Energy balance during active carbon uptake and at excess irradiance in three marine macrophytes

Doctoral dissertation
by
Herman Carr 2005

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Front cover: Oxygen tracing of *Laminaria saccharina* at the onset of light.
Back cover: Bohuslän coastline

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BOLD AND DISASTROUS, MY EARS CAN’T
HEAR HEAR WHAT YOU SAY TO ME

POSSESED. I FEEL A CONQUERING WILL DOWN
INSIDE ME
STRENGTH. THE STRENGTH OF MANY TO CRUSH
WHO MIGHT STOP ME.
MY STRENGTH IS IN NUMBER, AND MY SOUL
LIES IN EVERY ONE. THE RELEASING OF ANGER
CAN BETTER ANY MEDICINE UNDER THE SUN

THERE COMES A TIME WITHIN EVERYONE TO CLOSE YOUR EYES TO
WHAT’S REAL
NO COMPREHENSION TO FAIL
I VACUUM THE WIND FOR MY SAIL

Pantera (selected)
Table of contents

Included papers......................................................................................................................... 5
Abstract .................................................................................................................................. 6
Abbreviations .......................................................................................................................... 7
Preface..................................................................................................................................... 8
Aim and Macrophytes used..................................................................................................... 9
Introduction............................................................................................................................ 10
  Chlorophyll a fluorescence ............................................................................................... 11
  Carbon uptake and acquisition in a marine environment................................................. 14
Results and discussion.......................................................................................................... 16
  Some possible sources of energy involved in carbon uptake........................................... 16
  Ratios between Oxygen evolution and estimated ETR...................................................... 19
  The photosynthetic machinery itself and photo protection.............................................. 20
Comments on Analytic Protein methodology ..................................................................... 22
Conclusions........................................................................................................................... 24
Acknowledgement................................................................................................................. 25
References............................................................................................................................... 26
Included Papers


II Carr, H. Björk, M. Degradation of D1 protein and changes in photosynthetic capacity in the green macroalgae Ulva fasciata Delile during exposure to high irradiance. Damage or down regulation within an hour? (submitted)

III Axelsson, L. Carr, H. Photosynthetic utilization of HCO3- in Laminaria saccharina depends on ATP from respiration. (manuscript)

IV Carr, H. Axelsson, L. Utilisation of HCO3- in Zostera marina is supported by ATP from respiration. (manuscript)

My contributions to the papers were as follows: Paper I and II, performing all experiments and planning and writing together with co-author. Paper III and IV, performing and planning of experiments and part of the writing together with co-author.
Abstract

The marine environment is an important habitat where many processes occur that affect life conditions on earth. Macrophytes and planktonic oxygen evolvers are an essential component for almost all marine life forms and have developed in an environment that differs largely from the terrestrial habitats. For instance in regards to available ionic forms of inorganic carbon and moving water masses which affects incoming light. It is therefore relevant to examine the physiology of algae and marine plants to identify their unique features and differences to terrestrial plants that once orginated from algae. By using chlorophyll fluorescence measurements alone or combined with measurements of oxygen evolution and protein analysis photosynthetic strategies to withstand excess energy have been evaluated under a variety of experimental conditions. Furthermore metabolic pathways involved in energy transfer from photosynthesis to the site of active carbon uptake have been examined.

The following was found:

- The ratio between photosynthetic gross oxygen evolution and estimated electron transport rate varies in *Ulva* spp depending on previous history of light and dark exposures. To obtain P/I curves with ratios close to the theoretical 1:4 value, measurements should be performed on separate pieces of tissue at each irradiance level.

- Under carbon deficient conditions, the estimated ETR is larger than the gross oxygen evolution, which may be due to the so called “water-water” cycle and absorption changes in PSII which are not corrected for in the calculation of ETR.

- Upon exposure to high irradiances (1500 µmol photons m⁻² s⁻¹) the PSII core protein D1 is broken down with a concomittant reduction in ETR in *Ulva* spp. With the decrease in electron transport between PSII and PSI the acidification of the lumen decreases and the ability to dissipate excess energy as heat. At prolonged irradiance, an acclimation occurs with a lesser or no breakdown of D1 indicating an additional photo-protective strategy other than heat dissipation.

- *Laminaria saccharina* is dependent on mitochondrial respiration for active utilization of bicarbonate. By extruding protons outside the plasmalemma an acidification takes place that favors the conversion of bicarbonate into carbon dioxide that then can diffuse in to the cell. These proton pumps are driven by ATP supplied to a large degree from mitochondria, likely through the reductant NADPH produced photochemically.

- The marine angiosperm *Zostera marina* is dependent on mitochondrial respiration for utilization of bicarbonate in a manner similar to that in *Laminaria saccharina*. However, the water-water cycle may supply additional ATP to the proton pumps in *Zostera marina*. Both species exhibit a lag-phase at the onset of illumination after a dark incubation period and at least part of this lag-phase is due to a lag in an activation of mitochondrial supported bicarbonate utilization.

It is clear that the marine environment holds complex plants and algae and much is still to discover about the oxygen evolvers that grow beneath the water surface.
Abbreviations

ATP – adenosine 5’-trisphosphate, molecule involved in energy capture and transfer
AZ - acetazolamide – external inhibitor of carbonic anhydrase
CA - carbonic anhydrase
CCM - carbon dioxide concentrating mechanism
Ci - dissolved inorganic carbon
D1 - protein within photosystem two
ETR - electron transport rate at photosystem two and onwards to photosystem one
Fm - maximal chlorophyll fluorescence in a dark adapted sample
Fm’ - maximal chlorophyll fluorescence in actinic light
Fo - minimal chlorophyll fluorescence in a dark adapted sample
Fo’ - minimal chlorophyll fluorescence measured directly after an exposure to actinic light
Ft - steady state chlorophyll fluorescence in actinic light
HPLC - high performance liquid chromatography
LHCII- light harvesting complex two associated with photosystem two
NADP+/NADPH - nicotinic adenine dinucleotide phosphate, electron carrier
NPQ - non photochemical quenching in photosystem two
OEC - oxygen evolving complex
P680 - photosynthetic reaction center in photosystem two
PAM - pulse amplitude modulated
PSI - photosystem one
PSII - photosystem two
Preface

