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FLUORESCENCE PROPERTIES OF THE FRESHWATER PHYTOPLANKTON: THREE ALGAL CLASSES COMPARED

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The fluorescence properties of exponentially growing freshwater diatoms, green and blue-green algae were compared with a standard filter fluorometer and a scanning spectrofluorometer. Green algae and diatoms excited with blue light had *R* values (ratio of in vivo fluorescence to extractable chlorophyll *a*) up to 50 times higher than the cyanophytes. There were also considerable differences in *R* between species within each algal class—coefficients of variation were typically 35–50%—and this variation was slightly increased by treatment with the non-cyclic electron flow inhibitor, 3(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU). A major component of blue-green algal fluorescence was the red light emission from phycobilin accessory pigments. This contribution characterized their emission and excitation spectra and suggested a rapid assay for cyanophyte dominance, which was tested on natural plankton communities. Phycobilin fluorescence was insensitive to DCMU and resulted in highly wavelength-specified variations in CFC (cellular fluorescence capacity, the rise in fluorescence upon addition of DCMU). CFC values for blue-greens were consequently low when measured in the broad bandpass fluorometer, but were up to three times higher when measured with the spectrofluorometer set to the emission peak for photosystem II chlorophyll *a*. Similar results were obtained with natural populations of blue-green algae. The phycobilin content of blue-green species was influenced by nitrogen source and light quality during growth, and this resulted in variable *R* and CFC values. Fluorescence properties of diatoms and green algae were less responsive to environmental conditions during growth. Both diatoms and green algae exposed to bright light for 15 min demonstrated a strong reduction in *R*. Conversely blue-green algal fluorescence was depressed very little, or more commonly, was slightly enhanced by bright light. These observations underscore the importance of species composition and choice of fluorometer as critical determinants of measured algal fluorescence properties.

From the earliest application of in vivo fluorometry to aquatic ecosystems there has been a developing awareness that algal fluorescence varies widely for reasons unrelated to changes in extractable chlorophyll *a* (Chl *a*). Thus Lorenzen (1966) and subsequent advocates of field fluorometry as a synoptic or depth-profiling tool, recommend frequent calibration of readings against Chl *a* measured by traditional extraction procedures. These variations in apparent fluorescence yield (*R*, ratio of in vivo fluorescence to extractable Chl *a*) were later more precisely defined (e.g. Kiefer, 1973; Loftus & Seliger, 1975) and for the most part interpreted in terms of structural or photochemical adjustments by the photosynthetic apparatus.

Fluorescence is only one of several processes consuming excitation energy delivered to the reaction centres of photosystem II (RCII), and changes in any of the competing processes may alter the amount of energy fluoresced. Changes in photosynthetic capacity result in large and generally inverse shifts in fluorescence yield which can be eliminated by the non-cyclic electron flow inhibitor 3(3,4-

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dichlorophenyl)-1,1-dimethyl urea (DCMU) (e.g. Slovacek & Hannan, 1977). Bright light promotes a transfer of excitation energy from highly fluorescent RCII to weakly fluorescent RCI (reaction centre of photosystem I), thereby reducing R values by up to 80% (Vincent, 1979); this fluorescence depression can be fully reversed by incubating the algal cells in dim light or darkness (Heaney, 1978). Factors which control the absorption of light and transfer of excitation energy to RCII must also modulate in vivo fluorescence. Such factors include algal cell size and shape (e.g. Kirk, 1975), optical cross-section of the chloroplasts (e.g. Kiefer, 1973), as well as the relationship between antennae pigments and RCI and II—for example, current models describing thylakoid structure and function postulate a dynamic coupling between light harvesting chlorophyll proteins and photosystem reaction centres (Bennett, Steinback and Arntzen, 1980).

A second group of factors which can account for wide variation in phytoplankton fluorescence has been identified (Heaney, 1978; Yentsch, 1980) but less widely explored. The various classes of planktonic algae possess different accessory or "antennae" pigments and differ markedly in the organization of their light-capturing apparatus. The importance of these variations has been obscured by general statements in the ecological literature that are derived from measurements of a restricted array of organisms. For example, recent proponents of DCMU methodology note that in vivo fluorescence in various taxa comes primarily from Chl *a* (Slovacek & Hannan, 1977). This statement may not be correct for the ubiquitous marine and freshwater groups that contain highly fluorescent chromoproteins (cyanophytes, cryptophytes, rhodophytes). As will be shown below, the disparate behaviour of these algae has important implications for various fluorescence assays in the field.

DCMU-induced fluorescence has been widely commended as a rapid measure of photosynthetic capacity in natural waters (e.g. Samuelsson & Oquist, 1977; Roy & Legendre, 1978), but some evidence indicates that unlike other algae, the in vivo fluorescence of Chl *a* for certain blue-greens may be insensitive to this photosynthetic inhibitor (Bader & Schmid, 1980). Light-state transitions and the attendant shifts in fluorescence intensity have been proposed as measures of vertical mixing processes near the surface of lakes and oceans (Vincent, 1979), but blue-green algae may not respond to bright light in the same way as species from other algal classes (Papageorgiou & Govindjee, 1968; Heaney, 1978).

A third and most unwelcome source of variation in fluorescence properties of the plankton may lie in the type of fluorometer used. Prézelin & Ley (1980) reported two distinct fluorescence rhythms, each measured with a different fluorometer, in a marine phytoplankton community dominated by diatoms. They observed that these diel fluorescence shifts were wavelength specific and they concluded that differences in bandpass characteristics, and perhaps excitation intensity, between their types of fluorometer might explain the separate rhythms. Fluorescence induction curves are often seen with narrow-bandpass, high-intensity excitation beam equipment, but they are not observed with Turner fluorometers for reasons probably associated with the excitation characteristics of this type of instrument (Vincent, 1981).

The present study compares in vivo measurements from a standard filter fluorometer and a high-performance scanning spectrofluorometer. Phytoplank-

ton from three common marine and freshwater groups were analysed to determine whether differences in room-temperature fluorescence could be ascribed to differences between algal classes. I first review the excitation and emission characteristics of green algae, diatoms and blue-green algae and then examine the way these change in response to DCMU, bright light, and light quality during growth.

