Seagrass Respiration

An assessment of oxygen consumption patterns of temperate marine macrophytes

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List of papers

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


II Rasmusson LM, Lauritano C, Procaccini G, Gullström M, Björk M. Respiratory oxygen consumption in the seagrass *Zostera marina* is affected by light and varies on a diel basis: a combined gas exchange and gene expression study. (Manuscript)

III Rasmusson LM, Gullström M, Björk M. Effects of increased temperature on respiration and photosynthesis differ among different leaf parts and with tissue age of the seagrass *Zostera marina*. (Manuscript)

My contribution to the papers:

Paper I: Planning the study, analysing data and writing the paper with co-author, performing all experiments.

Paper II: Planning the study, performing experiments (CL helped with gene expression analyses), analysing data and writing the paper with co-authors.

Paper III: Planning the study, analysing data and writing the paper with co-authors, performing all experiments.

Paper IV: Participated in planning and in performing the experiments and writing together with co-authors.

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Abbreviations

AOX1 alternative oxidase
ATP adenosine triphosphate
CA carbonic anhydrase
CCM carbon concentrating mechanism
Ci inorganic carbon
CO₂ carbon dioxide
CO₃²⁻ carbonate
COX5B cytochrome C subunit 5b
DBL diffusive boundary layer
DO dissolved oxygen
e⁻ electron
FADH₂ flavin adenine dinucleotide
GCHS glycine cleavage system H protein
H⁺ hydrogen ion
H₂CO₃ carbonic acid
HCO₃⁻ bicarbonate
IDH isocitrate dehydrogenase
IRGA infrared gas analysis
L. Linnaeus
MDH malate dehydrogenase
mtETC mitochondrial electron transport chain
mtPDC mitochondrial pyruvate dehydrogenase complex
NADH nicotinamide adenine dinucleotide
O₂ oxygen
OGDH 2-oxo glutarate dehydrogenase
PCR polymerase chain reaction
PDHA pyruvate dehydrogenase alpha
PDHB pyruvate dehydrogenase beta
pH power of hydrogen
qRT-PCR quantitative Real-time PCR
R_D mitochondrial respiration in dark
R_L mitochondrial respiration in light
Rubisco ribulose-1,5-biphosphate carboxylase/oxygenase
RuBP ribulose-1,5-biphosphate
TCA tricarboxylic acid
Introduction

Fluxes of oxygen and inorganic carbon in coastal areas

Coastal areas support an exceptionally high diversity of marine life due to the broad variety of niches they are offering. The living conditions in coastal zones are determined by a variety of biotic- and abiotic factors where, for instance, temperature, light, salinity and currents strongly influence the distribution of species and life-forms. The levels of oxygen and inorganic carbon also play a crucial role in the dispersal pattern within habitats. In the water column, both oxygen and inorganic carbon content change in accordance to contemporary abiotic conditions, however to a large extent, these fluxes are driven by primary production, i.e. the fixation of carbon dioxide into organic molecules by photosynthetic organisms. During photosynthesis, carbon dioxide is taken up and oxygen is released. This process is counterbalanced by the reverse function of respiration where oxygen is instead consumed and carbon dioxide is evolved. Respiration is commonly referred to as the yin to the yang of photosynthesis, meaning that even though these processes might look opposing they are actually complementary and interdependent. Oceanic photosynthetic organisms are estimated to be responsible for approximately half of the annual global net primary production (Beer et al., 2014; Field et al., 1998; Geider et al., 2001). Marine primary production is by far dominated by the input from phytoplankton, especially in the open waters (Field et al., 1998). Nevertheless, in coastal areas, marine macrophytes (i.e. macroalgae and seagrasses) have substantial impacts on the fluxes of oxygen and inorganic carbon, especially in areas with dense plant cover and shallow waters (Buapet et al., 2013; Kaldy et al., 2002; Obrador and Pretus, 2012; Saderne et al., 2013).

