



Characterisation of estuarine intertidal macroalgae by laser-induced fluorescence



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ABSTRACT

The article reports the application of laser-induced fluorescence (LIF) for the assessment of macroalgae communities of estuarine intertidal areas. The method was applied for the characterisation of fifteen intertidal macroalgae species of the Tagus estuary, Portugal, and adjacent coastal area. Three bands characterised the LIF spectra of red macroalgae with emission maxima in the ranges 577–583 nm, 621–642 nm and 705–731 nm. Green and brown macroalgae showed one emission maximum in the red region (687–690 nm) and/or one in the far-red region (726–732 nm). Characteristics of LIF emission spectra were determined by differences in the main fluorescing pigments: phycoerythrin, phycocyanin and chlorophyll *a* (Chl *a*). In the green and brown macroalgae groups, the relative significance of the two emission maxima seems to be related to the thickness of the photosynthetic layer. In thick macroalgae, like *Codium tomentosum* or *Fucus vesiculosus*, the contribution of the far-red emission fluorescence peak was more significant, most probably due to re-absorption of the emitted red Chl *a* fluorescence within the dense photosynthetic layer. Similarly, an increase in the number of layers of the thin-blade green macroalgae *Ulva rigida* caused a shift to longer wavelengths of the red emission maximum and the development of a fluorescence peak at the far-red region. Water loss from *Ulva*'s algal tissue also led to a decrease in the red/far-red Chl fluorescence ratio (F_{685}/F_{735}), indicating an increase in the density of chloroplasts in the shrinking macroalgal tissue during low tide exposure.

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1. Introduction

Macroalgae (seaweeds) communities are some of the most productive and widespread marine plant communities in the world (Dawes, 1998). In estuarine intertidal ecosystems, macroalgae grow in an exceptionally dynamic environment experiencing stressful environmental conditions with respect to nutrient availability, irradiance, salinity or temperature (Davison and Pearson, 1996). Furthermore, macroalgae of the upper intertidal zone are subjected to frequent and prolonged emersion periods, and most species tolerate extreme water loss. Remote sensing techniques are extremely important tools to assay macroalgal community structure due to the large spatio-temporal variability of these communities and the hardly accessible conditions of the estuarine

intertidal habitat.

Laser-induced fluorescence (LIF) has been extensively used to infer taxonomic structure of phytoplankton communities (e.g. Babichenko et al., 1993; Barbini et al., 1998), to study changes in intertidal microphytobenthos biomass (Vieira et al., 2011), and to assess different stresses in higher plants (e.g. Subhash and Mohanan, 1997; Lavrov et al., 2012). Environmental monitoring related to the presence of oil slicks and chemical pollutants has also been addressed successfully using LIF technology (Utkin et al., 2002). However, the application of LIF techniques to assay macroalgal communities is scarce and, to the best of our knowledge, only two studies used fluorescence spectral signatures to discriminate between macroalgae groups (Topinka et al., 1990; Kieleck et al., 2001). Topinka et al. (1990) measured fluorescence emission at 685 nm and used fluorescence excitation ratios to distinguish between green, brown and red macroalgae. Kieleck et al. (2001) described a LIF imaging system using two excitation wavelengths to identify various macroalgal groups in the subtidal environment.

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The main objective of this work was to evaluate emission fluorescence spectra obtained with a 532 nm pulsed Nd:YAG laser for the three main groups of macroalgae (red, green and brown) present in the intertidal areas of the Tagus estuary, Portugal, and adjacent coastal zone. Obtained fluorescence spectra were related to the different photosynthetic antennae complexes (chlorophyll vs phycobiliproteins) and to the structure of the photosynthetic tissue of the different macroalgae. Furthermore, we investigated the effect of water loss occurring during low tide exposure in the LIF spectral signature of the macroalgae *Ulva rigida*. To our knowledge, this is the first application of LIF to the study of intertidal estuarine macroalgae.