METHODS

CULTURE PROCEDURES

All species of phytoplankton were grown in unialgal culture (non-axenic, but bacterial contamination always low) on either Woods Hole medium (MBL, Nicholls, 1973) or American Standard Medium (ASM, Gorham et al., 1964). The liquid cultures were incubated at 20°C under 80 μE m^{-2} s^{-1} cool white fluorescent light on a 16:8 light-dark cycle. All measurements were made on exponentially growing populations. The isolates selected and their sources are listed in Table 1.

TABLE I. Source of isolates used for fluorometric assays

Class	Species	Source
Cyanophyceae	*Microcystis aeruginosa* Kutz.	Oxidation pond, Orewa, New Zealand
	Anabaena variabilis Kutz.	Cambridge culture collection, B1403/4b
	Anabaena oscillarioides Bory	Waikato River, New Zealand
	Anabaena flos-aquae (Lyngb.) Bréb.	University of Texas culture collection (UTEX) 1444
	Nostoc muscorum Kutz.	Cambridge culture collection, B1453/12
	Nostoc calcicola Bréb.	Cambridge culture collection, B1453/1
Chlorophyceae	*Choricystis coccoides* Fott	Lake Tahoe, California-Nevada
	Stichococcus chodati (Bial) Heering	Lake Tahoe, California-Nevada
	Oocystis sp.	University of Texas culture collection (UTEX 80)
	Coelastrum microporum Naegeli	University of Texas culture collection (UTEX 281)
Bacillariophyceae	*Synedra ulna* (Nitzsch) Ehr.	Waikato River, New Zealand
	Nitzschia sp.	Waikato River, New Zealand
	Fragilaria sp.	Waikato River, New Zealand
	Navicula sp.	Waikato River, New Zealand

CHLOROPHYLL *a*

Samples for Chl *a* measurement were filtered through 25 mm glass fibre filters (Whatman grade GF/C) which were immediately plunged into boiling methanol and extracted for 30 s. The samples were allowed to stand for 5 min, stirred with a glass rod, inverted and then centrifuged clear. Levels of Chl *a* were measured in these extracts with an Aminco SPF 500 spectrofluorometer (excitation wavelength of 420 nm, emission wavelength 663 nm, bandpass 5 nm on each side) calibrated against commercial grade Chl *a* (spinach Chl *a*, Sigma Chemicals).

IN VIVO FLUORESCENCE

All samples for fluorescence measurements were incubated in dim light (<10 μE m^{-2} s^{-1}) prior to measurement. Fluorescence was recorded with either a Turner model 111 fluorometer (Turner Associates, 2524 Pulgas Ave, Palo Alto, California 94303) or an Aminco SPF 500 spectrofluorometer (American Instrument Company, 8030 Georgia Ave, Silver Spring, Maryland 20910). The Turner fluorometer was fitted with a blue filter (Corning CS5-60), an ex-

ternally operated shutter over the excitation beam and a red filter (Corning CS2-64) on the emission side. The transmission properties of each filter are shown in Fig. 1. The Aminco spectrofluorometer was usually operated at an excitation wavelength of 430 nm, an emission wavelength of 680 nm (bandpass of 5 nm on each side), and in the ratio mode which corrects for fluctuations in lamp intensity. For excitation or emission scans the spectrofluorometer was connected to an Aminco A85-7500 X-Y recorder operated at a wavelength scan speed of 90 nm min^{-1}.

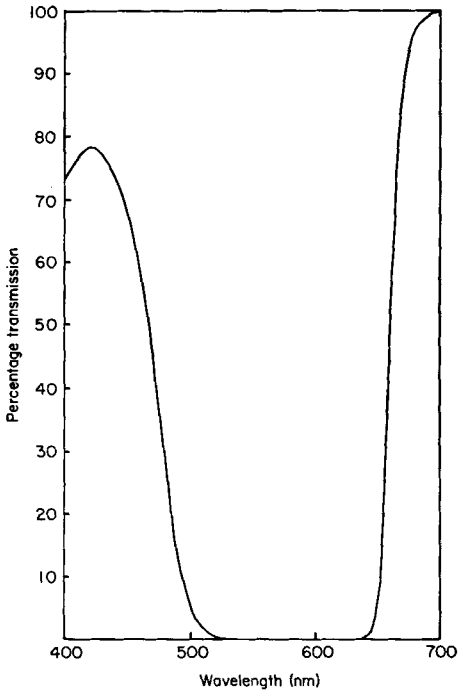

FIG. 1. Transmission properties of the standard Turner 111 filters. Lefthand curve—blue Corning filter CS5-60. Righthand curve—red Corning Filter CS2-64.

Samples were measured before (F_a) and after (F_b) addition of DCMU. Maximum fluorescence (F_b) was normally recorded within 60 s of addition of DCMU. These values were used to calculate the index "cellular fluorescence capacity" (CFC) defined by Vincent (1980) as $1-(F_a/F_b)$.

To examine the fluorometric response to bright light, samples were incubated in Spectrosil cuvettes in full sunshine (ca. 2000 $\mu E\ m^{-2}\ s^{-1}$) for 15 min. They were then immediately measured within the Aminco spectrofluorometer. At the end of each scan for these and other experiments, selected wavelengths were re-checked to ensure that there had been no major shifts in fluorescence over the course of measurement.

