Seagrass productivity
from plant to system
Diana Deyanova

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Abstract
Seagrasses form one of the most productive habitats on earth and are recognized as very efficient carbon sinks. The levels and patterns of productivity within and across different seagrass systems vary widely due to natural or human-induced factors. Seagrass plants, being the foundation species of seagrass meadows, have a substitutational role as primary producers in the overall productivity of their habitat. Clarifying the variation in the carbon capture potential of these plants on physiological and ecological levels is essential to understand of the whole system’s carbon balance. In this thesis, the photosynthetic performance and productivity of seagrass plants were studied in relation to factors that have large impact on productivity, such as tissue age, season and water depth. Furthermore, the seagrass response, in terms of capacity to capture and sequester carbon, to human-induced stress factors such as shading and simulated grazing was evaluated in a tropical seagrass meadow. The research has included a multitude of seagrass productivity assessments from plant- to system level.

The results showed that age has a significant effect on the photosynthetic performance of the temperate seagrass Zostera marina L., both within a single shoot and between shoots. When comparing leaves among the same shoot, the photosynthetic capacity and efficiency were highest in mature tissues and significantly reduced in very young tissues as well as in tissues undergoing senescence. In response to high light stress, very young tissues seemed to cope better with dissipating excess light energy, which was demonstrated by the higher values of non-photochemical quenching (NPQ) observed compared to mature and senescent tissues. Such an effect was also observed when comparing the oldest and youngest shoots from the same genet; the youngest shoot showed higher ability to dissipate excess light energy compared to the oldest one, and might thus be able to better withstand light stress.

On a larger spatiotemporal scale, the areal productivity of seagrass plants was significantly affected by light availability and temperature, leading to a strong seasonal variation. In addition, depth had a strong site-specific effect on plant productivity in terms of biomass. On a yearly basis, productivity rates varied substantially, reaching up to 20 g C m⁻² 24h⁻¹ in the summer months. This high carbon capture potential was, however, outbalanced by the high respiration rates of the benthic community. Overall, the whole system had a low but positive yearly carbon balance.

Both shading and simulated grazing negatively affected seagrass plants and the whole habitat after five months of experimental disturbance. On the plant level, photosynthesis, productivity and growth were all reduced. On the system level, a reduction in community productivity was recorded. The long-term refractory carbon was, however, not affected although erosion was observed in treatments subjected to simulated grazing.

In summary, this thesis has established that age, season, depth and exposure are factors highly responsible for natural variation in seagrass plant- and habitat productivity, and that seagrasses respond to human-induced stress by significantly reducing their productivity. Even though seagrass plants are generally capable of surviving stress periods, these results suggest that prolonged deteriorating stress conditions will lead to serious harm on the plants as well as the entire habitat, and thereby compromising the carbon burial capacity of the seagrass system.

Keywords: Seagrass, Chlorophyll a fluorescence, Ageing, Productivity, Seasonality, Stress response.

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Seagrass productivity
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Diana Deyanova
To my niece Katerina
List of papers

This thesis is a compilation of four manuscripts that will be referred to by their roman numerals.

I. **Deyanova, D.,** Gullström, M., Lyimo L. D., Sköld, H. N., Björk, M. Effect of ageing on the photosynthetic capacity of the seagrass *Zostera marina* Linnaeus (manuscript)


My contribution to the papers:

Paper I: Participated in the planning of the study, performing all experiments, analysis of data and writing the manuscript together with co-authors.

Paper II: Participated in the planning of the study, collection of all data and performing all laboratory measurements, analysis of data and writing the manuscript together with co-authors.

Paper III: Participated in the planning of the study, collection of all data and performing all laboratory measurements, analysis of data and writing the manuscript together with co-authors.

Paper IV: Participated in the planning of the study, collection of the data in field, commented on manuscript.

Papers III and IV are reprinted with the kind permission of the publishers.
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## Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AF</td>
<td>Absorption factor</td>
</tr>
<tr>
<td>AG</td>
<td>Aboveground</td>
</tr>
<tr>
<td>BG</td>
<td>Belowground</td>
</tr>
<tr>
<td>C</td>
<td>Carbon</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
</tr>
<tr>
<td>DW_{sp}</td>
<td>Specific tissue dry weight</td>
</tr>
<tr>
<td>ETR</td>
<td>Electron transport rate</td>
</tr>
<tr>
<td>ETR_{max}</td>
<td>Maximum electron transport rate</td>
</tr>
<tr>
<td>F_{v}/F_{m}</td>
<td>Maximum photosynthetic quantum yield</td>
</tr>
<tr>
<td>GPP</td>
<td>Gross primary productivity</td>
</tr>
<tr>
<td>GPP_{total}</td>
<td>Gross primary productivity of the whole community</td>
</tr>
<tr>
<td>IC+Rec</td>
<td>Induction curve followed by recovery period</td>
</tr>
<tr>
<td>NCP</td>
<td>Net Community production</td>
</tr>
<tr>
<td>NEP</td>
<td>Net ecosystem production</td>
</tr>
<tr>
<td>NPP</td>
<td>Net primary production</td>
</tr>
<tr>
<td>NPQ</td>
<td>Non-photochemical quenching</td>
</tr>
<tr>
<td>PAM</td>
<td>Pulse amplitude modulated (fluorometer)</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetically active radiation</td>
</tr>
<tr>
<td>P/I (curve)</td>
<td>Productivity vs irradiance curve</td>
</tr>
<tr>
<td>P_{g}</td>
<td>Gross photosynthesis</td>
</tr>
<tr>
<td>P_{n}</td>
<td>Net photosynthesis</td>
</tr>
<tr>
<td>P/R</td>
<td>Productivity to respiration ration</td>
</tr>
<tr>
<td>R_{D}</td>
<td>Respiration in darkness</td>
</tr>
<tr>
<td>R_{L}</td>
<td>Respiration in light</td>
</tr>
<tr>
<td>R_{total}</td>
<td>Respiration of the whole community</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>RLC</td>
<td>Rapid light curve</td>
</tr>
<tr>
<td>TSC</td>
<td>Total soluble carbohydrates</td>
</tr>
<tr>
<td>THAA</td>
<td>Total hydrolysable amino acids</td>
</tr>
<tr>
<td>$\Phi_{PSII}$</td>
<td>Photosynthetic yield (in the light adapted state)</td>
</tr>
</tbody>
</table>
Introduction

Primary productivity of plants and communities

As a main part of the carbon (C) cycle – primary productivity is responsible for incorporating inorganic C into organic compounds through the physiological process of photosynthesis of all autotrophic organisms. Ecosystems can be categorized in various ways, according to e.g. location, dominant species, biodiversity, and the services they provide. They can also be ranked based on their patterns and strength of productivity, expressed in amount of carbon fixed per area unit. For instance, tropical rain forests are ranked among the most productive ecosystems on the planet, whereas deserts among the least productive. The productivity of a specific habitat or of a whole ecosystem depends on many factors of which the green biomass of primary producers, turnover rates and their efficiency are among the most important. Controlled by temperature, amount of radiation received, and water- and nutrients availability, photosynthesis is the basis for the productivity of the autotrophs. Very productive systems are of high importance on a global scale since they contribute significantly to the sequestration of atmospheric carbon into soils and sediments. Therefore, in the current conditions of increasing carbon the understanding of ecosystem productivity fluctuations due to natural and human induced factors is of great importance.

