

Photosynthesis in relation to D1, PsaA and Rubisco in marine and brackish water ecotypes of *Fucus vesiculosus* and *Fucus radicans* (Phaeophyceae)

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Abstract The aim of this study was to investigate photosynthetic differences between the marine, Norwegian Sea ecotype and the brackish, Bothnian Sea ecotype of *F. vesiculosus* and *F. radicans* and to see whether photosynthetic differences could be connected with the relative amounts of D1 protein (PSII), PsaA (PSI) protein and/or Rubisco. For this purpose, we tested if a higher photosynthetic maximum (P_{\max}) in the Atlantic Ocean ecotype of *F. vesiculosus* relative to the Baltic Sea ecotype, and an increase of the P_{\max} in Baltic Sea ecotype of *F. vesiculosus* at higher salinity, could be due to an increase in the relative amounts of Rubisco. The proteins have been evaluated on a relative basis. Immunoblot signals showed that the amount of Rubisco was higher in both ecotypes of *F. vesiculosus* than in *F. radicans*, but no differences could be detected between the two ecotypes of

F. vesiculosus. The results suggest an uneven photosystem protein stoichiometry in *Fucus*, with more of the PSI protein PsaA relative to the PSII protein D1. The difference in P_{\max} between the two ecotypes of *F. vesiculosus* might be related to the difficulties for the algae to adapt to the environment in Bothnian Sea.

Keywords D1 · *Fucus radicans* · *Fucus vesiculosus* · Photosynthetic maximum capacity (P_{\max}) · PsaA · Rubisco

Introduction

The brown alga *Fucus vesiculosus* L. is mainly a marine, North Atlantic, intertidal species (Powell, 1963), but the alga also grows in the sublittoral of the brackish Baltic Sea, along a salinity gradient down to the range of 2–4 psu (Wærn, 1952). Before the recent discovery of *Fucus radicans* L. Bergström et al. (2005), *F. vesiculosus* was thought to be the only large belt-forming algal species in the Bothnian Sea, a northerly part of the Baltic Sea (Wallentinus, 1979). *F. radicans* is most certainly endemic to the Baltic Sea, grows side-by-side with *F. vesiculosus* and was earlier thought to be the same species.

The environmental factors differ greatly between the Norwegian Sea (part of the Atlantic Ocean) and the Bothnian Sea (part of the Baltic Sea). In contrast to the

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marine tidal Norwegian Sea (34 practical salinity units, psu), the Bothnian Sea has low salinity (4–5 psu), no tides and periodically an ice-cover. Environmental factors fluctuate rapidly for algae in the tidal zone of the Norwegian Sea. The differences in the local environments between the Norwegian Sea and the Bothnian Sea have given rise to differences in morphology as well as different physiological acclimation and/or adaptation capacities and mechanisms in the marine Norwegian Sea and the brackish Bothnian Sea ecotypes of *F. vesiculosus*. The brackish ecotype of *F. vesiculosus* is smaller with a thinner thallus (Kalvas & Kautsky, 1993), has greater respiration rates, lower maximum photosynthetic capacities (P_{\max}), lower mannitol concentration, lower tolerance of emersion stress and contains fewer water soluble organic compounds than the marine ecotype of *F. vesiculosus* (Bäck et al., 1992; Ruuskanen & Bäck, 1999; Pearson et al., 2000; Nygård & Ekelund, 2006; Gylle et al., 2009a, b).

An important protein, involved in linear electron transport in oxygenic photosynthesis, is the chlorophyll (Chl) *a*-binding core protein D1 (PsbA, 37 kDa) in the photosystem II (PSII) reaction centre. The D1 protein is rapidly cycled during illumination and disruption of D1 protein cycling or losses of D1 protein pools are central to the photoinhibition of photosynthesis (Nixon et al., 2005; reviewed by Nixon et al., 2010). One of the important proteins in the photosystem I (PSI) reaction centre is the Chl *a* binding core protein, PsaA (82–83 kDa; Hall & Rao 1999; reviewed by Santabarbara et al. 2010). PSI is involved in linear electron transport as well as cyclic electron transfer around PSI. The energy generated in the electron transport chain is stored in ATP and the reducing power is stored in NADPH. ATP and NADPH are used in the photosynthetic rate-limiting step of fixation of CO₂ by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). The most common structure of Rubisco consists of eight large (L) subunits (50–55 kDa) and eight small (S) subunits (15 kDa), L₈S₈ (~550 kDa; Raven, 1997).

P_{\max} is lower in the Baltic Sea ecotype compared to the Atlantic Ocean ecotype of *F. vesiculosus* and if the Baltic Sea ecotype is kept for 1 week or longer in higher salinity (10–35 psu) the P_{\max} increases considerably (Bäck et al., 1992; Nygård & Ekelund, 2006; Nygård & Dring, 2008). The reason for the higher P_{\max} in the Atlantic Ocean ecotype of *F. vesiculosus*

relative to the Baltic Sea ecotype, and for the increase of the P_{\max} in Baltic Sea ecotype of *F. vesiculosus* at higher salinity, have been suggested to be either an increase in the relative amounts of Rubisco, or an up-regulation of its activity (Nygård & Dring, 2008).

The aim of this study was therefore to investigate photosynthetic differences between the marine, Norwegian Sea ecotype and the brackish, Bothnian Sea ecotype of *F. vesiculosus* and to see whether the photosynthetic differences could be connected with the relative amounts of D1 protein (PSII), PsaA (PSI) protein and/or Rubisco.

Fucus radicans was also included in this study in order to investigate if the native Bothnian Sea species shows other adaptations to the environment than in the original marine *F. vesiculosus* ecotype in the Bothnian Sea. The proteins have not been evaluated in absolute terms and therefore the amounts of D1 (PSII), PsaA (PSI) and Rubisco are compared among the algae on a relative basis. Further to this, the aim was to see whether the photosynthetic differences could be connected with the relative amounts of D1 protein, (PSII), PsaA (PSI) protein and/or Rubisco when the brackish Bothnian Sea ecotype of *F. vesiculosus* was affected by varying salinities (5, 10, 20 and 35 psu) in the short-term.