RESULTS

EXCITATION SPECTRA

The three algal classes differ markedly in their excitation characteristics [representative curves in Fig. 2(a) (b)]. The green algae demonstrate two distinct peaks in the blue region corresponding to in vivo absorption maxima for Chl *a* (430 nm) and Chl *b* (480 nm). Fluorescence yield is low in the region 500–600 nm,

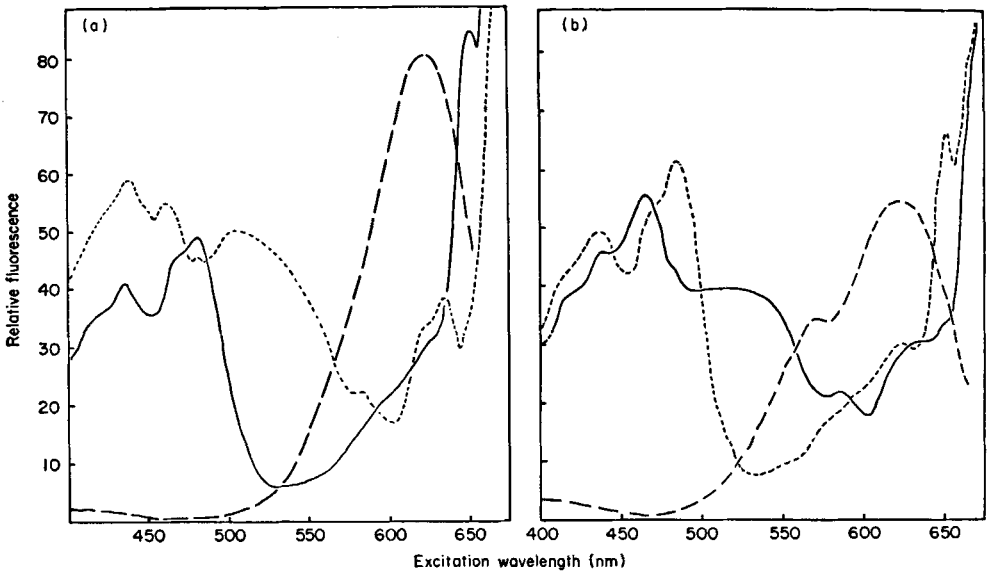

FIG. 2.(a) Excitation spectra for *Fragilaria* sp. (– – –), *Microcystis aeruginosa* (— —) and *Stichococcus chodati* (——). (b) Excitation spectra for *Oocystis* sp. (– – –), *Nostoc calcicola* (— —) and *Synedra ulna* (——). Emission measured at 680 nm on exponentially growing cultures.

but rapidly rises in the red waveband, with a further Chl *b* shoulder at 650 nm. For some diatoms [e.g. *Synedra*, Fig. 2(b)] the 430 nm Chl *a* peak is less pronounced than in the greens with a Chl *c* peak at 460 nm. A second Chl *c* peak is recorded at 640 nm, and there is a strong fluorescence response to light absorbed by fucoxanthin over the broad region 500–550 nm. A completely different excitation spectrum characterizes the blue-green algae. They have a very low fluorescence response to blue wavelengths, but show an increasing response above 500 nm to a peak at 635 nm associated with c-phycocyanin. Some blue-greens [e.g. *Nostoc calcicola*, Fig. 2(b)] have a secondary shoulder or peak at 570 nm associated with phycoerythin, the red-coloured phycobilin.

EMISSION SPECTRA

Both the green algae and diatoms have similar emission characteristics with a single peak at 680 nm associated with RCII Chl *a* [Fig. 3(a), (b)]. The blue-greens also demonstrate high fluorescence emission at this wavelength, but in addition they have a strong emission in the region 620–680 nm caused by direct fluorescence of phycocyanin [Fig. 3(c), (d)]. This phycocyanin fluorescence is greatly enhanced by excitation with wavelengths in the phycobilin absorption maximum [550 nm or greater, Fig. 3(c), (d)] whereas fluorescence by green algae and diatoms is markedly reduced by excitation at 550 nm [Fig. 3(a), (b)]. Those blue-green algal species with a high cellular phycoerythrin content demonstrate an additional fluorescence peak at ca. 570 nm when excited by 550 nm light [e.g. *Nostoc muscorum*, Fig. 3(d); *N. calcicola*, see below].

