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PHYSIOLOGICAL ADAPTATIONS IN TWO ECOTYPES OF *FUCUS VESICULOSUS* AND IN *FUCUS RADICANS* WITH FOCUS ON SALINITY

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The picture on the front cover page illustrates the sublittoral, brackish *Fucus vesiculosus* from the Archipelago Sea (6 practical salinity units, psu; Photo: 2005-10-03 FORSTSTYRELSEN). The picture on the back cover page illustrates the intertidal marine *F. vesiculosus* from the Norwegian Sea (34-35 psu) during low tide in January 2007 (Photo: DR. JON-ARNE SNELI).

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ABSTRACT

The in origin intertidal marine brown alga *Fucus vesiculosus* L. grow permanently sublittoral in the brackish Bothnian Sea, side by side with the recently discovered *F. radicans* L. Bergström *et* L. Kautsky. Environmental conditions like salinity, light and temperature are clearly different between *F. vesiculosus* growth sites in the Bothnian Sea (4-5 practical salinity units, psu; part of the Baltic Sea) and the tidal Norwegian Sea (34-35 psu; part of the Atlantic Ocean). The general aims of this thesis were to compare physiological aspects between the marine ecotype and the brackish ecotype of *F. vesiculosus* as well as between the two Bothnian Sea species *F. vesiculosus* and *F. radicans*.

The result in the study indicates a higher number of water soluble organic compounds in the marine ecotype of *F. vesiculosus* compared to the brackish ecotype. These compounds are suggested to be compatible solutes and be due to an intertidal and sublittoral adaptation, respectively; where the intertidal ecotype needs the compounds as a protection from oxygen radicals produced during high irradiation at low tide. The sublittoral ecotype might have lost the ability to synthesize these compound/compounds due to its habitat adaptation. The mannitol content is also higher in the marine ecotype compared to the brackish ecotype of *F. vesiculosus* and this is suggested to be due to both higher level of irradiance and higher salinity at the growth site.

77 K fluorescence emission spectra and immunoblotting of D_1 and PsaA proteins indicate that both ecotypes of *F. vesiculosus* as well as *F. radicans* have an uneven ratio of photosystem II/photosystem I (PSII/PSI) with an overweight of PSI. The fluorescence emission spectrum of the Bothnian Sea ecotype of *F. vesiculosus*

however, indicates a larger light-harvesting antenna of PSII compared to the marine ecotype of *F. vesiculosus* and *F. radicans*. Distinct differences in 77 K fluorescence emission spectra between the Bothnian Sea ecotype of *F. vesiculosus* and *F. radicans* confirm that this is a reliable method to use to separate these species.

The marine ecotype of *F. vesiculosus* has a higher photosynthetic maximum (P_{max}) compared to the brackish ecotype of *F. vesiculosus* and *F. radicans* whereas both the brackish species have similar P_{max}. A reason for higher P_{max} in the marine ecotype of *F. vesiculosus* compared to *F. radicans* is the greater relative amount of ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco). The reason for higher P_{max} in marine ecotype of *F. vesiculosus* compare to the brackish ecotype however is not due to the relative amount of Rubisco and further studies of the rate of CO_2 fixation by Rubisco is recommended. Treatments of the brackish ecotype of *F. vesiculosus* in higher salinity than the Bothnian Sea natural water indicate that the most favourable salinity for high P_{max} is 10 psu, followed by 20 psu. One part of the explanation to a high P_{max} in 10 psu is a greater relative amount of PsaA protein in algae treated in 10 psu. The reason for greater amount of PsaA might be that the algae need to produce more ATP, and are able to have a higher flow of cyclic electron transport around PSI to serve a higher rate of CO_2 fixation by Rubisco. However, studies of the rate of CO_2 fixation by Rubisco in algae treated in similar salinities as in present study are recommended to confirm this theory.

Keywords: Bothnian Sea, brackish, brown algae, D_1, 77 K fluorescence emission, *Fucus vesiculosus*, *Fucus radicans*, light-harvest antenna, mannitol, marine, NMR, Norwegian Sea, quantum yield, photosynthetic maximum capacity (P_{max}), photosystem, (PSI, PSII), PsaA, Rubisco, salinity.

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SVENSK SAMMANFATTNING (SUMMARY IN SWEDISH)

Fucus vesiculosus L. (Blåstång) är en brunalg som i huvudsak växer i tidvattenzonen i marint vatten men arten klarar också att växa konstant under ytan i det bräckta Bottenhavet. Norska havet och den del av Bottenhavet, där algerna är insamlade i denna studie, har salthalterna 34-35 psu (praktisk salthaltsenhet) respektive 4-5 psu. *F. radicans* L. Bergström *et* L. Kautsky (Smaltång) är en nyligen upptäckt art (2005) som har utvecklats i Bottenhavet. *F. radicans* och Bottenhavets ekotyp av *F. vesiculosus* växer sida vid sida och har tidigare ansetts vara samma art. Sett till hela Östersjön, så ändras ytans salthalt från 25 till 1-2 psu mellan Östersjöns gräns mot Kattegatt och norra Bottenviken. Den låga salthalten i Östersjön beror på det höga flödet av sötvatten från älvarna och på ett litet inflödet av saltvatten i inloppet vid Kattegatt. Salthaltsgradienten är korrelerad med antalet arter som minskar med minskad salthalt. Östersjön är ett artfattigt hav och de arter som finns är till stor del en blandning av söt- och saltvattenarter. Det finns bara ett fåtal arter som är helt anpassade till bräckt vatten och *F. radicans* är en av dem. Exempel på miljöskillnader för *F. vesiculosus* i Norska havet och i Bottenhavet är salthalten, tidvattnet, ljuset och temperaturen. Tidvattnet i Norska havet gör att algerna växlar mellan att vara i vattnet och på land, vilket utsätter algerna för stora ljusskillnader, snabba och stora temperaturväxlingar samt även torka. De alger som växer i Bottenhavet har däremot en jämnare och lägre temperatur, istäcke på vintern och mindre tillgång på ljus eftersom de alltid lever under vattenytan. Skillnaderna i miljön mellan växtplatserna leder till skillnader i fysiologiska anpassningar. Anledningen till att *F. vesiculosus* och *F. radicans* valdes som studieobjekt i denna avhandling är att de är viktiga nyckelarter i Bottenhavet. *F. vesiculosus* och *F. radicans* är de enda större bältesbildande alger som finns i det artfattiga ekosystemet och de används därför flitigt som mat, gömställe, parningsplats och barnkammare för t.ex. fisk. Att de är nyckelarter gör det angeläget att försöka förstå hur algerna är anpassade och hur de reagerar på miljöförändringar för att få veta hur de kan skyddas och bevaras. *F. radicans* inkluderades även för att se hur en naturlig art i Bottenhavet är anpassad i jämförelse med den invandrade *F. vesiculosus*. Marin *F. vesiculosus* inkluderades för att vara en artreferens från artens naturliga växtplats.

Studien visar att det finns fler vattenlösliga organiska substanser (finns vissa organiska substanser som har en proteinskyddande funktion) i den marina ekotypen av of *F. vesiculosus* än i Bottenhavets ekotyp. Anledningen till detta föreslås vara en anpassning till att växa i tidvattenzonen. Vid lågvatten utsätts *F. vesiculosus* från Norska havet för starkt ljus, uttorkning, och snabba temperatur-växlingar vilket gör att den kan behöva dessa organiska substanser som skydd mot

fria syreradikaler som bildas under lågvattenexponeringarna. *F. vesiculosus* från Bottenhavet har troligen mist förmågan att syntetisera dessa substanser på grund av anpassning till att hela tiden växa under ytan. Mängden mannitol (socker) är högre i den marina ekotypen av of *F. vesiculosus* än i Bottenhavets ekotyp. Detta föreslås bero på högre fotosyntetiskt maximum i *F. vesiculosus* från Norska havet jämfört med ekotypen från Bottenhavet. Skillnaden i fotssyntetiskt maximum är bland annat kopplat till ljus- och salthaltskillnaden på algernas växtplatser. Denna teori styrks av att både fotosyntesen och halten av mannitol ökar i Bottenhavets ekotyp när den behandlas i högre salthalt.

Studien visar även att båda ekotyperna av *F. vesiculosus* samt *F. radicans* har ett ojämnt förhållande mellan fotosystem II och I (PSII och PSI) med en dominans av PSI. Denna slutsats är baserad på fluorescens emissions mätningar vid 77 K (-196 °C) och mätning av den relativa mängden D_1 protein (motsvarar PSII) och PsaA protein (motsvarar PSI). *F. vesiculosus* från Bottenhavet visar ett emission spektrum som pekar mot en jämnare fördelning av PSII och PSI jämfört med den marina ekotypen och *F. radicans*. Detta stämmer dock inte med förhållandet mellan D_1/PsaA som indikerar att alla tre har mer PSI än PSII. Förklaringen till avvikelsen mellan metoderna antas vara att *F. vesiculosus* från Bottenhavet har större ljus-infångande antennpigment än marin *F. vesiculosus* och *F. radicans*. De tydliga skillnaderna i 77 K fluorescens emission spektra mellan Bottenhavets *F. vesiculosus* och *F. radicans* visar att denna metod kan användas som säker artidentifiering.

Den marina ekotypen av *F. vesiculosus* har högre fotosyntetiskt maximum än de båda arterna från Bottenhavet. Mätningar av den relativa mängden av enzymet Rubisco, viktigt för upptaget av koldioxid hos växter och alger, visar att mängden enzym är en sannolik förklaring till skillnaden i fotosyntetiskt maximum mellan den marina ekotypen av *F. vesiculosus* och *F. radicans* och detta är troligen en normal artskillnad. Mängden Rubisco kan dock inte förklara skillnaden i fotosyntetiskt maximum mellan de båda ekotyperna av *F. vesiculosus*. För att undersöka vad skillnaden mellan dessa två beror på så föreslås istället mätningar av Rubisco's koldioxidfixeringshastighet.

Det är en ökning av fotosyntetiskt maximum i Bottenhavets ekotyp av *F. vesiculosus* när den behandlas i högre salthalt (10, 20 och 35 psu) och det högsta fotosyntetiska maximumet uppmättes i alger som behandlats i 10 psu. Denna ökning beror inte på ökning i den relativa mängden av Rubisco. Ökningen i fotosyntesen speglas dock av en ökning av den relativa mängden PsaA. Detta antas bero på att det behövs mer energi i form av ATP och att en ökning av detta kan ske på grund av att mer PsaA kan driva den cykliska elektrontransporten i fotosyntesreaktionen. Ökat behov av ATP antas bero på en ökning av Rubisco aktiviteten men mätning av aktiviteten krävs för att bekräfta detta.

TABLE OF CONTENTS

PAPERS

Included Papers

The thesis is based on four **Papers** which are referred to in the thesis by Roman numerals (**Paper I-IV**):

Paper I Ecotype differentiation in qualitative content of water soluble organic compounds between marine and brackish *Fucus vesiculosus L.* (Phaeophyceae). Gylle AM, Isaksson D & Ekelund NGA. 2009. *Phycological Research,* 57: 127-130.

Paper II Desiccation and salinity effects on marine and brackish *Fucus vesiculosus* L. (Phaeophyceae). Gylle AM, Nygård CA & Ekelund NGA. 2009. *Phycologia*, 48 (3): 156-164.

Paper III Fluorescence emission spectra of marine and brackish-water ecotypes of *Fucus vesiculosus* and *Fucus radicans* (Phaeophyceae) reveal differences in light-harvesting apparatus. Gylle AM, Rantamäki S, Ekelund NGA & Tyystjärvi, E. 2011. *Journal of Phycology*, 47 (1): 98-105.

Paper IV Photosynthesis and relative amounts of photosynthetic proteins (D_1, PsaA and Rubisco) in marine and brackish water ecotypes of *Fucus vesiculosus* and *Fucus radicans* (Phaeophyceae). Gylle AM, Nygård CA, Svan IC, Pocock T & Ekelund NGA. *Manuscript*.

Contribution to Included Papers

Paper I: Took part in planning and field work, performed the experiment, laboratory work, did the most of the data analysis and wrote the paper.

Paper II: Took part in planning and field work, performed the experiment and the chlorophyll and mannitol part of the laboratory work, did the most of the data analysis and wrote the paper.

Paper III: Took part in planning and field work, performed the experiment and the laboratory work, except the kinetics measurements, did the most of the data analysis and wrote the paper.

Paper IV: Took part in planning and field work, performed the experiment and the SDS-PAGE and immunoblotting part of the laboratory work, did the most of data analysis and wrote the paper.

Related Papers not Included in this Thesis

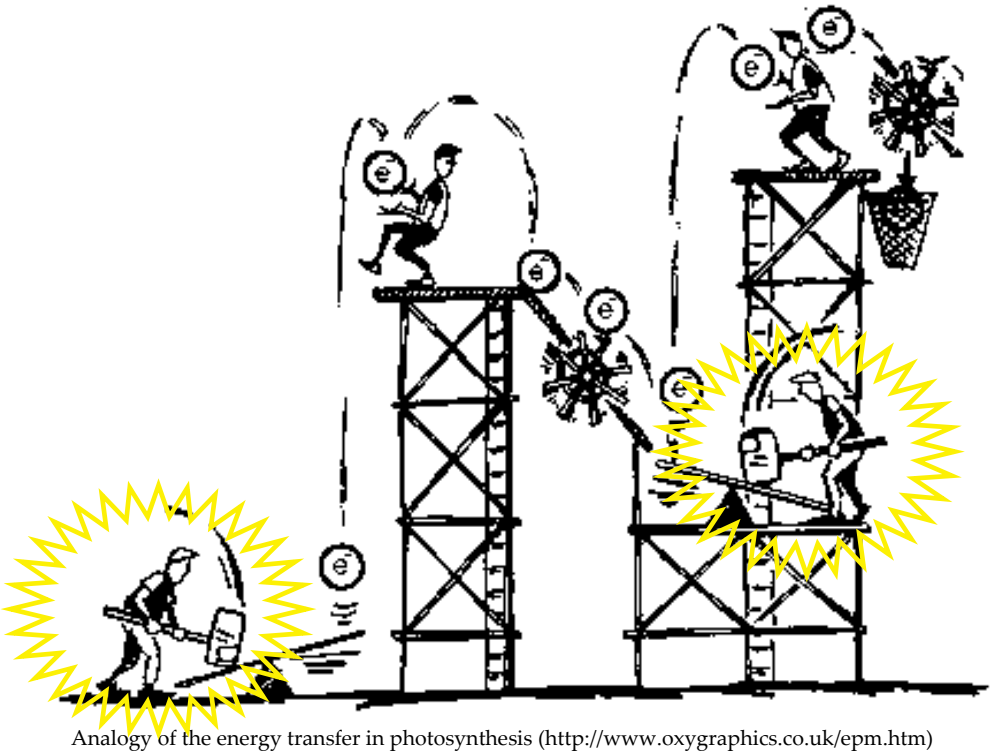
Impacts of UV radiation on photosynthesis of *Fucus vesiculosus* at low temperature and different salinities. Nyberg (Gylle) M, Nygård CA, Ekelund NGA (2002) V*erh Internat Verein Theor Angew Limnol* 28: 242-245.