Materials and methods

Algal material

Fucus vesiculosus samples were collected from the intertidal area of the Norwegian Sea at Trondhjem Biological Station (Norway; 63°43'N; 10°39'E; 34 psu) and from 2 to 4 m depth from the Bothnian Sea on the East side of Åstön (Sweden; 62°24'N; 17°45'E; 4–5 psu). *F. radicans* was collected from the Bothnian Sea at the same time and place as *F. vesiculosus*. The samples were collected in May 2006 and the water temperature was 6–8°C. The algae were transported in their natural water and darkness to the laboratory. Five individual samples of *F. vesiculosus* from the Bothnian Sea were prepared for an experiment as described below. For the aim of measuring the relative amounts of photosynthetic proteins from the collected algae groups, epiphytes were removed and tissue from the vegetative parts of the algal tips from ten individuals of each ecotype of *F. vesiculosus* and

from ten individuals of *F. radicans* were immediately ground separately with a mortar and pestle in liquid N₂ and frozen at −80°C until use. To establish the electron transport rates (ETR) in the three algal groups, fresh thallus from five individuals of each ecotype of *F. vesiculosus* and from five individuals of *F. radicans* were collected at the same places as above in February to March, 2010. The water temperature was 0–4°C. ETR was measured within 24 h after arrival to the laboratory and the samples were kept in darkness at 6°C until measurements.

Acclimation to changed salinity

For each experiment, an individual of *F. vesiculosus* from the Bothnian Sea was cleaned from epiphytes (approximately 10–15 cm long parts of thallus were used) and separated into five samples with two to four branches (depending on size) per sample. One of the samples was placed in Bothnian seawater (BSW, normal salinity 4–5 psu) and four samples in 5 psu artificial seawater (ASW), each in an individual 7 L aquarium. This was repeated with five individuals, so that in the salinity experiment each assay was done on five samples from different individuals. ASW was a mixture of deionised water, sea salt (Instant Ocean, United Pet Group, Cincinnati, OH, USA), inorganic compounds (0.344 mg L⁻¹ NH₄Cl, 2.05 mg L⁻¹ NaNO₃ and 0.016 mg L⁻¹ NaH₂PO₄) and bicarbonate (1 mM NaHCO₃, Sigma-Aldrich, St. Louis, MO, USA). The concentrations of inorganic compounds were chosen to be similar to those in BSW, according to an analysis done by LMI AB, Helsingborg, Sweden, and the concentration of bicarbonate was the same as in BSW (Raven & Samuelsson, 1988). Before changing salinity, algae were allowed to acclimate for 3 days to the irradiance, temperature and basic growth medium. Thereafter, samples incubated in ASW were placed in salinities of 5, 10, 20 or 35 psu; the sample in BSW was kept as a control. The growth medium was changed every 2.5 days and after 1 week of treatment, tissue from the vegetative parts of the algal tips (approximately three cm of each tip) was ground in liquid N₂ and frozen at −80°C until use. During the whole experiment, the algae were kept in 12-h dark-light rhythm at the irradiance of 90 μmol photons m⁻² s⁻¹ (Philips Master TL-D 36 W/840, Amsterdam, the Netherlands; measured with

LI-COR LI-189 quantum sensor, Lincoln, NE, USA) at 6–8°C.

Electron transport

For the measurements of the ETR from the five individuals of each ecotype of *F. vesiculosus* and *F. radicans*, tissues from the vegetative parts of the algal tips were used. ETR was estimated from Chl *a* fluorescence measurements by rapid light curves using a pulse amplitude modulated fluorometer (Diving PAM, Walz, Effeltrich, Germany). After an initial dark-adaptation of 20 min, nine steps with increasing irradiance between 0 and 700 μmol photons m⁻² s⁻¹ (30 s at each step) were used to induce photosynthesis, interrupted by dark periods of 2 min. The temperature was kept approximately at 8°C during the measurements. ETR was measured at three different points at each thallus tip ($n = 5$) and the mean of the three measurements was used as each individual ETR. The ETR was calculated as:

$$\text{ETR} = \text{PAR} \times \text{AF} \times \Delta F/F'_m \times 0.5$$

AF (the absorption factor) is the fraction of incident PAR absorbed by the thalli (plant tips, $n = 5$) and was estimated according to the method of Beer et al. (2000) and Nygård & Dring (2008). The AF obtained was 0.83 for *F. vesiculosus* from the Norwegian Sea, 0.78 for *F. vesiculosus* from the Bothnian Sea and 0.80 for *F. radicans*. $\Delta F/F'_m$ is the effective quantum yield measured in light. PAR was multiplied by 0.5 to allow for an equal involvement of the two photosystems (Beer et al., 2000; Nygård & Dring, 2008).

The x axis of the rapid light curve is presented as absorbed light ($\text{PAR} \times \text{AF} \times 0.5$) and the PSII photochemical efficiency was measured as the maximum quantum yield (Y_0) as recommended by Saroussi & Beer (2007). Y_0 can also be expressed as F_v/F_m where F_v is the variable fluorescence, difference between the maximum (F_m) and minimum fluorescence, and F_m is the maximum fluorescence reached on dark-adapted algae when all PSII reaction centres are closed (Krause & Weis, 1991). An investigation by Gylle et al. (2011) indicated that an assumption of equal involvement of the two photosystems in marine and brackish ecotype of *F. vesiculosus* and *F. radicans* is an incorrect simplification. However, more studies have to be done before the exact involvement of PSI

and PSII in the absolute ETR have been totally clarified. The P_{\max} was calculated with Photosyn Assistant software (Ver. 1.1.2, Dundee Scientific, Dundee, Scotland, UK).