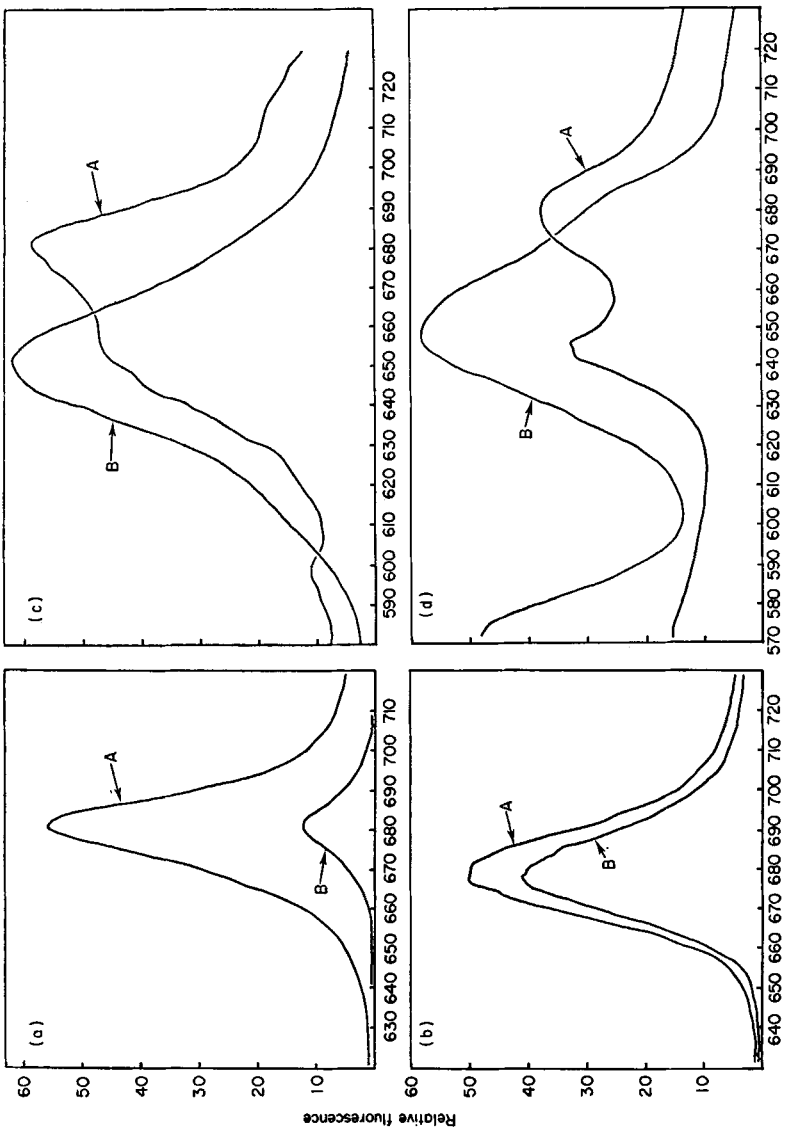

FIG. 3. Emission spectra for three algal classes, excitation at 430 nm (A) or 550 nm (B). (a) *Stichococcus chodati*, (b) *Synedra ulna*, (c) *Anabaena flos-aquae*; fluorescence scale for 550 nm scan is 11 × that for 430 nm; (d) *Nostoc muscorum*; fluorescence scale for 550 nm is 11 × that for 430 nm.

TABLE II. In vivo fluorescence per extractable Chl *a* before (F_a/Chl *a*) and after (F_b/Chl *a*) DCMU treatment. Each value is the mean±1 s.e. for triplicate assays from an exponential phase culture. Values are expressed as a percentage of F_a/Chl *a* for *Oocystis* sp. which gave the highest fluorescence yield in each machine. CV=coefficient of variation (s.d. as % of X)

		Turner 111		Aminco SPF-500	
Class	Species	F_a/Chl *a*	F_b/Chl *a*	F_a/Chl *a*	F_b/Chl *a*
Chlorophyceae	*Oocystis* sp.	100·0±1·2	196·7±4·7	100·0±2·4	218·5±6·3
	Choricystis coccoides	66·3±2·6	116·7±8·3	57·4±2·0	134·9±1·2
	Stichococcus chodati	60·7±0·7	110·3±2·4	63·4±1·0	149·1±1·1
	Coelastrum microporum	42·3±0·7	87·3±2·7	28·2±0·4	76·9±1·0
Bacillariophyceae	*Fragilaria* sp.	50·4±0·3	123·5±0·5	21·3±0·1	110·8±3·1
	Nitzschia sp.	33·1±2·4	57·6±2·1	36·9±7·7	61·5±6·7
	Synedra ulna	25·4±1·5	46·0±1·8	26·3±1·3	49·3±1·1
Cyanophyceae	*Microcystis aeruginosa*	12·5±0·1	22·5±0·1	6·5±0·4	14·4±0·9
	Anabaena flos-aquae	14·6±0·4	17·5±0·3	4·7±0·6	8·9±0·1
	Anabaena oscillarioides	13·0±0·2	19·3±0·6	12·1±0·2	27·5±0·5
	Anabaena variabilis	7·2±0·2	10·1±0·1	3·1±0·3	6·2±0·1
	Nostoc muscorum	7·3±0·2	8·7±0·2	2·6±0·1	8·7±0·2
Chlorophyceae	Mean (CV)	67·3 (35·7)	127·8 (37·3)	62·2 (47·4)	144·9 (40·2)
Bacillariophyceae	Mean (CV)	36·3 (35·3)	75·7 (55·2)	28·2 (28·1)	73·8 (44·1)
Cyanophyceae	Mean (CV)	10·9 (31·7)	15·6 (38·2)	5·8 (66·5)	12·3 (47·8)
Overall	Mean (CV)	36·1 (80·1)	68·0 (87·1)	36·8 (97·7)	80·9 (95·1)

FLUORESCENCE PER UNIT CHLOROPHYLL

The two fluorescence instruments were compared using fluorescence yield/Chl *a* characteristics of various algae (Table II). Three of the four green algae tested had significantly ($P<0{\cdot}05$) higher F_a/Chl *a* and F_b/Chl *a* values than either *Nitzschia* or *Synedra ulna* but there were no consistent differences between instruments for these two groups of species. A fourth green alga, *Coelastrum microporum*, demonstrated *R* values in each instrument that were closer to the mean for diatoms. A third diatom species, *Fragilaria*, had low F_a values, but F_b values comparable to the greens. Variation between diatom species was increased by DCMU addition, but for the chlorophyte species there was little change in the coefficient of variation.

Mean *R* values for the blue-green algae were significantly lower than either the greens (e.g. for F_b/Chl *a* in the Turner, $t=4{\cdot}66$, $P<0{\cdot}02$) or the diatoms (e.g. for F_b/Chl *a* in the Aminco, $t=4{\cdot}58$, $P<0{\cdot}02$). Unlike the green algae and diatoms, all cyanophyte fluorescence values in the Turner were significantly higher than in the Aminco; for a paired *t*-test comparison of F_a/Chl *a* means for each species $t=3{\cdot}55$, $P<0{\cdot}025$. The coefficient of variation was slightly increased by DCMU-treatment for both instruments.

NATURAL POPULATIONS

The large class-to-class variations in measured fluorescence properties for algae in culture suggest that in natural communities with major shifts in species composition over depth or horizontal space, fluorescence profiling may provide a misleading guide to the distribution of Chl *a* and biomass. Table III presents a set of data from Lake Vanda (Dry Valleys, Antarctica) where the euphotic zone—as measured by in situ ^{14}C-HCO_3 photofixation—extends from immediately under the 3·25 m permanent ice-cap to a depth of 58 m. Fluorescence F_b values (measured with a Turner 111) were correlated with extracted Chl *a*

TABLE III. Distribution of in vivo fluorescence (after DCMU treatment, F_b) and extractable Chl *a* in Lake Vanda, Antarctica, 13 December 1980

Depth (m)	Fluorescence (arbitrary units)	Chl *a* ($\mu g\ l^{-1}$)
3·25	9·0	0·07
5	6·2	0·06
10	5·2	0·04
15	5·0	0·04
20	5·8	0·05
25	7·2	0·10
30	6·9	0·09
35	5·1	0·05
40	6·8	0·06
45	7·2	0·07
50	14·5	0·18
55	7·5	0·23
57·5	10·5	0·62
60	6·0	0·04