There are several concepts and terms that need to be addressed for further clarification related to primary productivity. Gross photosynthesis ($P_g$) takes into account all the light-energy that flows in the photosynthetic apparatus and includes all the carbon fixed by the organism, irrespective of whether that carbon is subsequently respired or not. The net photosynthesis ($P_n$) is the sum of the gross photosynthesis and the respiration performed during the light period ($R_L$) (Falkowski and Raven 2007).

$$P_n = P_g + R_L$$

Those processes are expressed on a time basis and as rates, either as fixed CO$_2$ or as O$_2$ released, depending on the type of measurements. The Gross Primary Production (GPP) is essentially the same as $P_g$ (i.e. the sum of the net primary production and the respiration), whereas measurements of the Net Primary Production (NPP) should include a dark period (e.g. the time period should be at least 24h). Consequently, the NPP is a result of the sum of $P_n$ and the respiration during the dark period ($R_D$).
\[ NPP = P_n + R_D \]

NPP of plants can only be measured if the organism can be isolated so that the production of other photosynthetic organisms or the respiration of consumers can be excluded. Thus, on a system level, when not only primary producers are involved but also consumers, the net ecosystem production (NEP) equals the sum of the GPP of all primary producers (GPP\(_{\text{total}}\)) and the respiration of the whole system, including both non-photosynthetic and photosynthetic organisms (\(R_{\text{total}}\)).

\[ \text{NEP} = \text{GPP}_{\text{total}} + R_{\text{total}} \]

NEP can also be expressed on a time basis, which includes the dark period. Traditionally, NEP is expressed in mass C per area and time (for example g C m\(^{-2}\) y\(^{-1}\)). Finally, in this thesis the term “Net Community Production” (NCP) is used, which is similar to NEP but refers to the benthic community being studied, excluding the overlaying pelagic water.

**Seagrass meadows as highly productive and important marine habitats facing global decline**

Among the most productive systems are those formed by marine vascular plants referred to as seagrasses (Fig. 1). Distributed globally in all oceans and along all coasts except for Antarctica, seagrass habitats have rates of NPP comparable to those of tropical rain forests (Kennedy and Björk 2009). Seagrasses are a polyphyletic group, formed of about 60 species of marine and brackish monocots, which belong to four families – Posidoniacae, Zosteraceae, Hydrochariceae and Cymodoceaceae (Green and Short 2003). They have clonal morphology with aboveground green shoots, also called ramets, and belowground rhizomes and roots. One plant comprised of genetically identical shoots and connected via rhizomes forms a genet (Setchell 1929).

The high productivity of seagrass habitats is based on several key characteristics of the seagrass plants. These plants are foundation species (Hughes et al. 2009), they form dense erect and flexible canopies and their leaves serve as substrate for a variety of epiphytic algae (Larkum et al. 2006). The soft sediments they inhabit are covered by microphytobenthic communities; different macroalgal species are located among the seagrass shoots and in the overlaying water column there is phytoplankton. All of these primary producers contribute significantly to the total habitat productivity (McRoy and McMillan 1977). For instance, the contribution of the epiphytic communities varies from 9 to 60% of the total production in seagrass habitats (Larkum et al. 2006). Kaldy et al. (2017) showed that in a subtropical meadow, the relative contribution of autotrophs is dominated by benthic macroalgae responsible for up to 42% of the primary production, followed by seagrasses and microalgae. In
temperate systems, seasons have a great effect on community primary productivity influencing both species composition and abundance of primary producers as well as their photosynthetic activity.

The bulk of the primary fixed carbon is metabolized as part of the carbon cycle—it is either decomposed, grazed or exported from the seagrass habitat. According to Duarte and Cebrián (1996), an average of 50% of the fixed carbon is decomposed within the habitat, 19% is consumed by herbivores, 24% is exported and about 16% remains as mineralized matter in the sediments. Most seagrasses have extensive belowground organs—rhizomes and roots, which are buried in the soft sediment and serve as anchorage for the plants and have strap-like flexible leaf blades that move with the water currents (Kuo and Hartog 2006). Due to their morphological features, they form an environment with a characteristic “architecture” facilitating the direct trapping of suspended matter from the water column (Agawin and Duarte 2002, Hendriks et al. 2008) as well as keeping shed leaves and dead algal mats from resuspension. These features make seagrass habitats one of the most efficient carbon burial systems (Nellemann et al. 2009), responsible for the storage of about 10 to 15% of the mineralized carbon stock in the oceans (Duarte and Cebrián 1996, Duarte and Chiscano 1999, Laffoley and Grimsditch 2009), despite of their small areal cover (c. 0.1% of the ocean floor).

Figure 1. Seagrass (Zostera marina) meadow on the Swedish west coast during summer months. Overgrowth with filamentous epiphytic algae can be seen among the seagrass leaves. Photo: Diana Deyanova.
Additionally to their role as very productive habitats and one of the most efficient carbon sinks, the value of the ecosystem services seagrass habitats provide is estimated to be higher than that of rainforests (Costanza et al. 1998). They serve as shelter and nursery grounds for a variety of fish and invertebrates (Heck Jr. et al. 2003), support high biodiversity, stabilize sediments and prevent erosion (Orth 1977). Seagrass habitats also improve the water quality by enhancing sedimentation rates and reducing resuspension (Ward et al. 1984).

Despite all their positive qualities, seagrass habitats are declining worldwide mainly due to increasing pressure from human activities (Short and Wyllie-Echeverria 1996, Ort et al. 2006, Hughes et al. 2009, Waycott et al. 2009). The major reasons for their decline are attributed to urbanization of coastal areas as shown in many regions around the world (Baden et al. 2003, Green and Short 2003, Westphalen et al. 2005). As a result of coastal erosion, water quality deteriorates and leads to a substantial reduction in light availability. Sedimentation due to e.g. dredging or nutrient loading, leading to massive development of opportunistic algae, are additional reasons for seagrass disappearance (Short and Wyllie-Echeverria 1996). Other threats posed to seagrass habitats are due to uncontrolled fishing practices which lead to the reduction of key predators of seagrass grazers, whose populations can in turn grow to be responsible for overgrazing of vast areas of seagrass (Camp et al. 1973, Rose et al. 1999, Peterson et al. 2002, Eklof et al. 2008). In a field experiment at the Swedish west coast, Moksnes et al. (2008) showed that reduced top-down control due to overfishing can induce strong trophic cascades, eventually negatively affecting seagrass production. Causes for seagrass disappearance can also be of natural character such as the wasting disease caused by the pathogen Labyrinthula zosterae, which has affected many populations of seagrass in the Northern hemisphere (Short et al. 1986). Furthermore, volcanic eruptions, ice formation or natural coastal erosion due to wave action may also impact seagrass populations (Short and Wyllie-Echeverria 1996).