Net photosynthesis measured by oxygen evolution

When the Bothnian Sea ecotype of *F. vesiculosus* had been treated for 1 week in BSW, 5, 10, 20 or 35 psu, net photosynthesis was measured as oxygen evolution with an oxygen electrode (Rank Brothers Ltd, DM 10, Cambridge, England). Measurements were performed in a saturated inorganic carbon environment (3 mM NaHCO_3), using the vegetative tips of the thallus (pre-cut 24 h before measurement). The sample was placed in a vertical position above a magnetic stirrer and facing the light projector. Light periods (5 min) with increasing irradiance in eight steps between 0 and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were used, interrupted by dark periods of 5 min. In the salinity-treated Bothnian Sea ecotype of *F. vesiculosus*, efficiency of use of the absorbed light in photosynthesis at limiting irradiance (α) was calculated from the light-limited initial slope of the photosynthesis/irradiance curve (*PII* curve) between three data points from 0 to 35 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. P_{\max} was calculated with Photosyn Assistant software.

Protein extraction

For immunoblotting, proteins from 75 mg of tissue were extracted in 1.5 mL of buffer according to a protocol modified from Li et al. (1998). The buffer (pH 7.4) contained 100 mM sodium ascorbate (KEBO Lab, Stockholm, Sweden), 50 mM sodium 3-[*N*-morpholino] propane sulfonic acid (MOPS), 3 mM EDTA, 5 mM dithiothreitol (DTT), 2% polyvinyl-pyrrolidone (PVPP), 12 mM 3-[(3-cholamidopropyl) dimethyl-ammonio]1-propanesulfonate (CHAPS), 2% Triton X-100 and 10 μL protease Inhibitor Cocktail (Sigma-Aldrich). Aliquots of the protein extracts were stored at -80°C until SDS-PAGE and immunoblotting analysis. For spectrophotometric determination of Chl *a* and Chl *c* (c_1 and c_2) concentrations, 90% acetone was used and the absorbance was measured at 630, 664 and 750 nm (Varian Cary 50 Scan, Agilent Technologies, Santa Clara, CA, USA; Porra, 1991).

SDS-PAGE and immunoblotting

Proteins in the solubilised samples containing 5 μg Chl *a* were used for the detection of D1 and PsaA proteins and 1 μg Chl *a* for the detection of Rubisco. The proteins were electrophoretically separated in a 15% (w/v) polyacrylamide resolving gel containing 6 M Urea, 0.66 M Tris (pH 8.8) and a 5% (w/v) polyacrylamide stacking gel-containing 0.125 M Tris (pH 6.8) using the discontinuous Laemmli buffer system (Laemmli, 1970). Electrophoresis was performed at room temperature in a Mini-Protean II apparatus (Bio-Rad, Hercules, CA USA) for 1 h at 3 mA. Protein standards (Bio-Rad, item number 161-0304) and protein extracts for all treatments were separated by SDS-PAGE and transferred electrophoretically to 5% (w/v) BSA blocked nitrocellulose membranes (Bio-Rad) and probed using the PsaA polyclonal total IgY antibody (anti-D1, PSII), 1:50,000 dilution; PsaA polyclonal antibody (anti-PSI-A), 1:500 dilution and global RbcL polyclonal antibody (anti-Rubisco), 1:5,000 dilution (Agrisera, Umeå, Sweden). After incubation with the secondary antibody IgY, conjugated to peroxidase, for PsaA (Sigma-Aldrich, 1:10,000 dilution); IgG, conjugated to horseradish peroxidase, for PsaA and Rubisco (Agrisera, 1:50,000 dilution and Abcam, Cambridge, MA, USA, 1:20 000 dilution, respectively), the antibody-protein complexes were visualised using ECL chemiluminescence detection reagents (GE Healthcare, Waukesha, WI, USA) and developed on X-OMAT film (VWR, East Grinstead, United Kingdom). To obtain sharp bands, the optimal concentrations of Chl *a* and antibodies were determined in preliminary tests. The immunoblotting was repeated 5–7 times for each replicate and for each class of protein and the developed photos were analysed by using ImageJ software (developed by Rasband (1997–2011), National Institutes of Health, Bethesda, MD, USA; use reference, Shim et al., 2010).

Data analysis

To analyse the differences, a general linear model analysis of variance (GLM) or one-way ANOVA, followed by Tukey's pairwise comparison, were performed. All factors were categorical and fixed. Tukey's pairwise comparison was performed for a complete comparison of all parts included in the

experiments. The confidence intervals were set to 95%. The standard deviation values are given in the figures and tables.

Results

Photosynthesis and relative amounts of D1, PsaA and Rubisco proteins in *F. vesiculosus* and *F. radicans*

To analyse the photosynthesis and to see if photosynthetic differences were connected with the relative amounts of D1, PsaA and/or Rubisco in the *Fucus* strains, analyses of *P/II* curves (by ETR) and immunoblotting were performed (Figs. 1, 2; Table 1).

The results confirm that P_{\max} was significantly higher (57%) in the marine ecotype of *F. vesiculosus* compared to the brackish ecotype of *F. vesiculosus* ($P < 0.01$; Fig. 1; Table 1). The P_{\max} was also significantly higher (51%) in the marine ecotype of *F. vesiculosus* compared to *F. radicans* ($P < 0.01$; Fig. 1; Table 1). No differences between the brackish *Fucus* species were detected. The results did not indicate any differences in Y_0 between the *Fucus* strains (Table 1).

The results from the estimations of the relative amounts of the D1 protein on a Chl *a* basis did not indicate any significant differences between the two ecotypes of *F. vesiculosus* or between the two brackish *Fucus* species (Fig. 2a). Between the marine ecotype of *F. vesiculosus* and *F. radicans*, however, a significant difference could be detected with a greater relative amount of D1 protein in *F. vesiculosus* ($P < 0.01$; Fig. 2a). The measurements of PsaA protein showed that all three *Fucus* strains contained similar amounts of PsaA on a Chl *a* basis (Fig. 2b). No significant differences in the relative PSII/PSI ratios were detected between any of the *Fucus* strains (Table 1). The results from the immunoblotting of Rubisco indicated that the relative amount of Rubisco in both strains of *F. vesiculosus* was almost double that in *F. radicans* ($P < 0.001$; Fig. 2c).

