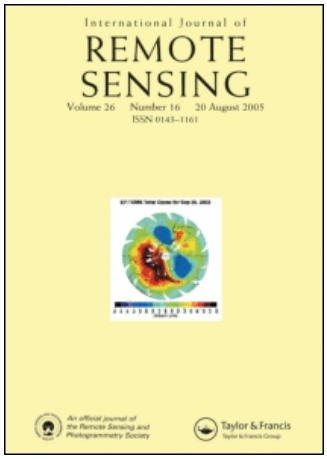

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Characterization of marine macroalgae by fluorescence signatures

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Abstract. An investigation of 20 marine macroalgae suggests that fluorescence emission could be used to distinguish macroalgal classes. Narrow waveband light was used to excite groups of accessory pigments in brown, red and green macroalgae and fluorescence emission measured at 685 nm, the wavelength at which chlorophyll *a* has its maximum fluorescence emission. Fluorescence induced by 540 and 465 nm wavelengths gave 540:465 fluorescence excitation ratios of 0·59 ±0·07 for seven brown algae, 3·67±0·56 for seven red algae and 0·28 ±0·07 for six green algae. These results suggest that fluorescence excitation signatures are relatively uniform within phylogenetic classes but differ substantially between classes. When *Ectocarpus siliculosus* (Dillw) Lyng (Phaeophyceae) was cultured over a large range of light and nitrate regimes, the 540:465 fluorescence excitation ratio showed little variation. Somewhat higher fluorescence excitation ratios were correlated with tissue C:N ratios of 6·1–15. The ability to distinguish macroalgal divisions on the basis of fluorescence emission may allow the type and abundance of subtidal macroalgae to be characterized by existing laser-induced fluorescence methodology from low-flying aircraft.

1. Introduction

The fluorescence characteristics of macroalgae may be used to distinguish phylogenetic classes. Algal pigments have long been employed to classify algal groups (Govindjee and Braun 1974). Each macroalgal class has different accessory pigments which may be excited by different wavelengths of light. Much of this light energy may be passed to chlorophyll *a*, causing it to fluoresce at a maximum emission wavelength of approximately 685 nm. The differential chlorophyll *a* fluorescence resulting from the excitation of different groups of accessory pigments has been used to characterize and quantify phytoplankton in discrete samples (Yentsch and Yentsch 1979).

More recently, efforts have been made to use remote sensing to examine the fluorescence characteristics of phytoplankton. A remote sensing laser-induced fluorescence system has recently been developed by the National Aeronautic and Space Administration and has been applied to the examination of natural phytoplankton populations (Mumola *et al.* 1975, Esaias 1980, Hoge and Swift 1981). In this system, a low-flying aircraft discharges light from a multiband laser, exciting algal antennae pigments which causes them to fluoresce. The fluorescence is detected telescopically by a photosensor and digitized on magnetic tape. The use of this instrument to detect and characterize subtidal macroalgal populations could provide an important synoptic perspective on macroalgal communities.

Owing to its emergence, the areal distribution of intertidal vegetation may be approached via several remote sensing and ground-level techniques (Cameron 1950, Baardseth 1970, Vadas and Manzer 1971, Jamison 1972, Michanek, 1975, Austin and Adams 1978, Topinka *et al.* 1981). With the exception of shallow water populations, however, subtidal macroflora do not easily lend themselves to quantification with

existing remote sensing methodologies (Kelly and Conrad 1969, Austin and Adams 1978, Schwenke 1972).

The major goals of this investigation were to examine the fluorescence excitation spectra of macroalgae; to determine the feasibility of using fluorescence signatures to distinguish macroalgal divisions; and to determine the stability of these signatures under extremes of light intensity and nitrogen availability, both of which are known to influence macroalgal growth and pigment composition.

2. Methods

Fluorescence signatures were determined for field-collected plant material. Healthy non-reproductive material was gathered from West Boothbay Harbor, Maine during the spring of 1981. Whole young plants were kept at 15°C in an illuminated tank for up to 24 h prior to analysis. Immediately before use, plants were wiped with paper tissue and rinsed in filtered seawater to reduce epiflora. For thalliod plants, 1 cm^2 sections of mid-blade areas were cut near distal ends. With filamentous plants, only distal ends were taken for analysis. A total of 7 Rhodophyta, 7 Phaeophyta, and 6 Chlorophyta were examined (table 1).