Adaptations of seagrass plants to a dynamic environment

Seagrasses inhabit a very dynamic environment. They experience varying conditions which challenge their survival daily and seasonally. These variations occur naturally, and therefore seagrass communities are adapted to, for instance, seasonal changes in light availability and temperature, tidal fluctuation, exposure to air, desiccation, and occasional grazing pressure. The main means of adaptation seagrass plants have to cope with such a variable environment is the possession of extensive belowground tissues, which can make up as much as 50% of the total biomass of the plant (Duarte and Chiscano 1999). Rhizomes are the main storage organ for carbohydrates in seagrasses. This stored carbohydrate can be allocated to support growth during unfavorable conditions (Alcoverro et al. 1999, Alcoverro and Mariani 2002) such as
during winter periods or episodes of occasional heavy grazing (Burke et al. 1996). The main stored carbohydrates are the soluble sucrose, which can make up to 90% of the total soluble carbohydrate pool and insoluble starch (Alcoverro et al. 1999, Touchette and Burkholder 2000). Maintaining nonproductive belowground organs such as rhizomes has a cost, and consequently seagrass plants have relatively high minimum requirements for growth; at least 11% of the surface irradiance has to reach the canopy in order to ensure the survival of the plants (Duarte 1991, Dennison et al. 1993, Cayabyab and Enríquez 2007). Therefore, permanent reduction of light availability has been shown to be devastating for seagrass survival (see review by Ralph et al. 2007). Reduced productivity of the main primary producers in the system, or their complete disappearance, can lead to serious consequences for the whole community and compromise the important services seagrass habitats provide.
Aim of the thesis

By using a combination of physiological approaches investigating the photosynthetic response of seagrass plants to various conditions, this thesis aims to assess a large-scale ecological process, system productivity, while also exploring possible natural and human-induced variation in productivity of the seagrass plants. In addition, the thesis gives an insights in the potential of seagrasses to cope with less than optimal conditions. Further, the thesis aims to provide an understanding of how plant productivity is linked to the productivity of the whole habitat and regarding the effects of stress on important ecosystem services such as carbon burial and carbon content in seagrass sediment.

In specific, the objectives were:

- To determine age-dependent variations in photosynthetic performance and resistance to light stress of seagrass leaves and shoots of different age
- To follow the natural yearly variation in productivity of seagrass plants and the associated community over different seasons and depths
- To study the effects of light reduction and simulated grazing on seagrass plant productivity, seagrass community productivity, sediment characteristics and carbon sequestration within a tropical seagrass meadow
Comments on the methods

Experimental sites

In this thesis, two of the studies – presented in Papers I and II – were performed on the Swedish west coast in the Skagerrak Sea (Fig. 2a and b). The experiments presented in Papers III and IV were part of a bigger manipulative field experiment performed in Chwaka Bay, Unguja Island, Zanzibar, Tanzania (Figs. 2a and c).

Figure 2. a) Map of the study locations in Europe and Africa. b) Magnification of the Swedish west coast with locations of the sites in Papers I and II; Site 1 is an exposed site entitled Kristineberg, and site 2 is a sheltered site entitled Getevik. c) The experimental site in Chwaka Bay, Unguja Island, Zanzibar, Tanzania used in the experiment presented in Papers III and IV.
The shallow soft bottoms of the Skagerrak Sea at the Swedish west coast provide suitable environments for the development of extensive underwater meadows dominated by the temperate seagrass *Zostera marina* L., which can coexist with other species of vascular plants like *Z. noltii* and *Ruppia maritima* or macroalgae such as *Fucus* spp., *Chorda filum* and *Ulva* spp. The seasonality in the area is pronounced, with long days in summer (up to 18 h of daylight) and water temperatures of up to 18-24°C and very short days in winter (down to 6 h 30 min of daylight) when the water temperature can drop to about -3°C and freezing of the surface water is common in sheltered bays.

Chwaka Bay, located on the east coast of Unguja Island, Zanzibar, Tanzania, is well recognized as a 'hotspot' for seagrass biodiversity, and research in the area has been extensive (de la Torre-Castro and Lyimo 2012). With up to eleven seagrass species present in a rather limited area of about 50 m² (Gullström et al. 2006, 2012), this embayment comprises one of the world’s most seagrass-rich areas. The bay is characterized by high semidiurnal tidal fluctuations varying from 0.9 m during neap tide to 3.2 m in spring tide (Cederlöf et al. 1995) and hence the bay is suitable for setting up experiments in an intertidal seagrass-dominated area. The studies presented in Papers III and IV were performed at a site located close to the shore in a well preserved meadow composed of *Thalassia hemprichii* and to some extent of the calcareous green macroalgae *Halimeda* spp.
Experimental designs

Seagrass plant material for the experiments performed in Paper I was taken from a shallow bay (also used in Paper II) located in close vicinity of the Sven Lovén Centre for Marine Sciences – Kristineberg, on the Swedish west coast (Fig. 2b). In this study, the effect of relative age on photosynthetic performance was tested on seagrass plants (Fig. 3a). Plant material was collected two times per day and fluorescence measurement were performed in laboratory conditions. Rapid light curves (RLC) and Induction curves followed by recovery (IC+Rec) were performed in order to compare between leaf parts – in the base, middle and top part of each leaf, between all leaves on the same shoot and between shoots of different age on the same genet.

Figure 3. Schematic representation of a Z. marina genet. Top view a) eight intact shoots are show, where shoot A is the oldest and shoot B1 is the youngest. Lateral view b) the locations of fluorescence measurements on shoots A and B1 are indicated with red circles. Illustrations: Diana Deyanova.
In Paper II the seasonal variations in productivity were studied over one year at two sites – one exposed and one sheltered (Fig. 2b), and at two stations within each site – a shallow station at 1.5 m depth and a deep station at 4 m depth (Fig. 4a). The exposed site is open to the northeast and faces predominantly easterly winds. It has coarse and sandy sediment due to its active wave dynamics. The sheltered site is well protected from wind and has fine-grained and muddy sediments due very low wave activity. Temperature, dissolved oxygen (DO) and photosynthetically active radiation (PAR) were logged every 30 minutes for one year – from June 2015 to June 2016. Benthic incubations were performed in flexible transparent chambers (Fig. 4b) every second month. Simultaneously, over a 24 hours period and at both depths at each station, diel fluorescence measurements with a pulse amplitude modulated (PAM) fluorometer were performed on the seagrass leaves (Fig. 4c).

Figure 4. a) Schematic drawing of the experimental design used in Paper II. Vertical profile (top) and horizontal profile (bottom). Black circles represent the incubation chambers, the line in the middle represents a transect with permanent PAR, DO and Temperature loggers at each end and a PAM fluorometer, with a logger in the middle (purple circle), connected via cables to three sensors at each depth, coupled with PAR loggers and attached to seagrass plants. b) Closed benthic incubation chamber. c) Seagrass leaf, attached to a holder, equipped with a PAR logger (blue instrument) and a fiber optic cable of the PAM fluorometer. Photos and illustration: Diana Deyanova.
In Papers III and IV, the effects of shading and simulated grazing on the performance of plants and the whole community, and on sediment characteristics and carbon sequestration, were analysed in a tropical seagrass meadow. In Paper III the design comprised of five treatments: two intensities of shading, performed by covering the seagrass canopy with a shading screen, two intensities of clipping (clipping all seagrass shoots half of their length and clipping the plants just above the meristematic region), which simulated grazing and a control. In paper IV additionally there was a sixed treatment – bare sand.