Effect of salinity on net photosynthesis and the relative amounts of D1, PsaA and Rubisco proteins in the brackish, Bothnian Sea ecotype of *F. vesiculosus*

To test if changes in photosynthetic variables were connected with the relative amounts of D1, PsaA and Rubisco when the brackish ecotype of *F. vesiculosus*

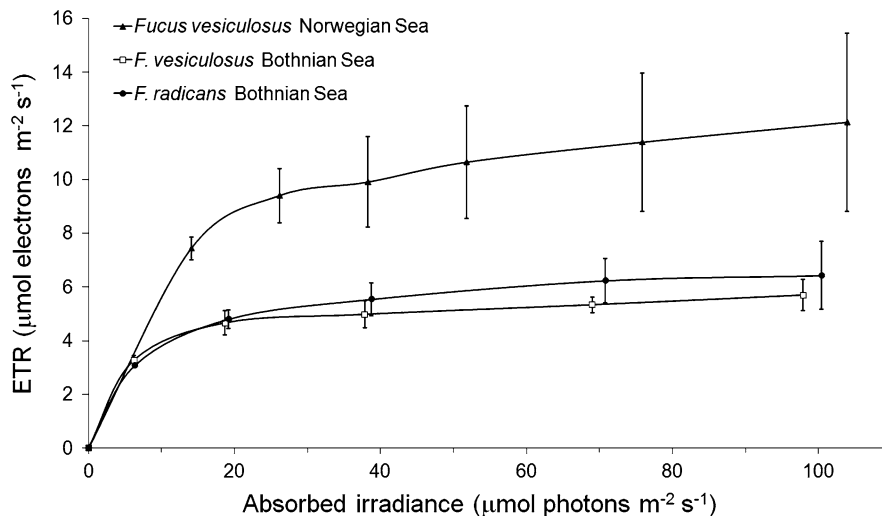


Fig. 1 Photosynthesis/irradiance curve measured by electron transport in marine *F. vesiculosus* from the Norwegian Sea (35 practical salinity units, psu) and brackish *F. vesiculosus* and *F. radicans* from the Bothnian Sea (4–5 psu). The measurements were completed after an initial dark-adaptation of 20 min

and nine steps with increasing irradiance between 0 and 700 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were used (points with incident irradiance above 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ are not shown). Data represent means of five replicates \pm standard deviation

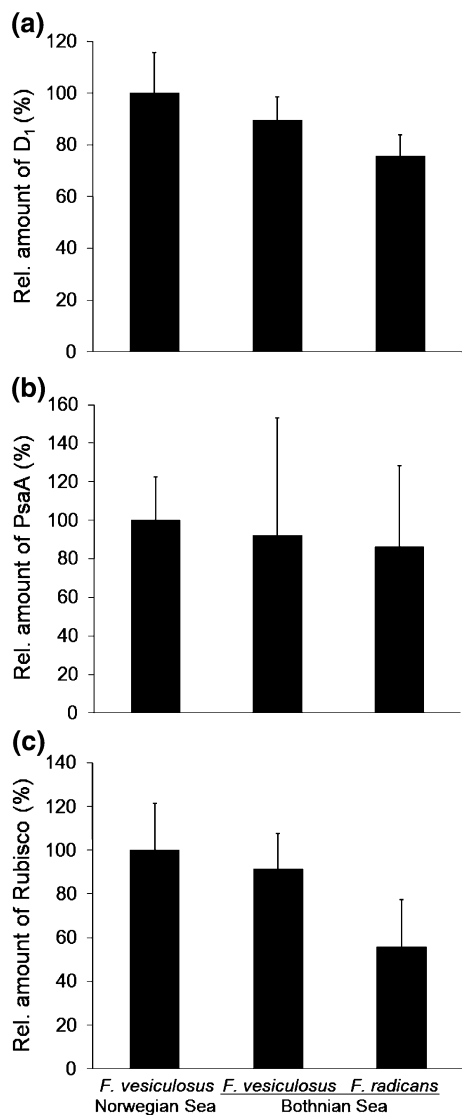


Fig. 2 The relative amounts of **a** D1 protein in photosystem II (PSII), **b** PsaA protein in PSI and **c** ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in marine *F. vesiculosus* from the Norwegian Sea (35 practical salinity units, psu) and brackish *F. vesiculosus* and *F. radicans* from the Bothnian Sea (4–5 psu) were determined by immunoblotting and by ImageJ software. Data were represent means of ten replicates blotted 5–7 times each (for each protein) \pm standard deviation. Different letters indicate significant ($P < 0.05$) difference between the *Fucus* strains and lack of letter means no differences compared to the other *Fucus* strains. The y axis presents the results in percent relative to the marine *F. vesiculosus*

was affected by salinity, the alga was treated in a range of salinities and analyses of *P/II* curves (by O_2 evolution) and immunoblotting were performed (Figs. 3, 4; Table 2).

Short-term salinity treatments had an effect on P_{max} . The algae treated at salinities of 10 and 20 psu had significantly higher P_{max} values than those at BSW or 5 psu ($P < 0.01$) and the algae treated at 35 psu had significantly higher P_{max} values than those at BSW ($P < 0.05$; Fig. 3; Table 2). The algae treated at 10 psu had also significantly higher P_{max} than algae treated in 35 psu ($P < 0.05$; for details in significant differences in P_{max} between the treatments see Table 2). Photosynthetic efficiency (α) was largely unaffected but it was significantly lower in algae treated in BSW compared to algae treated in 20 psu (Fig. 3; Table 2).

The immunoblotting did not indicate any effects of salinity on the relative amounts of D1 protein or Rubisco on a Chl *a* basis in the brackish ecotype of *F. vesiculosus* (Fig. 4a, c). In contrast, the relative amount of PsaA protein in PSI on a Chl *a* basis showed significantly greater amounts of PsaA in algae treated in 10 psu compared to algae treated in BSW and in 35 psu ($P < 0.05$; Fig. 4b). The PSII/PSI ratio, measured by immunoblot signals of D1/PsaA, was significantly lower in algae treated in 10 psu (0.45) than in algae treated in 35 psu (0.72; $P < 0.05$; Table 2).