Marine plants and algae, like *Laminaria* spp and *Zostera marina*, are important components of their ecosystem, for example as a nursing habitat for fishes and other animals. Such habitats are therefore of both ecological and economical importance. Apart from their ecological benefits both the green alga *Ulva* and the brown *Laminaria* spp can be used as human food without further processing than drying, and many species within the Laminariales are of industrial importance because of their content of alginic acid, which is abstracted from the alga and is used in food and cosmetic industries. However, some species are also associated with a negative impact on the ecosystem. Rapid growth of algal genera like *Ulva* is often an indication of eutrophication, and mass accumulation of such species can be inconvenient. Other genera like *Cladophora* can be directly harmful for the environment by shading and competing with more ecological important, slow growing genera. To be able to grow in a marine environment, aquatic plants and algae have developed special strategies for growth and photosynthesis that are substantially different from terrestrial plants. It is for the reasons mentioned above of importance to study these mechanisms to be able to accurately predict their productivity and possible effects on their physiology from anthropogenically induced threats.
Aim

The aim of this thesis was to achieve a better understanding of metabolic pathways involved in energy transfer from photosynthesis to the site of active carbon uptake and to evaluate which strategies can be applied to withstand excess energy. To achieve this, chlorophyll fluorescence measurements and oxygen evolution were used as tools to monitor photosynthesis and related processes, and this approach was evaluated under a variety of experimental conditions.

The following biological statements were tested and explained further in the thesis and papers:

1. Gross oxygen evolution and calculated ETR from chlorophyll fluorescence can give a ratio that differs from 1:4 at certain conditions
2. Chlorophyll fluorescence parameters that reflect the condition of photosystem II, such as Fv/Fm, follow D1 dynamics
3. *Laminaria saccharina* and *Zostera marina* receive partly their energy for utilization of HCO$_3^-$ from energy supplied through mitochondria.

**Macrophytes used**

The *Ulva* spp used in the experiments are cosmopolitan green macroalgae composed of two cell-layers sheet-like thalli. These algae are well suited for photosynthetic measurements due to their simple structure. Previous experiments have shown their ability to non-photochemically quench excess energy and to utilize bicarbonate. The algae were chosen mainly due to a long record of being used in chlorophyll *a* fluorescence measurements. *Laminaria saccharina* (L) is a brown alga with a length of up to 3 m with an undivided laminate blade. *L. saccharina* was chosen since previous experiments have shown a well-documented bicarbonate supported photosynthesis. This bicarbonate utilization most likely involves the plasma membrane as the active site of uptake. However, it was unknown whether the inorganic carbon uptake utilized energy from mitochondria or not. *Zostera marina* is a grass-like flowering plant with narrow shaped leaves. *Z. marina* was also chosen for its documented bicarbonate utilization. Moreover, *Z. marina* is a marine angiosperm that moved secondarily back to the Ocean, which makes it relevant to examine bicarbonate utilization mechanisms from an evolutionary point of view, mechanisms that most likely evolved first in algae.
Introduction

Light drives photosynthesis in all, as the name implies, photosynthetic organisms. Without the so familiar colors to the human eye, earth would be without life as we know it. Both marine and terrestrial plants capture the visible light from the sun and transform it into chemical bound energy that fuels growth and maintenance. Even when running a motor warm on gasoline the energy originates from chemically bound sun energy in the form of ancient plants that have been transformed to oil from pressure and age. The mixture of air and fuel has to be correct in a gasoline engine and it has to work under various frequencies of engine cycles within an effective range of an upper and a lower limit of rotations. Likewise, the binding of solar energy is a delicate process, the plant needs sufficient energy to perform the essential tasks and at the same time avoid poor performance and damage due to excess energy. The pathways of energy in plants are complex and created by nature and as processes in nature always change there is much to discover about the complexity of plants. A plant is usually fixed in one place and is forced to be flexible to cope with varying amounts of energy, one day or moment can be cloudy while the next can be with bright sunshine.

The work and performance of a vehicle can be measured as how fast it took you from one place to another. The performance and work of the plant and algae in the following papers have mainly been measured as oxygen evolution, the light driven splitting of water into molecular oxygen, protons and electrons (Fig 1).

**Figure 1.** Schematic illustration of the electron transport in green plants and algae. Light hits the antenna of PSII and the reaction center, P680, of PSII and charge separation of electrons occur at the reaction center. This separation is so strong that it enables to withdraw electrons from water assisted by the Oxygen Evolving Complex situated on PSII. The withdrawal of electrons leads to the splitting of water in to protons and molecular oxygen. The electrons are transported further to PSI generating a proton gradient and ATP and at PSI the electrons are excited again to finally produce NADPH. See text for further explanation.
In the photosynthetic reaction light hits the antenna of PSII and the reaction center, P680, of PSII and charge separations of electrons occur at the reaction center and electrons are withdrawn from water assisted by the Oxygen Evolving Complex situated on PSII (Fig 1). The electrons derived from water are transported down through an electron transport chain in a thylakoid membrane inside a chloroplast producing a proton gradient across the membrane and the electrons eventually reduce NADP+ to NADPH. Upon excitation at PSII the electrons are transferred via a primary plastoquinone, QA, to a secondary plastoquinone, QB, (both denoted Q in Fig 1, for Quencher of chlorophyll fluorescence, Govindjee, 1995) and when plastoquinone has become reduced by two electrons to plastoquinol (PQH2) it transfers two protons from the stroma to the lumen and the electrons from PSII to the cytochrome f/b complex (denoted Cyt). At the cytochrome complex an additional proton is transferred to the lumen. The electrons are transferred further with the soluble plastocyanin to PSI. At PSI the electrons are excited once again by light captured by antenna and the reaction center of PSI. The electrons are received by intermediate electron acceptors at PSI and then passed on to ferredoxin to finally reduce NADP+ to NADPH. The protons that have accumulated in the stroma create a proton gradient that drives the production of ATP at the F1F0 complex. (Aro et al 1993, Tenney 2000). Both ATP and NADPH are chemically bound energy.