Mitochondrial respiration

Respiration is fundamental for the overall function of a plant, as well as for all other organisms, as energy and carbon intermediates for biosynthesis are generated. Three main processes are part of respiration: glycolysis, the tricarboxylic acid (TCA) cycle and the mitochondrial electron transport chain
(mtETC) (Fig. 1). In glycolysis, the sugars produced through photosynthesis or from degradation of complex molecules, are split into smaller organic molecules and ultimately into pyruvate. The pyruvate is incorporated into the TCA cycle and through a series of reducing steps a small portion of energy in the form of ATP is produced, and NADH and FADH$_2$ needed to drive the mtETC are released. A by-product of the reduction steps in the TCA cycle is CO$_2$. NADH and FADH$_2$ function as electron carriers, bringing the fuel that will drive the electron transport chain from the mitochondrial matrix to the inner membrane of the mitochondrion, where a series of protein complexes are embedded and thus constitutes the actual electron transport chain. As the carriers become oxidized, electrons (e$^-$) and protons (H$^+$) are released. The electrons will be pulled through the mtETC until reaching oxygen, which is the terminal electron acceptor. This part of the process is the actual driving force of the whole chain; however it would not function if the protons were not dislocated over the membrane into the intermembrane space. This action is creating a chemiosmotic gradient that drives the pumping of the ATP-synthase creating ATP, and thus regulating the activity of the chain (e.g. Amthor, 1984; Millar et al., 2011; Plaxton and Podestá, 2006; Taiz and Zeiger, 2010).

Carbon dioxide is released through the reducing steps in the TCA cycle. As much as half of the carbon dioxide taken up by photosynthesis has been found to be respired back on a diel basis (Overgaard Mogensen, 1977; Ryan, 1991) Thus, respiration can highly affect the carbon balance within coastal water bodies. Determination of the respiratory quota, meaning the carbon dioxide released per oxygen molecule consumed (Falkowski and Raven, 2007), is important for estimations of coastal carbon budgets. These should ideally be based on gross photosynthetic rates compensated by all oxygen consuming processes in light, i.e. mitochondrial respiration and photorespiration. However as these rates are practically almost impossible to obtain, gross photosynthesis is commonly compensated merely by subtracting dark respiratory rates (Falkowski and Raven, 2007). The determination of mitochondrial respiration in light is quite complex due to the interactions with and masking of other ongoing processes such as photosynthesis, photorespiration, Mehler reactions and recycling of respiratory CO$_2$ (Hurry et al., 2005; Krömer, 1995; Pinelli and Loreto, 2003; Raghavendra et al., 1994; Ribas-Carbo et al., 2010) Therefore, values for respiration have traditionally been obtained during darkness and considered constant over the day. However, this can be misleading as respiration can be altered by a wide array of abiotic- and biotic factors. Elevated temperature generally has an increasing effect as enzyme activities and membrane fluidity become higher (Amthor, 1984; Atkin et al., 2005; Ryan, 1991). Light, on the contrary, seems to have suppressing effects on the rates of respiration (e.g. Brooks and Farquhar, 1985; McCashin et al., 1988; Sharp et al., 1984; Tcherkez et al., 2005; Villar et al., 1995). The mechanisms behind this inhibition is not fully unraveled, alt-
Though products from photosynthesis - and/or photorespiration seem to be involved by regulating the flow of substrates through the TCA cycle and thereby hampering respiration (Budde and Randall, 1990; Gemel and Randall, 1992; Igamberdiev and Gardeström, 2003; Krömer, 1995; Tovar-Méndez et al., 2003). Availability of nutrients, oxygen and carbon dioxide might also affect respiration rates as well as stressors such as drought (Millar et al., 2011; Ryan, 1991). Under conditions of anoxia, e.g. during fermentation, other terminal electron acceptors than oxygen might be used, which will lower the yield of ATP substantially (King, 2005; Pace and Prairie, 2005). Respiratory demands may also differ with tissue type, age and/or maturity (Amthor, 1984; Millar et al., 2011).

**Figure 1.** A highly simplified overview of the steps of the mitochondrial respiration. The reduction of NADH and FADH$_2$ with concomitant CO$_2$ release is highlighted in the TCA cycle. The production of ATP is seen in all three major steps; however the majority of energy production derives from the oxidative phosphorylation in the mitochondrial electron transport chain (mtETC). The major protein complexes (I-V) of the electron transport chain is shown in this figure and the most fundamental functions (i.e. electron transport towards oxygen and translocation of H$^+$ over the membrane) is emphasised, however e$^-$ are released by the oxidation of NADH by dehydrogenases embedded in the membrane as well. (Figure inspired by Taiz and Zeiger 2010).
Photosynthesis

The regulation of photosynthesis in the marine environment differs significantly from photosynthesis on land and is mainly restricted by two factors: light and carbon accessibility. Diffusion also plays an important role, since the rates are about ten thousand times slower in water than in air (Beer et al., 2014; Falkowski and Raven, 2007).