In situ study of relative electron transport rates in the marine macroalga *Fucus vesiculosus* in the Baltic Sea at different depths and times of year Ekelund NGA, Nygård CA, Nordström R, Gylle AM (2008). *J Appl Phycol* 20: 751–756.

Elucidation

1. The figures and tables reference written in **bold style** refers to figures and tables in this thesis, and not to figures or tables in the included **Paper I-IV** or other references.
2. The quantifications made in **Paper I-IV** are made in the aims to be a relative comparison between the included algae and not in the aims to be absolute quantifications.
3. The studies in **Paper I-IV** are made in laboratory environment.
4. The word adaptation and acclimatization are used in the way they are described in Henderson's Dictionary of Biology (abbreviations and dictionary below; Lawrence, 2008). The word adaptation is mainly used with respect to the third point in the explanations with the exception of "dark adaptation" which refers to a short treatment in dark (mostly ~20 min).

This doctoral thesis is dedicated to my husband with love – you are the "sun" in this analogy of photosynthesis:



Analogy of the energy transfer in photosynthesis (http://www.oxygraphics.co.uk/epm.htm)

ABBREVIATIONS AND DICTIONARY

ADP/ATP: adenosine-di/tri-phosphate, molecules involved in energy transfer
AF: absorption factor
ASW: artificial sea water
Chl *a*, Chl *c*: chlorophyll *a*, chlorophyll *c* (*c*1 and *c*2)
DW: dry weight
ETR: electron transport
F_m: maximum chlorophyll *a* fluorescence in dark adapted algae
F_o: minimum chlorophyll *a* fluorescence in dark incubated algae
F_v: variable chlorophyll *a* fluorescence (F_m-F_o)
F_v/F_m: maximum quantum yield of photosystem II photochemistry
$\Delta F/F'_m$: effective quantum yield of photosystem II photochemistry
FW: fresh weight
LHC: light-harvesting antenna complex associated to photosystem
M1PDH mannitol-1-phosphate dehydrogenase enzyme
$NADP^+$ / $NADPH_2$: nicotinamide adenine dinucleotide phosphate, carrier of reducing - power
NMR: nuclear magnetic resonance
BSW: Bothnian Sea water
P680; P700: photosynthetic reaction center in photosystem II and I, respective
PAR photosynthetic active radiation
P_{max}: photosynthetic maximum capacity
PS: photosystem (PSII and PSI)
psu: practical salinity units
Q_A; Q_B primary and secondary quinine electron acceptor on D_2 and D_1 protein, respective
Rubisco: ribulose-1.5-bisphosphate carboxylase/oxygenase
Acclimation: physiological habituation of an organism to a change in a particular environmental factor for example the onset of winter (Lawrence, 2008).
Acclimatization: physiological and/or behavioural habituation of an organism to different climate or environment (Lawrence, 2008).
Adaptation: 1) evolutionary process involving genetic change by which a population becomes fitted to its prevailing environment 2) structure or habitat fitted for some special environment or activity; 3) processes by which a cell, organ or organism becomes habituated to a particular level of stimulus then being needed to produce a response (Lawrence, 2008).

INTRODUCTION

Why *Fucus*?

In the brackish water of the Bothnian Sea, the brown algae *Fucus vesiculosus* L. and *Fucus radicans* L. Bergström *et* L. Kautsky (Bergström *et al*., 2005) grow side by side. The species belongs to the class Phaeophyceae and are the only large belt-forming algae in the Bothnian Sea (northerly part of the Baltic Sea). As the only large belt-forming algae, the species are important for the functioning in the ecosystem. The algae are key species and provide other species, such as some fish and invertebrate species, with habitats for feeding, sheltering and breeding (Kautsky *et al*., 1992; Engkvist *et al*., 2004; Råberg & Kautsky, 2007). One example is *Idotea baltica´s* (Baltic isopod, Tånggråsugga) use of the algae for grazing. *I. baltica* is even also a part of the structuring force in macroalgae communities in the southern Baltic Sea (Engkvist *et al*., 2004) which confirm that *Fucus* constitute a basis for food webs.

As a consequence of the importance of *F. vesiculosus* and *F. radicans* in the Bothnian Sea ecosystem it is of high interests to increase the understanding of the physiology of the algae in relation to the environment, and changes in the environment, to know how to protect these species from harmful anthropogenic disturbances. *F. vesiculosus* has an ability to survive and grow in a wide range of natural environmental conditions, for example a broad salinity gradient from the brackish waters in the Bothnian Sea to the normal marine salinity in the Atlantic Ocean. This makes it highly interesting to study the species from an ecophysiological point of view. To better understand the physiological adjustments for the *F. vesiculosus* in the Bothnian Sea, this ecotype has been compared to the *F. vesiculosus* ecotype growing in the algae's original environment of fully marine water. The physiological adjustment to salinity and the tolerance for changed salinities of the marine (Norwegian Sea) and brackish (Bothnian Sea) ecotype of *F. vesiculosus* are some questions addressed in this thesis. *F. radicans* was included in two of the studies in this thesis because of its importance for the ecosystem functions mentioned above but also in order to investigate how the native Bothnian Sea species is adapted to the environment compared to the Bothnian Sea ecotype of *F. vesiculosus*. *F. radicans* is a recently discovered species and not much is known about the physiology in the alga.

The Baltic Sea

The Baltic Sea has since the last ice age pass through several different stages and has only been in the present form for ~3000-3500 years. The Baltic Sea may

therefore be considered as a relatively young ecosystem (Voipio & Leinonen, 1984). The first weak marine influence in the Ancylus Lake stage is recorded about 10 100 calibrated years before present (BP) (*c.* 8900 ^{14}C BP), representing a complex transition to the later Littorina Sea with different phases of brackish-water inflow (Andrén *et al.*, 2000). The large fluctuations of the salinity in the area during these different phases have probably altered between 0 and 10–15 practical salinity units (psu; Gustafsson & Westman, 2002). The present Baltic Sea (**Figure 1**) has a lower salinity than the previous Littorina Sea (~8000-4000 years BP; Björk, 1995). The alternations in phases have formed the Baltic Sea´s ecology and biological diversity through time (Johannesson & André, 2006). The changes in environmental conditions from a fresh water lake to a marine environment occurred relatively fast and possessed a significant stress on the organisms. The present Baltic Sea is an ecologically marginal zone ecosystem for immigrated marine species and many species demonstrate signs of isolation and on the average the Baltic Sea populations, e.g. *F. vesiculosus*, have lost genetic diversity compared to the Atlantic Ocean populations (Johannesson & André, 2006). The present salinity in the Baltic Sea is regulated by freshwater inflow from precipitation and rivers and the marine contribution of water through the Baltic Sea entrance at the Kattegat (HELCOM, 2006). The Baltic Sea area has a surface salinity gradient between the range of 25 psu at the entrance from Skagerrak, 4-6 psu in the Bothnian Sea, and 1-2 psu in the most northern part of the Bothnian Bay (HELCOM, 1996).

The salinity in the Baltic Sea, as it put forward by some scientists, is expected to be even lower in the future due to an increase of precipitation and runoff in the northern part of the sea as a response to higher temperature due to climate changes (HELCOM, 2006). On the other hand, as it put forward by other scientists, it is not obvious how a climate change will influence the Baltic Sea salinity (Omstedt & Hansson, 2006). Due to several feedback mechanisms, a

Figure 1. The present borders of the Baltic Sea (map modified from the webpage HELCOM, 2011).

warmer atmosphere may reduce snow on land and ice cover on sea and increase the evaporation, which may cause reduced river runoff and net precipitation over the Baltic Sea. The Baltic Sea is influenced by large-scale atmospheric circulation and changes in the atmospheric circulation may cause a shift in the hydrological cycle (Omstedt & Hansson, 2006). The most recent results and calculations, due to climate changes and temperature rise, predict an increase of the salinity in the Baltic Sea with 2-3 psu. The reasons for this prediction are a greater reduces of river runoff in the southern part of the Baltic Sea compared to the expected increase in river runoff in northern part, which is a net-decrease of the fresh water inflow (Hansson *et al.*, 2010). The salinity increase is also due to the oncoming raise of sea level and thereby an enlarging of the marine water inflow into the Baltic Sea (Gustafsson, 2004). Climate change is also off interest for the temperature in the water and the ice cover of the Baltic Sea. Calculations indicate that the Baltic Sea will become almost completely ice free with an on average increased air temperatures of 2 °C. Beyond the ice cover and the sea temperature, the temperature also influence the stratification (Omstedt & Hansson, 2006).

Changes in the salinity have a great impact on the ecosystem in the Baltic Sea. If the prediction of 1) decreased salinity agrees, it will become a decline in, for the ecosystem functioning, important marine species diversity (**Figure 2**). The Baltic Sea is a species-poor ecosystem and the distribution of species are a mix of fresh water and marine organisms and only few species have been evolved to brackish specialists. Most of the species are believed to have colonised the area during the latest 8000 year (Snoeijs, 1999). A species-poor ecosystem is more vulnerable to disturbances, e.g. alien species, than a species-rich ecosystem (Kaiser *et al.*, 2006). If on the other hand the scenario with 2) increased salinity agrees it will be more favourable for the marine species. Thus, in either scenario transition in the salinity gradient is expected and makes the study of the ecophysiology of *Fucus* even more vital.

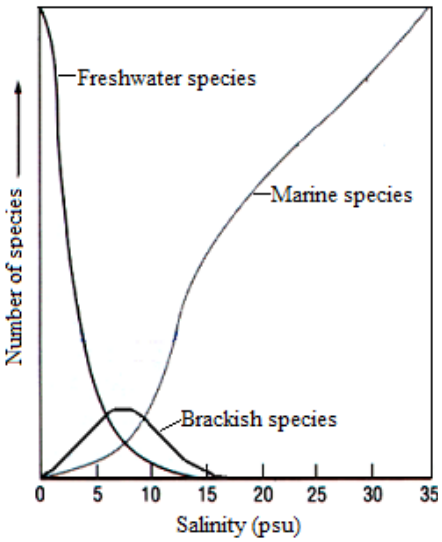


Figure 2. The made to order Remane diagram show the diversity trend in terms of number of species related to the salinity. The diagram is modified from Attrill &Rundle (2002).

Growth Conditions: Norwegian Sea versus Bothnian Sea

In general, the growth and distribution of algae are mainly controlled by competition, salinity, light, temperature, nutrients, substratum, sedimentation, ice scouring and strength in water movements (Ramus e*t al*., 1977; Wethey, 1985; Kautsky & Kautsky, 1989; Kirst, 1989; Malavenda & Voskoboinikov, 2009). These environmental conditions are highly diverse between the Norwegian Sea (part of the Atlantic Ocean; **Figure 3**) and the Bothnian Sea (part of the Baltic Sea; **Figure 1, 3**). Also the depth distribution of macroalgae is affected by the environmental conditions as e.g. light. The depth distribution of macroalgae in the Baltic Sea is mostly controlled by light, sediment cover and ice-scouring (Wærn, 1952; Bäck & Ruuskanen, 2000; Eriksson & Johansson, 2003) whereas the depth distribution of the marine macroalgae is highly affected by species competition (Ramus *et al*., 1977). The ongoing eutrophication, nevertheless, increase the competitive environment in the Baltic Sea. Ephemeral and fast-growing species benefit from the increased nutrient at the expense of perennial slow-growing species, such as *Fucus*. Reduced levels of light because of shad from epiphytic algae and decreased light penetration into the water column because of higher amount of phytoplankton and other particles force the algae to growth shallower. Several investigations demonstrate that *F. vesiculosus,* among other algae, growing shallower and shallower in the Baltic Sea (Eriksson *et al.,* 1998; Bergström, 2005; Torn *et al*., 2006; Korpinen *et al.,* 2007; Rhode *et al*., 2008; Schories *et al.,* 2009). Chlorophyll (Chl) *a* concentration has been observed to increase in *F. vesiculosus* in environments with reduced levels of light, but not enough to compensate for the light deficiency on growth (Rhode *et al*., 2008). Growing shallower in the Baltic Sea will make the *F. vesiculosus* belts less stable, because a larger part of the belts will be affected by disturbances such as ice-scouring, low-water events and strong wave actions. This in turn might change the overall productivity and ecology in the whole algae belt community (Eriksson *et al.,* 1998). Another negative effect from the eutrophication is the reduced opportunities for algae zygotes to establish. The establishment of zygotes from perennial macroalgae, as e.g. *Fucus* require bare rocks. Eutrophication leads to increased sedimentation, due to the higher amount of phytoplankton, as well as by fast-growing filamentous algae covered substratum and reduce the accessibility of bare rocks (Schramm, 1996; Kautsky & Serrao, 1997). So far however, the eutrophication in the more southern part of the Baltic Sea has not, to so great extent, affected the *Fucus* communities in the Bothnian Sea, where algae were collected in this study.

Salinity

In the areas for collection of algae in present study the salinity was 34-35 psu for the marine algae in the Norwegian Sea and between 4-5 psu for the brackish algae in the Bothnian Sea (**Figure 3**).

The ability to acclimate to changed salinity and occurrence of physiological responses because of changed salinity have been compared between marine and brackish ecotype of *F. vesiculosus* with respect to water soluble organic compounds (**Paper I**), relative amount of mannitol concentration and Chl *a*, c_1 and c_2 (Chl *c*) concentrations (**Paper II**). Salinity change effects on spectral features, Chl *a* and *c* content (**Paper III**) oxygen evolution, the relative amount of ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco), D_1 protein (core protein of photosystem II, PSII) and PsaA protein (core protein of photosystem I, PSI) in the brackish ecotype of *F. vesiculosus* was studied as well (**Paper IV**). Physiological differences, between the marine the brackish ecotypes of *F. vesiculosus* and between *F. vesiculosus* and *F. radicans* without any experimental influence were also studied. The studied parts were spectral features by Chl *a* fluorescence emission and Chl *a* and *c* content in **Paper III** and photosynthetic maximum capacity and relative amount of Rubisco, D_1 and PsaA protein in **Paper IV**.

Tide versus no Tide: Light, Temperature and Desiccation

The light and temperature are different between the algae's growth site in the Norwegian Sea compared to the growth site in the Bothnian Sea, mainly because of the tides in the Norwegian Sea but also because of the part time ice cover in the Bothnian Sea.

Light: The optical characteristics of the growth environment of the Bothnian Sea algae differ greatly from those of the Norwegian Sea algae grow in tidal zone as e.g. *F. vesiculosus*. The Norwegian Sea ecotype of *F. vesiculosus* alternate between exposure to unfiltered sunlight during low tide and lower irradiance and filtered sunlight during high tide. In sea water, wave scattering, dissolved substances, suspended sediments, and density of planktons reduce the depth of light penetration and diminish the amount of light availably for the photosynthesis (Dring, 1992). The productive zone of coastal sea water absorbs the blue and red parts of the visible spectrum at shallow water and allowing the green and green-yellow wavelengths to penetrate deepest (Dring, 1992). The constant sublittoral growing Bothnian Sea species receive much lower irradiance and on the average a narrower range of wavelengths, mainly the blue-green light, than the Norwegian Sea ecotype *F. vesiculosus.* In the present study, the ability to tolerate differences in salinity in both light and darkness, with respect to effects on relative amount of mannitol and Chl *a* and *c* content, have been compared between the marine and

brackish ecotype of *F. vesiculosus* (**Paper II**). Light is also an important part of the discussions in **Paper III** and **IV**.