Discussion

Photosynthesis and relative amounts of D1, PsaA and Rubisco proteins in *F. vesiculosus* and *F. radicans*

The results indicated a higher P_{max} in the marine ecotype of *F. vesiculosus* than in the brackish ecotype of *F. vesiculosus* and *F. radicans* (Fig. 1; Table 1). The lower P_{max} in the brackish water *Fucus* than in the marine ecotype of *F. vesiculosus* is in line with earlier investigations (Bäck et al., 1992; Nygård & Ekelund, 2006; Nygård & Dring, 2008). In this study we made a first attempt to reveal what causes the differences in P_{max} between the *Fucus* strains by immunoblotting of D1, PsaA and Rubisco. Immunoblot signals in the detections of D1 and PsaA showed a similar D1/PsaA ratio in all three *Fucus* strains. The results suggest, however, an uneven photosystem protein stoichiometry in *Fucus*, with more of the PSI protein PsaA relative to the PSII protein D1 (Table 1). This difference between PSI and PSII appears to be in agreement with 77 K Chl *a* fluorescence emission

Table 1 Photosynthetic maximum capacity (P_{\max}) measured by electron transport, the efficiency to use absorbed light in photosynthesis (maximum quantum yield, Y_0) and the relativeD1/PsaA ratio in marine *F. vesiculosus* from the Norwegian sea (35 practical salinity units, psu) and brackish *F. vesiculosus* and *F. radicans* from the Bothnian Sea (4–5 psu)

Algae	P_{\max}	Y_0	D1/PsaA
<i>F. vesiculosus</i> Norwegian Sea	13.0 ^A ± 4.33	0.70 ± 0.030	0.38 ± 0.077
<i>F. vesiculosus</i> Bothnian Sea	5.6 ^B ± 0.46	0.70 ± 0.017	0.40 ± 0.115
<i>F. radicans</i> Bothnian Sea	6.4 ^B ± 1.49	0.70 ± 0.010	0.35 ± 0.086

The mean and ± standard deviation values of P_{\max} and Y_0 are based on five replicates. D1 and PsaA were determined by immunoblotting and by ImageJ software and the data represent means of ten replicates blotted 5–7 times each (for each protein) ± standard deviation. Different superscript letters indicate significant ($P < 0.05$) difference between the *Fucus* strains and lack of superscript letter means no differences compared to the other *Fucus* strains. The expressed D1/PsaA ratio (PSII/PSI) is the ratio of the immunoblot signals, not the absolute molar ratio of the complexes and therefore, the comparison can only be interpreted as a relative indicator of PSII/PSI

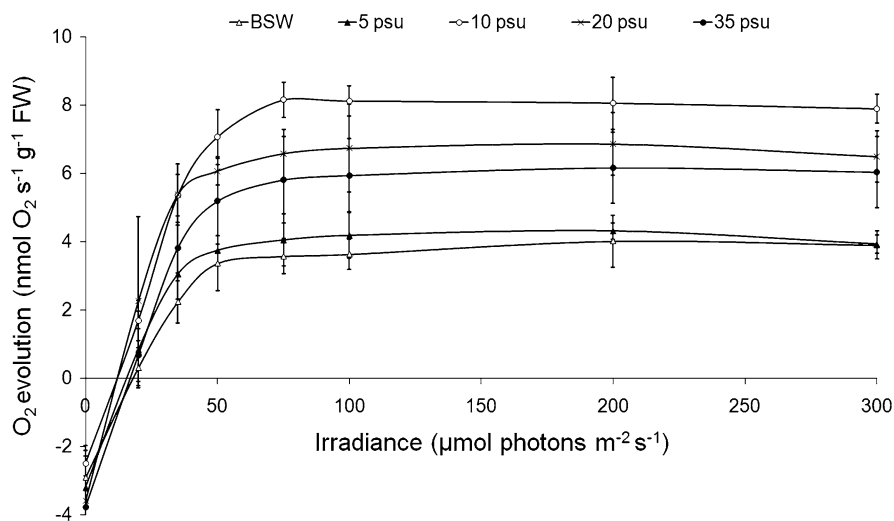


Fig. 3 Photosynthesis/irradiance curve measured by oxygen evolution in brackish *F. vesiculosus* from the Bothnian Sea (4–5 practical salinity units, psu) treated for 1 week in Bothnian seawater (BSW) or in artificial sea water at different salinities (5, 10, 20 or 35 psu). The light (90 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$); dark

period was 12:12 h. The measurements were completed after an initial dark-adaptation of 20 min and eight steps with increasing irradiance between 0 and 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were used. Data were represent means of five replicates ± standard deviation

spectra for the marine *F. vesiculosus* (PSII/PSI ~ 0.3) and *F. radicans* (PSII/PSI ~ 0.4; Gylle et al., 2011). 77 K fluorescence emission of the brackish ecotype of *F. vesiculosus*, however, showed a PSII/PSI ratio ~ 1 in Gylle et al. (2011). The reason for the higher fluorescence emission in PSII in the brackish *F. vesiculosus*, than in the other *Fucus* strains, was concluded to be due to larger light-harvesting antenna of PSII and not due to a truly higher PSII/PSI ratio (Gylle et al., 2011). This interpretation of the 77 K fluorescence emission of the brackish ecotype of *F. vesiculosus* in Gylle et al. (2011) appears

to be in line with the immunoblot signals of D1/PsaA (Table 1). The PSII/PSI ratio differ between different organisms and have been observed to be 1.43–1.72 in some terrestrial plants, 0.43 in blue-green algae and 0.84–1.03 in some green algae (Melis & Brown, 1980; Falkowski et al., 1981; Yamazaki et al., 2005; Fan et al., 2007).