Energy might be lost if the electrons are diverted from the above pathway into alternative pathways such as the water-water cycle that consume oxygen but do not produce any NADPH (Asada 2000) or energy can be lost via oxygen consumption in photorespiration (Kozaki and Takeba 1996). In the water-water cycle molecular oxygen act as an electron acceptor at PSI instead of NADP+ and the molecular oxygen is transformed to water. In photorespiration Rubisco fixes oxygen instead of carbon dioxide. Since both photorespiration and the water-water cycle dissipate energy and consume oxygen, the measurable amount of oxygen evolution with Clark type electrodes (with other words oxygen evolution at PSII minus oxygen consumed by alternative energy consuming processes) is likely to represent a measure of energy primarily used for carbon fixation and or nitrogen reduction. Measurement of oxygen evolution is therefore a suitable method to study carbon assimilation and acquisition, which is closely linked to the photosynthetic performance. If there is a lower consumption of NADPH by the Calvin cycle due to insufficient carbon dioxide, there is a decline in the photochemistry of PSII due to a lack of regeneration of the electron acceptor NADP+ (Peterson et al 1988) and this is also reflected in oxygen evolution.

Chlorophyll a fluorescence

Upon light exposure electrons of the chlorophyll molecules become excited to a higher energy orbital. If there are no suitable acceptors within range the electrons fall back to the ground state emitting heat and fluorescence (Fig 2). By increasing the irradiance also the fluorescence will increase since the higher photon pressure will excite more electrons.
When a molecule is hit by a photon with sufficient energy (1) to excite an electron to a higher energy level of the electron shell (2) the electron will fall back first emitting heat (3) and then fall back close to the initial level emitting light or fluorescence (4) (from data presented in Taiz and Zeiger 2002 p 114, Frackowiak 1988).

Also when plants and algae are irradiated, a certain amount of the absorbed light is re-emitted as fluorescence. At room temperature, nearly all of the fluorescence comes from PSII. The magnitude of this fluorescence can be measured with instrument termed fluorometers. Fluorescence emitted from PSII has been extensively used to study the state of PSII as well as for estimating the amount of electrons passing through PSII and onwards to PSI. Besides being of a large theoretical value, the fluorescence measurements are of practical advantage when conducting measurements in the field (Häder et al 1999) or when a large number of samples are to be tested or screened.

The fluorescence of plants and algae is a well-known phenomenon and was early recognized to originate from chlorophyll a (Kautsky and Franck 1943, Krause and Weis 1991). When dark acclimatized plants or algae are suddenly exposed to irradiance, there is a typical change in the fluorescence intensity. The phenomenon was studied by Kautsky and Franck (1943) in Ulva lactuca. At the onset of irradiation, the fluorescence starts at an initial low value. The low fluorescence value depends on the fact that a maximal amount of excited electrons is used to reduce the electron acceptors (and the plastoquinone pool of PSII) and few electrons will have the chance to fall back to the ground state. As the electron acceptors gradually become more reduced there is a rise in the fluorescence. When all electron acceptors eventually have been reduced, the fluorescence is at its maximal value and most of the electrons fall back to the ground state. With time, as the CO₂ assimilation becomes active, the acceptors will become more oxidized and fluorescence will decline. These changes in fluorescence are known as the Kautsky effect.

If a photosynthesizing plant or alga is exposed to light the fluorescence can increase due to two main reasons. The fundamental reason is the more photons chlorophyll a molecules absorb the more will the likelihood to emit fluorescence increase due to the increased number of
excitons than can fall back to the ground state. Secondly, more fluorescence will be emitted as a consequence of a reduction of the electron acceptors of the reaction center of PSII since less acceptors will be available and more electrons are forced to fall back to the ground state. A block between the transfer of the electrons from PSII to an acceptor will likewise cause an increase in fluorescence as a strong light pulse that reduces the electron acceptors. It is desirable to be able to measure the fluorescence as a consequence of available electron acceptors and not as a direct effect of the amount of absorbed light, since the fluorescence as a consequence of blocked acceptors can give a value of the quantum yield of PSII and how many electrons can be passed further from PSII (Horton and Bowyer 1990).

In low light there will be more acceptors available and fewer electrons will have the possibility to fluorescence since some are passed further to the acceptors. By having such a low light that hardly any reduction the electron acceptors of PSII occurs, it is possible to measure the fluorescence as dependent on the redox state of the acceptors. Moreover by Pulse Amplitude Modulate (PAM) this low irradiance light and only measure the fluorescence of the same pulse amplitude it is possible to measure the fluorescence as an effect of reduced electron acceptors at a high irradiance without measuring the overall increase in fluorescence due to the higher irradiance.

In a typical measurement, the PAM light is set constantly to a low irradiance, the so called measuring light, which gives a fluorescence value that relates to the redox state of the acceptors (F_t) depending on the surrounding light. Combining the PAM measuring light with approximately one second of strong light will reduce all acceptors and give a maximal fluorescence value (F_m in darkness and F_m’ in surrounding light). These two values, high and low fluorescence, can be used to estimate the portion of available acceptors and how many electrons that can go further in the electron transport chain (ETC) to produce photochemical energy as described by Genty et al(1989):

\[
\text{Electron Transport Rate} = \frac{(F_m' - F_t)}{F_m'} \times \text{absorbed light}
\]

The maximal fluorescence signal can also be used to estimate the tendency of the energy capturing system to loose energy as heat, a decrease indicating a higher degree of energy loss.

The equipment used for carrying out fluorescence measurements in these studies are Pulse Amplitude Modulated (PAM) fluorescence meters. The recent developments of diving-PAM make it possible to carry out chlorophyll fluorescence measurements and experiments also underwater and in the field (Schwarz et al 2000). Measurements at PSI have to be carried out at 77K and no such measurements were carried out in this thesis.

