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Evaluation of a portable chlorophyll optical meter to estimate chlorophyll concentration in the green seaweed *Ulva ohnoi*

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Abstract

In situ measurement of chlorophyll concentration, with an optical meter, in the green seaweed *Ulva ohnoi* is presented to estimate the absolute chlorophyll content. The MC-100 optical meter (Apogee Instruments) was used during the study; the optical meter gives chlorophyll content in relative units as Chlorophyll Content Index units (CCI). Absolute chlorophyll content, in μmol m^{-2}, was determined by extraction with dimethylformamide, and the chlorophyll concentration was measured with a spectrophotometer. Equations to convert CCI from optical meter to absolute chlorophyll content were presented for total chlorophyll, chlorophyll *a*, and chlorophyll *b*. Highly linear relationships (r^2 above 0.9, $n = 130$) were found for total chlorophyll, chlorophyll *a*, and chlorophyll *b*. In situ non-destructive estimation of *U. ohnoi* chlorophyll content using the MC-100 portable chlorophyll optical meter was considered convenient, fast, and accurate.

Keywords *Ulva ohnoi* · Chlorophyta · Chlorophyll content · Optical meter

Introduction

Pigments are colorful chemical compounds that reflect light of a specific wavelength and absorb other wavelengths. Chlorophyll *a* and *b* are green photosynthetic pigments found in plants, algae, and cyanobacteria, which absorb light wavelengths in the visible spectrum. Chlorophyll is vital for photosynthesis, since it traps the light energy from the sun. This light energy is used to combine carbon dioxide and water into sugars in the process of photosynthesis.

Many biotic and abiotic stresses cause leaf bleaching, which results from a loss of chlorophyll. Then, chlorophyll content in plants and algae are an important parameter in any research. These studies require the in vitro pigment extraction with organic solvents, and spectrophotometric readings at different wavelengths; finally, the absolute concentration of chlorophyll is determined by different model equations (Porra et al. 1989; Wellburn 1994). The most used organic solvents are acetone 80%, chloroform, diethyl-ether, dimethyl formamide DMF, dimethyl sulfoxide DMSO, and methanol. Excepting DMF and DMSO, the other solvents require grinding of the tissue for complete extraction. This extractive method is destructive and laborious and includes hazardous compounds and also requires trained personnel.

Non-destructive, in situ, optical techniques, which give a relative value of chlorophyll per unit of area of a leaf, have been used in terrestrial crops with high correlation coefficients between the optical (in situ) and the extractive method (in vitro) chlorophyll relationship (Parry et al. 2014). Among others, this optical technique has been used with paper birch *Betula papyrifera* (Richardson et al. 2002), sugar maple *Acer saccharum* (Cate and Perkins 2003; Berg and Perkins 2004), coastal wetland plant species (Biber 2007), and tropical tree species (Gonçalves et al. 2008). It has been concluded that there is no significant effect of environment on this optical/absolute chlorophyll relationship (Parry et al. 2014). A similar study has not been done on common seaweeds.

Since values output from the optical meters are a relative unit (a dimensionless numerical value which is related to the chlorophyll content), species-specific regression models for the values obtained from the optical method and absolute chlorophyll content from the extractive method are needed.

The aim of the present work is to evaluate the utility of a portable chlorophyll optical meter for the non-destructive determination of chlorophyll concentration, and to present the equations to convert relative units to absolute chlorophyll

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content in the seaweed *Ulva ohnoi*. In order to evaluate the effectiveness of the optical meter, the seaweed chlorophyll content per unit area was measured using standard extraction techniques (in vitro) and compared with the optical value (in situ).

Material and methods

Ulva ohnoi, used in the present study, was collected at the Ebro Delta from the bioremediation ponds of an aquaculture facility in Sant Carles de la Ràpita, Spain (latitude, 40.62 N; longitude, 0.66 E). This species was genetically identified by DNA extraction and PCR amplification of the chloroplast rbcl gene following the protocol described in Hayden et al. (2003) with the primers used by Manhart (1994). It was maintained at the Aquaculture Laboratories of the Universitat Politècnica de Catalunya in Castelldefels (Spain) for more than 3 years, in indoor tanks fed by water coming from a *Solea senegalensis* recirculation aquaculture system (RAS) equipped with biological and mechanical filter, water temperature control, aeration, and oxygen supply. *Ulva ohnoi* were cultivated in three circular tanks (28 cm water depth, 64 cm diameter) with bottom aeration to tumble the seaweeds, and tanks were illuminated by LED light sources (with photon flux densities on the water surface ranging from 162 to 886 μmol photons m^{-2} s^{-1}). Twice a week seaweed was weighed, and the weight of algae in the tank was adjusted to the previous stocking density (ranging from 0.4 to 2.4 kg FW m^{-2}, FW: fresh weight). The stocking densities and the photon flux densities on the water surface were combined in order to obtain seaweed fronds with a wider range of chlorophyll content.