Figure 5. a) Control, C, and disturbance treatments: low shading–LS, high shading–HS, low clipping–LC, and high clipping–HC. b) Experimental setting showing randomized complete block design (n = 4), with filling patterns corresponding to treatments presented in a). c) Schematic drawing of a shoot with rhizomes and roots of a Thalassia hemprichii plant. The circle (on the third fully developed leaf) indicates the location where PAM measurements were made.
Seagrass plant material

Papers I and II were focused on the temperate seagrass *Zostera marina* (Fig. 6), while Papers III and IV were focused on the tropical seagrass *Thalassia hemprichii* (Fig. 7).

*Figure 6. Zostera marina on the Swedish west coast. Photo: Diana Deyanova.*

*Zostera marina* L. is a temperate seagrass species, which is extensively studied. It has a mono-meristematic, leaf replacing type of growth with meristems located in the base of the green shoots (Short and Duarte 2001). Branching is monopodial and alternating. The formation of a new branch is coupled with new shoot development (Kuo and Hartog 2006). This species of seagrass is widely distributed in the northern hemisphere in both the Atlantic and Pacific oceans, and is also present in the Mediterranean and the Black Sea (Green and Short 2003). It inhabits various coastal areas, primarily shallow sheltered bays with soft bottom sediments, and can be found from about 0.5 m to 6-7 m depth (Green and Short 2003). Even though *Z. marina* is widespread, substantial loss has been documented all over its distribution range (Orth et al. 2006, Waycott et al. 2009), with the Swedish west coast being no exception (Baden et al. 2003).
Thalassia hemprichii (Ehrenberg) Ascherson is a tropical climax seagrass species distributed along the coasts of Eastern Africa, India, Southeast Asia and Northern Australia (Green and Short 2003). It forms mixed or monospecific meadows, usually dense in cover and with a canopy reaching 20 cm in length. It has di-meristematic, leaf-replacing morphology with meristems at the base of the leaves and at the tip of the rhizomes.

**Light**

Photosynthetically active radiation was measured in field with permanent loggers (Odyssey, Dataflow System, New Zealand), which proved to be easy to use and sensitive enough after appropriate calibration. Calibration was done once in the beginning of each experiment described in Papers II, III and IV. As a result of the prolonged deployment time during the experiment presented in Paper II, the data recorded by the loggers had to be corrected due to fouling. Even though the loggers were regularly taken out of the water (every second month due to logistical reasons) and cleaned, it was visible from the raw data that fouling obstructed the recordings about two weeks after deployment, and therefore a correction was needed. For this purpose, PAR data recorded in air and values recorded underwater prior to fouling were used to create regression
models. The obtained equations from the regression models were used to re-
place miscellaneous data with recalculated values based on the PAR measured
in air only.

**Dissolved oxygen and water temperature**

The natural fluxes of oxygen and water temperature were recorded during the
experiment described in Paper II. To get entirely accurate measurements of O₂
concentrations in water, usually Winkler titrations are performed in the labor-
atory (Winkler 1888, Beer et al. 2001). For the purposes of this study, how-
ever, permanent field loggers, requiring minimal maintenance and satisfactory
precision, were needed; therefore, optode loggers for DO (HOBO, Onset)
were used. This type of logger has an accuracy of 0.2 mg/L in DO levels and
can measure in the range from 0 to 30 mg/L. It is equipped with antifouling
copper housing, which protects the sensor and allows accurate, long-term
measurements. The water temperature was also measured (simultaneously
with oxygen) using permanent loggers, which were set to log every 30
minutes.

**Open water mass balance**

Measurements of Net Ecosystem Productivity (NEP) by a so called “open wa-
ter mass balance method” was first used for flowing waters by Odum (1956)
and was later reviewed by Staehr et al. (2010). This methods is acknowledged
as a good way for measuring fluxes on a community scale (Macreadie et al.
2014). In Paper II, this method was used to characterize the trophic status of
the seagrass meadows as well as to calculate NEP. The rationale of this
method is the assumption that changes in oxygen levels over time during day-
light hours represent the system’s net production rate and the changes in oxy-
gen level during the night account for the respiration of the system. Hence,
diel net community production is the difference between day and night values.
Accurate measurements of oxygen and water temperature are the core of these
estimates, but also other parameters are essential. In this study, barometric
pressure, wind speed and salinity parameters were taken from the observatory
station at the Sven Lovén Centre for Marine Sciences – Kristineberg, where
measurements are regularly logged in the vicinity of the study sites.
Chlorophyll $a$ fluorescence

Measurements of photosynthetic capacity using chlorophyll $a$ fluorescence were performed both in the field and in the laboratory. This is a well-recognized technique in plant research and commonly used by many scientists (Ralph et al. 1998, Beer et al. 2001). It allows a non-invasive and non-destructive way of measuring photochemical yield and the determination of photosynthetic kinetic parameters used to determine stress level and recovery of green tissues. Other techniques for determination of plant productivity are often performed on isolated plants, detached from the substrate and enclosed in incubation chambers, where the O$_2$ or CO$_2$ change over time is measured (Beer et al. 2014). This causes stress to the plants, and their performance is altered (Buapet et al. 2013, Olivé et al. 2016). With chlorophyll $a$ fluorescence estimations it is possible to measure photosynthetic yield directly on plants in situ, without any disruption. It is also possible to make such estimations over a prolonged period of time such as over 24 h.

Several photosynthetic parameters were derived with the use of chlorophyll $a$ fluorescence technique:

- Maximum photosynthetic quantum yield – $F_{v}/F_{m}$, is a ratio used to detect stress in the photosynthetic apparatus in a dark-adapted state. $F_{v}/F_{m}$ is estimated as follows:

$$
F_{v}/F_{m} = (F_{m} - F_{0})/F_{m}
$$

where $F_{v}$ is the maximum variable fluorescence yield, $F_{m}$ is the maximum fluorescence and $F_{0}$ is the minimum fluorescence.

- Alternative and more sensitive ratio according to Lichtenthaler et al. (2005) for estimating maximum photosynthetic quantum yield – $F_{v}/F_{0}$ was also used and estimated as follows:

$$
F_{v}/F_{0} = (F_{m} - F_{0})/F_{0}
$$

- Non-photochemical quenching – NPQ, is responsible for the dissipation of excess light energy received by the photosynthetic apparatus as heat, fluorescence or overflow of excitation energy from PSII to PSI (Müller et al. 2001, Cosgrove and Borowitzkai 2010). NPQ is estimated as follows:

$$
NPQ = (F_{m} - F_{m}^{'})/F_{m}^{'}
$$

where $F_{m}^{'}$ is the maximum fluorescence yield in actinic light.

- Photosynthetic yield – $\Phi_{PSII}$, is the efficiency of PSII in the light—adapted state. $\Phi_{PSII}$ is estimated as follows:
Electron transport rates – ETR, is the linear rate of electron transport at a given irradiance. ETR is calculated as follows:

\[ \Phi_{\text{PSII}} = \frac{(F_m' - F_o')}{F_m'} \]

- Electron transport rates – ETR, is the linear rate of electron transport at a given irradiance. ETR is calculated as follows:

\[ \text{ETR} = \text{PAR} \times \Phi_{\text{PSII}} \times \text{AF} \times 0.5 \]

where PAR is the photosynthetically active radiation, AF is the absorption factor of the leaf and 0.5 is an accepted factor that reflects the fraction of photons captured by both PSII and PSI.