The chlorophyll a fluorescence is believed to reflect the state of reaction center P680 in balance with surrounding chlorophyll molecules in the antenna or LHC and the fluorescence originates from the antenna (Horton and Bowyer 1990). Upon excitation and transfer of its electrons P680 receives electrons from the water splitting occurring in its vicinity (Barber et al 1997) at the Oxygen Evolving Complex (OEC). Both P680 and the OEC are situated or associated with the transmembrane protein D1 (Yamamoto 2001). The D1 protein has a fast turnover rate and is easily degraded by excess irradiance and its re-synthesis is essential for photosynthesis (Aro et al 1993).
Carbon uptake and acquisition in a marine environment

In marine plants and algae, like in terrestrial plants, CO$_2$ is the only inorganic carbon species that can enter the Calvin cycle. However, unlike a terrestrial environment, the marine water contains additional carbon species, HCO$_3^-$, H$_2$CO$_3$ and CO$_3^{2-}$. At normal pH 8.2 in ocean water at 25°C only a minor fraction of available carbon is present as CO$_2$ (10.2 mmol m$^{-3}$) and the majority is as HCO$_3^-$ (1732 mmol m$^{-3}$) and a lesser part as CO$_3^{2-}$ (234 mmol m$^{-3}$) (Raven et al 2002). The dissolved CO$_2$ in water is in equilibrium with the CO$_2$ of the atmosphere as well as with the ionic forms of inorganic carbon, and as outlined by formula 1. The equilibrium shifts depend on the pH of the seawater with more total carbon being present as carbon dioxide at lower pH and at higher pH more carbon is in the form of bicarbonate and carbonate:

\[
\begin{align*}
H_2O + CO_2 & \leftrightharpoons H_2CO_3 \leftrightharpoons H^+ + HCO_3^- \leftrightharpoons 2H^+ + CO_3^{2-}.
\end{align*}
\]

**Formula 1.** The hydration of carbon dioxide in ocean water in equilibrium with air levels of carbon dioxide results in various forms of carbon and the proportion of the different carbon species of the equilibrium reactions are dependent of pH. At lower pH a larger proportion is present in the form of CO$_2$ and the reactions to the left are favored. At pH 6.15 carbon dioxide is so abundant that it can drive high photosynthetic rates by sole diffusion in to the site of carbon fixation from the bulk water as demonstrated by paper III and IV. At a higher physiological pH that plants and algae can tolerate, like pH 9, the majority of carbon is present as bicarbonate and carbonate and hardly any carbon dioxide is present.

The combination of low CO$_2$ concentrations and low diffusion rates in seawater results in a low availability of CO$_2$ for photosynthesizing marine plants and algae. By developing mechanisms that makes a utilization of HCO$_3^-$ possible, the amount of inorganic carbon available for the plants and algae increases several folds. Carbonic anhydrase (CA) is a well know enzyme in plants, algae and cyanobacteria and it catalyses the reaction of the above described equilibrium formula and enables higher photosynthetic rates and CO$_2$ fixation than what would have been possible from spontaneous HCO$_3^-$ dehydration alone. Several types of CA exist and these are localized at different organelles and compartments of a cell. In terrestrial C4-plants CA is important since it catalyses the reaction of CO$_2$ to HCO$_3^-$ so that PEP carboxylase can incorporate carbon into oxaloacetate in the mesophyll cells (Badger 2003). Carbonic anhydrases have also been found in C-3 plants however their role is somewhat unsure or unknown; cytosolic CA may have a non-photosynthetic function (Fett and Coleman 1994) and it is possible that CA facilitate the movement of CO$_2$ in the chloroplast although its importance for carbon fixation is marginal (Price et al 1994). In marine angiosperms, algae and cyanobacteria CA plays a major role in mechanisms that concentrate inorganic carbon and to maintain the carbon within the cells (Hellblom and Axelsson 2003, Badger 2003, Axelsson et al 1995, Björk et al 1992). At the surface of the cell, at the plasmalemma, CA can catalyze and speed up the inter conversion between CO$_2$ and HCO$_3^-$ to reach a specific equilibrium for a particular pH which will enable higher photosynthetic rates. Either CO$_2$ or HCO$_3^-$, or both, can be transported into the cell, actively or passively by diffusion. There might also be transport proteins that aid passive diffusion by functioning like a gate in the plasma membrane. Within the cell a pool of carbon can accumulate as HCO$_3^-$, which may be transported into the chloroplast where CA can catalyze the conversion of HCO$_3^-$ to CO$_2$ for the incorporation of carbon into the Calvin cycle (Sültemeyer 1998).
Figure 3. Hypothetical model showing different possibilities for inorganic carbon to enter the chloroplast of marine plants and algae. Carbon in the form of bicarbonate and carbon dioxide can diffuse through the cell wall into the periplasmic space. Protons can be pumped out through the plasma membrane, likely fuelled by ATP (Paper III and IV), creating an acid environment that favors the conversion of bicarbonate to carbon dioxide through an equilibrium reaction. Carbon dioxide can thereafter diffuse through the plasma membrane. Bicarbonate may also be taken up across the plasma membrane through a proton pump or through an anion exchanger as likely in *U. lactuca*. For further explanations see text.

*Zostera marina* and *Laminaria saccharina* are believed to pump out protons that acidify at the cell wall; this will drive the conversion of HCO$_3^-$ to CO$_2$ that then may be transported actively or diffuse into the cell (Fig 3, paper III and IV). An active transport of HCO$_3^-$ driven by a proton gradient may also take place in *Zostera marina* but is then probably of minor importance (Hellblom et al 2001, Hellblom and Axelsson 2003). Direct HCO$_3^-$ uptake does probably not occur in *L. saccharina* due to the involvement of external CA activity, which is important for the conversion of bicarbonate to carbon dioxide outside the plasma membrane, and likely to interfere with a direct HCO$_3^-$ uptake (Axelsson et al 2000, cf. Price et al 1985).