As light hits the water surface, a fraction of it is reflected back to the atmosphere. Furthermore, light of the red wavelength will predominantly be absorbed by the water molecules while blue wavelengths are easily scattered and therefore spreading horizontally instead of penetrating into the depths. Therefore, the abundance of the wavelengths that most efficiently drive photosynthesis is much lower in water than what can be encountered on land (Falkowski and Raven, 2007). To be able to cope with the lack of light, submerged plants have orchestrated their pigment set up to harvest as much energy as possible from the available light. This is obtained both by altering the amount and type of chlorophylls but also by certain accessory pigments, e.g. phycobilins of the red algae and fucoxanthin of the brown algae (Beer et al., 2014; Larkum et al., 2006; Lobban and Harrison, 1994a).

Terrestrial plants take up carbon dioxide via their stomata; however, plants submerged in water lack stomata and are therefore depending on diffusion of carbon sources via the plant surface for their carbon utilization. Also the forms of inorganic carbon (Ci) present in water differ from in the air, as four different forms can be encountered; carbon dioxide, carbonic acid (H₂CO₃), bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) (Beer et al., 2014). However, H₂CO₃ is a fast living form that will instantly react with H₂O and dissociate into HCO₃⁻ (Millero et al., 2002) and will therefore not be discussed further. In seawater with a pH of about 8.1, ca. 90% of the total Ci is in the form of HCO₃⁻, about 9% in the form of CO₃²⁻ and only a mere fraction of about 1% in the form of CO₂ (National Research Council, 2010). Most submerged plants can take up both CO₂ and HCO₃⁻; however, carbon dioxide is the only form of Ci that can be reduced in the Calvin Benson cycle and is also the only form that can pass freely over the plasma membrane. Submerged plants have therefore evolved certain systems for carbon acquisition and carbon concentrating mechanisms (CCMs) to be able to overcome the constraints in carbon utilization (Giordano et al., 2005; Larsson and Axelssson, 1999; Reinfelder, 2011). A more efficient CCM for bicarbonate utilization might therefore work as an advantage in certain environments with less carbon dioxide present and could explain why some habitats are dominated by certain species (Beer and Koch, 1996; Björk et al., 2004).

The fraction of each Ci species in water is partly determined by e.g. temperature and salinity but mainly affected by the pH, i.e. the amount of hydrogen ions, in the water. Conversely, the pH of the water is regulated by the
addition and removal of carbon dioxide. Addition e.g. from respiration or enhanced atmospheric CO\(_2\) levels will lower the pH and removal e.g. by photosynthesis will elevate the pH (Fig. 2). Primary production can therefore have extensive effects on the forms of Ci of the water (Buapet et al., 2013; Frankignoulle and Bouquegneau, 1990; Kaldy et al., 2002; Obrador and Pretus, 2012; Saderne et al., 2013), especially in shallow areas. This, will in turn strongly affect the distribution of marine organisms (Beer and Koch, 1996; Bjöörk et al., 2004; Hendriks et al., 2014; Semesi et al., 2009a). When Ci, as either CO\(_2\) or HCO\(_3^-\), is consumed by the plants, pH increases (Buapet et al., 2013; Semesi et al., 2009a; Unsworth et al., 2012). At higher pH-values, the fractions of CO\(_2\) will be low or even absent, which will hamper further photosynthesis, with different extent depending on the type of CCM that the plant is using (Beer et al., 2014). Instead, at higher pH, there will be more CO\(_3^{2-}\) present; this is important for calcifying organisms as the carbonate together with calcium creates calcium carbonate essential for the build-up of their structures (Fabry et al., 2008; Hall-Spencer et al., 2008). pH reductions due to higher emissions of CO\(_2\) are referred to as ocean acidification and might impose threats to marine biodiversity as the proportion of carbonate is lowered (Doney et al., 2009; Fabry et al., 2008; Guinotte and Fabry, 2008; Hall-Spencer et al., 2008; National Research Council, 2010).

