR-MS spectra for *Nitzchia*, *Phormidium*, *Sphaerocystis* and *Scenedesmus* before (solid line) and after (dotted line) the re-supply of orthophosphate to P-limited cultures. The right hand panels are expanded regions from the areas enclosed in boxes in the left hand panels. In all species except *Scenedesmus*, re-supply of orthophosphate causes a marked decrease in absorbance in the spectral regions associated with carbohydrate and lipid (see text for details). In *Scenedesmus*, the absorbance in regions associated with carbohydrate increased one hour after addition of orthophosphate. Absorbances have been corrected for any differences in cell densities of suspensions before and after P re-supply

FTIR spectroscopy was capable of rapidly providing a comprehensive analysis of the major cellular biochemical fractions in algal cells under conditions of different P-supply. This approach has considerable future potential for studies of the physiological status of natural populations of phytoplankton in varying environmental conditions.

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