*Ulva* spp (green alga) are known to have an HCO$_3^-$ utilization mechanism, inducible by high pH (Carlberg et al 1990, Larsson et al 1990, Axelsson et al 1995). This HCO$_3^-$ mechanism can be blocked by DIDS, an inhibitor of an anion exchange (AE) protein likely present at the plasma membrane (Axelsson et al 1995, Beer and Björk 1994, Sharkia et al 1994). At normal and low pH *Ulva lactuca* is more dependent on CA (Axelsson et al 1995), while *U. intestinalis* in rockpools of high pH depend almost exclusively on the AE-mechanism (Larsson et al 1997, Björk et al 2004).
Results and discussion

Some possible sources of energy involved in carbon uptake

Although a few reports have suggested NADH or NADPH to be involved in the functioning of CCMs in the outer cell membrane (Nimer et al. 1998, Axelsson et al. 2000), ATP is the likely energy source for CCM in the plasma membrane of both *Zostera marina* (Hellblom et al. 2001) and *Laminaria saccharina* (Klenell et al. 2004). The ATP and NADPH that are produced by the photochemical reactions are used primarily for fixation of carbon by the Calvin cycle. The photo-produced energy may also be used for active carbon uptake in aquatic species that have a CCM. Such a use may either be direct (as ATP) or indirect as ATP produced from NADPH via mitochondria. Apart from the photosynthetic linear electron flow, starting with water splitting and ending up at NADPH as an electron acceptor, other energy producing pathways exist within the photosynthetic complexes that may be important for providing ATP to active carbon uptake. The water-water cycle has its name since an oxygen molecule at PSI receives the electrons derived from the water splitting and the oxygen is eventually transformed to water. The transport of electrons creates a proton gradient and ATP but no NADPH (Heber 2002) and discussed in paper IV. Cyclic electron flow around PSI also creates ATP but no NADPH. Such ATP can possibly be used in active carbon uptake, since the uptake can make use of ATP while both NADPH and ATP are required for carbon fixation in the Calvin cycle. Cyclic electron transport around PSI may be induced by a low concentration of carbon since the electron acceptors of PSII become reduced (Paper I), due to less regeneration of NADP+, which may trigger state-transition with the movement of LHCII to PSI (Allen 1995). More energy can thereby be transferred to PSI, which enables cyclic electron transport. State-transition as a function of carbon availability may be the cause of the lower than expected values of the ratio oxygen evolution/ETR in *Ulva* discussed in Paper I.

<table>
<thead>
<tr>
<th></th>
<th>Laminaria saccharina</th>
<th>Zostera marina</th>
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<tbody>
<tr>
<td>Oligomycin</td>
<td>16.5 % ±3.9</td>
<td>40.6 % ±20.9</td>
</tr>
<tr>
<td>Azide</td>
<td>21 % ±2.7</td>
<td>10.5 % ±2.3</td>
</tr>
</tbody>
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Table 1. The effect on net photosynthetic rates of mitochondrial respiratory inhibitors measured as percent remaining photosynthesis compared to full photosynthetic rates in NSW, all measurements were carried out at approximately pH 8.4 (n= 6, ± SD).

In both *Zostera marina* and *Laminaria saccharina* the ability to utilize HCO$_3^-$ was diminished when using inhibitors of respiration such as oligomycin and azide (Paper III & IV and Table 1, Fig 4). By supplying an adequate amount of CO$_2$ it was verified that these inhibitors did not interfere with the photochemical reactions and photosynthesis could be regained. In the presence of oligomycin and at carbon dioxide sufficient conditions photosynthetic rates were even higher than for the control in NSW (Fig 4). However, in *Zostera marina*, azide had a larger inhibitory effect on photosynthesis than oligomycin and this may be due to the fact that azide can interfere with the water-water cycle (Asada 1999). Therefore it may be that both respiration and the water-water cycle are important for supplying energy for active utilization of HCO$_3^-$ in *Zostera marina* (Paper IV). In comparison with Tris-buffer, azide caused a lesser difference between photosynthetic oxygen evolution and estimated electron transport in PSII (Fig 5). This may be explained if azide abolished or diminished the water-water cycle. In *Laminaria saccharina* both azide and oligomycin had similar inhibitory effects on photo-
synthetic rates (Paper III) and it therefore seems that the inhibition caused by blocking the ATP production in the mitochondria is sufficient to block the HCO$_3^-$ utilization. Azide is also known to interfere with some types of CA and inhibit CA catalytic properties (Innocenti et al 2004). Neither $Zostera marina$ nor $Laminaria saccharina$ appear to depend largely on internal CA for their photosynthetic performance, as suggested by similar inhibitory effects of AZ (which inhibits only external CA activity) and EZ (which inhibit both external and internal CA activity) under various conditions (Hellblom unpublished, Mercado et al unpublished). However, considering the large variation in sensitivity to different inhibitors between different CA types (Innocenti et al 2004) it cannot be ruled out that azide inhibit CA in $Zostera marina$ thereby causing some of the larger inhibitory effect of azide as compared to oligomycin on photosynthetic rates.

![Figure 4](image_url)

**Figure 4.** Net photosynthetic rates by *Laminaria saccharina* in natural seawater. Natural Seawater (NSW) pH 8.4, in NSW with oligomycin added (oligo), in the same sample under following CO$_2$ sufficient conditions, in the presence of buffer to lower the pH to 6.15 (mes) and after washing the sample with NSW with oligomycin still present (wash). All values given as percent of full photosynthetic rates in NSW. (n=6, bars represent SD).