Figure 2. Relative proportions (%) of the species of inorganic carbon at different pH. At today’s oceanic pH, (ca. pH 8.1), most of the Ci is in the form of HCO\(_3^-\). This balance can be shifted by photosynthetic Ci uptake that will drive the pH up, or by respiratory CO\(_2\) release that will lower the pH. Figure inspired by National Research Council (2010) and Beer et al. (2014).
Photorespiration

Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), the key enzyme in photosynthetic carbon assimilation, has dual functions as it has both carboxylase and oxygenase properties. The carboxylase function is when Rubisco is fueling the fixation process of CO$_2$ through the Calvin Benson cycle. Rubisco also, especially under conditions of greater temperatures and high oxygen availability, drive the oxygenation of ribulose-1,5-biphosphate (RuBP) through a series of steps known as the photorespiratory pathway (Lorimer, 1981; Ogren, 1984; Tolbert, 1997). Photorespiration is hampering carbon fixation and thereby plant productivity. Photorespiration, like mitochondrial respiration, is a process where oxygen is consumed and carbon dioxide released, however while mitochondrial respiration is producing ATP and carbon intermediates necessary for plant functioning, photorespiration is consuming energy and reducing equivalents making it a rather costly process (Lorimer, 1981; Ogren, 1984; Taiz and Zeiger, 2010; Tolbert, 1997). These drawbacks mentioned aside, photorespiration is still thought to have important functions in reducing electron overflow during conditions of high light and therefore preventing photo-inhibition (Wingler et al., 2000; Voss et al., 2013). Photorespiration in terrestrial plants is extensively studied although reports on the process for submerged macrophytes are existing (e.g. Beer, 1989; Frost-Christensen and Sand-Jensen, 1992; Giordano et al., 2005; Raven, 1997) although sparse.

Seagrasses and their influence on coastal carbon and oxygen dynamics

Seagrasses need high photosynthetic rates to be able to support their extensive network of belowground tissues, and therefore keeping a high proportion of their biomass in roots and rhizomes is a trade-off in terms of energy reserves used (Hemminga, 1998). Roots give an advantage in nutrient uptake, especially in oligotrophic systems, and are necessary for anchorage. The rhizomal extension is fundamental for the clonal reproduction (Hemminga, 1998; Marbà and Duarte, 1998; Olesen and Sand-Jensen, 1994). To build up and maintain the below ground tissues, large part of assimilated carbon needs to be allocated from the aboveground biomass. High photosynthetic rates, often combined with effective carbon burial rates (i.e. the removal of fixed carbon into the hypoxic-/anoxic sediments), make seagrass
meadows targets as marine hot spots for blue carbon storage as the carbon potentially can be locked away for long periods of time. Blue carbon is the term used for the carbon capture and sequestering of marine life forms and ecosystems (Björk et al., 2008; Duarte et al., 2013; Fourquarean et al., 2012; Kennedy et al., 2010; McLeod et al., 2011). On a diel scale, seagrass productivity has been seen to alter the inorganic carbon and oxygen levels to great extents (e.g. Buapet et al., 2013; Kaldy et al., 2002; Obrador and Pretus, 2012; Saderne et al., 2013; Semesi et al., 2009a; Unsworth et al., 2012). Both dissolved oxygen (DO) and pH is driven up during hours of high productivity and brought down by respiration during darkness or less productive hours, thus creating highly fluctuating environments. Seagrass photosynthesis can thus help oxygenating the water column (Borum et al., 2006; Caffrey and Kemp, 1991, Rasmussen et al, unpublished data), keeping levels much higher than what can be seen in unvegetated areas. The pH level has been recorded to be as high as pH 9.6 in shallow seagrass meadows (Setchell, 1924), and even though such extreme values might hamper photosynthesis, the relative proportion of carbonate will increase. Hence, seagrass meadows might work as refugias for calcifying organisms that would otherwise suffer from carbonate depletion in an ocean acidification scenario (Hendriks et al., 2014; Semesi et al., 2009a, 2009b). The positive effect may even spill over to adjacent ecosystems containing calcifying organisms such as coral reefs (Unsworth et al., 2012).