2. Material and methods

2.1. Sampling

Fifteen macroalgae species (see Table 1) were collected at the Tagus Estuary and adjacent coastal area on several occasions between December 2012 and April 2013 (Fig. 1). The Tagus estuary is located on the west coast of Portugal (38.75°N, 09.15°W) and has a broad shallow bay (10 m mean depth) covering an area of about 320 km². The estuary has extensive intertidal flats covering an area of approximately 100 km² (Brotas and Catarino, 1995), ranging from very fine muddy sediment to sand and oyster banks. Three sites in the Tagus Estuary were sampled: Alcochete, Banco do Cavalo and Ponta do Destrói. Alcochete sampling site is located at a small fishing harbour characterised by a muddy substrate and the latter two sites are oyster banks. Rocky and sandy shores characterise Tagus estuary adjacent coastal area. Two adjacent coastal area sites were sampled: Cabo Raso and Avencas. The first is a rocky zone, while the latter is a sandy beach with intertidal rock pools. Sampling was always carried out during low tide periods, and macroalgae species were collected at different sediment and substrata types. After sampling, all collected species were taken immediately to the laboratory, stored at *in situ* temperature, and frequently hydrated with collected water from each site. All experiments and measurements were carried out on the sampling day or the following day. Laser-induced fluorescence (LIF) spectra were determined for all macroalgae species in healthy and non-reproductive material. Each macroalgae LIF spectral signature was obtained from triplicate measurements in three different individuals per species.

2.2. Laser-induced fluorescence (LIF)

The LIF detector was developed on the basis of a commercial palm-size spectrometer Ocean Optics USB4000 and a frequency-doubled Nd:YAG laser, manufactured by Quantel (model Ultra 532-30-20-H-N). The laser provides 30 mJ, 7 ns radiation pulses at 532 nm, with a pulse repetition rate up to 20 Hz and spot diameter at the sample location of ~3 mm (about 1 m from the laser output aperture). The pulse energy is sufficiently low to prevent disturbing effects that could result from reaction centre closure and excitation annihilation (Rosema et al., 1998). A part of the fluorescence emission, reflected back to the instrument, is collected by a light gathering optical train based on low-cost components provided by Thorlabs, assembled within the Ø1-in lens-mounting tube SM1L30.

The train comprises an optical filter and a telescopic system, positioned and centred using three retaining rings SM1RR. The long-pass optical filter FEL0550, with the cut-off wavelength of 550 nm and transmission of ~80% in the region of 650–730 nm, protects the spectrometer from strong retroreflected laser light. The SMA fiber optics collimation package F810SMA-635 is installed immediately after the filter. Usually being intended for collimating a laser beam propagating from the tip of an SMA-connectorized fiber, here it operates in the reciprocal mode, playing a role of a principal light gathering element, which collects the fluorescence radiation over a Ø21 mm input pupil and transmits it into an optical fiber. This fiber transports the optical signal to the spectrometer optical bench, the f/4 asymmetrical crossed Czerny-Turner configuration with the diffraction grating Ocean Optics #9, providing nearly uniform efficiency at wavelengths from 450 to 800 nm.

The spectrometer was tuned and calibrated with the help of the mercury argon calibration source CAL 2000, demonstrating the sensitivity of ~55 photons per count and resolution of 0.19 nm per channel in the spectral range of interest (650–730 nm). Spectrometer synchronization with the laser pulse enabled the signal to be measured with minimum permissible exposure, which reduces the influence of the background radiation, allowing carrying out measurements in the daylight conditions.

2.3. Effects of the thickness of the photosynthetic layer on macroalgae laser-induced fluorescence spectra

The green macroalgae *U. rigida* morphology consists of a very thin single sheet-like blade that can be flat or ruffled. *U. rigida* LIF

Table 1

Classification of studied algal species and corresponding sampling site, date of collection and laser-induced fluorescence emission maxima.

Algal group/species	Site	Date (month)	Fluorescence λ_{\max} (nm)	
Chlorophyta (green algae)				
1 <i>Bryopsis plumosa</i>	Raso	Apr	–	690
2 <i>Codium tomentosum</i>	Raso	Apr	–	726
3 <i>Ulva intestinalis</i>	Avencas/B. Cavalo	Mar	–	687
4 <i>Ulva rigida</i>	P. Destrói	Dec	–	688
Phaeophyta (brown algae)				
5 <i>Colpomenia peregrina</i>	Raso	Apr	–	688
6 <i>Fucus vesiculosus</i>	Alcochete	Dec	–	726
Rhodophyta (red algae)				
7 <i>Calliblepharis jubata</i>	Raso	Apr	582	642
8 <i>Corallina officinalis</i>	Raso	Apr	583	639
9 <i>Cryptopleura ramosa</i>	Raso/P. Destrói	Apr	580	636
10 <i>Gelidium corneum</i>	P. Destrói	Feb	582	626
11 <i>Gracilaria gracilis</i>	B. Cavalo	Feb	582	640
12 <i>Lithophyllum incrustans</i>	Raso	Apr	583	640
13 <i>Lithophyllum tortuosum</i>	Raso	Apr	581	642
14 <i>Osmundea pinnatifida</i>	Raso	Apr	577	625
15 <i>Plocamium cartilagineum</i>	Raso	Apr	582	621

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