It has previously been shown that photosynthesis can be blocked by buffers in the media, especially at high pH, for both *Laminaria saccharina* (Axelsson et al 2000) and *Zostera marina* (Hellblom et al 2001). These macrophytes depend on an extrusion of protons that can acidify the area outside the plasmalemma (Axelsson et al 2000, Hellblom et al 2001), the acidification is diminished, since buffers react with the protons thus preventing the acidification (Price and Badger 1985, Price et al 1985). The importance of acidification for the conversion of HCO$_3^-$ to CO$_2$ in both *Ruppia maritima* and *Laminaria saccharina* is supported by the fact that external CA activity is required (Hellblom and Axelsson 2003, Axelsson et al 2000). The same mechanism is probably involved in the HCO$_3^-$ utilization by *Zostera marina* (Hellblom and Axelsson 2003), although the possibility that a proton symport could be (at least partly) involved via an active uptake of HCO$_3^-$ for this species cannot be excluded (Hellblom et al 2001, Hellblom and Axelsson 2003).
The active HCO$_3^-$ utilization constitutes a CCM located to the outer cell membrane (Axelsson et al 2000, Hellblom et al 2001). If Zostera marina and Laminaria saccharina have any CCM capability located to the chloroplast is not known. In for example the green macroalga Ulva lactuca there is probably an active transport of carbon at the chloroplast level, while the entry of HCO$_3^-$ is passive (Axelsson et al 1999). A CCM at the chloroplast may also to some extent occur in Zostera marina and Laminaria saccharina, but is then of minor importance (c.f. above concerning absence of detectable internal CA activity). This is supported by the fact that full photosynthesis could be restored with an adequate supply of carbon dioxide in the presence of oligomycin (Papers III and IV), which indicates that no hydration of carbon dioxide to bicarbonate occurred at the cytosol with no subsequent active transport (driven by ATP from mitochondria) of HCO$_3^-$ across the chloroplast membrane. Also if a dehydration of bicarbonate occurs outside the plasma membrane the need to store carbon as bicarbonate in the cytosol would likely diminish since the outward diffusion gradient of carbon dioxide would be abolished or diminished.

For both Laminaria saccharina and Zostera marina there was a period of constant low rate photosynthesis (a lag-phase) in the photosynthetic oxygen evolution after an incubation period in darkness (Paper III and IV). After the lag-phase, photosynthesis gradually increased to full rate. This lag-phase could be abolished by a sufficient supply of carbon dioxide, also in the presence of inhibitors of mitochondrial ATP production (Paper III and IV). Moreover the photosynthetic rate during the lag-phase was proportional to the concentration of carbon dioxide in the seawater (paper IV) and photosynthetic rates after the inhibition of azide and oligomycin were similar to the rates of the control samples during the lag-phases (Paper III and IV). It therefore seems likely that the lag-phase was caused by a lag in the activation of the bicarbonate utilization.

Also in terrestrial higher plants, respiration has been shown to be beneficial for photosynthesis, even if the processes involved are less obvious. The redox state in cytosol and chloroplasts is likely an important parameter that is regulated by mitochondria and which effects photosynthesis in pea (Padmasree and Raghavendra 1999a). Mitochondria are also important for glycine oxidation in photorespiration (Igamberdiev et al 2001). Moreover mitochondrial respiration is important for the activation of enzymes in the Calvin cycle and other enzymes of the chloroplast in pea (Padmasree and Raghavendra 2001). It seems unlikely that the mechanisms for full photosynthetic rates that are dependent on respiration in terrestrial plants are equally important for Laminaria saccharina and Zostera marina since full photosynthetic rates could be restored with an adequate supply of carbon, while respiratory ATP production was still blocked (Papers III and IV, Fig 4). In pea it has been shown that inhibitors of respiration have no or little effect on the photochemical reaction with artificial electron acceptors while it seemed to have an effect on activation on the amount of steady-state RuBP (Padmasree and Raghavendra 1999b). This was apparently not the case in Z. marina and L. saccharina since an increase of photosynthesis in vivo due to increasing carbon dioxide is not possible without the fixation of carbon dioxide into RuBP.
**Ratios between Oxygen evolution and estimated ETR**

Oxygen evolution measured with Clark-type electrodes and PAM chlorophyll fluorescence measurements have been the main tools of this study. Sometimes the two methods have been used in combination to either evaluate the methods at different conditions or to study different phenomena. For studying mechanisms closely related to carbon acquisition, such as possible energy sources involved in HCO$_3^-$ utilization, net oxygen evolution was chosen since it is closely associated with ETR in relation to carbon fixation. It is possible to estimate the number of electrons transported through PSII by using the fluorescence parameters, (Fm’-Ft)/Fm’ as mentioned in the introduction. By multiplying this $\Delta F’/Fm’$ with the incoming light and with the amount of light absorbed and assuming that half on the absorbed light is diverted to PSII, an estimate of ETR can be obtained (Krall and Edwards 1992; described in paper I). For the splitting of two water molecules and the release of one molecule oxygen, four electrons must be transported through PSII (Schiller et al 1998), which gives a ratio of oxygen evolution at PSII/ETR of 0.25. As shown by paper I the 0.25 ratio holds true, or as a constant ratio of O$_2$/ETR, measured at varying irradiances if measured in a fresh piece of alga for each new irradiance. A dark period between the different irradiance levels, with gross photosynthesis calculated after each dark period, as also done previously by Beer et al (2000), gives ratios closer to 0.25 than a light curve with constantly increasing irradiance, without inserted dark periods (Paper I). The decrease from the 0.25 ratio of net oxygen evolution/ETR with increasing irradiance (Paper I) may be due to oxygen acting as an electron acceptor as PSI (Asada 1999) or cyclic electron flow within PSII (Miyake and Okamura 2003) in species where photorespiration is low. In Ulva, photorespiration is likely low when CCM is activated or at ambient external carbon concentrations (Beer et al 1990, Björk et al 1993). ETR calculated from chlorophyll fluorescence may give erroneous values at limiting carbon conditions if there are absorption changes at such conditions that are uncorrected for in the calculation of ETR. There might be movement of LHCII to PSI from PSII, which is a process induced by a reduced ETC (Allen 1995). As shown by paper I at low carbon concentrations the electron acceptors of PSII are reduced and there are deviations from the O$_2$/ETR ratio of 0.25 at the same time. Also the electrons transported through PSII that is part of the water-water cycle and the rate of electrons at PSII that is higher than net-oxygen evolution due to photo-respiratory rates are not likely to reflect an ETR that is proportional to carbon fixation. However, when oxygen evolution and chlorophyll fluorescence are used together it is an excellent tool to monitor disequilibrium or deviations in the photosynthetic system that may be due to carbon limitation or excess photosynthetically absorbed energy (Paper I, Fig 3).