Temperature and desiccation: At low tide, the intertidal marine algae *F. vesiculosus* can be exposed to partly desiccation and high or low (freezing) temperatures during summer and winter, respectively. The temperature and the risk of desiccation for intertidal algae's fluctuate several times every day in the tidal rhythm. The Bothnian Sea species are not exposed to desiccation or to extreme and fast temperature changes but grows in a constant lower temperature compared to the marine ecotype of *F. vesiculosus*. In the present study, the ability to tolerate desiccation at different temperatures with respect to photosynthetic yield and mannitol content has been compared relatively between the marine and brackish ecotype of *F. vesiculosus* (**Paper II**).

Inorganic Carbon, pH and Nutrients

In seawater, dissolved inorganic carbon (DIC) is present as a mixture of, and equilibrium between, CO_2, HCO_3^- and CO_3^{2-}. The relative proportion of CO_2 and HCO_3^- and CO_3^{2-} in seawater depends on pH, salinity and temperature. At low pH most of the DIC occurs as CO_2 and at high pH most of the DIC occurs as CO_3^{2-}. In marine water (35 psu) with a pH around 8.2, 90 % of DIC is presented as HCO_3^- (Lobban & Harrison, 1997). The total concentration of DIC is higher in Norwegian Sea compared to the Bothnian Sea. In marine water the amount is ~2.0 mol m^{-3} (Surif & Raven, 1989) and in the brackish water the amount is ~1.0 mol m^{-3} (Raven & Samuelsson, 1988).

As mentioned above, the eutrophication in the Baltic Sea affects perennial algae, as *F. vesiculosus* and *F. radicans*, negatively by e.g. reducing light penetration in the water. It has also been confirmed that high level of nutrients limits the growth of perennial macroalgae, including *F. vesiculosus* whereas annual algae are stimulated by nutrient enrichment (Kraufvelin *et al*., 2010). However, greater amounts of nutrients have also been confirmed to contribute to an increase of photosynthesis in the Baltic Sea *F. vesiculosus* (Nygård & Dring, 2008).

The Species

Area of Distribution, Morphology and Reproduction

The brown algae *F. vesiculosus* is primarily a marine, North Atlantic, intertidal species (Powell, 1963) but the alga is also found in the sublittoral of the brackish Baltic Sea in areas with salinity down to approximately 4 psu (Wærn, 1952; **Figure 3**). *F. radicans* is a native brackish water species and in all probability endemic to

the Bothnian Sea and its immediate surroundings (Bergström *et al*., 2005; Pereyra *et al.,* 2009; **Figure 3**).

Comparison of the morphology between the marine and brackish ecotype of *F. vesiculosus* confirm that the ecotype from low salinity is smaller, have thinner thallus and lack bladders (Kalvas & Kautsky, 1993; Ruuskanen & Bäck, 1999; **Figure 4a-b**). The reasons for smaller size in low salinity are probably due to low photosynthetic rate, high respiration (Munda & Kramer, 1977; Nygård & Ekelund, 2006) and a constant regulation of the cellular osmotic potential (described below; Munda & Kramer, 1977; Kaiser *et al*., 2006).

There are also differences between brackish *F. vesiculosus* and *F. radicans* from the Bothnian Sea with smaller, thinner thallus and more branches at *F. radicans* (Bergström *et al*., 2005; **Figure 4b-c**).

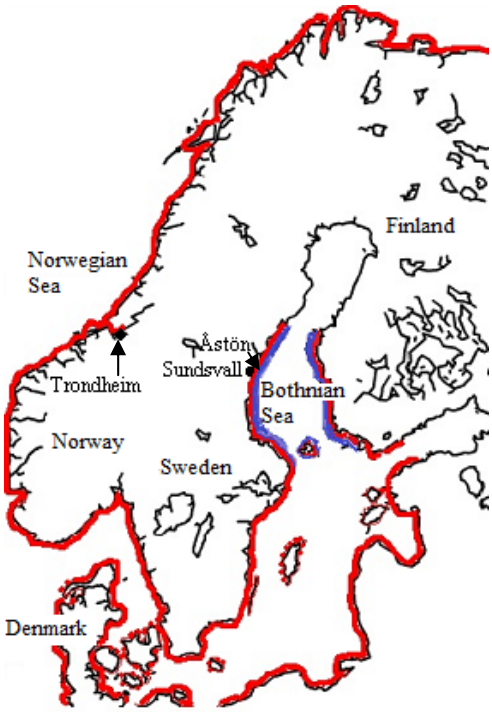


Figure 3. The range of distribution of *Fucus vesiculosus* (—) and the so far known range of distribution of *F. radicans* (—) around Scandinavia and Finland. Algae studied in present thesis were collected near Trondheim and at Åstön (map modified from the webpage Aqua-Scope, 2010).

The northern distribution limit of the Baltic Sea ecotype of *F. vesiculosus* is probably determined by the osmotic tolerance of the gametes (Serrão *et al*., 1996). According to Serrão *et al*. (1999), *F. vesiculosus* does reproduce sexually in salinities down to 4 psu but the reproduction is inhibited by physiological problems when the salinity becomes too low. However, *Fucus* in the low salinity part of the Baltic Sea have also evolved adaptive ecological characteristics by using of asexual reproduction by vegetative propagules (spores; Tatarenkov *et al*., 2005; Bergström *et al.,* 2005) and recent findings of genetic diversity of *F. vesiculosus* show 30% cloned individuals in the northern Baltic Sea (Johannesson & André, 2006). *F. radicans* reproduce sexually but only to an extent of 20% of the individuals, the rest of the individuals have asexual reproduction (Johannesson & André, 2006).

Relationship and Genetic Divergence

An analysis of highly polymorphic microsatellite DNA loci have been used to confirm genetic divergence between *F. radicans* and *F. vesiculosus* (Bergström *et al.*, 2005; Pereyra *et al.*, 2009). *F. radicans* has been revealed to emerging from a *F. vesiculosus* lineage in the Baltic Sea but is clearly genetically distinct from the brackish ecotype of *F. vesiculosus* and even more genetically distinct from the marine ecotype of *F. vesiculosus*. *F. radicans* and *F. vesiculosus* in the Baltic Sea started to diverge from a common population somewhere between 120 and 2500 years ago, probably as late as ~400 years ago. The exact mechanism of the *F. vesiculosus* – *F. radicans* speciation event is unknown but the extreme environmental stress forced by the low salinity water environment has most likely contributed to the development of *F. radicans* (Pereyra *et al.*, 2009). Apart from the genetic analysis of *F. vesiculosus* and *F. radicans* in the Baltic Sea, studies of genetic divergence between populations of *F. vesiculosus* from the Baltic Sea and Skagerrak (Tatarenkov *et al.*, 2007) and between populations at the east coast of North America and Greenland (Muhlin & Brawley, 2009) have been performed. The genetic differentiations and the effects of isolation by distance between populations from the Baltic Sea and Skagerrak were confirmed to be substantial (Tatarenkov *et al.*, 2007).

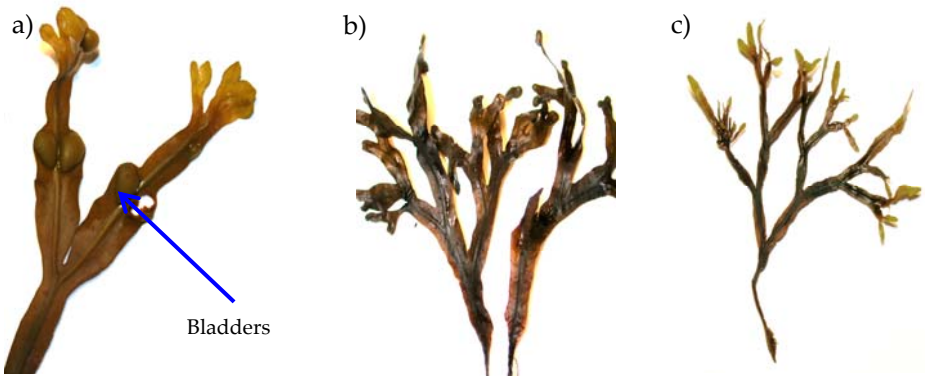


Figure 4. The distal parts of *Fucus vesiculosus* from the Norwegian Sea (34-35 practical salinity units, psu) a) and both distal and proximal parts of *F. vesiculosus* b) and *F. radicans* c) from the Bothnian Sea (4-5 psu; Photo: Maria Gylle).

Physiology

Distinctions in the environment between the marine growth sites and the brackish growth site have beyond given rise to differences in morphology, also

given rise to differences in physiological acclimatization and/or adaptation mechanisms between the marine and brackish ecotype of *F. vesiculosus*.

In earlier studies, *F. vesiculosus* from the Baltic Sea areas has been confirmed to have a lower growth rate, lower mannitol content, lower photosynthetic maximum capacity (P_{max}), greater dark respiration, lower ability to tolerant emersion stress, lower tolerance threshold for heavy metals and a lower tolerance to ultraviolet-b radiation and high level of irradiance compared to *F. vesiculosus* from marine areas (Bäck *et al.*, 1992a; Bäck *et al.*, 1992b; Pearson *et al.*, 2000; Nygård, 2005; Nygård & Ekelund, 2006; Nygård & Dring, 2008). The most important reasons for lower growth rate and lower P_{max} for the brackish ecotype of *F. vesiculosus* compared to marine ecotype were confirmed to be the low salinity followed by lower concentration of DIC (Nygård & Dring, 2008). For the recently discovered species *F. radicans* there are only few physiological investigations made. These studies however indicated that *F. radicans* has similar maximum quantum yield of PSII photochemistry as both ecotypes of *F. vesiculosus* and similar dark respiration and mannitol concentration as the Bothnian Sea ecotype (Nygård, 2005; Gylle, 2007).

Photosynthesis

Photosynthesis is the energy source for almost all life. Light energy is absorbed as photons by pigments in the light-harvesting antenna protein-pigment complex (LHC). LHC is located in the thylakoid membranes of the chloroplasts. The light absorbing pigments in *Fucus* are mainly Chl *a*, fucoxanthin and Chl *c*. Chl's absorb red and blue light whereas fucoxanthin mainly absorb in the green region of light (Dring, 1992). Photon capture by the LHC's and the excitation transfer to PSII and PSI provide the energy for oxidation of water (water split) and electron movement to electron acceptors (Lawlor, 2001). The photon energy is transferred between pigment molecules by resonance energy (a non-radiative physical process) until it reaches the core Chl *a* and the reaction centers (Taiz & Zeiger, 2006). When the absorbed energy reaches the reaction centers, an electron is excited to a higher energy level. In the excited stage of P_{680} (P_{680}*) in PSII reaction center the energy is 1) used for electron transport in the photochemical reaction where the light energy is converted to chemical energy (**Figure 5**), 2) re-emitted as photon energy through Chl *a* fluorescence when the excited electron falls back (**Figure 6**) or 3) dissipated as heat. The relative sum of the energy is constant, so if the probability for fluorescence increases the probabilities for photochemistry and/or heat dissipation has to decrease (Taiz & Zeiger, 2006).

Electron Transport, $NADPH_2$ and ATP in Photosynthesis

The electron transport system is found in the thylakoid membranes in the chloroplast and might be considered in five parts: 1) the water-splitting complex; 2) the PSII protein-pigment complex; 3) an electron carrier chain; 4) the PSI protein-pigment complex and 5) a group of electron carriers (reduce electron acceptors: $NADP^+$, O_2; Lawlor, 2001; **Figure 5**). These multisubunit complexes convert the light energy into chemical energy by catalyse of linear electron transport for a production of reducing power, $NADPH_2$, and carrier of energy, ATP. The energy from the electron transport chain and $2H^+$ reduce $2NADP^+$ to $2NADPH_2$ at the stroma side of PSI. The protons, produced at the water split generate ATP via ATP-synthase (catalyse ADP into ATP). ATP and $NADPH_2$ are used in the further steps of the photosynthesis reaction when CO_2 is reduced to carbohydrates by the Calvin cycle and for some other energy demanding processes such as nitrogen and sulphur metabolism (Taiz & Zeiger, 2006). The photosynthetic status can be determined by measuring of e.g. oxygen evolution or Chl *a* fluorescence. A usual way to present the data is by photosynthesis/irradiance curves (P/I curves; **Paper IV**). The initial slope (α) of the curve indicates the efficiency to use the absorbed light in the photosynthesis at limiting irradiance and the point where higher level of irradiance no longer increase the photosynthesis, light saturation, indicate P_{max}.

Photosystem II

Most of the electron transfer in PSII is coordinated by the core subunits proteins, D_1 and D_2 in the reaction center (Mattoo *et al*., 1999). D_1:D_2 contains all the primary reactants for charge separation within the PSII reaction center and are structurally organized in five parts with binding sites for Chl´s, pheophytins (Pheo), iron, caretenoids and plastoquinones where Q_A bounds to D_2 and Q_B bounds to D_1 (McEvoy & Brudvig, 2006). Among components of PSII, the D_1 protein is the most vulnerable for environmental stress. The D_1 protein is rapidly cycled during illumination and disruption of D_1 protein cycling or losses of D_1 protein pools are central to the photoinhibition of photosynthesis. The damage of the D_1 subunits requires D_1 re-synthesis and D_1 replacement within PSII (Dasgupta *et al*., 2008). Photoinhibition occurs by production of singlet oxygen, which modifies the Chl *a* binding part of D_1, under certain conditions as e.g. excess light, ultraviolet radiation, low or high temperatures and salt (Sudhir & Murthy, 2004; Nixon *et al*., 2005; Allakhverdiev *et al*., 2008; Dasgupta *et al*., 2008; Nixon *et al*., 2010).

