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Anatomical Differentiation and Photosynthetic Adaptation in Brown Algae

David J. Garbary and Kwang Young Kim[1]*

Department of Biology, St. Francis Xavier University, Antigonish, Nova Scotia, B2G 2W5, Canada
[1]Department of Oceanography, Chonnam National University, Gwangju 500-757, Korea

The photosynthetic parameters of dark- adapted minimum fluorescence (F_o) and maximum quantum yield of charge separation in PSII (F_v/F_m) were measured in transverse sections of eight species of marine Phaeophyceae (species of Laminariales, Fucales, Desmarestiales, Chordariales) using pulse amplified modulation (PAM) fluorometry. Within each transverse section fluorescence was measured in three regions corresponding to outer cortical and meristoderm cells, inner cortical cells and innermost medullary cells. Minimum fluorescence declined from 19-74% (mean of 39%) from outermost to innermost cells. Maximum quantum yield varied from 0.51-0.59 in outermost cell layers and this was reduced to 0.23-0.40 in innermost cell layers, with an average reduction of 50%. Despite the reduction Fo in medullary cells (inner), medullas of all species showed maximum quantum yields consistent with a photosynthetic role in carbon fixation. These results show that medullary cells of complex brown algae have more than a role in structure, storage or transport, and may also provide an important role in carbon fixation.

Key Words: anatomy, brown algae, medullas, PAM, Phaeophyceae, photosynthesis

INTRODUCTION

Brown algae (Phaeophyceae) are often morphologically complex with considerable anatomical differentiation based on parenchymatous or syntagmatic development (Fritsch 1945; Christensen 1980; Bold and Wynne 1985). The level of differentiation approaches that in vascular plants in that tissue level differentiation occurs in many groups, but especially in members of Fucales and Laminariales and their relatives. It has long been known that different morphological parts of the same thallus can have very different photosynthetic properties (King and Schramm 1976; Arnold and Manley 1985; Kilar *et al.* 1989). Thus even the same tissue in different part of the same thallus can be cytologically differentiated (e.g., Davies *et al.* 1973; Fagerberg *et al.* 1979; Clayton and Ashburner 1990), suggesting differentiation of function associated with morphology and development. Specialized cells are morphologically differentiated, and numerous cytological and ultrastructural studies suggest functional differentiation of cells associated with photosynthesis, storage of photosynthate, transport, reproduction etc. (e.g., Oates 1988; Lobban and Harrison 1994).

Virtually all cells within complex brown algae have plastids. The outer tissues (meristoderm and cortex) have cells that are typically heavily pigmented with well-developed chloroplasts and perform a primary role in photosynthesis. Deeper into the thallus there are fewer chloroplasts and these tend to have different arrangements and be morphologically differentiated (e.g., McCully 1966; Schmitz and Srivastava 1974, 1975, 1976; Rüffer *et al.* 1978; Katsaros and Galatis 1985; Clayton and Ashburner 1990). The extent to which these modified chloroplasts have different photosynthetic properties remains to be determined. Thus, given the cytological differentiation, it is of interest to evaluate if the photosynthetic process in these interior tissues is qualitatively or quantitatively different from those on the outside.

Here we use Microscopy-PAM (pulse amplitude modulation) fluorometry of chlorophyll *a* fluorescence to evaluate photosynthetic physiology within different tissues of the same thallus part. The use of fluorescence analysis for this general application is well established

*Corresponding author (kykim@chonnam.ac.kr)

Table 1. List of brown algal species, their taxonomic affiliation and plant portions examined

Species	Order	Portion of Plant Used
Alaria esculenta (L.) Grev.	Laminariales	Midrib of blade
Ascophyllum nodosum (L.) Le Jol.	Fucales	Main axis of frond
Chordaria flagelliformis (O.F. Müll.) C.Agardh	Chordariales	Main axis of frond
Desmarestia aculeata (L.) J.V. Lamour.	Desmarestiales	Main axis of frond
Fucus distichus ssp. *distichus* L.	Fucales	Main axis of frond
Fucus vesiculosus L.	Fucales	Main axis of frond
Laminaria digitata (Huds.) J.V. Lamour.	Laminariales	Mid portion of blade
Laminaria saccharina (L.) J.V. Lamour.	Laminariales	Mid portion of blade

(Schreiber *et al.* 1994); however, technology to allow for fluorescence on spatial scales of single cells or tissues is more recent and carried out using Microscopy-PAM (Walz GmbH) (Schreiber 1998). Here we examine photosynthetic processes in adjacent cell and tissue types in a variety of parenchymatous and syntagmatic brown algae.