ETR and PAR were further used to construct production to irradiance curves (P/I). A fit suggested by Platt et al. (1980) was used to derive the maximum ETR (ETR$_{\text{max}}$) and the slope of the alpha curve (further referred to as “alpha slope”). ETR$_{\text{max}}$ is the maximum photosynthetic capacity of plants, whereas the alpha slope is the photosynthetic efficiency.

Chlorophyll $a$ fluorescence measurements were performed with two pulse amplitude modulated (PAM) fluorometers – a Dive PAM (Walz, Germany) and a Multi-sensor PAM (Aquation, Australia). The Dive PAM was used mostly for the performance of RLC. The Multi-sensor PAM has six separate sensors that can operate simultaneously. This instrument was used for prolonged measurements (up to 24 h) of $\Phi_{\text{PSII}}$ in the field. The optical cable transferring the signal of the sensor to the leaf is shown in Fig. 4c.
Plant productivity model

Primary productivity of seagrass plants is often estimated indirectly by measuring growth, most commonly by the so called leaf puncturing technique – a method in which the plants are punctured with a needle in the meristematic region of the shoot and growth is measured after several days; the hole made by the needle moves along the length of the shoot and the distance is measured (Short and Duarte 2001). Plant growth or the accumulation of biomass are not equal to CO₂ fixation; therefore, for the purpose of estimating carbon capture, which also reflects carbon accumulation and sink capacity in the sediments, measurements of fixation rates of CO₂ are a reliable way to evaluate the contribution of primary producers. In Paper III, a model was proposed for the estimation of net plant productivity, and in Paper II, this model was further developed and expanded (Fig. 8, Table 1). In this model, the basis for estimating GPP is the direct link between ETR, O₂ evolution and CO₂ fixation during photosynthesis (Beer et al. 1998, Beer and Björk 2000, Silva and Santos 2004). In order to determine the NPP over the year several parameters were considered – respiration rates of the whole plant, photorespiration induced by oxygen levels in the water (Buapet et al. 2013), light availability, the absorption factor of the leaves, the ratio between dry weight and leaf area specific dry weight (DWₛₚ) and above- and belowground biomass. All these factors were incorporated in the model.
Table 1. Summary of all measured and derived parameters together with equations used in the proposed model for estimating yearly NPP of seagrass plants.

<table>
<thead>
<tr>
<th>Measurements frequency</th>
<th>Measured parameters</th>
<th>Derived parameters</th>
<th>Equation</th>
<th>n.d.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Every second month</td>
<td></td>
<td>Electron transport rates</td>
<td>ETR&lt;sub&gt;l&lt;/sub&gt; μmol e&lt;sup&gt;-&lt;/sup&gt; m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;</td>
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<td></td>
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<td></td>
<td></td>
<td>Maximum quantum yield</td>
<td>ETR&lt;sub&gt;l&lt;/sub&gt; = ETR&lt;sub&gt;l&lt;/sub&gt;&lt;sup&gt;max&lt;/sup&gt; x PAR&lt;sub&gt;l&lt;/sub&gt; x AF x 0.5</td>
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<td></td>
<td></td>
<td>Photosynthetically active radiation</td>
<td>PAR&lt;sub&gt;l&lt;/sub&gt; μmol photon m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>Photosynthetic efficiency</td>
<td>Φ&lt;sub&gt;PSII&lt;/sub&gt;</td>
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<td></td>
<td></td>
<td>Absorption factor</td>
<td>AF</td>
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<td></td>
<td>Abovement ground biomass</td>
<td></td>
<td>A&lt;sub&gt;b&lt;/sub&gt; g dry m&lt;sup&gt;-2&lt;/sup&gt;</td>
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<td></td>
<td>Belowground biomass</td>
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<td></td>
<td>Specific dry weight of seagrass tissue</td>
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<td>DW&lt;sub&gt;cells&lt;/sub&gt;</td>
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<td></td>
<td>Photosynthetically active radiation</td>
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<td>PAR&lt;sub&gt;l&lt;/sub&gt; μmol photon m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;</td>
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<td></td>
<td>Dissolved oxygen</td>
<td></td>
<td>C&lt;sub&gt;l&lt;/sub&gt; mg L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td></td>
<td></td>
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<td></td>
<td>Gross primary production of aboveground tissue</td>
<td></td>
<td>GPP&lt;sub&gt;g&lt;/sub&gt; g CO&lt;sub&gt;2&lt;/sub&gt; g tissue 30 min&lt;sup&gt;-1&lt;/sup&gt;</td>
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<td></td>
<td>Respiration of aboveground tissue</td>
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<td>R&lt;sub&gt;g&lt;/sub&gt; g CO&lt;sub&gt;2&lt;/sub&gt; g tissue 30 min&lt;sup&gt;-1&lt;/sup&gt;</td>
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<td>Respiration of belowground tissue</td>
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<td>R&lt;sub&gt;b&lt;/sub&gt; g CO&lt;sub&gt;2&lt;/sub&gt; g tissue 30 min&lt;sup&gt;-1&lt;/sup&gt;</td>
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<td></td>
<td>Net primary productivity of aboveground tissue</td>
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<td>NPP&lt;sub&gt;g&lt;/sub&gt; g CO&lt;sub&gt;2&lt;/sub&gt; g tissue 30 min&lt;sup&gt;-1&lt;/sup&gt;</td>
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<td></td>
<td>Net primary productivity of belowground tissue</td>
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<td>NPP&lt;sub&gt;b&lt;/sub&gt; g CO&lt;sub&gt;2&lt;/sub&gt; g tissue 30 min&lt;sup&gt;-1&lt;/sup&gt;</td>
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</table>
Figure 8. A schematic representation of the mathematical model used to calculate NPP of seagrass plants over a full year. The numbers in circles correspond to equation numbers in Table 1. The abbreviations in the boxes are measured and derived parameters, explained in Table 1. Colors correspond to measured parameters frequency over the year: blue – parameters measured every two months, red – parameters measured every 30 minutes and green – parameters measured once.
**Benthic incubations**

Benthic incubations performed in enclosed chambers are commonly applied for estimating productivity in marine soft bottom communities. It has been argued that several issues need to be considered when applying this method. Stagnation of the water in the chambers can lead to an increase in thickness of the diffusion boundary layer next to the surface of the leaves. Elements trapped in this layer can only diffuse in the plant cells through molecular diffusion, which is a slow process in water. This can restrict the CO₂ diffusion and consequently reduce photosynthesis (Westlake 1967, Champenois and Borges 2012). Moreover, prolonged incubation time may lead to both supersaturation of oxygen and depletion of inorganic carbon in the water, which increases photorespiration of the plants (Buapet et al. 2013), which in turn leads to underestimation of productivity. Therefore, short incubation times and good mixing of the water in the chambers are recommended (Olivé et al. 2016). In the experiments performed in Papers II and IV, conditions were improved by the use of flexible incubation chambers made of transparent plastic that allows movement of the enclosed community together with the natural water motion. In Paper II, the incubations lasted for two hours, which is an optimal time period according to Olivé et al. (2016). Incubations were repeated three times over 24 hours – in the morning, afternoon and night (in full darkness).