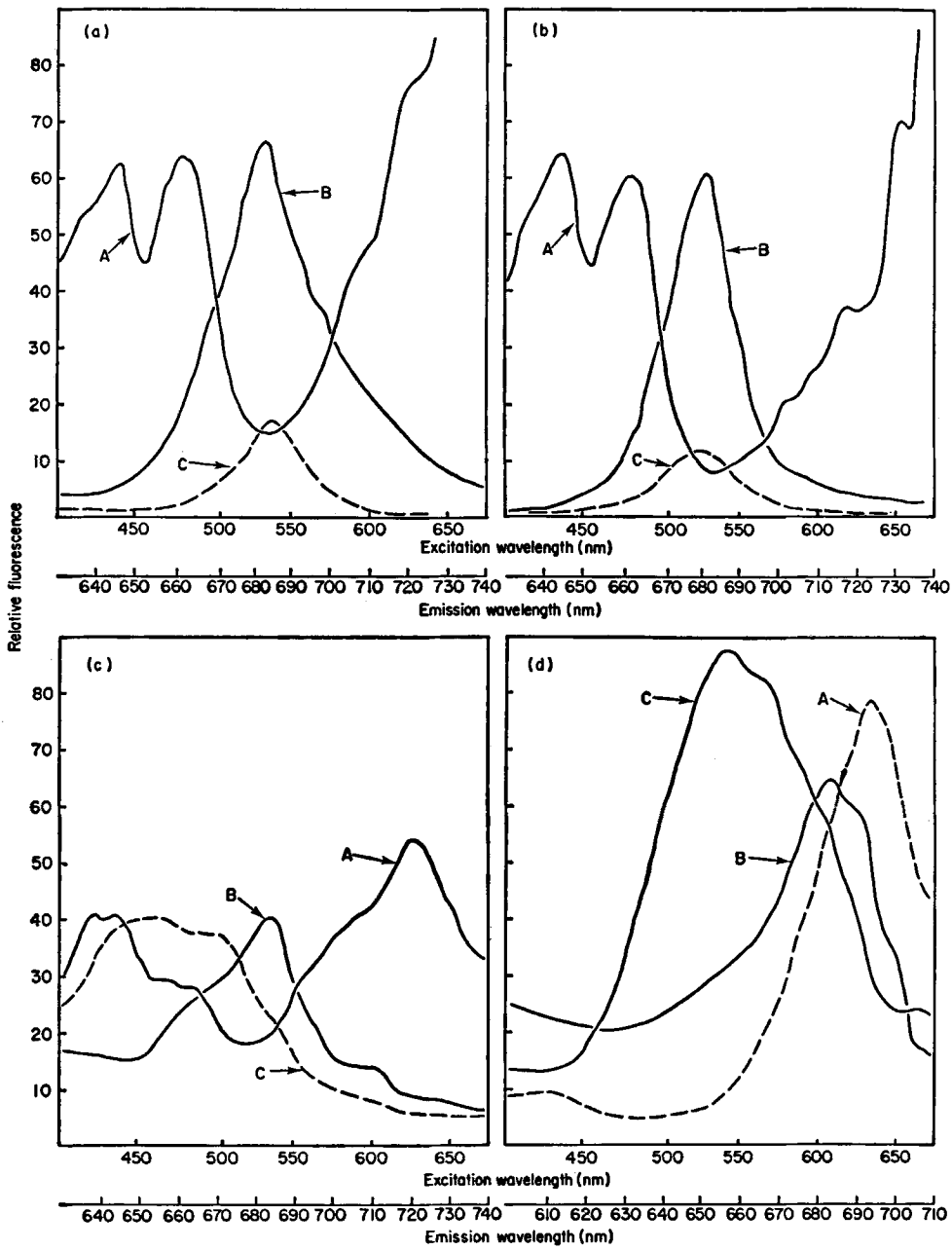

FIG. 4. Fluorescence scans for natural populations of phytoplankton. (a) Lake Rotokawa, (b) Lake Rotowhero, (c) Lake Rotoehu, (d) Lake Rotongaio, A=excitation curve, emission measured at 680 nm; B=emission curve for excitation at 430 nm; C=emission curve for excitation at 550 nm.

(r=0·548, P=0·025–0·05), but the linear fit was imprecise (r^2=0·30). The fluorescence profile failed to demonstrate the pronounced biomass maximum at 57·5 m where both Chl *a* and photosynthetic carbon fixation were maximal for the water column.

The anomalous fluorescence profile in Lake Vanda can be entirely accounted for by the changing species composition with depth. Immediately beneath the ice the community contained chrysophytes (e.g. *Ochromonas* sp.) and chlorophytes (e.g. *Chlorella*) and fluorescence was predictably high per extractable Chl *a* (F_b/Chl *a*=129). By 30 m the assemblage was a mixture of chlorophytes (e.g. *Chlamydomonas*) and blue-green algae (*Phormidium* sp.) and F_b/Chl *a* was 77. At the bottom of the euphotic zone (57·5 m) the biomass maximum was entirely composed of blue-green algae and F_b/Chl *a* was at its water column minimum of 17. Thus in Lake Vanda the standard excitation bandpass in the Turner became less appropriate with depth as the assemblage shifted towards blue-green algal dominance.

Although the distinctive excitation and emission characteristics of each algal class can lead to incorrect estimates of Chl *a* by standard in vivo fluorometry, they also lend themselves to a rapid assay for species composition of natural assemblages. Figure 4(a)–(d) presents excitation and emission scans for various natural populations. Blue-green algal dominance is particularly conspicuous from the emission scans at 430 versus 550 nm excitation wavelengths. An appropriate ratio which widely separates the cyanophytes from other algal classes is fluorescence at 650 nm (excitation at 550 nm) to fluorescence at 680 nm (excitation at 430 nm). This provides a rough measure of phycobilin fluorescence relative to RCII Chl *a*. For the tested assemblages dominated by blue-greens it exceeded 0·9, but was less than 0·05 for assemblages dominated by other algal classes (Table IV).