MATERIALS AND METHODS

Specimen collection and processing

All brown algal species (Table 1) were collected at low tide from Tor Bay, Guysborough Co, Nova Scotia in August 2004. Thalli were removed from rocks or collected from the drift, placed in plastic bags and transported on ice. Upon return to the laboratory (2 h) thalli were placed in seawater (18-20°C) with circulation and aeration under fluorescent lights (10 μmol photons $m^{-2} \cdot sec^{-1}$) until processing (within 48 h).

Portions of plants were removed with a razor blade and hand sections were cut (ca. 0.5 to 1 mm thick) and placed in a well slide in seawater. Sections were cut from the blades of kelp species (the midrib in *Alaria esculenta*), or from mature, undamaged axes of mature fronds. A transverse section from each three plants was used for each species.

Fluorescence Measurement

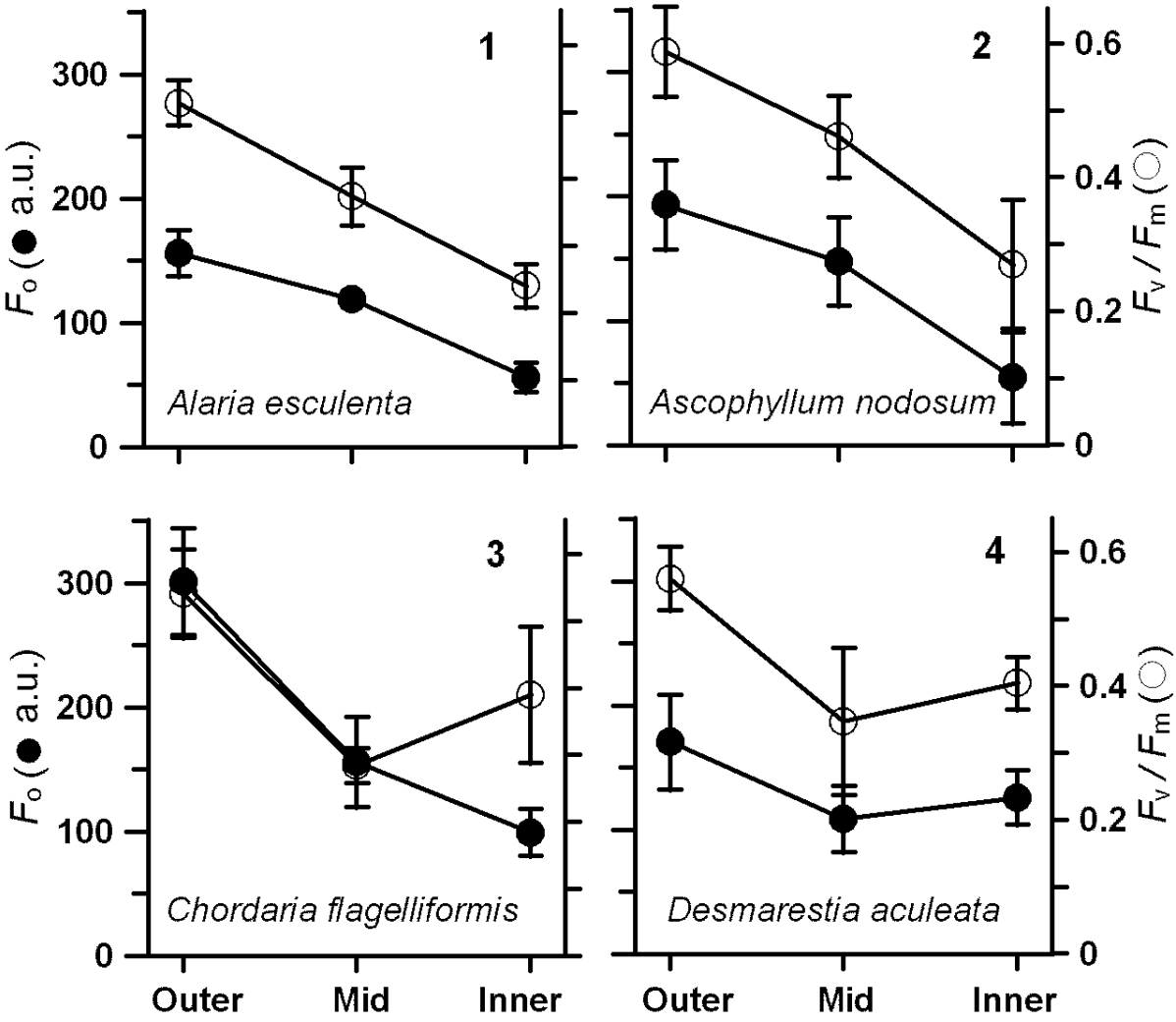
Fluorescence measurements were performed using a Microcopy-PAM facility (Microscopy-PAM, Walz GmbH, Effeltrich, Germany). This consists of a modified epifluorescence microscope, the PAM control unit and a notebook computer with dedicated Windows-software (WinControl) for system operation and fluorescence analysis. Using visual inspection and an iris diaphragm the active field is narrowed so that the fluorescence characteristics of a small area (i.e. 200 μm diameter) can be assessed. The apparatus is equipped with a pin-hole micro quantum sensor (MC-MQS) to assess quantum flux density of the blue excitation light in the object plane.