**Respiration**

Belowground tissues of seagrasses have generally lower respiration rates per weight compared to photosynthetic green tissues (Kraemer and Alberte 1993, Dunton and Tomasko 1994, Masini et al. 1995). The separate estimation of respiration in underground tissues is often omitted in the estimation of plant productivity due to methodological difficulties (Hemminga 1998). Instead, the respiration rate of aboveground tissues, such as part of a leaf, is commonly used to extrapolate respiration of the whole plant. However, the biomass of roots and rhizomes makes up a substantial part of the total seagrass biomass and using the respiratory rates derived from green tissue can lead to a significant overestimation of the total respiration: This may lead to an underestimation of whole plant productivity. In Paper III, existing literature data on reported tissue respiration rates of above- and belowground biomass for *T. hemprichii* organs was combined. These data naturally had a large intrinsic variation, which led to a high variation in the modelled productivity. Therefore, there was a need for the development of a complex measuring system in the study presented in Paper II. A custom-made double compartment chambers was used (Fig. 9) in order to estimate the respiration of both belowground non-photosynthesizing tissue and aboveground green tissue – which ultimately allowed for the use of whole uprooted undamaged plants. Each chamber was made up of two plastic jars (200 ml in volume) attached to each other.
via a rubber stopper with a hole. The meristematic region of the plant was held in the rubber stopper and sealed with a spongy foam material that did not allow any gas leakage between the two chamber compartments. Two types of chambers were used, one with two dark compartments for estimating respiration of both above- and belowground tissues, and another with one dark and one transparent compartment for estimating aboveground production and belowground respiration. Control incubations were performed using chambers filled only with sea water. A glass ball (d = 1 cm) was placed inside each of the chamber compartments to improve water mixing by freely moving inside the compartment. This is a less invasive method compared to the incubation of leaf and roots parts, and allows for the determination of production to respiration (P/R) ratios. Such kind of ratio can later be incorporated in a model for the estimation of productivity.

Figure 9. Schematic representation (left) of the double compartment incubation chambers used to estimate P/R ratios of whole seagrass shoots. A photo (right) showing the chambers during underwater incubation, while attached to a raft with a coupled PAR logger (blue device to the left in the photo). Photo and illustration: Diana Deyanova.
Results and discussion

The effects of tissue age on productivity and ability to cope with excess light (Paper I)

In this study, the age related variation of photosynthetic performance and the response to high light stress of tissues of different age were tested using chlorophyll \( a \) fluorescence measurements. The age of the seagrass tissues had an effect on both productivity and the recovery capacity of the plants. Differences were detected within leaves, i.e. between the bottom and top parts, as well as between the leaves of the same shoot from the youngest leaf (L1) to the oldest leaf (L6). Along the genet, when comparing shoots of different age, only the youngest shoot, formed on the most recently branched rhizome, differed from the other shoots.

The leaves of a fully developed shoot are shed on regular intervals and have gradually aging tissues. \( ETR_{\text{max}} \) and alpha were similar for the tissues of the youngest leaves (L1 and L2), which are still undergoing greening and are located just above the meristems, and for those of senescing leaves (L4 and L5) located at the top of the leaves. Mature tissues however, had higher values of \( ETR_{\text{max}} \) and alpha compared to those mentioned above (Fig. 10a). On the contrary, very high energy dissipation capacity was observed in the very young tissues, which is demonstrated by the significantly higher NPQ in the base of leaves L1 and L2, compared to the top of L4 to L6 (Fig. 10b). This, however, resulted in a lower percentage of recovery from light inhibition of the youngest tissues compared to the oldest tissue (Fig. 10c). The reason for the observed high NPQ in very young tissues could be the difference in pigment composition. Higher concentrations of carotenoids and xanthophyll cycle pigments compared to chlorophyll \( a \) and \( b \) can facilitate more efficient photo-protective mechanisms (Thiele et al. 1997). The senescing sections are located in the top parts of the oldest leaves and senescence seems to begin in L4 where lower \( F_{v}/F_{0} \), \( ETR_{\text{max}} \) and alpha slope can be seen in the top part. In L5, the effect of senescence is seen from the middle to the top and L6 can be defined as a leaf which undergoes senescence along the whole length. To better illustrate the general performance of tissues, they were categorized based on values of \( F_{v}/F_{0} \), \( ETR_{\text{max}} \) and the alpha slope (Fig. 11).
Figure 10. a) ETR\textsubscript{max} calculated from RLC, performed on the base, middle and top parts of the leaves of shoot A (the oldest one). Values are mean ± SE, n=10. b) Mean NPQ during exposure to strong light intensity. Comparison between shoots A and B1, n=10. c) Percent recovery of F\textsubscript{v}/F\textsubscript{0} in the end of the recovery period after exposure to light stress. Comparison between the base, middle and top parts of the leaves of shoot A (left) and between shoot A and shoot B1 (right). Values are mean ± SE, n=10. The different letters above bars in a) and c) represent significant differences between the different parts of each leaf with p<0.05 or between shoots of different age, respectively. Numbers 1 to 6 on the horizontal axes correspond to number of leaves L1 (youngest) to L6 (oldest).
Even when shoots are of different ages, their leaves will be shed in regular similar intervals and will therefore be of equivalent age. The youngest shoot on a *Zostera marina* genet is shorter than the rest of the shoots, but nevertheless its leaves will reach their terminal height and undergo senescence. When comparing leaves of the same age between the youngest and the oldest shoot, a significant difference was observed in their recovery capacity but not in their photosynthetic performance (Fig. 11c). The reasons for this differences are not yet clear.

**Figure 11.** Performance map of the different leaves within an average *Z. marina* shoot. a) $ETR_{\text{max}}$ and the alpha slope of a rapid light curve are presented on the same graph since they have the same performance in the different tissues, and b) $F_v/F_o$. Green indicates high performance tissue, yellow indicates medium performance tissue (with ETR 19.9 % lower than high performance) and red indicates low performance tissue (43.6 % lower than high performance). The map is based on Tukey’s post-hoc analysis, and comparisons are performed among leaves and parts of leaves within shoot A, respectively, $n=10$, $p<0.05$. 
The effects of season, depth and exposure on plant- and habitat productivity (Paper II)

The seasonal variation in productivity of seagrass plants at the two study sites was mainly driven by the amount of light and the water temperature. The productivity was highest in the summer months and gradually decreased towards the winter when it was very close to zero degrees Celsius at both sites. The productivity was negative at the exposed shallow station during November and December (Fig. 12, NPP_{seagrass}).

![Graph showing NPP, NCP, and NEP for sheltered and exposed stations.]