The addition of Tris buffer in Zostera marina causes carbon deficient conditions, as the HCO$_3^-$ utilization is inhibited. This carbon deficient situation may cause or increase photorespiration, the water-water cycle or state-transitions and cause a lower O$_2$ to ETR ratio (see Fig 3A). As shown by paper III, azide inhibits bicarbonate utilization in Zostera marina. Azide is also known to inhibit the ascorbate specific peroxidase of the water-water cycle (Asada 1999). The addition of azide does not cause such a pronounced decrease in O$_2$/ETR as Tris addition (Fig 3B), which indicates that operation of the water-water cycle is responsible for the larger discrepancies between gross oxygen evolution and ETR obtained with Tris (Fig 3A). Since the water-water cycle produces ATP it may be important for providing energy for active bicarbonate utilization in Zostera marina.
Figure 5. The effect of Tris buffer (A) and Azide (B) on the relationship between gross oxygen evolution (y-axis) and ETR estimated from PAM-fluorescent measurements (x-axis), in *Zostera marina* (n = 6).

The photosynthetic machinery itself and photo protection

The photosynthetic systems in the semi-autonomous chloroplasts have developed a large flexibility to deal with varying conditions of light in relation to available electron acceptors and substrates (Rosenqvist 2001). If too much solar energy reaches the photosynthetic apparatus in the thylakoid membrane, excess energy can be dissipated as heat before the excited electron travel further in the ETC (Casper-Lindley and Björkman 1998). The relative amount of energy from excitons given away as heat or quenched non-photochemically around PSII and the efficiency of PSII can be measured with chlorophyll PAM-fluorescence (Horton and Bowyer 1990, Paper II). As also described in introduction by implying a strong saturating pulse of light that reduces the plastoquinone pool a maximal fluorescence value is obtained denoted Fm in darkness and Fm’ in actinic light. The fluorescence caused by the measuring light only is denoted Fo in darkness, Ft in actinic light and Fo’ in darkness immediately after a light period and measured when ETC is in an oxidized state (Paper II). By using these values it is possible to calculate several parameters that reflect the functionality of PSII. The quantum efficiency Fv/Fm, =(Fm-Fo)/Fm, is a value of the capacity of PSII in transforming captured solar energy in to chemical energy and it follows the dynamics of oxygen evolution and the essential PSII membrane protein D1 (Table 2, Paper II).

A decrease of maximal fluorescence of light compared to a previous maximal fluorescence in darkness is often considered to reflect non-photochemical quenching i.e. a process in which excess energy is given away as heat in the antenna or the PSII reaction center (Horton and Bowyer 1990). A decrease in maximal fluorescence as compared to a previous maximal value means that less energy is given away as fluorescence when all primary electron acceptors are reduced. This means that some energy has been lost non-photochemically or the amount of fluorescence emitting compounds has decreased in amount or functionality as compared to the previous value. In this case a decrease of Fo is likely to occur as well (Franklin et al 1992) since a decrease of the amount of fluorescence emitting compounds or a decrease of energy transmitted to fluorescence emitting compounds would decrease the overall fluorescence signal. Often several processes are occurring that affects fluorescence and it can be difficult to distinguish small changes if a large counteracting effect occurs. After a period
of high irradiance there can be an increase of Fo due to photo damage and at the same time there can be a tendency to a drop in Fo due to NPQ as shown in *Ulva rotundata* (Franklin et al 1992).

Two different ways of calculating non-photochemical-quenching have been used in paper II, NPQ = (Fm-Fm’)/Fm (Niyogi et al 1997) and qN = (Fm-Fm’)/(Fm-Fo) (Heinz Walz GmbH, 1993). The non-photochemical quenching parameter qN reflects the quenching of the variable fluorescence (Rohácek 2002), which may be correlated to the reaction center itself. Damaged reaction centers still connected to the antenna might act as permanent excitation quenchers (Cleland et al 1986). NPQ is believed to reflect non-photochemical quenching in the antenna and dropped considerably at high irradiances while qN remained high (paper II). The decline in NPQ at high irradiance can possibly be explained if NPQ reflected the overall ability of non-photochemical quenching of the tissue and decreased as the number of functional PSII decreased. Such a decrease of functional PSII units was demonstrated as a decrease of photosynthetic oxygen evolution and D1 protein content. On the contrary qN may have reflected the non-photochemical quenching of the remaining active PSII (Table 2, Paper II). An acidification of the lumen is important for the activation of quenching in the antenna (Kanazawa and Kramer 2002) and with the breakdown of D1 and decline of ETR, the ability to acidify the lumen was likely reduced, which may have affected NPQ. Photo inhibition has also been partly attributed to non-photochemical quenching (Krause and Weis 1991). The fast degradation of D1 may be a way of avoiding the generation of excess energy in an ETC that lack NADP+ as an acceptor at PSI. Damaged PSII centers that still are functional quenchers may protect the thylakoid membrane from further damage (Aro et al 1993), however the exact mechanism seems unclear considering that P680 likely dissociate from PSII upon breakdown of D1. The fluorescence parameter 1/Fo-1/Fm have been shown to correlate linearly with the numbers of active PSII (Lee et al 1999) and in *Ulva* spp the parameter followed the same trend as D1 dynamics and the ability of light saturated oxygen evolution, even though the fluorescence parameter gave a lower value of activity than oxygen evolution and D1 amount (Tab 2, Paper II).