Experimental material was placed in a plastic support, on glass fibre filters that were moistened to prevent the drying of plants. The support was placed in a Beckman scanning spectrofluorometer equipped with a xenon light source, a high sensitivity Hamamatsu photomultiplier tube and an X-Y recorder. The excitation spectrum was

Table 1. Macroalgal fluorescence at 685 nm.

Algae		n	540/465 excitation ratio x	S.E.
Rhodophyceae	*Dumontia incrassata* (O.F. Muller) Lamouroux	6	4·13	0·43
	Polysiphonia urecolata (Lightfoot) Greville	6	3·02	0·12
	Ceramium deslongchampsii Chauvin	6	3·42	0·10
	Cystoclonium purpureum (Hudson) Batters	6	3·01	0·20
	Polysiphonia lanosa (Linnaeus) Tandy	6	3·57	0·15
	Chondrus crispus Stackhouse	6	4·19	0·33
	Palmaria palmata (Linnaeus) Greville	6	4·43	0·45
Phaeophyceae	*Laminaria agardhii* Kjellman	6	0·65	0·03
	Fucus spiralis Linnaeus	6	0·63	0·04
	Fucus vesiculosus Linnaeus	6	0·53	0·02
	Elachista fucicola (Velley) Areschoug	6	0·69	0·04
	Ascophyllum nodosum Linnaeus	6	0·52	0·03
	Fucus disticus Linnaeus subsp. *edentatus* (Dela Pylaie)	6	0·57	0·05
	Fucus disticus Linnaeus subsp. *evanescens* (C. Agardh)	6	0·54	0·01
Chlorophyceae	*Monostroma fuscum* (Postels et Ruprecht) Wittrock	6	0·32	0·03
	Chaetomorpha melagonium (Weber et Mohr) Kutzing	6	0·23	0·00
	Ulothrix flacca (Dillwiyn) Thuret in LeJolis	6	0·27	0·03
	Spongomorpha arcta (Dillwiyn) Kutzing	6	0·22	0·01
	Enteromorpha sp.	6	0·26	0·02
	Ulva lactuca Linnaeus	6	0·41	0·03

350–650 nm and the resulting fluorescence emission was recorded at 685 nm, the maximum fluorescence wavelength for chlorophyll *a*. Relative fluorescence was measured at excitation wavelengths 540 and 465 nm.

Further investigation involved the culture of *Ectocarpus siliculosus* (Phaeophyta) under varying regimes of light intensity and nitrogen availability to examine the stability of the 540:465 fluorescence excitation ratio. *Ectocarpus siliculosus* (Dillw) Lyng (UTEX LB2008) was obtained from the University of Texas Culture Collection. Plants were grown in batch culture at 15°C in constant light. The growth medium was f/50 (Guillard and Ryther, 1962) with a salinity of 31‰, as determined by a refractometer, but with nitrate concentrations of 0, 2, 8 or 30 μM NO^{3-}. Light was provided by cool-white fluorescent lamps, screened with neutral density filters to give growth intensities of 1, 6, 26 and 73 μEin $m^{-2} s^{-1}$. Light intensities were measured with a 4π detector (Lamba quantum meter) over PAR (400–700 nm). There were thus 16 treatments of light and nitrate concentration.

In an attempt to maintain constant NO^{3-} concentrations in the cultures, approximately half of the medium was drained and replaced with fresh medium every 4 days. After 40 days, cultures were collected onto glass fibre filters, and fluorescence determinations made as previously described. Total carbon and nitrogen analysis of the algal tissue was determined on dried material with a Hewlett Packard 185b CHN analyser.

A long-term batch culture experiment was also conducted to examine the influence of severe nitrogen deficiency on fluorescence signatures. Plants were grown at high light (73 μEin $m^{-2} s^{-1}$) with no added nitrogen and examined over a 12-week period at approximately 2-week intervals. The culture methodology and analytical determinations were as described above.

3. Results

Representative fluorescence excitation spectra for the three classes of algae are shown in figure 1. When fluorescence was measured at 685 nm, pronounced spectral differences were found between *Fucus vesiculosus*—a brown alga, *Dumontia incrassata* —a red alga, and *Spongomorpha arcta*—a green alga. A comparison of these excitation spectra demonstrated that *Fucus* had high excitation activity at both 540 and 465 nm, *Dumontia* had high activity near 540 nm but low activity at 465 nm, while *Spongomorpha* exhibited low fluorescence excitation at 540 nm but high activity at 465 nm.