TABLE IV. Fluorescence ratios in natural populations of algae. F_1=ratio of emission at 650 nm (excite 550 nm) to 680 nm (excite 430 nm)

Lake	Species dominant	F_2
Rotongaio	*Anabaena oscillarioides*	1·417
Okaro	*Anabaena spiroides*	1·32
Rotoehu	*Anabaena* sp.	0·976
Rotokawa	*Euglena* sp.	0·015
Rotowhero	*Chlorella, Coccomyxa*	0·020

RESPONSE TO DCMU

In species from all algal classes there was a rapid rise in fluorescence to a maximum (F_{max}) upon addition of DCMU. The response time between DCMU addition and maximal emission was generally less than 60 s for all species tested, with the exception of *Oocystis* which did not achieve F_{max} in either machine until 5 min incubation. Much longer times have been recorded for other species (e.g. 30 min for certain flagellates, Prézelin & Ley, 1980).

The magnitude of the relative fluorescence increase was highly dependent on the type of fluorometer used (Table V). Although the Aminco and Turner pro-

duced similar results for the diatoms (for the two sets of CFC readings a paired t-test gave $t=1{\cdot}06$, $P>0{\cdot}2$), CFC values were significantly higher for all green ($t=5{\cdot}57$, $P<0{\cdot}025$) and blue-green ($t=5{\cdot}77$, $P<0{\cdot}005$) species measured in the Aminco. This difference was particularly striking for the blue-greens which in some instances more than doubled their CFC in the Aminco relative to the Turner.

TABLE V. DCMU-induced fluorescence for algae from three classes. Each CFC value is the mean±1 s.e. for triplicate assays from an exponential phase culture. CV=coefficient of variation (s.d. as % of X)

Class	Species	Turner 111	Aminco SPF-500
Chlorophyceae	*Oocystis* sp.	0·49±0·01	0·54±0·00
	Choricystis coccoides	0·44±0·02	0·57±0·01
	Stichococcus chodati	0·45±0·01	0·58±0·00
	Coelastrum microporum	0·51±0·01	0·63±0·01
Bacillariophyceae	*Fragilaria* sp.	0·59±0·00	0·81±0·00
	Nitzschia sp.	0·42±0·03	0·41±0·06
	Synedra ulna	0·45±0·02	0·47±0·02
Cyanophyceae	*Microcystis aeruginosa*	0·45±0·00	0·55±0·02
	Anabaena flos-aquae	0·16±0·01	0·51±0·02
	Anabaena oscillarioides	0·33±0·01	0·56±0·01
	Anabaena variabilis	0·29±0·02	0·51±0·05
	Nostoc muscorum	0·17±0·01	0·42±0·05
Chlorophyceae	Mean (CV)	0·47 (7·0)	0·58 (6·5)
Bacillariophyceae	Mean (CV)	0·49 (18·5)	0·56 (38·5)
Cyanophyceae	Mean (CV)	0·28 (43·0)	0·51 (10·8)
Overall	Mean (CV)	0·40 (33·5)	0·55 (19·3)

In part these differences between instruments result from the spectral response to DCMU. A wavelength-specific fluorescence response to DCMU is particularly marked for blue-green algae. Emission at 680 nm by cells excited with either 430 or 550 nm light is greatly enhanced by DCMU addition [Fig. 5(a), (b)]. However, at higher and lower wavelengths the effect of DCMU is much reduced (e.g. 640–680 nm emission) or negligible (>690 emission, <640 nm emission). For samples excited by 550 nm light DCMU changes the overall shape of the curve. Without DCMU the dominant peak is for phycocyanin fluorescence at 640 nm [plus for some species phycoerythrin fluorescence at 570 nm, Fig. 5(a)]. Upon DCMU treatment the increased RCII fluorescence broadens out the peak to 680 nm, but both phycoerythrin and phycocyanin fluorescence is little affected.

The high background phycobilin fluorescence that is unaffected by DCMU has important implications for CFC and other in vivo assays. This emission component is a major contributor to overall fluorescence, particularly in the Turner, which records emission wavelengths in excess of 640 nm (Fig. 1). Since much of this band is enhanced either slightly or not at all by DCMU, CFC values are low for blue-greens relative to other algae, especially when measured in the Turner.

DCMU-induced fluorescence values for blue-green algae are further reduced when these species are grown under conditions of abundant nitrogen (Table VI). Phycobilin levels rise relative to RCII Chl *a* (as measured by e.g. the ratio of

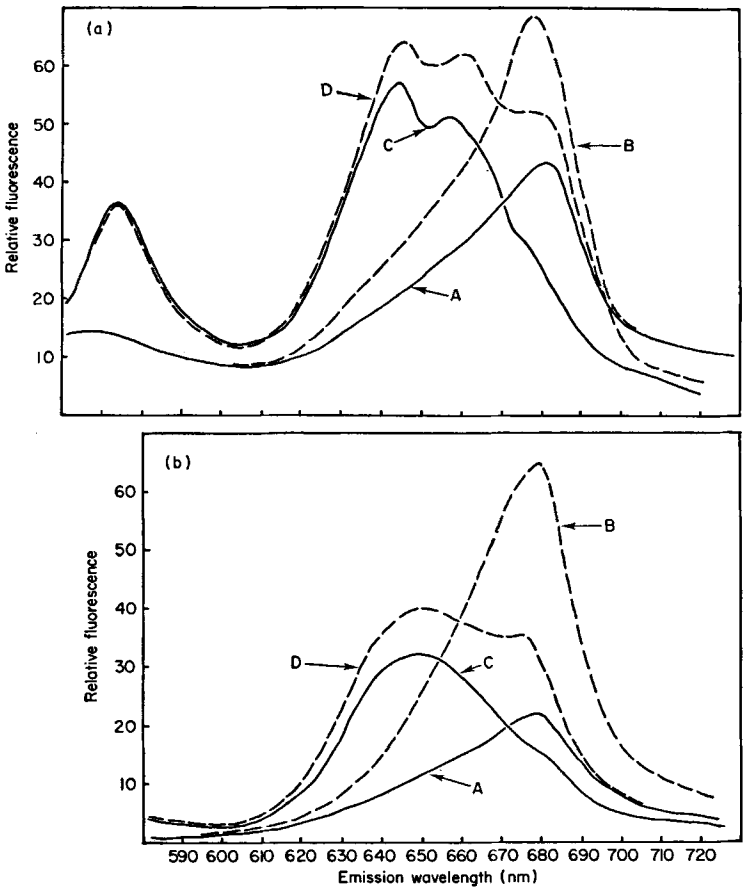

FIG. 5. Emission response to DCMU by blue-green algae. (a) *Nostoc calcicola* (b) *Anabaena oscillarioides*. A=excitation at 430 nm, without DCMU; B=excitation at 430 nm, with DCMU; C=excitation at 550 nm, without DCMU; D=excitation at 550 nm, with DCMU.