Even though seagrass photosynthesis is quite well documented, both in terms of the physiological properties and on a community level, the relative importance of mitochondrial respiration, and photorespiration, is not yet as extensively studied. The respiration of seagrasses has in most cases been considered constant over the day and the values used have been obtained during darkness (Barron et al., 2006; Duarte et al., 2010; Middelburg et al., 2005), hence potential fluctuations over the day and alterations during various conditions have not always been taken into consideration. However diel fluctuations of the community metabolism in seagrass meadows have been reported (Erftemeijer et al., 1993). Moreover Greve et al. (2003) measured the internal oxygen pressure in the lacunae of the meristems and rhizomes on a diel basis, where the oxygen saturation could vary from 0% (during night) to 200% (during high photosynthesis) of air saturation. However the study was more relating to if low internal O2 could be responsible for tissue degradation and consequential seagrass die-off events than to respiration per se. Photorespiration has been thought to exist, although to be insignificant in seagrasses (Beer, 1989; Frost-Christensen and Sand-Jensen, 1992). This might be true in many cases. However, in shallow vegetated bays with high photosynthetic activity, where low CO2 and high O2 levels will be encountered, the potential for photorespiration might be different than for the conditions previously examined.
Seagrasses have evolved certain traits to be able to cope with a marine lifestyle. Salt tolerance is attained by keeping surplus of ions (mainly Na\(^+\), K\(^+\) and Cl\(^-\)) in the vacuole and by osmolyte accumulation (primarily K\(^+\) and organic osmolytes e.g. sugars) in the cytosol where the differences in ion concentrations is mainly obtained by selective ion flux and portioning of ions between the vacuole and the cytosol (Touchette, 2007). To satisfy and maintain the internal oxygen supply, seagrasses have lacunae. This is a specialised type of aerenchyma tissue that works as channels leading oxygen (and to some extent other gases) from the photosynthesising leaves down to the below ground tissues (Fig. 3) (Kuo and den Hartog, 2006). The lacunae can also work as short term storage units for oxygen (Borum et al., 2006; Greve et al., 2003), something that might interfere with productivity measurements and should be kept in mind when performing such experiments (Beer et al., 2001; Borum et al., 2006; Rasmusson et al. unpublished data). Although a large part of the internal oxygen balance in seagrasses are depending on photosynthetic oxygen evolution, direct diffusion from the surrounding water bodies are occurring during insufficient photosynthesis and during night (Borum et al., 2006). Surplus of photosynthetically produced oxygen are oxygenating coastal waters but might also diffuse out from the roots and rhizomes creating oxic zones within sediments that may improve living conditions for many organisms and also prevent sulphide intrusion into the seagrasses (Caffrey and Kemp, 1991; Greve et al., 2003; Pedersen et al., 1998). Seagrasses are therefore not only contributing to fluctuations of inorganic carbon and oxygen in the water column through their photosynthesis and respiration, but they also have an impact on the conditions in sediments.
Figure 3. Lacunae tissue (i.e. voids) in leaf (top image) and root (lower image) of Zostera marina seen in cross section. Photos: Lina Rasmusson
Objectives

The overall aim of this thesis has been to investigate oxygen consumption patterns of temperate macrophytes commonly distributed along the Swedish west coast. Primarily the central interest has been to observe the functions of *Zostera marina*. In complementary studies, *Ruppia maritima* and the macroalgae *Ulva intestinalis* have been examined as well. The main research focus has been to understand if and how respiration is changing in accordance to different abiotic and biotic factors.

To be able to do this I set out to:

- Examine the respiration rates under differing conditions and in tissues of different age and developmental stages.

- Examine whether photorespiration occurs and under what conditions this process might act on ecological relevant levels in seagrass meadows.
Methods used

Model organisms – seagrasses

Seagrasses are a polyphyletic group consisting of about 60 species belonging to 13 different genera and are grouped together due to common traits rather than phylogenetic relations (den Hartog and Kuo, 2005; Waycott et al., 2014). Their most characteristic trait is that they are the only submerged angiosperms capable of sexual reproduction in saline environments (Björk et al., 2008; Hemminga and Duarte, 2000). Algae can also reproduce sexually in marine environments although they use spores instead of seeds. Moreover most algae are not considered true plants since some of them belong to the kingdom Protista (Beer et al., 2014). Unlike algae, seagrasses have extensive networks of below ground tissues where roots are used for nutrient uptake and anchorage and rhizomes mainly for horizontal clonal dispersal (Hemminga, 1998; Kuo and den Hartog, 2006). Seagrasses are found in most coastal areas of our planet; although they have certain requirements to thrive (Short et al., 2001). To be able to support the build-up of above- and below-ground biomass, seagrass distribution is highly depending on light availability. Turbidity is therefore an important factor as too turbid waters will alter light scattering. On the other hand, if the water is too calm, sedimentation rates will increase and insolation will not be sufficient enough (Koch, 2001).