First, minimum fluorescence, F_o, was induced by low irradiation pulses in dark-adapted samples. Following a saturating flash, maximal fluorescence, F_m, was detected. Variable fluorescence, F_v, was calculated as the difference between F_o and F_m.

One-way analysis of variance (ANOVA) tests were used to determine if significant differences were present among the regions of transverse sections of each species for the fluorescence parameter. The non-parametric Kruskal-Wallis test was performed on data from each species, whereas a general linear model was used on pooled data. These analyses were performed using the software SPSS Release 11.0.1 (SPSS Inc., Chicago, IL, USA).

RESULTS

The photosynthetic parameters F_o (minimum fluorescence) and F_v/F_m (maximum quantum yield) were determined in the outer, middle and inner portions of transverse sections for eight species brown algae (Figs 1-8) representing four orders (Table 1). These corresponded to meristoderm-outer cortex, inner cortex and medullary portions of transverse sections, respectively. In all species F_o was always greatest in the outermost cell/tissue layers. For most species F_o was lowest in the innermost cells (medulla) and significantly lower than in the outermost cells (outer cortex or meristoderm) (ANOVA, $p < 0.05$). The exceptions were *Desmarestia aculeata* and *Laminaria digitata* where there was a rise or consistency in F_o between inner cortical and medullary cells (ANOVA, $p = 0.051$). Within a given species, mean values for F_o from outer to inner layers ranged from 1:0.7 in *D. aculeata* to 1:0.2 in *Laminaria saccharina*. Across all eight species, values for F_o for