620 to 680 nm emission Table VI) and the DCMU-sensitive component of overall fluorescence is proportionately reduced.

Light quality during growth also affects the fluorescence characteristics of each algal class. Phytoplankton grown under light of the same quantum irradiance, but of different colours, had very different growth rates and, for some algal classes, different R and CFC values (Table VII). The green alga tested grew least under red light and these cells had the lowest CFC and highest R. The diatom *Nitzschia* demonstrated highest CFC and R values under green light but rates of chlorophyll increment were lowest in this treatment. The blue-green alga *Anabaena flos-aquae* grown under blue light had a conspicuously higher R and CFC, and chlorophyll increment rates were ca. 25% of those under white light of the same irradiance. Most rapid rates of chlorophyll growth were recorded under red light. The spectral characteristics of each class are also sub-

TABLE VI. Fluorescence properties of nitrogen-fixing blue-green algal growth with and without combined inorganic nitrogen (as NO_3). F620/680 is the ratio of emission at 620 nm to that at 680 nm for cells excited by 430 nm light measured in the Aminco. CFC values measured in the Turner

Species	Nitrogen source	620/680	CFC
Nostoc calcicola	N_2	0·13	0·17
	NO_3	0·25	0·09
Anabaena flos-aquae	N_2	0·05	0·34
	NO_3	0·10	0·16
Anabaena oscillarioides	N_2	0·15	0·39
	NO_3	0·17	0·33

TABLE VII. Effect of light quality on photosynthetic parameters. Algae were grown for 7 days under 20 μE m^{-2} s^{-1} white, blue, green or red light (16:8 light–dark cycle) at 20°C and then DCMU-induced fluorescence (CFC) and in vivo fluorescence per unit Chl *a* (*R*) measured with the Turner 111. *K* values express the specific rate of chlorophyll increment over the 7 days

	R	CFC	*K* (day^{-1})
Stichococcus chodati			
White	0·67	0·48	0·20
Red	0·76	0·40	0·09
Green	0·39	0·55	0·22
Blue	0·49	0·50	0·23
Nitzschia sp.			
White	0·24	0·46	0·19
Red	0·21	0·44	0·08
Green	0·47	0·51	0·10
Blue	0·38	0·40	0·16
Anabaena flos-aquae			
White	0·14	0·23	0·19
Red	0·11	0·23	0·20
Green	0·12	0·21	0·12
Blue	0·18	0·37	0·05

ject to changes in response to light quality (Table VIII). The ratio of Chl *b* to *a* in green algae appears least variable; more variable is the ratio of phycocyanin to Chl *a* in cyanophytes, which markedly increases under red irradiance. The ratio of fucoxanthin to Chl *a* in the diatom *Nitzschia* increased with decreasing wavelength towards blue light; the ratio of Chl *c* to *a* was also highest under blue light.

TABLE VIII. Effect of light quality during growth on fluorescence excitation spectra. Ratios are presented for the major peaks in each excitation scan: 435 nm —Chl *a*; 485 nm—Chl *b*; 465 nm—Chl *c*; 540 nm—fucoxanthin; 630 nm—phycocyanin

Light treatment	*Stichococcus chodati* (485/435)	*Nitzschia* sp. (540/435)	(465/435)	*Anabaena flos-aquae* (630/435)
White	1·23	0·78	0·98	54·17
Red	1·10	0·88	1·02	65·45
Green	1·19	0·96	1·04	46·50
Blue	1·18	1·03	1·10	45·45

TABLE IX. Effect of light quality on blue-green algal fluorescence. Cultures were grown for 8 days under different colour filters. CFC—DCMU-induced fluorescence; F_1–F_{620}/F_{680}, both excited at 430 nm; F_2–F_{650} excite at 550 nm; F_{680} excite at 430 nm

Species	Light regime	CFC (Aminco)	CFC (Turner)	F_1	F_2
Anabaena oscillarioides	Blue	0·571	0·417	0·18	0·97
	Green	0·566	0·345	0·24	1·27
	Red	0·549	0·220	0·21	1·18
Nostoc muscorum	Blue	0·679	0·397	0·16	1·31
	Green	0·536	0·306	0·21	1·87
	Red	0·533	0·241	0·22	1·90
Microcystis aeruginosa	Blue	0·585	0·375	0·23	3·64
	Green	0·545	0·304	0·24	5·81
	Red	0·567	0·222	0·27	6·00

This experiment was repeated with three isolates of blue-green algae under the three light-quality regimes. For these isolates CFC values were significantly less in the red incubation than in the blue incubation (Table IX, for paired t-test comparisons of red versus blue in the Turner $t=11{\cdot}88$, $P<0{\cdot}01$). A much reduced and non-significant ($t=1{\cdot}48$, $P>0{\cdot}2$) shift in CFC was recorded with the Aminco. These effects were probably the result of significantly increased ratios of phycocyanin to Chl *a* under the red light regime (for ratios F_1 in Table X, $t=4{\cdot}91$, $P<0{\cdot}05$).