Although a few species (e.g. Phyllospadix and Amphibolis) are able to colonize areas of rocky substrates, most seagrass species are depending on sandy- or muddy soft bottom areas (Hemminga and Duarte, 2000).

Seagrasses are highly important in economic and ecological terms as they are supporting marine biodiversity and function as ecosystem engineers (Barbier et al., 2011; Costanza et al., 1997). Many of our commercially important fish and crustacean species spend parts or the whole of their lifecycles within seagrass meadows (Jackson et al., 2001), hence these meadows provide a large number of people with their livelihood and daily protein intake (de la Torre-Castro and Rönnbäck, 2004). Seagrass leaves have strong filtering capacity, which improves water clarity. Through the extensive root-rhizome system of seagrasses, sediment can be retained and erosion is prevented (Koch, 2001). Seagrasses can also be seen as natural carbon dioxide filters as they take up vast amounts of CO₂ through their photosynthesis, and parts of the carbohydrates produced is then translocated to the sediments for
long term burial, which means that seagrasses might be excellent carbon sinks (Björk et al., 2008; Duarte et al., 2010; Fourqueuran et al., 2012; McLeod et al., 2011).

Unfortunately seagrass ecosystems are declining substantially all over the planet mainly due to the impact of anthropogenic stressors (Baden et al., 2003; Orth et al., 2006; Waycott et al., 2009). The most severe threats come from eutrophication and sediment loading (Orth et al., 2006; Short and Neckles, 1999; Short and Wyllie-Echeverria, 1996; Waycott et al., 2009), reducing light availability and thus hampering photosynthesis. Overfishing can also impose an indirect threat as the food web dynamics is altered (Eklöf et al., 2008; Moksnes et al., 2008). A decline in top predators increases the amount of smaller predators that might impose a threat on grazers keeping filamentous algae under control (Jephson et al., 2008; Moksnes et al., 2008). As seagrasses thrives in coastal areas, spatial competition with e.g. harbors and human recreation activities, are reducing available habitats, which has also led to substantial loss of seagrass (Ruiz and Romero, 2003). Furthermore, the usage of destructive fishing methods such as trawling may also destroy seagrass meadows (Neckles et al., 2005). To counteract the decline of seagrass meadows there are nowadays numerous more or less successful seagrass conservation and restoration programs (Kenworthy et al., 2006; Orth et al., 2006; van Katwijk et al., 2009) and in some places marine protected areas have been implemented (Kenworthy et al., 2006). Nevertheless, the most effective strategy to prevent seagrass loss would probably be to reduce the pressure from anthropogenic stressors.

Species studied

**Zostera marina** L.

This medium-sized seagrass is also known as eelgrass. It is the main species studied in all four papers (I-IV) and the main focus is on its functions regarding productivity. *Zostera marina* has high, yet declining coverage in Swedish coastal waters where it is highly important in building up feeding- and nursery areas for fish (Baden et al., 2003; Nyqvist et al., 2009). It is by far the most well studied seagrass species in temperate waters of the Northern hemisphere, where it can be found in the waters of e.g. northern Europe, North America and the North Pacific (Short et al., 2001; Waycott et al., 2014).
Ruppia maritima L.

*Ruppia maritima* L., known as widgeon grass, can be found in highly variable environments in regards of temperature, pH and salinity and seems highly tolerant to environmental cues (Setchell, 1924; Waycott et al., 2014). As *Ruppia* can reproduce in limnic environments it was previously debated whether this genus should be considered a seagrass or not (Waycott et al., 2014), something that nowadays is generally accepted. This fairly small-sized seagrass can build up extensive monospecific meadows or grow intermixed with *Z. marina*. *R. maritima* was used in studies presented in papers I and IV in this thesis.

Figure 4. *Zostera marina*. Photos: Thomas Staveley (right and top left photo) and Mats Björk (lower left photo)

Figure 5. *Ruppia maritima*. Photo: © Diane Littler (AlgaeBase, Guiry and Guiry)
*Ulva intestinalis* L.