The reasons for generally lower PSII/PSI ratio in marine algae compared to terrestrial plants have been suggested to be: (1) a need of greater ATP/NADPH ratio as energy to import various nutrients and therefore more PSI to mediate cyclic electron

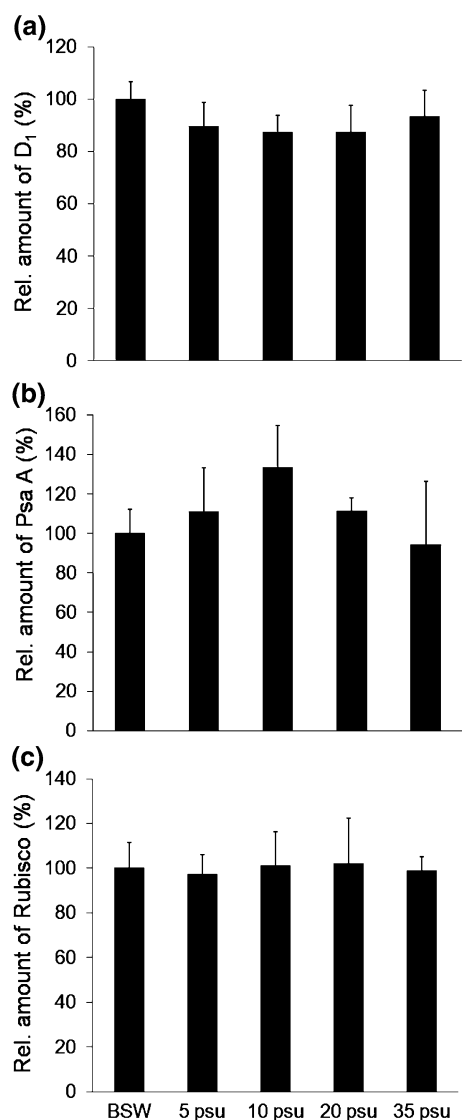


Fig. 4 The relative amounts of **a** D1 protein in photosystem II (PSII), **b** PsaA protein in PSI and **c** ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in brackish *F. vesiculosus* from the Bothnian Sea (4–5 practical salinity units; psu) were determined by immunoblotting and by ImageJ software. The algae were treated for 1 week in Bothnian seawater (BSW) or in artificial sea water at different salinities (5, 10, 20 or 35 psu). The light ($90 \mu\text{mol photons m}^{-2} \text{s}^{-1}$):dark period was 12:12 h. Data represent means of five replicates blotted 5–7 times each (for each protein) \pm standard deviation. Different letters indicate significant ($P < 0.05$) difference between the treatments and lack of letter means no differences compared to the other treatments. The y axis presents the results in percent relative to the algae treated in BSW

transport, and (2) the imbalance in the photosystem stoichiometry protects algae from photoinhibition in the blue–green light that preferentially excites PS II (Yamazaki et al., 2005).

Because of the differences in P_{max} (Fig. 1; Table 1), it was hypothesised that the marine ecotype of *F. vesiculosus* would have more Rubisco than both the brackish *Fucus* species. Rubisco is accumulated in parallel with high photochemical activities (Björkman, 1981; Maayana et al., 2008). The results, however, showed approximately the same relative amount of Rubisco in both ecotypes of *F. vesiculosus* whereas *F. radicans* had about half the amount (Fig. 2c). These results indicated that the reasons for the lower P_{max} in the brackish ecotype of *F. vesiculosus* and *F. radicans* than in the marine ecotype of *F. vesiculosus* might originate from different underlying causes. Whereas the relatively lower amount of Rubisco may be an important part of the explanation of the low P_{max} in *F. radicans*, there may be another explanation for the low P_{max} in the brackish ecotype of *F. vesiculosus*. The difference in P_{max} between the two ecotypes of *F. vesiculosus* might be related to the difficulties for the algae to adapt to the environment in Bothnian Sea or to normal differences in environmental adaptations. Further investigations are needed in order to understand the low P_{max} in the brackish ecotype of *F. vesiculosus* and we suggest measuring the rate of CO_2 fixation by Rubisco as a first step. Only few investigations have been done on the recently discovered *F. radicans* and it is important to keep in mind that *F. radicans*, unlike the brackish ecotype of *F. vesiculosus*, is a native species in the Bothnian Sea environment. It is interesting to note the similarity in P_{max} between the Bothnian Sea *Fucus* species despite lower relative amount of Rubisco in *F. radicans* pointing towards a more efficient CO_2 fixation in *F. radicans*. A speculation worth take in consideration is that the brackish ecotype of *F. vesiculosus* compared to the marine ecotype and *F. radicans* might have a less efficient C4-like pathway to accumulate CO_2 . This may increase a need of higher amount of Rubisco, relative to the P_{max} , in the brackish ecotype of *F. vesiculosus*. Wang et al. (2011) showed higher abundance and activity of Rubisco in gametophytes compared to sporophytes and assumed that a more marked C4-like pathway supply the sporophytes

Table 2 Photosynthetic maximum capacity (P_{\max}) measured by oxygen evolution ($\text{nmol O}_2 \text{ s}^{-1} \text{ g}^{-1} \text{ FW}$), the efficiency to use absorbed light in photosynthesis at limiting irradiance (α : calculated between 0 and $35 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and therelative D1/PsaA ratio in the brackish Bothnian Sea (4–5 practical salinity units, psu) *F. vesiculosus* after 1 week treatment in Bothnian seawater (BSW) or in artificial seawater at different salinities (ASW of 5, 10, 20 or 35 psu)

Treatment	P_{\max}	α	D1/PsaA
BSW	$4.2^{AA} \pm 0.92$	$0.15^A \pm 0.017$	$0.69^A \pm 0.111$
5 psu	$4.6^{AD} \pm 0.49$	$0.18^B \pm 0.038$	$0.56^A \pm 0.088$
10 psu	$8.8^{BB} \pm 1.35$	$0.22^B \pm 0.064$	$0.45^A \pm 0.071$
20 psu	$7.7^{BC} \pm 1.08$	$0.26^B \pm 0.043$	$0.52^A \pm 0.097$
35 psu	$6.4^{CD} \pm 1.20$	$0.22^B \pm 0.070$	$0.72^B \pm 0.239$

The light ($90 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$):dark period was 12:12 h. The values are mean values \pm standard deviations from five replicates. D1 and PsaA was determined by immunoblotting and by ImageJ software and each of the five replicates were blotted 5–7 times (for each protein). Different superscript letters indicate significant ($P < 0.05$) difference between the treatments and lack of superscript letter means no differences compared to the other treatments. The expressed D1/PsaA ratio (PSII/PSI) is the ratio of the immunoblot signals, not the absolute molar ratio of the complexes and, therefore, the comparison can only be interpreted as a relative indicator of PSII/PSI

with CO_2 and made it unnecessary to have a high abundance of Rubisco.