TABLE X. Fluorescence emission after 15 min exposure to full sunlight (S15) before and after treatment with DCMU. All values are expressed as a percentage of the fluorescence by samples adapted to dim light (F_o). Excitation was at 430 nm, bandpass 5 nm on each side

Species	Treatment	Emission wavelength (nm) 620	640	660	680	700
Chlorophyceae						
Stichococcus chodati	S15	—	40	35	42	43
	DCMU	—	150	176	173	170
	DCMU+S15	—	40	53	54	56
Bacillariophyceae						
Synedra ulna	S15	—	80	74	67	77
	DCMU	—	192	250	309	203
	DCMU+S15	—	96	83	94	74
Cyanophyceae						
Microcystis aeruginosa	S15	100	90	83	75	82
	DCMU	100	122	166	179	140
	DCMU+S15	100	92	82	71	78
Anabaena oscillarioides	S15	100	106	114	108	101
	DCMU	150	184	236	218	242
	DCMU+S15	117	119	127	132	153

RESPONSE TO BRIGHT LIGHT

For many, but not all of the species tested, a 15 min incubation in full sunlight markedly reduced in vivo fluorescence. Unlike DCMU treatment, however, with bright light treatment there were no obvious differential effects on the emission spectrum.

Table X compares the effects of three treatments—15 min bright light.

DCMU, and DCMU followed by 15 min bright light—on fluorescence yield from representatives of each algal class. Greatest fluorescence depression by bright light was recorded for the green algae—up to a three-fold reduction in F_a for *Stichococcus chodati*. DCMU-treated cells had similarly increased fluorescence emissions at all wavelengths, and bright light reduced fluorescence by these cells to values only a little above the bright light treatment without DCMU.

The diatoms tested were less sensitive to bright light. Fluorescence was enhanced by DCMU with a much stronger response by the 660 and 680 nm emissions. When these DCMU-treated cells were exposed to bright light, their fluorescence was reduced at all wavelengths to values a little above the unpoisoned bright light treated samples.

The blue-greens behaved very differently. The fluorescence of some species (e.g. *Microcystis aeruginosa*) was slightly reduced by bright-light at all wavelengths. As noted above there was a strong wavelength specific enhancement by DCMU, and fluorescence by these treated cells was reduced to intensities similar to those in samples treated with bright light only.

A second and more typical response by the blue-greens (e.g. *A. oscillarioides*) was a small increase in fluorescence induced by bright light in the RCII Chl *a* band 660–680 nm. DCMU-induced fluorescence, however, was almost eliminated by the full-sunlight incubation. Similar results for each cyanophyte species were recorded at excitation wavelengths of both 430 and 550 nm.

DISCUSSION

For four algal species in pure culture Slovacek & Hannan (1977) noted that "fluorescence becomes maximal and a constant function of cellular chlorophyll *a*" upon addition of DCMU. This constancy does not appear to hold over a wide array of freshwater phytoplankton species. The 12 isolates examined here showed very different F_a/Chl *a* ratios both within and between algal classes. Upon addition of DCMU this variability was either little affected (chlorophytes and cyanophytes) or conspicuously increased (diatoms). However, DCMU-treatment remains potentially useful as a means of increasing the absolute fluorescence readings, thereby reducing the lower limit of detectable chlorophyll, and possibly as a means of reducing fluorescence variations due to changing photochemical capacities. In the present study all cultures were in exponential growth phase and such variations might be expected to be minimal. Even so, a considerable range of CFC values was recorded (e.g. 0·41–0·81 for the diatoms), perhaps reflecting genetic differences in the maximum attainable P_{max} (maximum photosynthesis per unit chlorophyll). A chlorococcalean species has been isolated from Lake Tahoe, for example, which appears genetically incapable of adjusting to high light intensities; for this species both P_{max} and CFC values always remain low, irrespective of light conditions during growth (Vincent, 1982).

The highly fluorescent phycobilin pigments of blue-green algae dictate their distinctive spectral characteristics. As a consequence of this red light emission from chromoproteins, both *R* and CFC values for cyanophytes are particularly low relative to other species—the minimum F_b/Chl *a* in the Aminco was for *N. muscorum*, and this was one fiftieth the equivalent ratio for *Oocystis* sp. Phyco-

bilin pigmentation is responsive to light quality during growth and to nitrogen source, and the resultant shifts in phycobilin to RCII fluorescence affects excitation and emission spectra, F_a, F_b and CFC.

The distinct excitation and emission spectra of blue-greens pose problems for vertical or horizontal profiling through heterogeneous communities of algae. However, these distinctive properties may lend themselves to a rapid in vivo assay for phytoplankton species composition in general (e.g. Yentsch & Yentsch, 1979) and specifically for blue-green algal dominance. For example, the ratio of fluorescence emission at 620 to that at 680 nm is greater than 0·25 for cyanophytes but is very low (<0·01) for other algae. The best ratio for maximum separation of blue-greens from other components is that of phycocyanin fluorescence to RCII fluorescence. Appropriate wavelength settings appear to be 550 nm excitation, 650 nm emission and 430 nm excitation, 680 nm emission, respectively.

The wide array of blue-greens tested appeared not to react to bright light in the same way as diatoms and green algae. After incubation under this regime fluorescence yield increased in several species, perhaps reflecting a direct depression of photochemical capacity induced by bright light, and hence of excitation energy draw-off for non-cyclic electron flow. In cyanophyte-dominated waters the typical R/R_{max} curves reported by Vincent (1979) will not occur and cannot be used to measure light history of the plankton in surface waters.

For the three groups tested the measured fluorescence characteristics varied markedly with the type of fluorometer used, and between and within algal classes. Thus interpretation of the increasingly popular fluorescence assays in natural waters requires knowledge of both the species composition of the assemblage under test and the type of instrument selected to make the measurement. The large-scale variations in excitation and emission spectra, even in the presence of DCMU, underscore the need for frequent calibration of in vivo fluorescence measurements against extractable Chl *a*. Changes in DCMU-induced fluorescence (CFC) cannot be attributed simply to shifts in photosynthetic capacity, but must also be viewed in relation to changes in the relative proportion of individual species within the community. On the more positive side, these pronounced differences lend themselves to rapid fluorometric tests for dominance by specific algal groups. Such assays may prove particularly useful in high-intensity sampling programmes which examine spatial and temporal variation in phytoplankton community structure, and more specifically, experimental investigations of the factors controlling blue-green algal dominance.

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