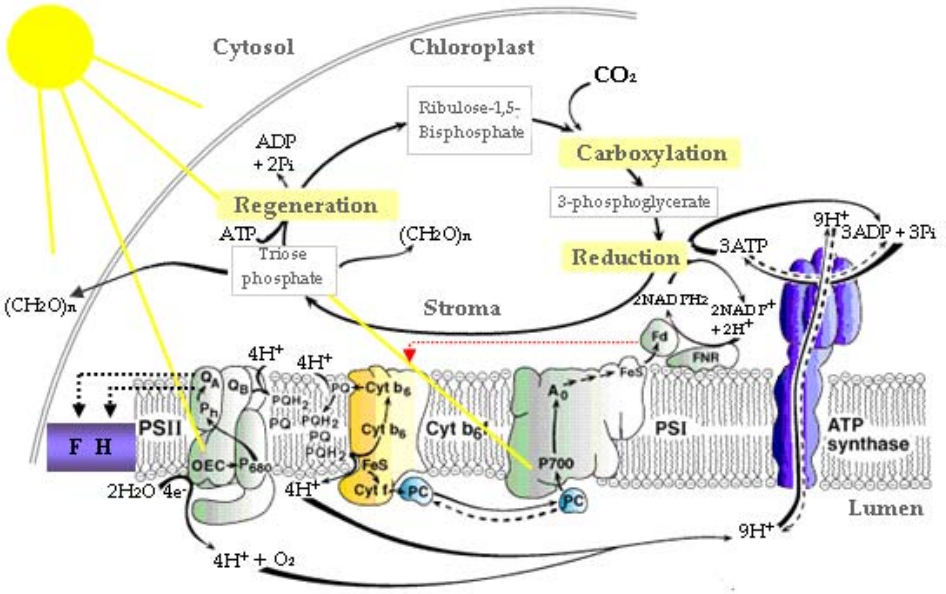


Figure 5. Photosynthetic electron transport in O_2-evolving organism's as plants, algae and blue-green bacteria. P_{680} is a chlorophyll molecule in the reaction center in photosystem II (PSII) which absorb light mainly at 680 nm. P_{700} is a chlorophyll molecule in the reaction center in PSI which absorbs light mainly at 700 nm. The primary step in oxygenic photosynthesis, the light induced charge separation, is when P_{680} absorb photons the molecule become excited to P_{680}* and transfer electrons to pheophytin (Ph). The oxidized P_{680}+ is re-reduced by the primary electron donator H_2O via Mn_4 in the oxygen evolution complex (OEC) and Y_z. When H_2O become oxidized the H_2O molecule split and ½ O_2 and $2H^+$ are released. On the reducing side of PSII, the electrons are transferred from pheophytin to the quinons, Q_A and Q_B, and further to the platstoquinon (PQ). The energized electrons pass through the electron transfer chain from molecules with more negative potential to molecules with less negative potential. When the PQ transfers electrons to the cytochrome b_6f complex they also bring H^+ from stroma to lumen and create a proton gradient over the thylakoid membrane, witch is used as energy in the ATP synthesis by the ATP synthase complex. The electrons from PQ are transferred further from cytochrome b_6f complex to plastocyanin (PC) and to PSI where the electrons reach the oxidized P_{700}+. Here the electrons is enegized again by excitation energy derived from photon energy trapped in the Chl. Electrons convert P_{700} to exited P_{700}* and the electrons transfers further from P_{700}* via the quinones, A_0 and A_1, membrane-bound iron-sulfur protein and ferredoxin (Fd) to the flavoprotein ferredoxin-NADP reductase (FNR) that reduces $NADP^+$ to NADPH. The dashed red line represent cyclic electron flow around PSI and the F and H, in the purple box at the left side of the figure, represent energy dissipation as fluorescence and heat, respectively (Lawlor, 2001; Taiz & Zeiger, 2006). The figure is modified from a webpage of Ort (2007).

Photosystem I

Reaction center in PSI contains up to 14 subunits of which two are the large core protein subunits PsaA and PsaB. PsaA and PsaB bounds most of reaction centers Chl *a* and all the cofactors involved in light induced electron transfer from the special Chl pair P700 to the electron acceptor ferredoxin (Hall & Rao, 1999; Nelson & Ben-Shem, 2006; Santabarbara *et al.,* 2010). In addition to the linear electron transfer involvement of PSI, there is a cyclic electron transfer around PSI. At least two pathways of cyclic electron transport have been introduced: 1) the ferredoxin-plastoquinone reductase dependent route and 2) the NAD(P) dehydrogenase dependent route (Johnson, 2005).

In the present study, P_{max} and the relative amount of D_1 protein (reflects relative amounts of PSII) and PsaA protein (reflects relative amounts of PSI) with respect to salinity, have been studied in the Bothnian Sea ecotype of *F. vesiculosus* (**Paper IV**). P_{max} and the relative amount of D_1 protein and PsaA protein have also been compared between the Norwegian Sea ecotype of *F. vesiculosus*, the Bothnian Sea ecotype of *F. vesiculosus* and *F. radicans* (**Paper IV**).

Light Absorption Balance between PSII and PSI

Acclimation to light regimes is one of the most important and complex responses of photosynthetic organisms to varying environmental conditions. Both the level of irradiance and the quality of light influence the PSI and PSII stoichiometry.

At low levels of irradiance, there are fewer PSII reaction centers in relation to PSI reaction centers (Anderson *et al*., 1995; Hihara *et al.,* 1998; Huang, 2006). Overall a decrease in irradiance results in an increase of LHC in both PSII and PSI. The increase of LHC might be achieved by 1) an increase in the size of existing photosynthetic units or 2) by an increase in the number of photosynthetic units (Dring, 1992; Lobban & Harrisson, 1997). In high levels of irradiance, however, there are a decrease of cellular pigment content, photochemical activities (per-cell basis) and LHC size of PSII. There is also an increase of the PSII to PSI ratios, Rubisco maximum photosynthetic rate (Anderson *et al*., 1995; Hihara *et al*., 1998; Huang, 2006). Acclimations of the photosystems stoichiometry serve to regulate the distribution of excitation energy between the photosystems and allow plants to maintain a high quantum efficiency of photosynthesis under diverse light quality (Chow *et al*., 1990; Anderson *et al*., 1995). In water, the light that reaching the photosynthetic species depends on the degree of sunshine but also of the waters optical absorbance, wave scattering, dissolved substances, suspended sediments, density of plankton, growth depth and if the water is tidal or not.

Calvin Cycle, Rubisco and Carbon Supply

The energy stored in ATP and reducing power stored in $NADPH_2$ is used in the Calvin cycle when CO_2 is reduced to carbohydrates through a series of reactions and intermediates. The Calvin cycle consists of three major parts: 1) CO_2 carboxylation of ribulose-1.5-bisphosphate (RuBP) catalyzed by Rubisco, 2) formation of triose phosphate by reduction of 3-phosphoglycerate catalyzed by 3-phosphoglycerate kinase and 3) regeneration of RuBP by several enzymatic reactions steps (Taiz & Zeiger, 2006; **Figure 5**). In plants, the triose phosphate is converted to fructose 6-phosphate and further to starch in the chloroplasts or sucrose in the cytosol. In *Fucus* and other brown algae, the triose phosphate is 1) converted to fructose 6-phosphate and further to laminaran in the cytosol or 2) converted to mannitol in the chloroplast and/or cytosol. The products are thereafter stored in the cytosol (Bidwell, 1958; Yamaguchi *et al.*, 1966; Kremer, 1985; Michel *et al.,* 2010). It has been suggested that the synthesis of laminaran and mannitol in the chloroplast are connected to the pyrenoids (Davis *et al*., 2003). Michel *et al. (*2010) identified the genes for the enzymes involved in carbon storage in the brown alga *Ectocarpus siliculosus* and confirmed that the alga has a complete set of enzymes for synthesis of mannitol, laminaran and trehalose but missing the pathways for sucrose, starch and glycogen.

Calvin cycle is the photosynthetic rate-limiting step because of the rate-limited CO_2 fixation by Rubisco. Rubisco is activated by Mg^{2+}, CO_2, light and specific Rubisco activase (Lobban & Harrison, 1997). The most common structure of Rubisco consists of eight large (L) subunits (50-55 kDa) and eight small (S) subunits (15 kDa), L_8S_8 (~550 kDa; Raven, 1997). The synthesis of Rubisco is regulated by light on both transcriptional and post-transcriptional levels (Berry *et al*., 1986). Reduced dark transcription rate of mRNA is compensated by an increase in the stability of the already available mRNA (Shiina *et al*., 1998). The levels of irradiance impact on the regulatory mechanisms on Rubisco synthesis has been suggested to be connected to the redox state in the chloroplasts (Salvador & Klein, 1999). In algae the carbon supply is important in the regulation of the mechanisms behind synthesis of Rubisco (Giordano *et al.,* 2005). *Fucus* use HCO_3^- and CO_2 as DIC sources. Rubisco, however, requires CO_2 as a substrate in catalysis of RuBP to 3-phosphoglycerate. Therefore, it has been suggested that HCO_3^- is converted to CO_2 by acidification or by carbonic anhydrases in the cell wall (Raven, 1997).

In the present study, the relative amount of Rubisco with respect to salinity has been investigated in the Bothnian Sea ecotype of *F. vesiculosus* (**Paper IV**). The relative amount of Rubisco has also been compared between marine ecotype of *F. vesiculosus*, brackish ecotype of *F. vesiculosus* and *F. radicans* (**Paper IV**).

Photosynthetic Apparatus - Plants versus Brown Algae

In general the photosynthesis is similar in Chl *a*/*b* plants and brown algae but there are some differences in photosynthetic apparatus between the organisms: e.g. 1) plants only use atmospheric CO_2 as DIC source while *Fucus* use both HCO_3^- and CO_2 (Lobban & Harrison, 1997), 2) plants have mainly Chl *a* and *b* as light-harvesting pigments whereas *Fucus* have Chl *a, c* and fucoxanthin (Dring, 1992), 3) the plant chloroplasts have two membranes whereas *Fucus* have two membranes plus two membranes of chloroplast endoplasmatic reticulum surrounding the organelle (Davis *et al*., 2003), 4) plants has grana thylakoids in the chloroplasts whereas the brown algae thylakoids are arranged in groups of three in the chloroplasts (Gibbs, 1970; Berkaloff *et al*., 1983), 5) CO_2 fixation by Rubisco and formation of carbohydrates in plants occurs in the chloroplast stroma whereas some brown algae have pyrenoids connected to the chloroplast where the reduce of CO_2 occurs (Davis *et al*., 2003), and 6) plants synthesis sucrose and starch as primary photosynthetic products while brown algae synthesis mannitol and laminaran (Bidwell, 1958; Yamaguchi *et al*., 1966; Davis *et al*., 2003).

Chlorophyll a Fluorescence

When an electron is excited to a higher level of energy state, by absorbed light photons in PSII, the energy has three different pathways to transform: 1) light energy convert to chemical energy by photochemical electron transport (described above), 2) Chl *a* fluorescence and/or 3) dissipate as heat. The Chl *a* fluorescence arise when the excited electron falls back and the energy is re-emitted as photon (light) energy through Chl *a* emission. The Chl *a* fluorescence emission can be analysed and used to determine the status of photosynthesis by e.g. measuring of maximum quantum yield of PSII photochemistry or electron transport (ETR). Chl *a* fluorescence emission can also be used for identification of spectral features, analysis of the photosynthetic apparatus and PSI/PSII stoichiometry.

The minimum fluorescence (F_o) is the emission from Chl *a* antenna before the photochemical events take place and all reaction center of PSII are in the open state (**Figure 6**). This reflects the size of PSII Chl antennae (Krause & Weise, 1991). Reaction center II is considered as open when charge separation between electron donator P_{680} and the primary electron acceptor pheophytin can occur; if not the reaction center II is closed (Lazár, 2003). F_o can be induced and measured by giving dark adapted algae a pulse of week red light and register the signal Chl *a* sends back as fluorescence (**Figure 6**). The maximum fluorescence (F_m) is the fluorescence when all reaction center II are closed (no further transfer of electrons from the reaction center Chl because all electron acceptors after pheophytin, quinon A, are reduced) and the fluorescence is emitted from excited state of the reaction center. F_m can be induced and measured by giving dark adapted algae a pulse of

saturating light (**Figure 6**). Variable fluorescence (F_v) is the difference between F_m and F_o. F_v indicate photochemical quenching of the fluorescence (photochemical electron transport). A combination of fluorescence variables expressed as $(F_m-F_o)/F_m = F_v/F_m$ indicate the maximum quantum yield of PSII photochemistry (Krause & Weis, 1991) or the ability for PSII reaction centers to use the available excitation energy for photochemistry (Falkowski & Raven, 1997). F_v/F_m is lowered by all effects that cause decrease in the rate of linear electron transport like inhibition of PSII reaction center and increase of heat dissipation. Both an increase of F_o and/or a decrease of F_m may contribute to a decrease of F_v/F_m. The level of emission of Chl *a* fluorescence in the pigment bed is affected by optical properties (e.g. thallus structure), size of Chl antenna, Chl *a* concentration, functionality of PSII reaction center, size of the core antenna versus peripheral antenna, rate of State-I-State-II transition, the efficiency of the photosynthetic protection system (e.g. xanthophylls cycle) and stress level of the algae (Björkman & Demmig, 1987; Krause & Weis, 1991; Hall & Rao, 1999; Pearson *et al*., 2000; Zhu *et al*., 2005).

Determination of the photosynthetic status by measuring of electron transport can be done by an initial measurements of F_v/F_m and then repeated measurements of $\Delta F/F'_m$ during stepwise increase of irradiance. $\Delta F/F'_m$ is the effective quantum yield of PSII photochemistry when the photosynthetic apparatus is hit by light. ETR is calculated as:

$$ETR = PAR * AF * \Delta F/F'm * 0.5$$

AF is the absorption factor and can be measured as the fraction of incident PAR absorbed by the thalli (Beer *et al.,* 2000; Nygård & Dring, 2008). PAR is the photosynthetic active radiation used at the stepwise increase of irradiance and 0.5 is used for allow of equal involvement of PSII and PSI (Beer *et al*., 2000; Nygård & Dring, 2008).

For identification of spectral features and analysis of differences in the photosynthetic apparatus and PSII/PSI stoichiometry in photosynthetic organisms, Chl *a* fluorescence emission spectra at 77 K (–196 °C) can be used. 77 K emission spectra provide information on the distribution of excitation energy between PSII and PSI. The emission spectrum of leaves at 77 K demonstrates three major peaks with maxima at around 685, 695 and 735 nm (Pospíšil *et al*., 1989) and it is confirmed that peaks at 685 and 695 nm originating in PSII and a peaks at 720-735 nm originating in PSI (Papageorgiou, 2004). The main emitter of the 685 nm peak in higher plants and cyanobacteria is the CP43 reaction center chlorophyll-binding protein while the 695 nm shoulder is emitted by the Chl's of the CP47 protein (Keränen *et al*., 1999).

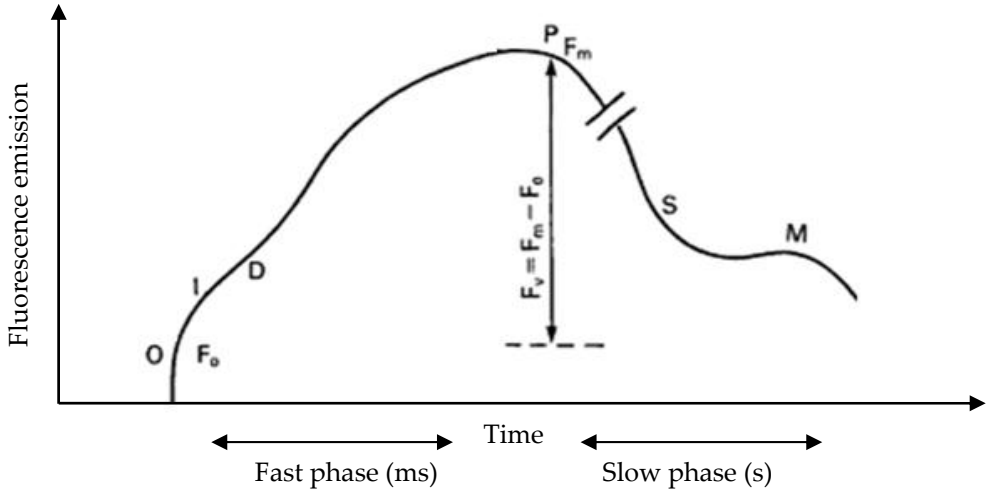


Figure 6. Schematic figure of chlorophyll *a* fluorescence induction from photosystem II (PSII) during illumination after a dark adaption. F_o, (0) is the minimum fluorescence when photosystem II (PSII) reaction centre's are open. Once the reaction centre chlorophylls are excited and quinon A (Q_A) becomes reduced (via pheophytin), the fluorescence rapidly rises to an inflection point, I. When electrons are transferred from reduced Q_A to Q_B the rate of increase in fluorescence decrease, D. F_m, (P) is the maximum fluorescence level when PSII reaction centre's are closed for photon capture because all Q_A are reduced. The S phase is mainly due to non-photochemical quenching because the Calvin cycle CO_2 assimilation is not fully activated. The M phase is caused by a temporary accumulation of $NADPH_2$ due to suboptimal rates of CO_2 reduction. F_v is the variable fluorescence. The figure is modified from Hall & Rao (1999).