Chlorophyll determination

Over the course of the study, 130 fronds of seaweed were used to determine chlorophyll content by both methods (optical and extractive) between January 2020 and May 2020.

First, seaweed fronds were randomly taken from the tanks, rinsed with distilled water to remove epiphytes and salt, and blotted dry on absorbent paper. From each frond, three measurements were made with the optical meter (in situ measurements). Once these measurements were completed, three circular disks were punched from each frond using a cork borer. The total procedure takes less than 3 min per frond. In the three punched disks, the chlorophyll extraction (in vitro measurements) was conducted.

In situ measurements with an optical meter

The chlorophyll content measurements in seaweeds were carried with the MC-100 Chlorophyll Concentration Meter (Apogee Instruments Inc. Logan, UT, USA) (Fig. 1). The optical meter measures the ratio of radiation transmittance from two different wavelengths (653 and 931 nm) and outputs chlorophyll concentration as Chlorophyll Content Index units (CCI), which is calculated internally from the transmittance ratio measurement (MC-100 user manual). In the MC-100, the measurement area is 63.6 mm^2 (9 mm diameter), the resolution 0.1 CCIs units, and the sample acquisition time less than 3 s.

Fig. 1 The MC-100 optical meter (left) and the example of CCI determination with a terrestrial plant (right). Both images from MC-100 user manual (with permission Apogee Instruments)

Once fronds were rinsed and blot dry on absorbent paper, three non-overlapping chlorophyll content measurements were made with the optical meter in each seaweed frond. These three data yielded an average CCI for each frond sample. Neither seaweed tissue nor pigments were harmed by sampling with the optical meter.

In vitro measurements (chlorophyll extraction with DMF)

Chlorophyll concentration can have significant spatial variation, and it is necessary to extract the samples from the same location where the optical measurement was made (Parry et al. 2014). In our case, the three disks used for the in vitro measurements were punched from each frond in a point as close as possible to the measuring point with the optical meter.

Immediately following the CCI measurements, three disks were punched from each frond using a 5-cork borer with an area of 100 mm^2. The three disks were placed in a vial containing 5 mL of DMF (dimethylformamide), and maintained at 4 °C and in darkness for 72 h. Finally, the chlorophyll concentration of the extracts was measured with a spectrophotometer (Shimadzu UV-1800 UV).

The concentrations of chlorophyll *a*, chlorophyll *b*, and chlorophyll *a* + *b* (total chlorophyll) were calculated using the equations described in Porra et al. (1989):

$$\text{Chl } a = 12.00\, A_{663.8} - 3.11\, A_{646.8}$$

$$\text{Chl } b = 20.78\, A_{646.8} - 4.88\, A_{663.8}$$

$$\text{Chl } a + b = 17.67\, A_{664.8} + 7.12\, A_{663.8}$$

where *A* is the absorbance measurement at the indicated wavelength.