The difference in fluorescence with excitation at 540 and 465 nm is expressed as a ratio, 540:465. The measured values of the 540:465 ratio for 20 species of macroalgae are given in table 1. There was little variation of the ratio between individuals of the same species. The mean ratios for the three classes studied are given in table 2. For the twenty species examined, there was no overlap in the ranges of 540:465 excitation ratio between the three divisions.

Variation in the 540:465 fluorescence excitation ratio for *Ectocarpus siliculosus* culture under varying regimes of light intensity and nitrate availability is shown in figure 2. There was no significant difference between treatments ($p = 0.05$).

Some association was also found between fluorescence excitation and carbon and nitrogen content of algal tissue. The fluorescence excitation ratio is plotted against C:N ratio in figure 3. The light and nitrogen gradient culture produced a wide variation in nitrogen status. Although the wide data spread did not allow the relationship between C:N ratios and fluorescence to be defined clearly, it appears that C:N ratios below approximately 15 result in higher fluorescence excitation ratios, while C:N ratios above 15 do not appear to influence fluorescence. Even at lowest

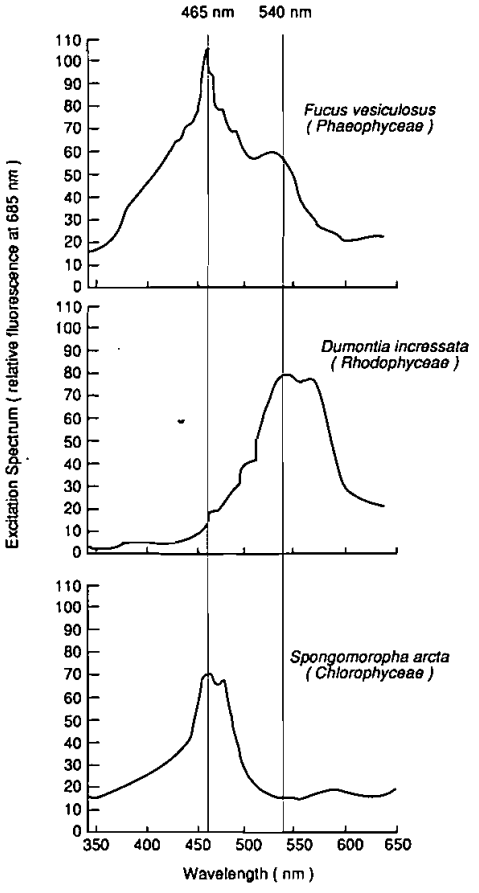

Figure 1. Representative fluorescence excitation spectra for three classes of macroalgae.

C:N ratios, however, the fluorescence ratios were not elevated to an extreme degree and remained within the range expected for brown algae (see table 2).

The long term batch culture of *Ectocarpus* at high light and low nitrogen, which was designed to examine the effect of severe nitrogen deficiency on the fluorescence excitation ratio, produced similar results. The 540:465 fluorescence ratios remained in the range of 0·4–0·6, despite pronounced alteration of nitrogen status.

Table 2. Fluorescence of major macroalgal divisions at 685 nm.

		540/465 nm excitation ratio		
Algae	*n*	*x*	S.E.	Range
Rhodophyceae	7	3·67	0·21	3·04–4·34
Phaeophyceae	7	0·59	0·03	0·52–0·69
Chlorophyceae	6	0·28	0·03	0·22–0·41

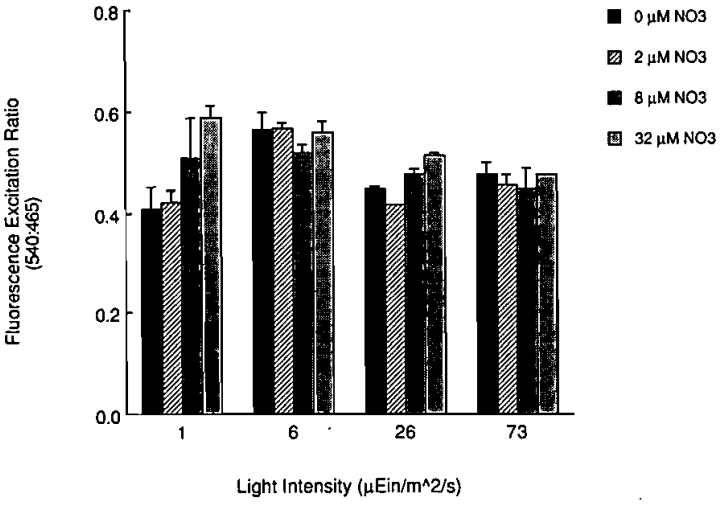

Figure 2. Fluorescence excitation ratios for *Ectocarpus siliculosus* cultured under varying regimes of light intensity and nitrogen availability.

