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Short communication

The impact of different preservation methods on macroalgal tissue light absorptance values: A case study with *Ulva australis*

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ABSTRACT

Calculations of absolute ETR require accurate measurements of plant tissue Photosynthetic Active Radiation light absorption (A_L(PAR)). The default leaf-specific A_L(PAR) value of 0.84 estimated from and applicable to nearly all higher plants does not apply for marine macroalgae which are phylogenetically, structurally and chemically very different from higher plants and quite variable among themselves. Consequently, to date there is no default A_L(PAR) value for all macroalgae, and hence A_L(PAR) values always need to be recalculated from live specimens on every new study. This study compared A_L(PAR) values from thalli of *Ulva australis* (Chlorophyta) preserved under different methods to test whether usable A_L(PAR) data can be obtained from samples other than live ones. Light absorption measurements were made using an Integrating Sphere attached to a spectrophotometer. No statistically significant differences in A_L(PAR) values were observed among live and pressed material whether recently collected (=4 days old) or stored more than seventy years but there were significant differences among live, frozen, formalin-preserved and bleached material. No significant differences in absorptance at wavelength of 675 nm were observed between fresh, frozen and formalin preserved material. A_L(PAR) = 0.74 appeared to be an appropriate A_L(PAR) default value for this species. This study demonstrated that accurate A_L(PAR) can be obtained from some pressed herbarium preserved species while some commonly used methods of field tissue preservation in macroalgal photochemical studies fails to do so.

1. Introduction

The development of chlorophyll *a* (chl *a*) fluorescence measurements via pulse amplitude modulation (PAM) fluorometers for assessing photosynthetic activity, provided a non-destructive, fast and popular method of measuring a range of photosynthetic parameters (Schreiber et al., 1994; Runcie & Durako, 2004). One of the most important photosynthesis parameters calculated from chl *a* fluorescence data is the electron transport rate (ETR; Genty et al., 1989): $ETR = (\Delta F/Fm') \times A_L(PAR) \times PAR \times 0.5$.

Therefore calculations of absolute ETR require accurate measurements of A_L(PAR) if data are to be compared between plant species and distinct experiments (Schreiber et al., 2011). Furthermore, A_L(PAR) depends on both the spectral transmittance and reflectance characteristics of the photosynthetic and non-photosynthetic tissue of the plant (Runcie & Durako, 2004) and, will influence maximum quantum yield, growth and ultimately macroalgal primary productivity (Beach et al., 2006).

ETR is often and traditionally calculated using the conventional averaged A_L(PAR) value of 0.84 (Björkmann & Demming, 1987). Expected A_L(PAR) variation from a range of distinct vascular plants, is quite small, varying between 0.83 and 0.87 (Knapp & Carter, 1998). But the range of variation affecting A_L(PAR) in macroalgae far surpasses those observed in higher plants (Beer & Axelsson, 2004; Beach et al., 2006), what renders the 0.84 A_L(PAR) default unrepresentative for macroalgal studies.

In the absence of a single standard macroalgal A_L(PAR) value, A_L(PAR) always needs to be calculated *de novo*, from live specimens, on every new eco-physiological study where absolute ETR values are analyzed. However, precise A_L(PAR) determinations from

Abbreviations: SHSA, State Herbarium of South Australia; A_L(PAR), the light absorptance values at the fraction of incident PAR absorbed by a plant.

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live specimens using the integrated sphere (IS) method (Elterman, 1970; Baurle et al., 2004), are often neglected in macroalgal studies. The use of IS can be fiddly, requires proper instrumentation and minimum laboratorial infrastructure, making field measurements impractical and new measurements time consuming. Researchers end up using relative ETR, rETR (=ETR calculations without A_L(PAR)), less conventional methods (e.g. Longstaff et *al.*, 2002), or preserving and storing specimens (e.g. Enríquez et al., 1994).

The ability to preserve and store the original light absorption properties of macroalgal tissue is a very appealing idea so accurate A_L(PAR) measurements can be performed later in the laboratory, using proper IS setup. However no studies have specifically tested how different preservation methods, including those most commonly used in macroalgal research, influence estimates of light absorption properties in marine macroalgae. The 'rule of thumb' has been never to assess A_L(PAR) from non-live material assuming that results would either not be the same as those obtained from live material nor usable, but without providing a rigorous empirical test for such assumptions. Consequently, information whether preserved specimens can be used to assess A_L(PAR) and which methods are the most appropriate for that purpose represent valuable information to help researchers calculate accurate values of A_L(PAR) in the absence of live specimens.