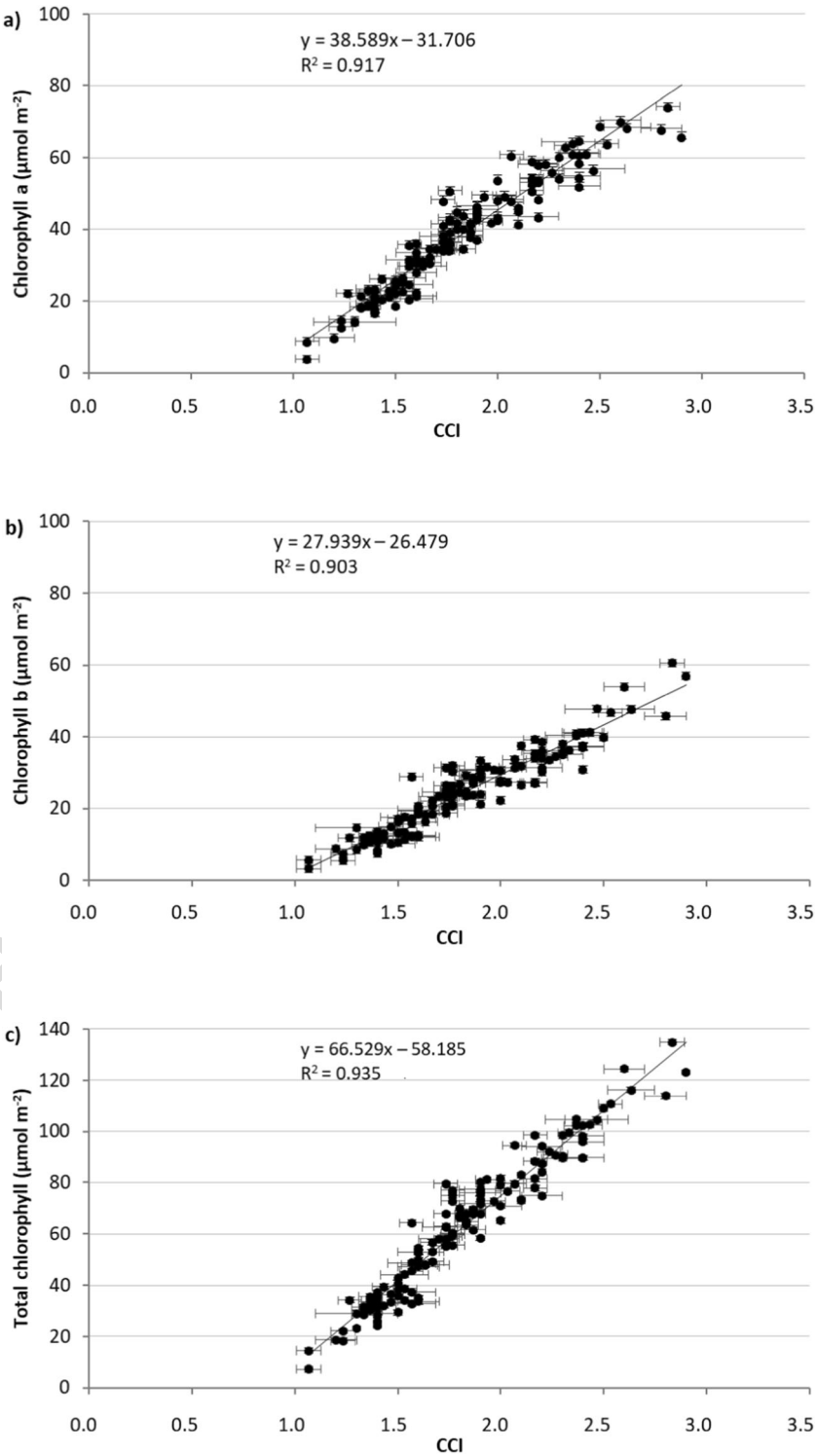

Fig. 2 Relationship between chlorophyll content index CCI measured with MC-100 optical meter ($n = 3$) and **a** Chlorophyll *a*, **b** Chlorophyll *b*, and **c** total chlorophyll concentration (µmol m^{-2}) determined by extraction with DMF. Error bars are the standard deviation of the CCI measured of each frond ($n = 3$)

Data analysis

Regression analysis was used to evaluate the strength of the relationship between chlorophyll determined by extraction with DMF (in vitro measurements) and by the optical meter (in situ measurements). Linear regression of CCI as the independent variable against chlorophyll content (µmol m^{-2}) as the dependant variable was performed. In the regression analysis, the average of the three optical measurements (CCI, $n = 3$) was used.

Results and discussion

From 130 samples, the total chlorophyll content ranged from 7.20 to 134.82 μmol m^{-2}. These values are within the published range for *Ulva* spp. (Fortes and Lüning 1980; Henley et al. 1991; Pérez-Lloréns et al. 1996; Vergara et al. 1998). Samples presented a narrow range of chlorophyll in CCI units: 1.07 to 2.90 from averaged measurements, and standard deviation ranged from 0.00 to 0.20 (n = 3). Two fronds, with an evident bleaching (completely white), were removed from the analysis since the three individual CCI measurement was 1.0, which is the optical meter minimal display measurement.

The range of values for the CCI in the present work was lower than those found in different terrestrial plants, which can reach a CCI value above 70 CCI units (Cate and Perkins 2003; Berg and Perkins 2004; Gonçalves et al. 2008; Padilla et al. 2018). The lower CCI values are a consequence of the fact that *Ulva* sp. contain less chlorophyll than terrestrial plants.

The relationship between total chlorophyll content determined in vitro and the readings with the optical meter (in situ measurement) was well explained by a linear equation (Fig. 2) with an r^2 of 0.935. Chlorophyll *a* and chlorophyll *b* determined by extraction also presented high r^2 (0.918 and 0.901, respectively).

Conclusions

The high positive linear correlation observed between the chlorophyll measured from the MC-100 optical meter and the chlorophyll concentration extracted by traditional methods suggests that the optical meter is suitable for a fast and non-destructive estimation of relative chlorophyll content in the green seaweed *Ulva ohnoi*.

The optical method allows making rapid chlorophyll determinations without harming the seaweed fronds and reduces the solvent waste stream which is environmentally responsible. *Ulva*, which is commonly used in environmental studies, is the seaweed employed in the present study, but the method can be adapted for the chlorophyll determination in other green seaweeds with an appropriate equation for each specie and solvent used in the extraction.

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