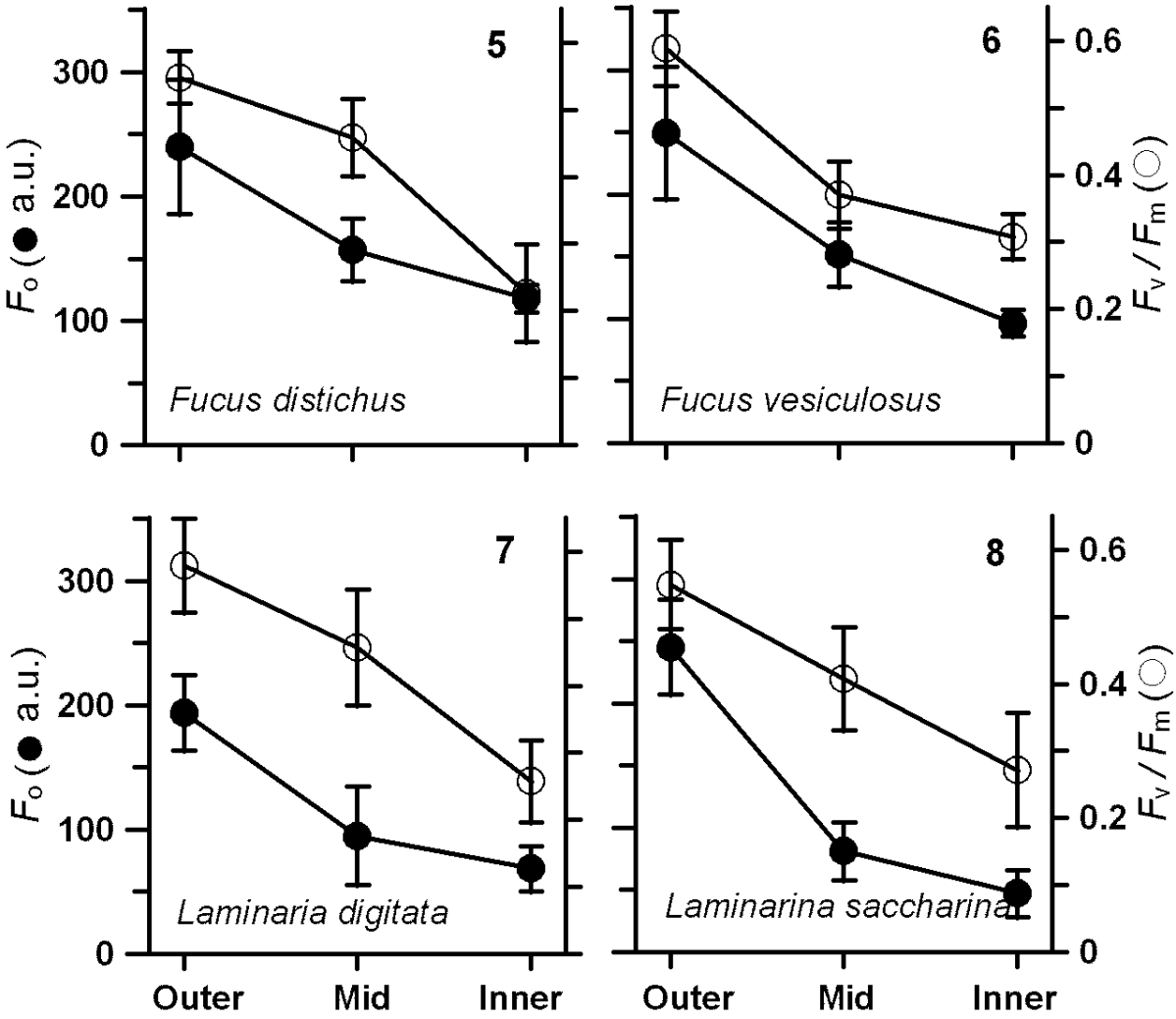


Figs 1-4. Photosynthetic parameters F_o and F_v/F_m in meristoderm and outer cortex (outer), inner cortex (mid) and medulla (inner) portions of transverse sections from four species of Phaeophyceae. Values indicate mean ± s.d. (n = 3). **Fig. 1.** *Alaria esculenta*. **Fig. 2.** *Ascophyllum nodosum*. **Fig. 3.** *Chordaria flagelliformis*. **Fig. 4.** *Desmarestia aculeata*.

medullary cells were 39 ± 16% of outer cells. These corresponded to the conspicuous change in pigmentation visible with the unaided eye across transverse sections of most species.

Levels of F_o showed positive correlations when the three areas of sections were compared across all species (r values from 0.317 to 0.463). Only the highest of these values (between outer and mid portions of sections) was significant ($p = 0.023$). When the two syntagmatic species were deleted from the analysis, the other two correlations (i.e., between inner and outer and between middle and inner parts of sections) became significant at $p < 0.05$ ($r = 0.503$ and 0.588, respectively).

There was less variation in maximum quantum yield between outer and inner cell types than for F_o. Values for medullary cells ranged from 42 to 72 % of outer cells (50 ± 9%). When all species were considered together, maximum quantum yield (F_v/F_m) varied significantly across transverse sections. Average values for the outer, densely pigmented cells fell within a narrow range from 0.513 (*Alaria esculenta*) to 0.589 (*Fucus vesiculosus*) (0.559 ± 0.026, X ± s.d.). This declined in inner cortical cells to 0.395 ± 0.063 and again in medullary cells to 0.296 ± 0.067. Values for the two syntagmatic species (*C. flagelliformis* and *D. aculeata*) were not consistent with the overall pattern, and there was no difference between mid and inner portions of sections. When the parenchymatous taxa were considered alone (i.e., with the two syntagmatic species removed) all comparisons of cell-tissue types were significant at $p < 0.001$. Correlations of values for maximum quantum yield between cell layers were not significant. Across all species and cell types F_o and F_v/F_m were highly correlated ($r = 0.687$, $p < 0.001$).