**Figure 12.** Average (± SE) monthly values of diel Net Primary Productivity of seagrass plants (NPP_{seagrass}), Net Community Productivity (NCP) and Net Ecosystem Productivity (NEP). White bars show shallow stations and dark bars show deep stations.
The difference in plant productivity between the two depths was prominent at the sheltered site but not at the exposed site. The reason can be found in the specific characteristics of the plant communities at both sites. The calculated seagrass productivity is based on photosynthetic activity, specific tissue dry weight (DW\textsubscript{sp}), above- and belowground respiration and areal biomass. The photosynthetic activity was similar at the two sites, following the patterns in light availability which as expected was higher at both shallow stations than at the deep stations. However, DW\textsubscript{sp} and the biomass differed significantly between sites as well as between depths. At the exposed shallow station, the DW\textsubscript{sp} was higher than at the deep station, while at the sheltered site it was similar at both depths. This effect can be attributed to the development of more sturdy leaves, resistant to intensified water motion (Cambridge and Lambers 1998), at the exposed shallow station. Higher DW\textsubscript{sp} leads to a decrease in gross primary productivity due to an overall decrease in area of photosynthetic surface per gram tissue. Therefore, the productivity at the deep stations remained similar, whereas at the exposed shallow station, it was reduced compared to the sheltered shallow station. The changes in biomass had an additional influence on productivity, which is illustrated in Fig. 13. At the sheltered site, the plant community had proportionally higher above- and belowground biomass at the shallow station compared to the deep one, especially in the summer months. At the exposed site, an opposite pattern in biomass was observed, as more aboveground biomass was found at the deep station in the summer months, while belowground biomass was the same at both depths. The belowground biomass does not photosynthesize, but respires and thus reduces net plant productivity. As a result, the P/R ratio of both shallow and deep communities was the same at the sheltered site, but the availability of more light for photosynthesis and more biomass were the reason for the observed high values of NPP. However, at the exposed site the higher aboveground biomass at the deep station counteracted the lack of light and brought the productivity to a similar level at both depths.

**Figure 13.** Schematic representation of the effects of exposure, depth and biomass on the NPP of seagrasses. See text for explanations.
The main effects of exposure, depth and season on ETR, NPP\textsubscript{seagrass}, NCP and NEP are presented in Fig. 14. The productivity of the benthic community was at all times lower compared to that of seagrass plants, reaching very low or even negative values at the sheltered site in August (Fig. 12, NCP). Seagrass habitats are believed to be autotrophic ecosystems (Duarte et al. 2010) but according to the observed results of community metabolism, this could depend on specific conditions. The productivity of seagrass plants varies greatly (Watanabe et al. 2005). Together with other primary producers in the system such as macroalgae, benthic microalgae and phytoplankton, seagrasses act as substantial contributors to carbon fixation. Nevertheless, often they could not compensate for the respiratory demands of the consumers at the two study sites. The NEP (Fig. 12) measured based on the change in oxygen concentrations, varied less than the NCP. It was positive throughout the whole year at the exposed site at both depths and also at the shallow station at the sheltered site. Only in the summer months at the deep station of the sheltered site, the NEP became negative. Even though the benthic community could have a negative carbon balance and be heterotrophic, the whole system was autotrophic. This is perhaps due to exchange of water mass with surrounding habitats and good mixing of water due to currents.

Figure 14. Main effects of site (sheltered or exposed), depth (shallow or deep) and season (spring, summer, autumn and winter) on ETR, NPP, NCP and NEP. Black dash – no difference, green dash – increase, red dash – decrease, blue curve – seasonal variation (corresponding to seasons indicated above). All lines represent overall averages.
Stress in a seagrass meadow – effects on seagrass plants, communities and carbon sequestration (Papers III and IV)

In this experimental field-study, the effects of two intensities of shading and simulated grazing (clipping) were tested on a tropical seagrass community with a focus on seagrass plants and sediments (Fig. 15). A clear effect of the high intensity treatments on productivity, growth and stored resources of seagrass plants was observed (Fig. 16). Effects were seen at the community level; the effect on the plants led also to an effect on the sediment, although the long-term refractory carbon pool was not affected.

Figure 15. The experimental site during advancing high tide. Wooden sticks mark the edges of the plots and the shading nets are visible above water level.

Plants go through a cascade of reactions when exposed to stress, which involves both physiological and morphological adaptations (Ruiz and Romero 2001). As an adaptation to reduced light, plants increase the chlorophyll concentration in their photosynthetic apparatus (Silva et al. 2013), which in our study resulted in an increase of their photosynthetic efficiency (measured as a reduced alpha slope of the RLC). Even though the efficiency of shaded plants generally increased, the high-intensity shading treatment led to an ultimate reduction in maximum photosynthetic capacity. On the other hand, in plants exposed to intensive clipping, the photosynthetic efficiency was reduced. This was perhaps due to exposure of the remaining tissues to too much light, since the effect of self-shading was no longer present and also by the end of the experiment, intensive clipping had led to reduced shoot density.

When physiological adaptations cannot counteract the stress from disturbances such as shading and grazing, and the stress factor is persistent, plants need to utilize stored resources in order to support growth. Seagrasses are to some extend well equipped to survive variable conditions in their environment and sustain moderate stress periods (Belsky et al. 1993). They have well-de-
veloped storage organs in terms of rhizomes, which serve as a depot for soluble carbohydrates and starch (Larkum et al. 2006) that can be relocated and mobilized where needed along the clone of the plants (Marbà et al. 2006, Collier et al. 2010). The prolonged support of growth and compensation for unfavorable conditions significantly reduced the total carbohydrate pool in rhizomes and leaves. This was evident both in percent carbon and in total soluble carbohydrates. The concentration of starch was only significantly reduced in the rhizomes of plants subjected to low shading and both intensities of clipping. As a response to shading, plants mobilize nitrogen to the leaves, in order to invest in growth (Makino et al. 1997). In this study, a higher nitrogen level was found in the rhizomes of the plants in both shaded treatments, which perhaps is due to intensified intake in order to support growth. Nevertheless, growth was reduced in all treatments except for the low clipping treatment.

Seagrasses, as other plants adapted to moderate grazing pressure, are somewhat stimulated in growth by clipping. If the grazing pressure is occasional and moderate, it will lead to intensified growth in plants (Belsky et al. 1993). However, after five months of persistent clipping, no increase in growth in the low clipping treatment was seen. The combination of resource use and persistent stress resulted not only in reduced growth rates but also in morphological modifications in the plant body, as an acclimation to the new conditions. Reduced number of leaves in the shoots in both shading treatments and reduced leaf width in all treatments except for low shading was observed. Leaf shedding has been documented for other species of seagrass as well (Carlson and Acker 1985, Czerny and Dunton 1995, Lee and Dunton 1997, Ruiz and Romero 2003).

The combined effect of all physiological and morphological changes of plants in the different treatments led to a substantial reduction of the plant net aerial productivity even at the low level of the treatments. As seen in all treatments, except in the low shading plots, the reduced productivity of the seagrass plants contributed to the reduction of the community productivity, which might alter the carbon sink capacity of the seagrass habitats. This important ecosystem function was also compromised due to the direct reduction of biomass as a result of tissue removal. This led to altered ability for sediment trapping by the seagrass leaves. It also possibly led to the observed reduced total hydrolysable amino acids (THAA) and carbon content in the top sediment layer, which could be seen in both intensity clipping treatments. This could be explained by the observed erosion, caused by the removal of plant tissue, which started to wash out the top layer of sediment.
Figure 16. Summary of observed significant effects after five months of treatments in the field experiment as a result of the imposed treatments – low shading (LS), high shading (HS), low clipping (LC) and high clipping (HC) vs the control (C). Red arrows indicate reduced values compared to the control, black arrows indicate increased values compared to the control, and dashes indicate no significant change compared to the control. AG – aboveground, BG – belowground.
Conclusions and future perspectives

This work has contributed to a deeper understanding of seagrass productivity in relation to natural and human-induced stress factors by the combination of physiological and ecological approaches.