<table>
<thead>
<tr>
<th>High Light minutes</th>
<th>0 min</th>
<th>15 min</th>
<th>110 min</th>
<th>Recovery 3 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>100 %</td>
<td>71 % (±33)</td>
<td>30 % (±9)</td>
<td>71 % (±10)</td>
</tr>
<tr>
<td>O2 evolution</td>
<td>100 %</td>
<td>67 % (±20)</td>
<td>42 % (±9)</td>
<td>74 % (±9)</td>
</tr>
<tr>
<td>Fm/Fv</td>
<td>0.77 (±0.01)</td>
<td>0.41 (±0.14)</td>
<td>0.13 (±0.06)</td>
<td>0.58 (±0.02)</td>
</tr>
<tr>
<td>1/Fo-1/Fm</td>
<td>100 %</td>
<td>39 % (±18)</td>
<td>13 % (±7)</td>
<td>62 % (±9)</td>
</tr>
<tr>
<td>NPQ</td>
<td>0.51 (±0.03)</td>
<td>0.23 (±0.05)</td>
<td>0.06 (±0.03)</td>
<td>0.33 (±0.12)</td>
</tr>
</tbody>
</table>

*Table 2.* The effect of high irradiance on several photosynthetic parameters. Control was samples exposed to only the irradiance of cultivation, 0, 15 and 110 minutes was exposure to approximately 1500 μmol photons m⁻² s⁻¹ and recover is in dim light after exposure to high irradiance. Gross oxygen evolution was at saturating non-photo inhibitory irradiances measured after the different treatments.
Comments on Analytic Protein methodology

Methods to analyze hydrophobic proteins in *Ulva* were developed in order to be able to screen for differential protein expressions of well-defined physiological states of *Ulva* (e.g. different activation levels of AE protein or CA, Axelsson et al 1995). Chloroplasts were prepared as described in Paper II by breaking the tissue to release the chloroplasts and isolating them through differential centrifugation. Thereafter the chloroplasts were incubated directly in 80% acetone for 48 hours in -20°C for precipitation of proteins. After the precipitation the chloroplast proteins were incubated in different solutions; Hexafluor-2-propanol (HFIP), Chloroform/Methanol (1:1 ratio), 1% Triton-X 100 (in 5 mM Na₂HPO₄), 1 % octaethylene glycol monododecyl ether (C₁₂E₁₈) in water or acetonitril for two hours. Insoluble material was centrifuged to a pellet and discarded and the supernatant was collected. Thereafter the supernatant was dried in a vacuum centrifuge and the proteins were dissolved in sodium dodecyl sulfate (SDS) buffer and insoluble material was removed through centrifugation. Thereafter the protein solutions were run on SDS polyacrylamide gel electrophoresis.

HFIP gave the highest amount of proteins as measured as the intensity of Coomassie blue visible on a SDS-gel. Chloroplasts were dissolved directly in HFIP, Chloroform/Methanol, Triton-X 100, C₁₂E₁₈ and acetonitril and then run on an HPLC (Merck Hitachi LaChrome). Reversed Phase chromatography was used to separate the proteins on a column (Chromatolit SpeedROD RP-18e) starting with 100% 10 mM HCl diluted to 40 % acetonitril in 10 minutes and to 100 % acetonitril after additional 2 minutes in a flow of 2 ml/min. HFIP gave the highest peaks measured with UV sensor (at 280 nm) corresponding to the highest intensity of stained proteins on the SDS-gel. Approximately 15 peaks could be detected with HFIP and several of the peaks were strongly hydrophobic (Fig 6). Also with Chloroform/Methanol approximately 15 peaks were detected and most of the proteins were less hydrophobic than the proteins separated from HFIP. When proteins were solubilized in Triton X-100 approximately 20 peaks were detected, both less and more hydrophobic proteins, though most of the peaks were smaller compared to HFIP and Chloroform/Methanol. C₁₂E₈ gave similar peaks as Triton-X 100 in terms of the hydrophobic nature of proteins, however only 13 peaks were detected. With acetonitril only four peaks were detected. From fractions collected from HPLC with chloroplast sample solubilized in HFIP several peaks could be detected with Mass-spectrophotometer (Theres Redeby). By dissolving the protein samples in the different solutions, HFIP, Chloroform/Methanol and Triton X-100 will give several peaks on HIC detected with UV-sensor. The different solutions when used together serve as an excellent tool to screen for differential protein expression and will be used to screen for proteins that can be involved in different carbon uptake strategies such as an ATPase complex for transport of carbon into the chloroplast.
Figure 6. The amounts and number of peaks of chloroplast proteins dissolved in HFIP and run on RP chromatography with elution of more hydrophobic proteins with increasing time (concentration of acetonitril). Y-axis is in relative absorbancy unit at 280 nm.

These experiments were carried out together with Theres Redeby, Analytical Chemistry, KTH.
Conclusions

- For measurements of ETR that will reflect primary production rates it is important that ETRs correlate to gross oxygen evolution. A discrepancy of ETR and O₂ evolution rates can indicate consumption of oxygen by the water-water cycle or photorespiration that will cause an ETR that do not produce any NADPH for the fixation of carbon. A constant ratio between O₂/ETR in a light curve is best obtained when measured in separate tissue for each irradiance level.

- Deviations of O₂/ETR ratios at carbon deficient conditions can be an indication of energy disequilibrium of photosynthetic processes.

- Breakdown of the PSII protein D1 leads to the loss of linear ETR and thereby the ability to acidify the lumen decreases, which is reflected as a decrease in heat dissipation in *Ulva spp*. This breakdown of D1 may protect the photosystems from further damage at prolonged illumination.

- There are large physical and environmental differences between land and sea that are reflected in the difference in physiological mechanisms between photosynthetic organisms, like the ability to make use of the additional carbon that are present in seawater. *Laminaria saccharina* and *Zostera marina* depend on mitochondrial respiration for photosynthetic utilization of bicarbonate.

- After a period of dark incubation both *Laminaria saccharina* and *Zostera marina* have a photosynthetic lag-phase at the start of illumination. At least a large part of this lag-phase is likely caused by the lag in the activation of ATP driven bicarbonate utilization.

Future challenges

Proteins involved in bicarbonate utilization will be screened for by comparing two physiological modes in *Ulva lactuca*, as reflected in its ability to utilize inorganic carbon.
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References


Björk, M. Axelsson, L. Beer, S. 2004. Why is Ulva intestinalis the only macroalga inhabiting isolated rockpools along the Swedish Atlantic coast? MEPS. (in press)


Rosenqvist, E. 2001. Light acclimation maintains the redox state of the PS II electron acceptor QA within a narrow range over a broad range of light intensities. Photosynthesis Research. 70: 299 – 310.