In present study, Chl *a* fluorescence has been used as an indicator of photosynthetic status after experimental treatments (**Paper II**) and also in the aim to compare the photosynthetic status between marine ecotype of *F. vesiculosus*, brackish ecotype of *F. vesiculosus* and *F. radicans* (**Paper IV**). 77 K Chl *a* fluorescence emission spectra have been used to identify differences in the spectral features and PSII/PSII stoichiometry between the marine ecotype of *F. vesiculosus,* the brackish ecotype and *F. radicans.* The method has also been used to analyse effects of salinity changes at spectral features in the brackish ecotype of *F. vesiculosus* (**Paper III**).

Compatible Solutes, Osmolytes and Mannitol

Compatible solutes are organic molecules that sustain osmotic potential and protecting and stabilizing enzymes, membranes and structural macromolecules in the cell during salt stress conditions (Kirst, 1989). The protein protection effect probably occurs by maintaining a hydration shell around the protein surface

(Arakawa & Timasheft, 1983) or by direct interaction with the proteins (Schobert, 1977). Commonly accumulated compatible solutes in algae are e.g. sugar alcohols as mannitol and glycerol and the quaternary amine glycine betaine and the tertiary sulphonium compound β-(dimethylsulphonio)-propionate (DMSP) (Kirst, 1989).

A relevant example of osmoregulation is when the marine *F. vesiculosus* grows in the brackish, low salinity, water in the Bothnian Sea. In low salinity the algae are exposed to hypoosmotic stress and this leads to a need of reducing the influx of water into the cell. The algae adapt to low salinity by tolerating an expansion of the cell volume or by a reduction of osmotically active solutes. In higher salinity the opposite would occur. In the first phase of an osmotic acclimatization, a rapid change of cellular turgor pressure caused by water flow in or out of the cell takes place. The direction of the water flow depends on the osmotic gradient. In the second phase, an osmotic adjustment by cellular concentration of osmolytes occurs and this will continue until a new steady state is achieved (Kirst, 1989). The osmolytes consist of inorganic ions that accumulate in the vacuole and organic compounds accumulating in the cytosol (Kirst 1989; Bäck *et al*., 1992b). Brown algae are capable of osmotic adjustment by changed concentrations of inorganic ions, mostly K^+, Cl^- and NO_3^- in the vacuole (Kirst & Bisson, 1979; Davison & Reed, 1985) as well as changes in mannitol content in the cytosol (Munda & Kremer, 1977; Davison & Reed, 1985; Reed *et al*., 1985; Bäck *et al*., 1992b).

Mannitol is one of the most widely occurring sugar alcohol compounds and found in bacteria, fungi, algae and plants. In these organisms the compound acts as a compatible solute and has multiple functions, including osmoregulation, storage, regeneration of reducing power and scavenging of active oxygen species (Iwamoto & Shiraiwa, 2005). In *Fucus* mannitol is also one of the primary photosynthetic products (Bidwell, 1958; Yamaguchi *et al*., 1966; Davis *et al*., 2003). The synthesis of mannitol in brown algae has been suggested to occur in the cytosol (Kremer, 1985) and/or in the chloroplast connected pyrenoid (Davis *et al*., 2003). The pathway for synthesis of mannitol is revealed to be from the photosynthesis product triose phosphate via fructose-1.6-2phosphate, fructose-6-phosphate and mannitol-1-phosphate. An increase in the activity of the enzyme mannitol-1-phosphate dehydrogenase (M1PDH), catalysing the step between fructose-6-phosphate and mannitol-1-phosphate, has been confirmed as a response to increased salinities (Davison & Reed, 1985; Ivamoto *et al*., 2003). Davison & Reed (1985) suggest that an increase of mannitol in algae as a response to higher salinity also is an acclimation connected to photosynthesis which enables brown algae to accumulate mannitol for turgor adjustment. This suggestion is in agreement with an increase of photosynthetic products built-in to mannitol at higher salinities (Munda & Kremer, 1977). Lower mannitol content in the algae during the winter (Stewart *et al*., 1961; Davison *et al*., 1984; Gómez & Wiencke, 1998; Gylle, 2007) gives a lower capacity

for osmotic adjustment and it has been suggested that a variety of minor nitrogenous substances are part of cytoplasmic osmolytes during the winter (Davison & Reed, 1985).

In the present study, the qualitative contents of water soluble organic compounds and the compounds connection to salinity have been studied in marine and brackish ecotypes of *F. vesiculosus* (**Paper I**). The relative quantitative contents of mannitol and mannitol´s connection to salinity changes in light and dark have been studied in marine and brackish ecotypes of *F. vesiculosus* (**Paper II**).

The Aims of the Thesis

The general aims of this thesis were to compare physiological aspects between the Norwegian Sea ecotype and the Bothnian Sea ecotype of *F. vesiculosus* as well as between the two Bothnian Sea species *F. vesiculosus* and *F. radicans*. Experimental influences were above all to investigate the physiological ability of *F. vesiculosus* to tolerate different salinities and also to investigate the algae's responses to light and dark. More specific, the aims in the included **Papers** were:

- To compare the qualitative content of different types of water soluble organic compounds in the Norwegian Sea ecotype of *F. vesiculosus* with the Bothnian Sea ecotype and to evaluate whether qualitative content of compounds is related to salinity at their respective growth sites (**Paper I**).
- To investigate the response to temperatures and desiccation of the brackish ecotype of *F. vesiculosus* in comparison to the marine ecotype. In addition, the aim was to investigate the importance of salinity and light for mannitol as available energy reserve and osmotic adjuster, and also the importance of salinity and light for Chl content, in the two ecotypes of *F. vesiculosus* (**Paper II**).
- To investigate if the light conditions in the Bothnian Sea have caused compensatory changes in the photosynthetic apparatus of the Bothnian Sea ecotype of *F. vesiculosus* and *F. radicans* compared to the Norwegian Sea ecotype of *F. vesiculosus*. The aims were also to identify spectral features of both ecotype of *F. vesiculosus* and *F. radicans* and to analyse differences in the photosynthetic apparatus between the algae. In addition, the aim was to investigate if the native Bothnian Sea species *F. radicans* had different spectral features adaptations compared to the Bothnian Sea ecotype of *F. vesiculosus*. Finally, there was also an aim to analyse the short-term (1 week) effect of salinity (5, 10, 20, and 35 psu) on the photosynthetic apparatus of the Bothnian Sea ecotype of *F. vesiculosus* (**Paper III**).

- To investigate photosynthetic differences between the marine ecotype of *F. vesiculosus* and the brackish ecotype of *F. vesiculosus* and *F. radicans* and to study if photosynthetic differences were correlated to the relative amounts of photosynthetic proteins, as Rubisco, D_1 (reflects PSII) and PsaA (reflects PSI). In addition, the aim was to investigate if the native Bothnian Sea species *F. radicans* had other adaptations to the environment than the, in origin marine intertidal *F. vesiculosus* ecotype in the Bothnian Sea. Further to this, the aim was to investigate if photosynthesis and the relative amounts of Rubisco, D_1 and PsaA were correlated when the Bothnian Sea ecotype of *F. vesiculosus* was affected by varying salinities (5, 10, 20, 35 psu) in the short-term (1 week; **Paper IV**).

MATERIALS AND METHODS

Collections and Cultivations

The marine ecotype of *F. vesiculosus* was collected from the intertidal area at the Norwegian Sea in Norway (34-35 psu; Trondhjem Biological Station; 63°43´N; 10°39´E; **Paper I-IV; Figure 3**). Brackish *F. vesiculosus* (**Paper I-IV**) and *F. radicans* (**Paper III, IV**) were collected from 2-4 m depth in the Bothnian Sea in Sweden (4-5 psu; East side of Åstön; 62°24´N; 17°45´E; **Figure 3**). The algae were transported in plastic bags in darkness during the trips to the laboratory. Replicates were defined individuals with own holdfast and replicate and controls were used in all studies (n = 5 or 10). In the laboratory the algae were cultivated in natural salt water or in artificial salt water in a cold room. For detailed culture conditions see **Table 1** and **Paper I-IV**.

Experimental Procedures and Analyses

The experiments in **Paper I** and **II** were designed for studies of the Norwegian Sea and the Bothnian Sea ecotypes of *F. vesiculosus* (n = 5) responses to different salinities. The salinities used in the experiments were 5 and 35 psu. In **Paper II** salinity responses in both dark and light cultivated algae were included. In **Paper III** and **IV** only the brackish *F. vesiculosus* was tested in different salinities and the salinities were 5, 10, 20 and 35 psu. A study of the response of the Norwegian Sea and the Bothnian Sea ecotypes of *F. vesiculosus* to desiccation at different temperatures was included in **Paper II**. Samples for analyses were collected before (named initial), during and after experimental treatments and only healthy vegetative part of the tips without epiphytes were used. Studies of the Norwegian Sea ecotype of *F. vesiculosus* and the Bothnian Sea ecotype of *F. vesiculosus* and *F.*

radicans without any experimental influence are presented in **Paper III** and **IV** (n = 10). Details in materials and methods are presented in respective **Paper**.

Table 1. Compilation of the pre-experimental and experimental conditions used in **Papers I-IV**. The time of the year is the period for collection and the acclimation time is the period of pre-experimental procedures before the experiments started. N is the number of replicates. The same temperature and level of irradiance were used during the acclimation and experiments with exception of **Paper II** where some algae were treated in darkness during the experiment.

Papers	Time of the year	Acclimation (days)	n	Temp (°C)	Irradiance (μmol m^{-2} s^{-1})
Paper I	May	7	5	6-11	45-50
Paper II	September	7	5	0; 6-10; 20	80
	May	3		6-9	100
Paper III, IV	May	2.5	5 or 10	6-8	90

Analysis of Water Soluble Organic Compounds and Chlorophyll

For analyses of the differences in the content of water soluble organic compounds (**Paper I**) and mannitol concentration (**Paper II**), ^{13}C nuclear magnetic resonance (NMR) and ^{1}H NMR were used. The shift values were determined by using acetone (215.94 ppm) as an internal reference for the ^{13}C spectra and by using 3-(Trimethylsilyl)-1-propanesulpfonic acid sodium salt (TMSPS; 0 ppm) as an internal reference for the ^{1}H spectra. For determination of mannitol concentrations in **Paper II**, TMSPS was added in known concentrations and the relative amount of mannitol is presented in mmol kg^{-1} DW (dry weight).

For determination of Chl *a* and *c* content (**Paper II, III**), the Chl's were extracted by 90 % acetone and the absorbance was measured at 630, 664 and 750 nm. The relative amount of Chl's are presented in mg g^{-1} FW (fresh weight) or DW.

Chl a Fluorescence and Oxygen Evolution

The ratio of F_v/F_m (**Paper II**) and relative ETR (**Paper IV**) were measured as an indicator of differences in photosynthetic status by use of pulse amplitude modulated fluorometer (Diving PAM, WALZ, Germany). The fluorescence was measured after an initial 20 min dark adaptation. In the ETR measurements, nine steps with increasing irradiance between 0 and 700 μmol m^{-2} s^{-1} (30 s at each step) were used to induce photosynthesis, interrupted by dark periods of 2 min.

In order to identify spectral features, analyse differences in the photosynthetic apparatus and PSI/PSII stoichiometry in the algae, Chl *a* fluorescence emission spectra at 77 K (-196 °C; liquid nitrogen) and 193 K (-80 °C; carbon acid ice) were determined (**Paper III**).

Oxygen evolution by Clark-type oxygen electrode (Rank Brothers Ltd, DM 10, England) was measured in order to analyse photosynthetic status after treatments in different salinities (**Paper IV**). Inorganic carbon was added at the measurements (3 mM $NaHCO_3$). Light periods (5 min) with increasing irradiance in eight steps between 0 and 300 μmol m^{-2} s^{-1} were used to induce photosynthesis, interrupted by dark periods of 5 min.

Relative Amount of D_1 protein, PsaA protein and Rubisco

For measuring of the relative amount of D_1 protein, PsaA and Rubisco, SDS-PAGE and immunoblotting were used (**Paper IV**). Proteins in the extracted samples contained 5 μg ml^{-1} Chl for detection of D_1 and PsaA protein and 1 μg ml^{-1} Chl for detection of Rubisco. The proteins were electrophoretically separated by SDS-PAGE and then transferred to nitrocellulose membranes. After the transferring, the proteins were probed by incubation in: 1) primary antibodies PsbA (anti-D_1, PSII), PsaA (anti-PSI-A) and RbcL (anti-Rubisco) in dilution 1:50 000, 1:500 and 1:5000, respectively and 2) secondary antibodies for PsbA, PsaA and RbcL in dilution 1:10 000, 1:50 000 and 1:20 000, respectively. The antibody-protein complexes were visualized using ECL chemiluminescence detection reagents and developed on X-OMAT film. The blotting was repeated 5-7 times for each replicate and for each class of protein and the developed photos of the blots were analysed by using ImageJ software (developed by Rasband, 1997-2011; use reference, Shim *et al*., 2010).

Data Analysis

In order to analyse differences and define effects of treatments a general linear model analysis of variance (GLM ANOVA) or a one-way analysis of variance (ANOVA), followed by Tukey's pairwise test for a complete comparison, have been performed in **Paper II-IV**. Before the ANOVA tests were preformed the data's were tested for normality by Anderson-Darling goodness-of-fit test. All factors have been categorical and fixed. The confidence intervals were set to 95%. The standard deviation values are given in the **Papers** figures and tables. **Paper I** is a qualitative investigation and does not include statistic.

RESULTS AND DISCUSSION

Content of Water Soluble Organic Compounds and the Concentration of Mannitol in *Fucus vesiculosus* (Paper I, II)

For qualitative analyses of differences in water soluble organic compounds between marine ecotype and brackish ecotype of *F. vesiculosus* ^{13}C NMR and ^{1}H NMR were used. The methods were also used to try to evaluate whether the qualitative content of compounds was related to salinity at the algae's respective growth site (**Paper I**). ^{1}H NMR was also used for quantification of mannitol concentration (mmol kg^{-1} DW) in the marine and the brackish ecotypes of *F. vesiculosus* and to investigate differences between the algae (**Paper II**). The aims for the quantifications of mannitol were 1) to investigate differences in mannitol content responses to desiccation at different temperatures (0, 10 and 20 °C) and 2) to investigate the importance of salinity and light for the available energy reserve and osmotic adjustment in both ecotypes of *F. vesiculosus*.