Effect of salinity on net photosynthesis and relative amounts of D1, PsaA and Rubisco of the brackish, Bothnian Sea, ecotype of *F. vesiculosus*

In general, there were higher P_{\max} values in the brackish ecotype of *F. vesiculosus* treated in the higher salinities, 10, 20 and 35 psu, compared to P_{\max} in algae treated in BSW and 5 psu. The most favourable salinity was 10 psu followed by 20 psu (Table 2). These results were in line with earlier ETR measurements when the alga was treated in different salinities together with low or high concentrations of dissolved inorganic carbon and nutrients (Nygård & Dring, 2008).

In this first attempt to explain the differences in the P_{\max} of the brackish *F. vesiculosus* treated in different salinities we did not observe any differences in the relative amount of either D1 or Rubisco (Fig. 4a, c). The relative amount of PsaA protein (Fig. 4b) and the D1/PsaA ratio (Table 2), however, indicated a salinity effect. The relative amount of PsaA was greatest in algae treated in 10 psu and it was significantly greater in 10 psu compared to algae treated in BSW and 35 psu. The greater amount of PsaA in algae treated in 10 psu was in line with the photosynthetic measurements (Fig. 3; Table 2). The explanation might be that the algae need to produce more ATP, and greater amount of PsaA may increase the possibility to have a greater flow of cyclic electron transport around PSI to serve a higher rate of CO_2 concentrating mechanisms

and/or a higher rate of CO_2 fixation by Rubisco. It has earlier been confirmed that greater relative amounts of PSI and greater cyclic electron flow around PSI might occur in conditions with an increased need for ATP (Mandori & Melis, 1984; Anderson et al., 1995; Tanaka et al., 1997; Hall & Rao, 1999; Yamazaki et al., 2005). Gao et al. (2011) have also showed that the electron flow around PSI was significantly elevated in *Ulva* sp. during desiccation which indicates that PSI-driven cyclic electron flow might provide some protection of the photosynthetic apparatus and extra ATP under stress conditions. As a consequence of more PsaA, the D1/PsaA ratio was lowest in algae treated in 10 psu and significantly lower relative to the algae treated in 35 psu (Table 2). Earlier studies have confirmed a change in PSII/PSI ratio as a response to CO_2 supply, irradiance, nitrogen supply and because of salinity shock (Mandori & Melis, 1984; Kim et al., 1993; Berges et al., 1996; Tanaka et al., 1997). It is tempting to speculate that an increased amount of ATP might be used to serve a more efficient C4-like pathway and thereby an accumulation of CO_2 .

The indicated lower D1/PsaA ratio in algae treated in 10 psu in Table 2 was not in line with 77 K fluorescence emission spectra (Gylle et al., 2011) and in order to understand what causes the differences between the studies we suggest an investigation of the light-harvesting antenna proteins as a first step.