The overall aim of this study was to test how different preservation methods impact A_L(PAR) using *Ulva australis*. More specifically, the objectives of this study were to: (1) establish how close A_L(PAR) for *U. australis* is to the higher plant standard 0.84 value; (2) produce a range of A_L(PAR) standard values for *U. australis* preserved under several frequently used storage conditions; (3) compare A_L(PAR) values between live and herbarium pressed tissues to test whether A_L(PAR) values decay over time and; (4) assess differences between the fractions of incident PAR absorbed by photosynthetic (pigments) versus non-photosynthetic tissue components in *U. australis*.

2. Materials and methods

2.1. Light absorption measurements

Light absorption measurements were made via a IC2 Integrating Sphere that has a 1.6 cm open port diameter (StellarNet Inc., Tampa, FL, USA) attached via UV-VIS-SR StellarNet fiber optic cables to a Black Comet CXR-SR-25 StellarNet spectrophotometer set with a 25 μm light slit, and a light source composed of a collimated beam from a dual deuterium and halogen light source that covers the 200–1100 nm range (SL5 StellarNet light source with Hamamatsu Photonics K.K. lamps). As suggested by Ramus (1978), absorptance is defined here as: 1 − reflectance + transmittance (absorptance + reflectance + transmittance = 100%) and were calculated by SpetracWiz software (StellarNet) as follows: absorptance = 100 − (% reflectance + % transmittance). All spectra were corrected for electrical noise using dark and light references. The light reference was 50 mm diameter white reflectance standard made of Halon with a reflectance of >97% between 300 and 1700 nm (model RS-50, StellarNet).

Algae-specific light absorption spectrum was measured between 400 and 700 nm at 1.0 nm resolution and determined by averaging all values (=301 data points). In addition, light absorption values at 675 nm (a_{675}), which are strongly correlated with PAR absorption (Enríquez et al., 1994) and where interference by other pigments is minimal on chl *a* absorption peak (Agustí and Phillips, 1992), were also analysed. Light absorption at 675 nm allows comparisons among plants with diverse pigments and among other studies (Agustí and Phillips, 1992; Enríquez et al., 1994).

2.2. Species used

The use of *U. australis* was chosen based on its manageable size, simple morphology, high abundance and widespread distribution in temperate Australia (Womersley, 1984). Furthermore, *Ulva* species are known for their ecological and economic significance (see Li et al., 2013; Ryu et al., 2013; Vijayaraghavan & Joshi, 2013). *Ulva* specimens are also often used as model organisms in PAM PSII fluorescence, photochemistry and macroalgal productivity studies (e.g. Beer et al., 2000; Longstaff et al., 2002).

2.3. Specimen treatments

2.3.1. Wet treatments

Live, formalin-fixed, frozen and bleached preservation methods were termed 'wet storage treatments'. A single healthy population was collected in West Beach, Adelaide, South Australia, on October 10th 2012. Live specimens had their light absorption values taken within 4 h of collection. The others treatments were obtained from live specimens. Frozen specimens treatment was placed damp in plastic bags, than frozen in a −20 °C freezer. Prior to A_L(PAR) determination, frozen specimens were thawed at room temperature. Formalin-fixed specimens treatment was placed in non-buffered 4% formalin/seawater solution. Bleached specimens treatment was soaked in a 3% (v/v) sodium hypochlorite/seawater solution until all visible photosynthetic pigments were eliminated, then rinsed with clean seawater prior to measurements. Light absorption spectra from bleached thalli were obtained to decouple the light absorption components produced by photosynthetic pigments and non-pigment cell constituents. The analyses were conducted in three to six replicates per treatment (Table 1). Specimens were placed on top of the RS-50 reflectance standard (StellarNet) prior to measurement.

2.3.2. Dry treatment

Light absorption data were observed from historical herbarium pressed specimens (herein '*pressed*') dating back to seven decades ago (from 1940 to 2010), totaling seven pressed treatment levels. Historical collections were obtained from specimens deposited at SHSA. Each decade had a minimum of four samples, a maximum of six, totaling 33 samples. The maximum number of samples per decade was a result of the total available number of specimens in the SHSA. For the absorptance measurements, pressed specimens were not removed from the herbarium paper. The putative influence of the paper on light absorption was controlled by obtaining background and zero readings from the herbarium paper and using that information to pre-set up the spectrophotometer. Preliminary tests measuring dried specimens either directly pressed onto their own herbarium paper or placing onto RS-50 reflectance standard disk showed no significant differences in A_L(PAR) (data not shown).

2.4. Statistical analysis

A one-way multivariate analysis of variance by permutation using the entire 400–700 nm spectrum as samples, and thallus treatment as variables was implemented. A p level less than 0.05 was considered statistically significant. Absorption spectra shape analyses were performed using the software PRIMER 6.1.10 with PERMANOVA add-on (Anderson, 2001), pairwise Euclidean dissimilarities, untransformed data and 999 permutations. Two distinct PERMANOVA tests were conducted. One analysis tested for statistical significance between differences of A_L(PAR) data among all seven *dry treatment* levels and the *live specimen* treatment.