Water Soluble Organic Compounds

The results indicate more water soluble organic compound/compounds in the Norwegian Sea, marine ecotype of *F. vesiculosus* compared to the Bothnian Sea, brackish ecotype of *F. vesiculosus* (**Paper I; Figure 7**). Experimental treatments with the marine ecotype in low salinity (5 psu) and the brackish ecotype in high salinity (35 psu) did not affect the number of water soluble organic compounds in neither of the ecotypes (**Paper I**). The results from the experiments suggest that the differences between the marine and the brackish ecotype is related to something else than the salinity at the growth sites. Earlier results have confirmed that mannitol is the only organic compound in *Fucus* that occurs in enough concentrations to act as osmolyte (Reed *et al.*, 1985) and the high numbers of scan for detection of the compounds in present study also indicate that the content is too low to have any impact on the osmotic pressure.

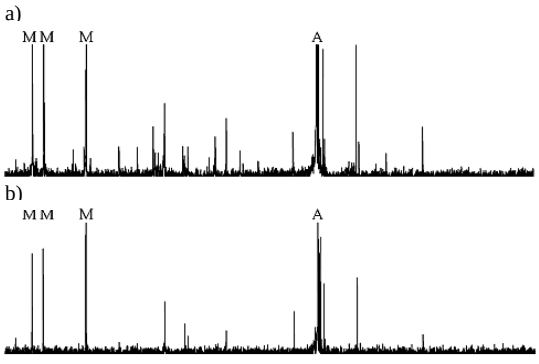


Figure 7. Qualitative analysis by ^{13}C nuclear magnetic resonance (NMR) spectra of a) marine *Fucus vesiculosus* (34-35 psu, practical salinity units) and b) brackish *F. vesiculosus* (4-5 psu) The spectra are representative of five replicates. A is acetone, the internal standard for calibrating of shift and M is mannitol.

An alternative explanation to the differences in water soluble organic compound/compounds between the algae is a greater need of the compounds in the intertidal environment for the marine ecotype of *F. vesiculosus.* One of the differences in compounds between the ecotypes is suggested to be due to glycine betaine, so far only detected in the intertidal marine ecotype of *F. vesiculosus* (**Paper I**). Glycine betaine is a compatible solute and has been concluded to be involved in the protection of protein complexes and membranes during drought and high and low temperatures (Sakamoto & Murata, 1998; 2000). Therefore, it is possibility that glycine betaine, or other compounds, synthesised in the marine ecotypes of *F. vesiculosus*, but not in the brackish ecotype, have complementary functions in the cells as compatible solutes to protect the algal cells from damaging oxygen radicals formed during low tide. Production of reactive oxygen radicals has been confirmed to increase after exposure to high levels of irradiance, ultraviolet radiation, desiccation and/or rapid temperature changes (Collén & Davisson, 1999). It has earlier been suggested that the brackish, sublittoral, Bothnian Sea ecotype of *F. vesiculosus* has evolved reduced ability to grow in the tidal zone (Pearson *et al*., 2000) and the results in **Paper I** suggest that lost of ability to synthesize certain types of water soluble organic compounds could be a part of the explanation.

Mannitol

The results indicate a much higher mannitol concentration in the marine ecotype compared to the brackish ecotype of *F. vesiculosus* (**Paper II**). This result is in agreement with earlier measurements (Munda, 1967; Bäck *et al*., 1992b). Experimental treatment of the algae in desiccation at different temperature did not cause any changes in the mannitol content. It has previously been reported that the tidal rhythm might be too short to achieve a new steady state of organic osmolyte concentrations (Kirst, 1989). Therefore, 5 h of desiccation is probably too short time to observe if there are any significant changes in mannitol content as a response to desiccation (**Paper II**). The temperature however, indicates a mannitol content response in the marine ecotype of *F. vesiculosus*. In the experiment the algae were treated in 0, 10 and 20 °C two days before the desiccation treatment and this was enough time to change the mannitol content. The marine ecotype of *F. vesiculosus* had higher level of mannitol in algae treated in 0 °C compared to higher temperatures (**Paper II**), and this is likely due to a decrease in respiration rate at low temperatures observed by Atkin & Tjoelker (2003). A consequence of higher mannitol concentration is a lowering of the freezing point in the algae, so this increase of the mannitol might be an acclimation to protect intertidal *F. vesiculosus* at low tide during the winter.

In **Paper II** there was also an experiment for investigation of the importance of salinity and light for the available energy reserve and osmotic adjustment in both

ecotypes of *F. vesiculosus* included. The mannitol content was studied as a response to salinity changes (5 and 35 psu), in both short time (6 h) and longer time (72 h), and the algae were treated in both light and darkness. The measuring times used in the experiment were chosen to resemble the tidal rhythm, but it was clear that 6 h was too short for significant changes in mannitol content to occur (also discussed above) in neither of the marine or the brackish ecotype of *F. vesiculosus.* Brown algae use both mannitol and changing in inorganic ions concentration for osmotic adjustment (Kirst & Bisson, 1979; Davison & Reed, 1985) and during short time tidal rhythm the ions is probably the most important osmolytes (Kirst, 1989). However, 24–72 h was enough time to confirm that salinity is an important factor when explaining why the marine ecotype of *F. vesiculosus* has more mannitol than the brackish ecotype. The experiment indicated that each ecotype approached the other's mannitol content when both ecotypes were treated at 35 psu and light. When the marine *F. vesiculosus* was treated at 5 psu and the brackish ecotype was treated at 35 psu, their mannitol contents became almost the same. It has earlier been revealed that marine ecotype has a higher P_{max} and lower respiration compared to the brackish ecotype of *F. vesiculosus* and that the brackish algae increase their P_{max} under higher salinities (Nygård 2005; Nygård & Ekelund, 2006; Nygård & Dring, 2008; **Paper IV**). These ecotypes differences in P_{max} and the brackish ecotype response in higher P_{max} at higher salinity are most certainly an important part of the explanations for mannitol differences between the two ecotypes and the increase of mannitol in the brackish ecotype in higher salinity.

The results in **Paper II** also indicate that mannitol content in the brackish ecotype of *F. vesiculosus* increases as a response to salinity in light but not in dark, where the concentration was stable during 24 h and then decreased. This demonstrates that light and continuous photosynthesis are necessary for osmotic adjustment by mannitol, at least at a time scale longer than 24 h. An earlier study demonstrated that the brown algae *Pilayella littoralis* increased its mannitol content during the first 24 h at higher salinity in darkness (Reed *et al*. 1985). The suggestion is therefore that the mechanism for osmotic adjustment by mannitol is complex and due to increased activity by mannitol synthesizing enzymes (Davison & Reed, 1985; Iwamoto & Shiraiwa, 2005) and also acclimation connected to photosynthesis (Munda & Kremer, 1977; Davison & Reed, 1985). A rapid decrease in mannitol content in the marine ecotype of *F. vesiculosus* incubated in dark, regardless of salinity; indicate that energy utilization is an important part of mannitol regulation as well.

Generally, the results in **Paper II** point to that the differences in mannitol content between the marine ecotype and the brackish ecotype *F. vesiculosus* are due to differences in both salinity and light conditions in the growth environments. *F. radicans* growth side by side with *F. vesiculosus* in the Bothnian Sea, in the same

level of irradiance and same salinity condition and earlier results has confirmed similar mannitol concentration in these brackish *Fucus* species (Gylle, 2007).

Quantum Yield of PSII Photochemistry (Paper II, IV)

Maximum quantum yield of PSII photochemistry (maximum efficiency to use the absorbed light in the photosynthesis), F_v/F_m values, were used to compare the brackish ecotype and the marine ecotype *F. vesiculosus* response to desiccation at different temperatures (0, 10 and 20 °C; **Paper II**). In addition, F_v/F_m was used to analyse the differences in PSII photochemical efficiency between the marine ecotype of *F. vesiculosus*, the brackish ecotypes of *F. vesiculosus* and *F. radicans* (**Paper IV**). In order to analyse differences in PSII photochemical efficiency in the brackish ecotypes of *F. vesiculosus* treated in different salinities, the initial slopes (α) were calculated from P/I-curves measured by oxygen evolution (**Paper IV**).

F_v/F_m as a Measure of Tolerance to Desiccation

Results from initial measurements of F_v/F_m indicated that both ecotypes of *F. vesiculosus* have the same potential to use the available excitation energy for photochemistry in PSII reaction center (**Paper II**). It has also earlier been confirmed that this similarity in the photochemical efficiency between the ecotypes is independent of season (May or November; Gylle, 2007). In the following temperature- and desiccation experiments in **Paper II**, no changes of F_v/F_m could be detected in the marine ecotype of *F. vesiculosus.* In the brackish ecotype, however, there were a significant reduction of F_v/F_m as a response to desiccation in all temperature but the decrease was most prominent at 20 °C. These results indicated that the brackish ecotype has lower potential to use the available excitation energy for photochemistry at desiccation compared to the marine ecotype of *F. vesiculosus*, especially at higher temperatures. Suggested reason for this is a higher rate of water loss in the brackish ecotype because of lower amounts of mannitol and because of a thinner thallus (**Paper II**). More mannitol has earlier been suggested as a reason to increased tolerance to desiccation (Pearson *et al*., 2000). An alternative explanation for lower desiccation tolerance in the sublittoral Bothnian Sea ecotype of *F. vesiculosus* might be the lack of some protective compatible solutes for the photosynthesis apparatus, discussed above (**Paper I**).

Quantum Yield of PSII Photochemistry in Fucus vesiculosus and F. radicans

The measurements from **Paper IV** indicated similar results as the results in **Paper II,** that both ecotypes of *F. vesiculosus* have the same photochemical efficiency. Also *F. radicans* has the same photochemical efficiency as *F. vesiculosus*

and this result was in agreement with earlier findings (Gylle, 2007). Earlier measurements of oxygen evolution also confirmed similar initial slopes (α) values for the two ecotypes of *F. vesiculosus* (Nygård & Ekelund, 2006). These findings are not expected from the point of view of sun and shade adapted algae, where the photochemical efficiency, as well as the Chl *a* concentration, used to be higher in shade adapted algae (Ramus *et al.*, 1977; Figueroa *et al.,* 2003). The amount of Chl *a* (discussed below) was only higher in the sublittoral ecotype of *F. vesiculosus,* compared to the intertidal marine algae in **Paper II** (related to DW; **Table 2**) and not in **Paper III** (related to FW; **Table 2**). Also *F. radicans* had similar Chl *a* concentration as both ecotypes of *F. vesiculosus* (**Paper III; Table 2**). P_{max} is also related to the shad and sun acclimatization and/or adaptation of algae and use to be lower in shade growing algae (Figueroa *et al.,* 2003). This is in agreement with the observed differences in **Paper IV** (**Table 3**) and also with earlier findings of a higher P_{max} in the intertidal marine ecotype of *F. vesiculosus* compared to the sublittoral growing brackish ecotype (Bäck *et al*., 1992b; Nygård & Ekelund, 2006; Nygård & Dring, 2008). The result in **Paper IV** also confirms a higher P_{max} in the marine ecotype of *F. vesiculosus* compared to *F. radicans* (**Table 3**). The fact that the Bothnian Sea ecotype of *F. vesiculosus* and the Norwegian Sea ecotype have different light regime at the growth sites however, can not entirely explain the observed differences in P_{max} because for the brackish ecotype of *F. vesiculosus* it is clear that also the salinity at the growth site affect the P_{max} (discussed more below).

77 K Fluorescence Emission Spectra in Marin and Brackish Ecotype of *Fucus vesiculosus* and *Fucus radicans* (Paper III)

Low-temperature (77 K) Chl *a* fluorescence emission spectra were used to identify PSII and PSI in *F. vesiculosus*. The method was also used to identify spectral features of the marine ecotype and the brackish ecotype of *F. vesiculosus* and *F. radicans* and to analyse differences in the photosynthetic apparatus between the algae. In addition, low-temperature emission spectra were used to investigate whether the optical conditions of the brackish have led to compensatory changes in the photosynthetic apparatus of the brackish ecotype of *F. vesiculosus* and *F. radicans*. Also short-term effects of salinity (1 week in 5, 10, 20, and 35 psu) on the photosynthetic apparatus of the brackish ecotype of *F. vesiculosus* were analysed by the emission spectra (**Paper III**).

77 K Fluorescence Emission Spectrum for Identifying of PSII and PSI

To identify PSII and PSI in *Fucus*, measurements of fluorescence emission spectrum at 77 K in intact thallus of *F. vesiculosus* were performed. The results

indicate a strong peak in the far-red region and only little emission <700 nm. To confirm that the weak emission at <700 nm originates in PSII the effect of light-induced closure of PSII reaction centers at 77 K was measured. The results confirm an increase in the emission yield <700 nm, peaking at 688 nm which indicates that in intact thallus, PSII emits at 688 nm and the far red peak originates in PSI (**Paper III**). These wavelengths are similar to emission peaks of cyanobacteria and green algae (Murakami, 1997; Keränen *et al*., 1999), and the wavelengths of the identified emission peaks are in agreement with earlier measurements from brown algae (Barrett & Thorne, 1980; Berkaloff *et al*., 1990).