4. Discussion

Members of the Phaeophyta, Rhodophyta and Chlorophyta had distinctive fluorescence excitation spectra when emission was measured at 685 nm, the chlorophyll α peak. The fluorescence excitation spectra of these macroalgae were very similar to those found for corresponding groups of phytoplankton (Jarrett *et al.* 1973, Yentsch and Yentsch 1979). The differences can be attributed to differences in accessory pigments between these classes. The relatively high fluorescence stimulated in the brown algae by excitation at 540 nm corresponds with absorption by fucoxanthin, and at 465 nm with absorption by chlorophyll *c* and fucoxanthin. High

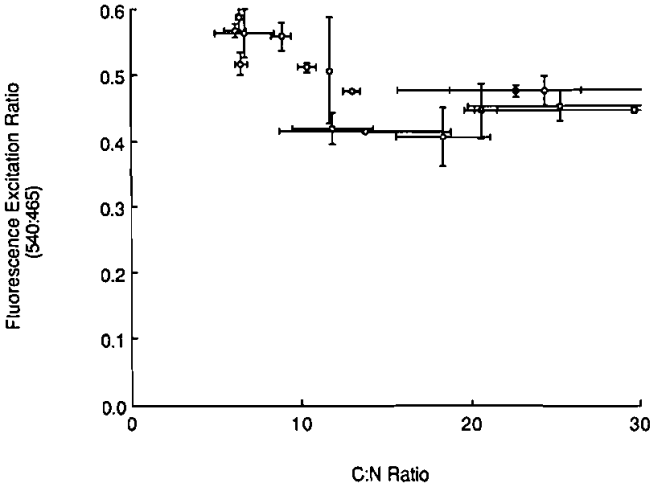

Figure 3. Fluorescence excitation ratios of *Ectocarpus siliculosus* as a function of tissue C:N ratios.

fluorescence in response to excitation at 530–570 nm in the red algae was probably due to phycobilins, principally phycocyanin. The high excitation peak with 465 nm in the green algae is associated with chlorophyll *b* and carotenoids.

By expressing the ratio of fluorescence observed at 540 nm to that observed at 465 nm it was possible to describe major differences between classes based on their excitation spectra. Each species had an excitation signature which varied very little between individuals. While absolute fluorescence intensities varied with the part of the plant used and/or positioning on the cuvette, the 540:465 ratio remained relatively constant. The stability of this fluorescence ratio is an important factor determining its utility in remote sensing applications. It was anticipated that many species within each class would possess similar excitation ratios. The ratios of all plants surveyed remained within narrow limits in each class. Perhaps more importantly, the range of each phylogenetic group differed significantly with no overlap between ranges.

Physiological state has long been known to affect pigment concentration in algae (see the review of Yentsch 1980). Alteration of fluorescence intensity often results from extreme variations in light intensity or nutrient availabilities (Flemer 1969, Berman 1972, Kiefer 1973 a, b, Blasco 1973, Schimura and Fujita 1975, Heaney 1978). Such extremes did not, however, drastically alter the 540:465 fluorescence excitation ratio of *Ectocarpus* in this investigation. Fluorescence ratios under all culture conditions remained between 0·41 and 0·59, which was typical for the Phaeophyta. The conservative nature of the 540-465 fluorescence ratio over a wide range of culturing conditions suggests that under normal environmental extremes the fluorescence excitation ratio for *Ectocarpus* may remain within a narrow range. Where fluorescence changes were observed, these changes were associated with low C:N ratios in the algal tissue, suggestive of luxury consumption and storage of nitrogen. The low variation of fluorescence ratios evident at high C:N ratios suggests that the degree of nitrogen deficiency attained here did not influence this fluorescence ratio. The results of this investigation indicate that the Phaeophyta, Rhodophyta and Chlorophyta exhibit distinctive 540:465 fluorescence excitation ratios which are probably stable under extremes of light and nitrogen availability. This suggests that major macroalgal divisions may be distinguished and quantified by existing laser-induced fluorescence techniques from low-flying aircraft.

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