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References

- Anderson, J. M., W. S. Chow & Y. I. Park, 1995. The grand design of photosynthesis: acclimation of the photosynthetic apparatus to environmental cues. *Photosynthesis Research* 46: 129–139.
- Beer, S., C. Larsson, O. Poryan & L. Axelsson, 2000. Photosynthetic rates of *Ulva* (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. *European Journal of Phycology* 35: 69–74.
- Berges, J. A., D. O. Charlebois, D. C. Mauzerall & P. G. Falkowski, 1996. Differential effects of nitrogen limitation on photosynthetic efficiency of photosystems I and II in microalgae. *Plant Physiology* 110: 689–696.
- Bergström, L., A. Tatarenkov, K. Johannesson, R. B. Jönsson & L. Kautsky, 2005. Genetic and morphological identification of *Fucus radicans* sp. Nov (*Fucales*, Phaeophyceae) in the brackish Baltic Sea. *Journal of Phycology* 41: 1025–1038.
- Björkman, O., 1981. Responses to different quantum flux densities. In Lange, O. L., P. S. Nobel, C. B. Osmond & H. Ziegler (eds), *Encyclopedia of plant physiology*, vol 12. Physiological plant ecology. I. Responses to physical environment. Springer, New York, NY: 57–107.
- Bäck, S., J. C. Collins & G. Russel, 1992. Comparative ecophysiology of Baltic and Atlantic *Fucus vesiculosus*. *Marine Ecology Progress Series* 84: 71–82.
- Falkowski, P. G., T. G. Owens, A. C. Ley & D. C. Mauzerall, 1981. Effects of growth irradiance levels on the ratio of reaction centers in two species of marine phytoplankton. *Plant Physiology* 68: 969–973.
- Fan, D.-Y., A. B. Hope, P. J. Smith, H. Jia, R. J. Pace, J. M. Anderson & W. S. Chow, 2007. The stoichiometry of the two photosystems in higher plants revisited. *Biochimica et Biophysica Acta* 1767: 1064–1072.
- Gao, S., S. Shen, G. Wang, J. Niu, A. Lin & G. Pan, 2011. PSI-driven electron flow allows intertidal macro-algae *Ulva* sp. (Chlorophyta) to survive in desiccated conditions. *Plant Cell Physiology* 52: 885–893.
- Gylle, A. M., C. A. Nygård & N. G. A. Ekelund, 2009a. Desiccation and salinity effects on marine and brackish *Fucus vesiculosus* L. (Phaeophyceae). *Phycologia* 48: 156–164.
- Gylle, A. M., D. Isaksson & N. G. A. Ekelund, 2009b. Ecotype differentiation in qualitative content of water soluble organic compounds between marine and brackish *Fucus vesiculosus* L. (Phaeophyceae). *Phycological Research* 57: 127–130.
- Gylle, A. M., S. Rantamäki, N. G. A. Ekelund & E. Tyystjärvi, 2011. Fluorescence emission spectra of marine and brackish-water ecotypes of *Fucus vesiculosus* and *Fucus radicans* (Phaeophyceae) reveal differences in light-harvesting apparatus. *Journal of Phycology* 47(1): 98–105.
- Hall, D. O. & K. K. Rao, 1999. *Photosynthesis*. Cambridge University Press, Cambridge.
- Kalvas, A. & L. Kautsky, 1993. Geographical variation in *Fucus vesiculosus* morphology in the Baltic and North Seas. *European Journal of Phycology* 28: 85–91.
- Kim, J. H., R. E. Click & A. Melis, 1993. Dynamics of photosystem stoichiometry adjustment by light quality in chloroplasts. *Plant Physiology* 102: 181–190.
- Krause, G. H. & E. Weis, 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Physiology and Plant Molecular Biology* 42: 313–349.
- Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680–685.
- Li, R., S. H. Brawley & T. J. Close, 1998. Proteins immunologically related to dehydrins in Fucoid algae. *Journal of Phycology* 34: 642–650.
- Maayana, I., F. Shayaa, K. Ratnera, Y. Mania, S. Laveeb, B. Avidana, J. Shahaka & O. Ostersetzer-Birana, 2008. Photosynthetic activity during olive (*Olea europaea*) leaf development correlates with plastid biogenesis and Rubisco levels. *Physiologia Plantarum* 134: 547–558.
- Mandori, A. & A. Melis, 1984. Photochemical apparatus organization in *Anacystis nidulans* (Cyanophyceae); effect of CO₂ concentration during cell growth. *Plant Physiology* 74: 67–71.
- Melis, A. & J. S. Brown, 1980. Stoichiometry of system I and system II reaction centers and of plastoquinone in different photosynthetic membranes (photoreaction/electron transport/spectrophotometry/chloroplast function/P700-chlorophyll). *Proceedings of the National Academy of Sciences of the United States of America* 77: 4712–4716.
- Nixon, P. J., M. Barker, M. Boehm & R. de Vries, 2005. FtsH-mediated repair of the photosystem II complex in response to light stress. *Journal of Experimental Botany* 56: 357–363.
- Nixon, P. J., F. Michoux, J. Yu, M. Boehm & J. Komenda, 2010. Recent advances in understanding the assembly and repair of photosystem II. *Annals of Botany* 106: 1–16.
- Nygård, C. A. & N. G. A. Ekelund, 2006. Photosynthesis and UV-B tolerance of the marine alga *Fucus vesiculosus* at different sea water salinities. *Journal of Applied Phycology* 18: 461–467.
- Nygård, C. A. & M. J. Dring, 2008. Influence of salinity, temperature, dissolved inorganic carbon and nutrient concentration on the photosynthesis and growth of *Fucus vesiculosus* from the Baltic and Irish Seas. *European Journal of Phycology* 43: 253–262.
- Pearson, G., L. Kautsky & E. Serrão, 2000. Recent evolution in Baltic *Fucus vesiculosus*: reduced tolerance to emersion stresses compared to intertidal (North Sea populations). *Marine Ecology Progress Series* 202: 67–79.
- Porra, R. J., 1991. Recent advances and re-assessments in chlorophyll extraction and assay procedures for terrestrial, aquatic, and marine organisms, including recalcitrant algae. In Scheer, H. (ed.), *Chlorophylls*. CRC Press, Boca Raton, FL, USA: 31–57.
- Powell, H. T., 1963. Speciation in the genus *Fucus* L. and related genera. In Harding, J. P. & N. Tebble (eds), *Speciation in the sea*. Systematic Association Publication, London, UK: 63–77.
- Rasband, W. S., 1997–2011. ImageJ, U. S. National Institutes of Health, Bethesda, MD, USA. <http://rsb.info.nih.gov/ij/>. Accessed on 02 Dec 2011.

- Raven, J., 1997. Putting the C in phycology. *European Journal of Phycology* 32: 319–333.
- Raven, J. A. & G. Samuelsson, 1988. Ecophysiology of *Fucus vesiculosus* L. close to its northern limit in the Gulf of Bothnia. *Botanica Marina* 31: 399–410.
- Ruuskanen, A. & S. Bäck, 1999. Morphological variation of northern Baltic Sea *Fucus vesiculosus* L. *Ophelia* 50: 43–59.
- Santabarbara, S., L. Galuppini & A. P. Casazza, 2010. Bidirectional electron transfer in the reaction centre of photosystem I. *Journal of Integrative Plant Biology* 52: 735–749.
- Saroussi, S. & S. Beer, 2007. Alpha and quantum yield of aquatic plants derived from PAM fluorometry: uses and misuses. *Aquatic Botany* 86: 89–92.
- Shim, J. S., Y. Matsui, S. Bhat, B. A. Nacev, J. Xu, H.-E. C. Bhang, S. Dhara, K. Chung Han, C. R. Chong, M. G. Pomper, A. So & J. O. Liu, 2010. Effect of nitroxoline on angiogenesis and growth of human bladder cancer. *Journal of the National Cancer Institute* 102: 1855–1873.
- Tanaka, Y., S. Katada, H. Ishikawa, T. Ogawa & T. Takabe, 1997. Electron flow from NAD(P)H dehydrogenase to photosystem I is required for adaptation to salt shock in the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiology* 38: 1311–1318.
- Wallentinus, I. 1979. Environmental influences on benthic macrovegetation in the Trosa-Askö area northern Baltic Proper. II The ecology of macroalgae and submersed phanerogams. PhD Thesis, University of Stockholm, Stockholm, Sweden.
- Wang, C., X. Fan, G. Wang, J. Niu & B. Zhou, 2011. Differential expression of Rubisco in sporophytes and gametophytes of some marine macroalgae. *PLoS ONE* 6: e16351.
- Wærn, M., 1952. Rocky-shore algae in the Öregrund archipelago. *Acta Phytogeographica Suecia* 30: 1–298.
- Yamazaki, J., T. Suzuki, E. Maruta & Y. Kamimura, 2005. The stoichiometry and antenna size of the two photosystems in marine green algae, *Bryopsis maxima* and *Ulva pertusa*, in relation to the light environment of their natural habitat. *Journal of Experimental Botany* 56: 1517–1523.