77 K Fluorescence Emission Spectrum – Comparison between Fucus Strains

For identifications of difference between the three *Fucus* strains, powdered *Fucus* samples were used. The emission spectra measured at 77 K were characterized with PSI emission at 717–728 nm and PSII emission peak at 692 nm in all three *Fucus* strains (**Paper III; Figure 8**). The ratio of emission between PSII and PSI was different between the *Fucus* strains. In the marine ecotype of *F. vesiculosus*, the PSII emission peak was ~0.3 time lower than the PSI emission peak, and in *F. radicans*, the PSII peak was ~0.5 time lower than the PSI emission peak. In the brackish ecotype of *F. vesiculosus* on the other hand, the ratio of PSII/PSI emission peaks was ~1. A simple explanation of these data would suggest a more even photosystem stoichiometry in the Bothnian Sea ecotype of *F. vesiculosus* compared to the other algal strains and that PSII absorbs the same number of photons as PSI. This in turn would suggest that electron transport is much faster in the Bothnian Sea ecotype of *F. vesiculosus* than in *F. radicans* and the Norwegian Sea ecotype of *F. vesiculosus*. Faster electron transfer rate in the Bothnian Sea ecotype of *F. vesiculosus* however, would disagree with the data indicating faster oxygen evolution in the marine ecotype (Bäck *et al*., 1992b; Nygård & Ekelund, 2006). Therefore, the conclusion from the data was that strong PSII fluorescence in the brackish ecotype of *F. vesiculosus* reflects better ability of PSII to harvest the blue excitation light used in the fluorescence measurements, rather than a high PSII/PSI ratio, because of larger light-harvest antenna of PSII compared to the marine algae and also *F. radicans*. This conclusion is supported by the findings of almost equally ratio of D_1/PsaA proteins (reflects PSII/PSI) between all three *Fucus* stains (**Paper IV**) and also by the finding that much lower level of irradiance is required to saturate photosynthetic electron transport in the brackish ecotype than in the marine ecotype (Nygård & Dring, 2008). Chl *c* has a much higher absorbance in blue wave-lengths than Chl *a* and less absorbance in red wavelengths (Büchel *et al*., 1998). Therefore, if either biosynthesis or targeting of LHC proteins favours PSII at the expense of PSI, the PSII emission peaks will increase in height. Thus, it is tempting to speculate that the strong PSII fluorescence in the brackish ecotype of *F.*

vesiculosus actually indicates that these algae produce LHCI proteins, which may harvest light for both PSI and PSII or occur free in the thylakoid membranes. If so, more light-harvesting protein-pigments might serve PSII in the brackish ecotype of *F. vesiculosus* than in the two other strains. This speculation is supported by the high biochemical similarity and similar Chl *a*/*c* ratios of LHCI and LHCII proteins in brown algae (De Martino *et al*., 2000). If the light-harvest antennae not are organized into specific PSI and PSII LHCs and the PSI and PSII are supplied with the same antenna system, no imbalance in the energy distribution between the photo-systems would occur (De Maritino *et al*., 2000). An alternative explanation to the speculation of LHCI proteins harvesting light for both PSI and PSII in the Bothnian Sea *F. vesiculosus* could be that LHCII instead contribute as antenna to PSI in the marine ecotype of *F. vesiculosus* and *F. radicans* but fail to do so in the brackish *F. vesiculosus*. A study by Yamazaki *et al*. (2005) confirmed a larger PSI antenna size compared to the PSII antenna in the marine green alga *Ulva pertusa* and demonstrated that a substantial amount of LHCII served as antenna at PSI. *F. radicans* does not show the similar large PSII antenna fluorescence as the Bothnian Sea ecotype of *F. vesiculosus*, despite similar light conditions at the growth site. This fact rather support the later explanation that LHCII contribute as antenna to PSI in the marine ecotype of *F. vesiculosus* and *F. radicans* than the speculation above that LHCI proteins harvesting light for PSII in the brackish ecotype of *F. vesiculosus*. The differences between the Bothnian Sea species also suggest that factors other than low-light intensity contribute to the properties of PSII antenna in the Bothnian Sea ecotype of *F. vesiculosus*. In addition, the Chl *a*/*c* ratio confirm more antenna pigment in *F. radicans* compared to the Bothnian Sea ecotype of *F. vesiculosus* (**Paper III; Table 2**). The 77 K fluorescence emission and Chl *a*/*c* ratio results combined implies another distribution of the fucoxanthin-Chl *a*-*c*-proteins and a

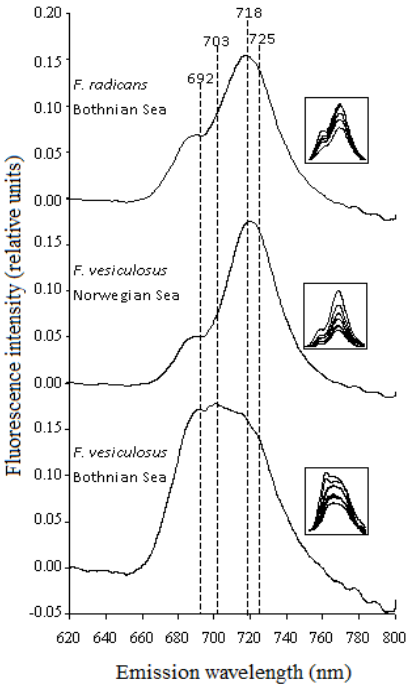


Figure 8. The 77 K fluorescence emission spectra of powdered *Fucus radicans* and *Fucus vesiculosus* from the Bothnian Sea (4–5 psu, practical salinity units) and *F. vesiculosus* from the Norwegian Sea (34-35 psu), as indicated. Blue light between 400 and 450 nm was used for excitation. The insets show the variation in the spectral shape in all 10 replicates.

better low light adaptation in *F. radicans* compared to the Bothnian Sea ecotype of *F. vesiculosus*.

The brackish ecotype of *F. vesiculosus* has a 703 nm peak, missing in the 77 K emission spectra of the other two *Fucus* strains. In *F. serratus* embryos and the rhodophyte *Rhodella violacea*, peaks at 702–705 nm has been assigned to uncoupled LHCI dimers (Doan *et al*., 2003; Lamote *et al*., 2003). The 703 nm peak of the brackish ecotype of *F. vesiculosus* likely has the same origin and energetically uncoupled antenna complexes may show a high fluorescence yield.

The differences in 77 K fluorescence emission between the brackish ecotype of *F. vesiculosus* and *F. radicans* deserves further attention The clear differences in the fluorescence emission spectra between the two Bothnian Sea species of *Fucus* were not only observed in the averaged emission spectra but also in all individual replicates (n = 10; **Paper III;** insets of **Figure 8**). The two algal species differ morphologically (Bergström *et al*., 2005), but the identification of these two species is not entirely reliable without a DNA test. The results of the study in **Paper III** suggest that 77 K fluorescence emission spectra can be used as a reliable method to distinguish the brackish ecotype of *F. vesiculosus* from *F. radicans* as well.

Effect of Salinity on the 77 K Fluorescence Emission Spectrum of the Bothnian Sea Ecotype of F. vesiculosus

The short-term (1 week) salinity experiment of the brackish ecotype of *F. vesiculosus* did only cause minor changes in the spectral form, indicating that the emission spectra of the brackish and the marine ecotypes of *F. vesiculosus* are different because of persistent differences in the photosynthesis machinery, and not because salinity has a crucial effect on PSII/PSI ratio (**Paper III**). However, worth mention is the salinity effect on the 703 nm emission peak, even if not discussed in **Paper III**. The 703 nm peak was less visible in algae treated in 10 psu compared to algae treated in the other salinities and the reason might be a reduced amount of uncoupled LHCI dimmers.

The heights of the peaks were affected by the salinity. The results indicated a higher 77 K Chl *a* fluorescence emission peak from both PSI and PSII in algae treated in 10 psu compared to algae treated in BSW, 5, 20 and 35 psu ($p < 0.001$ for all; **Paper III**). The higher fluorescence emission peaks in the brackish ecotype of *F. vesiculosus* treated 10 psu were not pointed out in **Paper III** because the absolute intensities of fluorescence depends on various optical properties of the sample,, difficult to control precisely in 77 K fluorescence (e.g. frost formation on the surface, size and patterns of ice crystals). However, in the light of high agreements between the fluorescence emission results, especially the PSI emission, and results in **Paper IV**, discussed below, the distinction in fluorescence emissions spectra between algae treated in different salinities is worth notice.

Concentration of Chlorophyll *a* and *c* in *Fucus vesiculosus* and *Fucus radicans* (Paper II, III)

Analyses of the Chl *a* and *c* content (mg g^{-1} DW) were performed in the aim to investigate the importance of salinity and light for the concentration of Chl in the marine and the brackish ecotype of *F. vesiculosus* (**Paper II**). The Chl's were measured initially and after 24 and 72 h during an experiment where both ecotypes of *F. vesiculosus* were treated in 5 and 35 psu in both light and darkness (**Paper II**). The Chl concentrations (mg g^{-1} FW) were also measured as a complement to the 77 K fluorescence emission spectra (**Paper III**).

The results in **Paper II** indicates higher Chl *a* and *c* content in the brackish ecotype of *F. vesiculosus* compared to the marine ecotype (**Table 2**). In **Paper III**, the results indicate higher Chl *c* content in both the brackish *Fucus* strains compared to the marine ecotype of *F. vesiculosus* (**Table 2**). Higher Chl content in the brackish ecotype of *F. vesiculosus* compared to the marine ecotype is contradictory to results from other measurements (Nygård & Ekelund, 2006; Ekelund *et al.*, manuscript in preparation). The differences observed between the present study (**Paper II, III**) and the other studies are most certainly due to the time of collections, May vs. January-February. The brackish ecotype of *F. vesiculosus* and *F. radicans,* have probably a seasonal acclimation with a resting period at winter because of the low levels of irradiance under the ice-cover (below the compensation point; Lehvo *et al.,* 2001) and a synthesis of much pigment would be a waste of energy.

The reason for higher Chl *c* in the brackish ecotype of *F. vesiculosus* and *F. radicans* in May compare to the marine ecotype of *F. vesiculosus* (**Table 2**) is probably because of the differences in the levels of irradiance at the growth sites. The Bothnian Sea species growth in a dark environment relative to the marine *F. vesiculosus* and therefore the algae need more light-harvesting pigments. The result from the experiments in **Paper II** confirm that *F. vesiculosus* is capable of synthesizing Chl *a* in complete darkness, at least within certain time-frames. The brackish ecotype of *F. vesiculosus* is also capable of synthesizing Chl *c* in darkness. It has earlier been confirmed that pine and cyanobacteria synthesise Chl in darkness (Mannan & Pakrasi, 1993; Schoefs & Franck, 1998).

Nygård (2005) confirmed higher photosynthetic efficiency (α) based on fresh weight in the brackish *F. vesiculosus* but not based on Chl *a* and at the same time higher P_{max} based on Chl *a* but not based on fresh weight. These findings indicate that an increase in pigment because of low irradiance originates from increased size of the photosynthetic units that is antennae size (Dring, 1992; Nygård, 2005). In brown algae the pigments are organized in fucoxanthin-Chl *a*-c_2-protein and Chl *a*-c_2 + c_2-protein complexes (Dring, 1992). Therefore, increases in Chl *c* and/or fucoxanthin relative to Chl *a* in low light must be accompanied by increases in the

Table 2. Initial chlorophyll (Chl) *a* and *c* (c_1 and c_2) measurements of the Norwegian Sea (34-35 practical salinity units, psu) ecotype of *Fucus vesiculosus*, the Bothnian Sea (4-5 psu) ecotype of *F. vesiculosus* (**Paper II, III**) and *F. radicans* (**Paper III)**. Different raised letters indicate significant ($p < 0.05$) difference between the *Fucus* strains within the **Paper**. No raised letter means no differences compared to the other *Fucus* strains.

	Chl (mg · g^{-1} DW*) %**		**Chl (mg · g^{-1} FW*) %****		
	Paper II		**Paper III**		
Algae	***a***	***c***	***a***	***c***	***a* / *c***
***F. vesiculosus* Norwegian Sea**	1.739^{A} 100	0.041^{A} 100	0.733 100	0.047^{A} 100	15.6^{A} 100
***F. vesiculosus* Bothnian Sea**	2.326^{B} 134	0.097^{B} 237	0.724 99	0.058^{B} 123	12.5^{B} 80
***F. radicans* Bothnian Sea**			0.675 92	0.066^{B} 140	10.3^{C} 66

* DW, dry weight; FW, fresh weight

** % Chl related to the Norwegian Sea *F. vesiculosus*

proportion of light-harvesting protein-pigment in the photosynthetic units and a consequence of this is an increase in the size of existing photosynthetic units (Gallagher *et al*., 1984).

It was no clear salinity effect on Chl *a* and *c* concentration in *F. vesiculosus* in neither of the experimental studies in **Paper II** and **III**. These results indicate that salinity has no effect on Chl concentration at short time (1 week). These results however, do not exclude that salinity have effect on Chl concentration at longer time as observed by Nygård & Ekelund (2006).

Photosynthesis and the Relative Amount of Photosynthetic Proteins in *Fucus vesiculosus* and *Fucus radicans* (Paper IV)

P/I-curves and immunoblotting were performed in the aim of studying if P_{max} was connected to the relative amounts of photosynthetic proteins (D_1, reflects PSII; PsaA, reflects PSI; Rubisco) on a Chl basis and if there were any differences between the three *Fucus* strains (**Paper IV**). *F. radicans* was included in he study in order to investigate if the native Bothnian Sea species has other adaptations to the environment than the in origin marine *F. vesiculosus* ecotype in the Bothnian Sea.

Further to this, the aim was to investigate if photosynthesis and the relative amounts of Rubisco, D_1 and PsaA on a Chl basis were correlated when the brackish ecotype of *F. vesiculosus* was affected by salinity (5, 10, 20, 35 psu) in the short-term (1 week; **Paper IV**).

P_{max} and the Relative Amounts of D_1, PsaA and Rubisco Proteins in F. vesiculosus and F. radicans

The results indicate a significantly higher P_{max} in the marine ecotype of *F. vesiculosus* compared to the brackish ecotype of *F. vesiculosus* and *F. radicans* whereas no differences between the both Bothnian Sea *Fucus* strains were detected (**Table 3**). Low P_{max} in the brackish water ecotype of *F. vesiculosus* compared to the marine ecotype of *F. vesiculosus* is in agreement with earlier investigations (Bäck *et al*., 1992b; Nygård & Ekelund, 2006; Nygård & Dring, 2008).

The study in **Paper IV** was a first attempt to reveal what cause the differences in P_{max} between the marine ecotype of *F. vesiculosus* and the brackish *Fucus* species. The results indicate that neither of the relative amounts of PsaA or the PSII/PSI (D_1/PsaA) ratio appears to contribute to the differences in P_{max} between the *Fucus* strains (**Paper IV; Table 3**).

The PSII/PSI ratio however indicates an uneven photosystem stoichiometry within *Fucus*, with an overweight of PSI (**Paper IV**; **Table 3**). PSII/PSI ratios differ between different organisms and have e.g. been observed to be 1.43-1.72 in terrestrial plants as *Spinacia oleracea* (spinach), *Cucumis sativus* (cucumber) and *Populus deltoides* (poplar), 0.43 in blue-green algae, and 0.90, 1.03, and 0.84 in the green algae *Dunaliella tertiolecta, Bryopsis maxima* and *Ulva pertusa,* respectively (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 mediates cyclic electron transport and 2) the imbalance in the photosystem stoichiometry protects algae from photoinhibition in the blue-green light that preferentially excites PSII (Yamazaki *et al*., 2005). PSII/PSI ratio has also been confirmed to fluctuate with a plants developing state, seasons and between different parts of the plants as in *Macrocystis pyrifera* (Giant Kelp) with PSII/PSI 1.8 in the surface blades and 2.2 in -20 m blades (Melis & Brown, 1980; Major & Dunton, 2000; Smith & Melis, 1987). The confirmed uneven photosystem stoichiometry within *Fucus*, with an overweight of PSI observed by protein blotting (**Paper IV**) are in agreement with 77 K Chl *a* fluorescence emission spectra for the marine *F. vesiculosus* and in *F. radicans* (**Paper III; Figure 8**). 77 K fluorescence emission spectra of the brackish ecotype of *F. vesiculosus* however, indicated a PSII/PSI ratio ~1 (**Paper III; Figure 8**) whereas the results by protein blotting indicate a PSII/PSI ratio ~0.4 (**Paper IV**; **Table 3**). The reason for the high fluorescence emission in the brackish *F. vesiculosus* was concluded to be due to larger light-harvesting antenna of PSII and not because of a higher PII/PSI ratio at ~1. This interpretation of the 77 K fluorescence emission of the brackish ecotype of *F. vesiculosus* in **Paper III** is in agreement of the PSII/PSI ratio measured by D1/PsaA (**Paper IV; Table 3**).