Figs 5-8. Photosynthetic parameters F_o and F_v/F_m in meristoderm and outer cortex (outer), inner cortex (mid) and medulla (inner) portions of transverse sections from four species of Phaeophyceae. Values indicate mean ± s.d. (n = 3). **Fig. 5.** *Fucus distichus.* **Fig. 6.** *Fucus vesiculosus.* **Fig. 7.** *Laminaria digitata.* **Fig. 8.** *Laminaria saccharina.*

DISCUSSION

Our data from brown algae demonstrate the usefulness of Microscopy-PAM for showing changes in photosynthetic parameters on very small spatial scales. Although minimum fluorescence and maximum quantum yield are quantitative parameters that are indirect measures of actual O_2 production, they do provide a perspective on the physiological status of the different thallus tissues pertaining to carbon fixation. In this study we used only a representative portion of each species, and this work could be continued to evaluate developmental changes (e.g., young versus old branch segments in *Ascophyllum*) or tissue-organ differences (e.g., blade versus stipe in kelps).

The cutting of transverse sections might be considered a major tissue trauma that would considerably increase stress and reduce quantum yields. This does not appear to be the case in the three fucoids examined here at least over the short-term duration of our measurements. Kim and Garbary (in preparation) used imaging PAM to examine age and organ related changes in photosynthetic parameters in the three fucoids studied here. Those data showed maximum quantum yields in the range of 0.6 to 0.7 that are only slightly higher than the values reported here for meristoderm-outer cortex tissues of the same fucoids.

Of the eight species examined, two stand out as different in terms of photosynthetic differentiation, i.e., *Chordaria flagelliformis* and *Desmarestia aculeata.* These species are entirely syntagmatic whereas the remaining fucoid and kelp species, have a parenchymatous organization (Christensen 1980). *Chordaria flagelliformis*

was the only species in which F_o and F_v/F_m were not well correlated, with the former increasing and the latter declining between inner cortex and medulla. *Desmarestia aculeata* was also anomalous in that maximum quantum yield was the lowest of any species and it showed the smallest changes in both F_o and F_v/F_m. This may reflect cytological deterioration as a consequence of damage incurred during its time in the drift and short exposures to air. Additional studies of syntagmatic brown algae, in general, and *Desmarestia* species, in particular, would be useful to extend our observations.

Despite the apparent lack of pigmentation in the inner cortex and medulla of most brown algae, considerable photosynthetic capacity is retained. Thus the apparently colourless medulla of all these brown algae retain substantial chlorophyll with F_o in medullary cells 20 to 74% of maximum levels (mean of 39%) in meristoderm or outer cortex. This suggests at least the possibility of significant photosynthetic capacity in medullary tissue. Our data for maximum quantum yield supports this contention. Indeed, for six of the eight species, the ratio of quantum yield in medullary cells to epidermal or outer cortical cells was 42 to 72% (mean of 50%). This suggests that despite clearly lower pigment levels, that photosynthetic capacity may be compensated by higher photosynthetic potential in these cells. Thus medullary cells in brown algae may be physiologically differentiated from meristoderm / outer cortical cells with respect to photosynthetic function. This could be resolved with Imaging-PAM in which greater magnification was possible than with current instrumentation. In *Laminaria saccharina* Grevby *et al.* (1988) concluded that chlorophyll amount and chloroplast area were not necessarily proportional to photosynthetic capacity. This is similar to our conclusion that maximum quantum yield in inner cortical and medullary cells may be elevated relative to chlorophyll amounts in different tissues.

Elsewhere we show organ level differences in photosynthetic fluorescence parameters in fucoid algae (Kim and Garbary, in preparation). This is analogous to previous work on kelp using more traditional O_2 evolution and CO_2 fixation approaches that demonstrated organ level variation (e.g., Arnold and Manley 1985). Those studies were primarily focused on developmental changes during growth and senescence. Microscope-PAM has allowed us to examine physiological performance at the cell and tissue level, and to relate anatomy and cell differentiation to photosynthetic capacity.

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