Tissue age was shown to clearly affect the photosynthetic performance of seagrass plants, which leads to variation in photosynthetic efficiency, maximum photosynthetic capacity and the potential to recover from high light stress within a single shoot as well as between shoots of different age. Very young undeveloped tissues had similar response to stress as tissues undergoing senescence; the younger tissues had lower photosynthetic efficiency and lowered maximum photosynthetic capacity compared to the mature tissues. It was also shown that mature leaves belonging to shoots of different age perform differently. For instance, leaves of a younger shoot displayed a higher capacity for heat dissipation than leaves of an older shoot. Efficiency and maximum capacity for photosynthesis depended on leaf age, whereas shoot age had no effect. In this study, only differences in photosynthetic performance of tissues with different age was observed, but the reasons behind the observed differences are not understood. Within a single shoot, the differences could be attributed to maturation and senescence of tissues and the processes which accompany those developmental stages. The differences observed between shoots of different age but with tissues of same relative age were hard to explain with the methods applied in this study and need further investigation.

The suggested performance map of seagrass productivity can be used in productivity models. The values of productivity can be corrected for the effect of age. This will improve the accuracy of such estimates. More samples from different areas are needed for the development of a general age-related productivity profile of the whole species Zostera marina since it has a very wide range of distribution and variations are possible. The same method can also be applied to various species of seagrass.

Seagrasses are important primary producers in the habitats they dominate but even when they have very high productivity rates, they cannot always outcompete the respiratory demands of heterotrophs in the system. Variation in seagrass productivity is caused by light, temperature and other environmental
factors, which are also factors that influence variation in community productivity.

A limitation of the presented model for the calculation of seagrass primary productivity was the use of single measurements for respiration of above- and belowground tissues. Respiration varies diurnally and seasonally (Rasmusson and Björk 2014, Rasmusson et al. 2017). Therefore, more estimates with the constructed double compartment chambers are needed to clarify these variations and improve the model. Additional productivity measurement similar to those done on seagrass can be further performed on other primary producers in the system – such as the dominant macroalgal species, microphytobenthic films and phytoplankton – to clarify the contribution of each of those primary producers for the carbon metabolism of the whole system.

These results can serve as a basis for an up-scalable model of whole system carbon balance in combination with the estimation of export and import dynamics, contribution of consumers on different trophic levels and sediment dynamics. Such a model would be very important for the understanding of seagrass system dynamics in a changing climate.

Seagrasses have proven to be very persistent and well equipped to survive prolonged periods of stress; nevertheless permanent stress conditions will seriously reduce their chances for survival. The effect of shading and simulated grazing negatively affected essential functions of the plant but also the system as a whole. Photosynthesis, productivity and growth of seagrass plants were all reduced and the community net productivity was also negatively affected. The long-term refractory carbon pool was not harmed during the time of the experiment and seagrass plants managed to survive at both levels of treatment. Nevertheless, the results showed that a prolonged period of stress could, depending on the stress level, eventually lead to the destruction of the community or to a severe impairment of the important ecosystem services it provides.

The proposed method for the estimation of productivity can potentially be applied in more areas around the world where experimental data are insufficient. In combination with estimates of carbon storage the method could be used to pinpoint hotspots for conservation efforts.
Sammanfattning

Sjögräsängar utgör en av de mest produktiva livsmiljöerna på jorden och har visat sig vara mycket effektiva på att lagra atmosfärisk koldioxid (i form av organiskt kol) vilket bidrar till att motverka klimatförändringar. Produktivitetsnivån varierar emellertid kraftigt inom och mellan olika sjögrässekosystem på grund av naturlig variation och mänsklig påverkan. Sjögräs bygger upp stora ängar och plantorna utgör de basala primäraproducenterna för den totala produktiviteten inom dessa ängar. Att kartlägga variationen i sjögräsplantornas förmåga att binda kol med fokus på både fysiologiska och ekologiska skalar är nödvändigt för att förstå koldioxidbalansen i systemet. I denna avhandling studerades hur fotosyntes och produktivitet hos sjögräs påverkas av olika faktorer såsom ålder, säsong och vattendjup. Vidare utvärderades effekterna av mänsklig störning, såsom skuggning och simulerad betning, på sjögräsens kapacitet att ta upp och lagra kol i en tropisk sjögräsäng. Forskningen har inkluderat en rad olika analyser och utvärderingar av sjögräsproduktivitet från plant-till system-nivå.

Resultaten visade att sjögräsplantans ålder har en betydande inverkan på fotosyntesefektiviteten hos det tempererade sjögräset *Zostera marina* L., både inom ett skott och mellan skott av olika ålder. Vid jämförelse av blad inom samma skott var fotosyntesefektiviteten högst i mognas, äldre vävnader och avsevärt mindre i mycket unga vävnader samt i döende vävnader. När plantorna utsattes för hög ljusstress visade det sig att de mycket unga vävnaderna hade en högre kapacitet att leda bort överflödig ljusenergi, vilket demonstrerades av högre nivåer av mindre skadlig värme (så kallad "non-photochemical quenching, NPQ") jämfört med hos mognas och döende vävnader. En sådan effekt observerades också när de äldsta och yngsta skotten från samma rhizom jämfördes. Det yngsta skottet visade sig ha bättre förmåga att avleda överflödig ljusenergi jämfört med det äldsta skottet och kan därför troligen bättre klara ljusstress.

På en större rums- och tidsskala påverkades den areala produktiviteten hos sjögräsplanter signifikant av ljustillgänglighet och temperatur vilket ledde till en stark säsongssvariation. Dessutom hade djupet en lokal-specifik effekt på växtproduktiviteten i form av förändrad växtbiomassa. Under året varierade
produktiviteten väsentligt och nådde upp till 20 g C m⁻² 24 h⁻¹ under sommarmånaderna. Denna förmåga att effektivt ta upp koldioxid motverkades dock av den höga respirationen hos det bentiska samhället. Generellt sett visade systemet på en låg men positiv årlig kolbalans.

Fem månaders experimentell störning i form av skuggning och simulerad betning påverkade sjögräsen och hela deras livsmiljö negativt. På plantnivån minskade fotosyntesen, produktiviteten och tillväxten. På systemnivån observerades en minskning av samhällets produktivitet. Den långsiktiga lagringen av kol påverkades dock inte, även om erosion av sedimenterades i de experimentområden som utsattes för simulerad betning.

Sammanfattningsvis har denna avhandling visat att ålder, årstid, djup och exponering är faktorer som styr den naturliga variationen i produktivitet hos sjögräs och deras livsmiljöer samt att sjögräs påverkas av antropogen stress genom att kraftigt reducera sin produktivitet. Trots att sjögräsplanter i allmänhet kan överleva långa perioder av stress visar resultaten från denna avhandling på att långvariga försämrade livsförhållanden kommer att leda till allvarliga skador på såväl växterna som hela livsmiljön och därigenom starkt reducera sjögräsängarnas kolsänkekapacitet.
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