However, the similarities in light conditions at the growth site and the similarities in PSII/PSI ratio, observed by D1/PsaA between *F. radicans* and the brackish ecotype *F. vesiculosus* but not in the 77 K fluorescence emission and Chl *a*/*c* ratio (even lower in *F. radicans*) in **Paper III** strengthen the suggestion that other factors than low levels of irradiance contribute to the properties of PSII antenna in the brackish ecotype of *F. vesiculosus*.

Table 3. Photosynthetic maximum capacity (P_{max}: measured by electron transport) and the relative PSII/PSI (D_1/PsaA) ratio in the Norwegian Sea (34-35 practical salinity units, psu) ecotype of *Fucus vesiculosus* and the Bothnian Sea (4-5 psu) ecotype of *F. vesiculosus* and *F. radicans* (**Paper IV**). Different raised letters indicate significant (p < 0.05) difference between the *Fucus* strains and lack of raised letter means no differences.

Algae	P_{max}: %*	PSII/PSI
***F. vesiculosus* Norwegian Sea**	13.0^{A} ± 4.33: 100	0.38 ± 0.077
***F. vesiculosus* Bothnian Sea**	5.6^{B} ± 0.46: 43	0.40 ± 0.115
***F. radicans* Bothnian Sea**	6.4^{B} ± 1.49: 49	0.35 ± 0.086

* % of the Norwegian Sea *F. vesiculosus* result

From the point of view of P_{max} (**Paper IV; Table 3**) it was hypothesized that the marine ecotype of *F. vesiculosus* would have more Rubisco than both the brackish strains of *Fucus* because Rubisco accumulates in parallel with high photochemical activities (Björkman, 1981; Maayana *et al.,* 2008). The results in **Paper IV** however indicated an almost equal amount of Rubisco in both ecotypes of *F. vesiculosus* whereas *F. radicans* had approximately half of the relative amount compared to both ecotypes of *F. vesiculosus.* These results indicate that the reasons for lower P_{max} in the brackish ecotype of *F. vesiculosus* and *F. radicans* compared to the marine ecotype of *F. vesiculosus* originates from different underlying causes. Whereas the relative lower amount of Rubisco probably is an important part of the explanation of the low P_{max} in *F. radicans* there has to 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 because of problem for the algae to adapt to the environment in Bothnian Sea or to normal differences in environmental adaptations. It is clear that further investigations are needed to be done to understand the low P_{max} in the brackish ecotype of *F. vesiculosus*, because something disturbs the photosynthetic apparatus in the algae, and the suggestion is to 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* but 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. Therefore, differences between the two ecotypes of *F. vesiculosus* and *F. radicans* in relative amount of Rubisco, as well as the lower relative amount of D_1 in *F. radicans* compared to the marine ecotype of *F. vesiculosus* (**Paper IV**), are probably due to typical differences between species. To notice is also that the similarity in P_{max} between the Bothnian Sea species despite lower relative amount of Rubisco in *F. radicans* points toward a more efficient CO_2 fixation in *F. radicans*.

In a discussion of P_{max} and Rubisco it is also important to consider the amount of DIC (dissolved inorganic carbon) in the water. There is a substantially greater concentration of DIC in fully marine waters (~2.0 mmol m^{-3}; Surif & Raven, 1989) compared with brackish waters as in the Bothnian Sea (~1.0 mmol m^{-3}; Raven & Samuelsson, 1988). I addition to the instant effect of the DIC concentration, because Rubisco use of CO_2, the carbon supply is also important in the regulation of mechanisms behind synthesis of Rubisco in algae (Giordano *et al*., 2005). Nygård & Dring (2008) however, demonstrated that salinity is a more important factor than DIC for the photosynthetic difference between the marine and brackish ecotype of *F. vesiculosus.*

Effect of salinity on P_{max} and the Relative Amounts of D_1, PsaA and Rubisco Proteins in the Bothnian Sea ecotype of F. vesiculosus

Overall, there were higher P_{max} 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 and the most favourable salinity was 10 psu followed by 20 psu (**Paper IV; Figure 9**). These results are in agreement with earlier ETR measurements of the brackish ecotype of *F. vesiculosus* treated in different salinities together with low or high concentration of DIC and nutrients (Nygård & Dring, 2008).

In this first attempt to explain the differences in the P_{max} of the brackish ecotype of *F. vesiculosus*, treated in different salinities, the results did not indicate any differences in the amount of either D_1 or Rubisco (**Paper IV**). It has earlier been confirmed by Northern analysis of transcript abundance for Rubisco that Rubisco demonstrate variations as a response to light and hydration status of *F. vesiculosus* (Pearson *et al*., 2001). Those variations do not appear to count for salinity changes in the range used in present study (5-35 psu) or in the used time scale (1 week; **Paper IV**). However, the P_{max} results indicate some salinity effects on Rubisco, even if no changes are observed on the relative amount of Rubisco. Therefore, measurements of the CO_2 fixation rate by Rubisco in algae treated in different salinities are needed.

The measurements of the relative amount of PsaA proteins from PSI and the PSII/PSI ratios however indicate a salinity effect in the brackish ecotype of *F.*

vesiculosus (**Paper IV**). 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 great amount of PsaA in algae treated in 10 psu is in agreement with P_{max} (**Paper IV; Figure 9**). At light saturation, P_{max}, it is the rate of return of ADP and NADH from the CO_2 fixation in the Calvin cycle that prevents the rate of the ETR from increasing any further (Dring, 1992). Consequently, an increase in the rate of CO_2 fixation would return more ADP to use for ATP production and give the ETR rate an opportunity to increase. Furthermore, it has earlier been confirmed that greater relative amounts of PSI and greater cyclic electron flow around PSI could occur in conditions with an increased demand for ATP (Mandori & Melis, 1984; Anderson *et al*., 1995; Tanaka *et al*., 1997; Hall & Rao, 1999; Yamazaki *et al*., 2005). Therefore, the explanation for the greater amount of PsaA in algae treated in 10 psu might be that the algae need to produce more ATP, and are able to have a greater flow of cyclic electron transport around PSI, to serve a higher rate of CO_2 fixation by Rubisco.

As a consequence of more PsaA, the PSII/PSI ratio was lowest in algae treated in 10 psu and significant lower relative to the algae treated in 35 psu (**Paper IV**). It has earlier been confirmed a change in PSII/PSI ratio as a response to CO_2 supply,

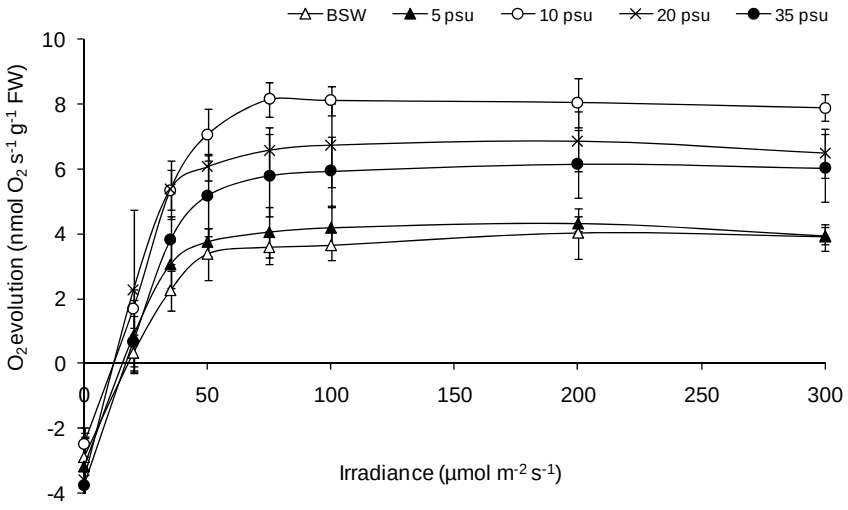


Figure 9. The photosynthesis light response curve of *Fucus vesiculosus* from the Bothnian Sea (4-5 practical salinity units, psu) treated for one week in natural Bothnian seawater (BSW) or in artificial water of 5, 10, 20 or 35 psu. Data represent means of 5 replicates ± standard deviation.

irradiance, nitrogen supply and because of salinity shock (Mandori & Melis, 1984; Kim *et al*., 1993; Berges *et al*, 1996; Tanaka *et al*., 1997). The salinity effect on the PSII/PSI ratio in **Paper IV** however is not in agreement with the 77 K Chl *a* fluorescence emission spectra (**Paper III**). In **Paper III** the salinity only caused minor changes (the changes rather indicated a higher PSII/PSI in10 psu instead of the opposite as in **Paper IV**), and the conclusion was that the emission spectra of the brackish and the marine ecotypes of *F. vesiculosus* were different because of persistent differences in the photosynthesis machinery, and not because salinity has a crucial effect on PSII/PSI ratio. In light of both studies (**Paper III, IV**) it appears that this conclusion is perhaps premature. An explanation for the difference between the studies might be that fluorescence emission from PSII and PSI in **Paper III** origin from both the antenna Chl and the core Chl whereas the PSII/PSI ratio measurements by immunoblotting in **Paper IV** only involve the core of the photosystems (D_1/PsaA). Therefore further investigations are needed in order to understand what cause the difference in PSII/PSI ratio between the studies and what salinity might affect in the PSII/PSI ratio and a suggestion is to investigate the light-harvesting antenna proteins as a first step.

CONCLUSIONS AND SUMMARY

The general aims of this thesis were to compare physiological aspects between the marine ecotype and the brackish ecotype of *F. vesiculosus* as well as between the two Bothnian Sea species *F. vesiculosus* and *F. radicans*.

- The marine ecotype of *F. vesiculosus* has a higher number of water soluble organic compounds compared to the brackish ecotype of *F. vesiculosus*. These compounds are suggested to be compatible solutes and be due to an intertidal and sublittoral adaptation, respectively. The sublittoral ecotype might have lost the ability to synthesize these compound/compounds due to habitat adaptation.
- The mannitol concentration is higher in the marine ecotype compared to the brackish ecotype of *F. vesiculosus* and the higher mannitol concentration in the marine ecotype is suggested to be due to both higher level of irradiance and higher salinity at the growth site.
- Both ecotypes of *F. vesiculosus* as well as *F. radicans* have an uneven PSII/PSI ratio with overweight of PSI. The brackish ecotype of *F. vesiculosus* however has larger light-harvesting antenna of PSII compared to the marine ecotype of *F. vesiculosus* and *F. radicans*.

- 77 K fluorescence emission spectra are a reliable method to separate the brackish ecotype of *F. vesiculosus* and *F. radicans*. The two algal species differ morphologically but the identification of these two species is not completely reliable without a DNA test or as this study indicates, by 77 K fluorescence emission spectra.
- The marine ecotype of *F. vesiculosus* has higher P_{max} compare to the brackish ecotype of *F. vesiculosus* and *F. radicans* whereas both the brackish species have similar P_{max}. One reason for higher P_{max} in the marine ecotype of *F. vesiculosus* compare to *F. radicans* is more relative amount of Rubisco. The reason for higher P_{max} in marine ecotype of *F. vesiculosus* compared to the brackish ecotype of *F. vesiculosus* however is not due to the relative amount of Rubisco.
- P_{max} in the brackish ecotype of *F. vesiculosus* indicates a most favourable salinity at 10 psu followed by 20 psu. One part of the explanation of a high P_{max} in 10 psu might be the confirmed greater relative amount of PsaA protein. The reason for greater amount of PsaA is suggested to be that the algae need to produce more ATP to serve a higher rate of CO_2 fixation by Rubisco. Nevertheless, more studies of the rate of CO_2 fixation by Rubisco in algae treated in similar salinities as in present study are needed to confirm this theory

FUTURE PERSPECTIVES

As a consequence of the importance of, the in origin marine *F. vesiculosus* and the native *F. radicans* in the Bothnian Sea ecosystem it is of high interests to continue to increase the understanding of the physiology in the algae in relation to the environment, and changes in the environment, to know how to protect these key species from harmful anthropogenic disturbances. It is already known that *F. vesiculosus* in the Baltic Sea is vulnerable for eutrophication. Eutrophication force the algae to grow shallower and thereby the *F. vesiculosus* belts become less stable because a larger part of the belts will be affected by ice-scouring, low-water events and strong wave actions (Eriksson *et al.,* 1998; Bergström, 2005; Torn *et al*., 2006; Korpinen *et al.,* 2007; Rhode *et al*., 2008; Schories *et al.,* 2009). *F. radicans* is possibly even more vulnerable to anthropogenic changes, as e.g. eutrophication with the following decrease in light penetration, than other *Fucus.* This is because *F. radicans* does not growth in the shallow water and a declines in *Fucus* populations as a response to decreased levels of irradiance are generally first visible at their lower depth limits (Råberg & Kautsky, 2007). Climate changes and the following changes in temperature, precipitation, runoff and salinity will probably affect the distribution of species in the Bothnian Sea and cause significant ecological changes.

However, it is still different opinions concerning how the climate changes will affect the ice-cover, precipitation, runoff and salinity (Gustafsson, 2004; Omstedt & Hansson, 2006; HELCOM, 2006; Meier, 2006; SOU, 2007; Hansson *et al.*, 2010) and therefore it is not possibly to know if the future benefits the marine or the fresh water flora and fauna in the Bothnian Sea.

In studies involving both marine and brackish ecotypes of *F. vesiculosus* it is vital to remember the differences in their adaptations and/or acclimatization's if experimental studies are planned. In comparison between the marine and the brackish ecotype of *F. vesiculosus*, it is also important to be careful with conclusions based on the Chl content because it appears to be dependent of season if the marine or the brackish ecotype has most Chl. In salinity experiments for studies of the physiology in the brackish ecotype of *F. vesiculosus* it is recommended to use at least one more salinity between 10 and 20 psu. Suggestions for the first steps in future studies of the photosynthetic apparatus in *Fucus* are:

- P_{max} is lower in the brackish ecotype of *F. vesiculosus* compared to the marine ecotype and further investigations to discover the explanation for this should include the rate of CO_2 fixation by Rubisco. The studies should included salinity, light, DIC and nutrient affects of the rate of CO_2 fixation by Rubisco. To considering in studies involving salinity is also the causes and effects of the rate of CO_2 fixation by Rubisco. Mannitol content increases in the brackish ecotype of *F. vesiculosus* as a response to higher salinity. An increase of mannitol could be due to increased carbon supply from photosynthesis and/or a raise of the activity of M1PDH as a direct response to higher salinities (Davison & Reed, 1985; Ivamoto *et al*., 2003; Iwamoto & Shiraiwa, 2005). An increase of the activity of M1PDH in higher salinities raise the questions; 1) could it be a salinity induced increase of M1PDH activity because of a need of osmotic adjustment by mannitol, that increase the photosynthesis because of a higher utilization of triose phosphates? or 2) is it a direct positive salinity effect of on Rubisco that increase the P_{max} and thereafter the mannitol increases?
- Further studies of the adaptations to different light environment should include investigations of the size and distribution of light-harvesting antenna in both ecotypes of *F. vesiculosus* as well as in *F. radicans*. The experimental treatment of the Bothnian Sea *Fucus* indicated that salinity might have effect at the light-harvesting antenna and thus salinity experiments should be included as well.

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“A person who never made a mistake never tried anything new.”

Albert Einstein

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Mario Gylle

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