*Ulva intestinalis* is a common cosmopolitan green algae (Chlorophyta) that can inhabit a wide array of habitats and successfully withstands abiotic stresses caused by fluctuations in, for instance, temperature, carbon availability and salinity (Björk et al., 2004; Fong et al., 1996; Lobban and Harrison, 1994b). Due to its adaptive capabilities and the knowledge about this species, *U. intestinalis* was used as a reference organism in papers I and IV. As its photosynthetic- and respiratory responses are fast, which will alter the pH and available inorganic carbon, this species was also used as a natural pH moderator in paper IV.

![Image](urll)

*Figure 6. Ulva intestinalis. Photo: Mats Björk*

**Gas exchange measurements**

All four papers in this thesis are based upon gas exchange techniques where the fluxes of oxygen have been used to determine changes in the rates of photosynthesis- and respiration. Oxygen consumption and production can be used as a valid proxy to estimate carbon fluxes, and is commonly used as measurements of carbon can be quite complex in aqueous media (Beer et al., 2001; Pedersen et al., 2013). Alternative approaches could have been to measure carbon uptake/release by infrared gas analysis (IRGA) (Leuschner and Rees, 1993; Leuschner et al., 1998). The limitation of this technique is that it is mainly designed for CO₂ measurements in air and is therefore hard
to implement in water since its highly sensitive to changes in pH (Beer et al., 2014). Thus, oxygen measurements were chosen as a more reliable and practical way of measuring the rates of photosynthesis and respiration. Whether oxygen or carbon dioxide measurements are performed some kind of chambers or enclosures need to be used simultaneously, which might impose negative influences on the results (Beer et al., 2014; Falkowski and Raven, 2007). One of the drawbacks is that usually the incubation chambers used are quite small and hence segments of the plants need to be used instead of full shoots, and the cutting of seagrass shoots or algal thalli might stress the plant and alter the actual rates (Beer et al., 2014; Rasmusson et al. unpublished data). Both respiration and photosynthesis will modify the oxygen- and available Ci conditions within the enclosure more dramatically than in the free water bodies (Beer et al., 2014, 2001; Pedersen et al., 2013). High photosynthetic rates might enhance the oxygen levels to the extent that higher photorespiration is occurring than in natural environments. This will lower the photosynthetic rates and the faster carbon dioxide uptake in small volumes might increase carbon stress as less Ci is available for photosynthesis (Beer et al., 2014). Thus, it is of high importance to keep incubation times restricted, use appropriate amounts of plant biomass, provide stirring to reduce the diffusive boundary layer (DBL) and also control the temperatures in the chambers (Beer et al., 2001).

As mentioned above, oxygen exchange measurements have been extensively used to better understand the different processes that together result in productivity (Beer et al., 2014; Borum et al., 2006; Pedersen et al., 2013). Traditionally, respiration in aquatic environments has been measured using the highly accurate Winkler method (e.g. Williams and del Giorgio, 2005). To be able to get the dissolved oxygen (DO) concentration using this method, the water samples needs to be fixated and go through a series of titration steps (Williams and del Giorgio, 2005). This would be logistically unfavorable for the purposes of this thesis since we were interested in continuous recording of O₂ values over time and the amount of samples that needed to be taken would be highly time consuming. Thus, the Clark type oxygen electrodes were used in the experiments (Fig.7). These electrodes (here, the origin from Hansatech, UK) are highly sensitive to shifts in temperature and increased diffusive boundary layer, but as long as thermoregulation and stirring are under control they give precise readings of oxygen evolution and consumption over time (Beer et al., 2014).
Figure 7. Experimental set up: water filled incubation chamber connected to a Clark type oxygen electrode containing a 3cm long seagrass segment. Photo: Lina Rasmusson
The Kok method

Measuring mitochondrial respiration in light ($R_L$) is a quite complex task as other oxygen consuming and evolving processes occur at the same time (Hurry et al., 2005; Krömer, 1995; Raghavendra et al., 1994; Ribas-Carbo et al., 2010). When measuring $R_L$ using gas exchange techniques, two different methods are widely used: the Laisk method (Laisk 1977) and the Kok method (Kok 1948), both measuring changes in the net photosynthetic rate with irradiance (Hurry et al., 2005; Ribas-Carbo et al., 2010; Villar et al., 1994). The Kok method was found the most appropriate for our purposes. The Laisk method was discarded based on three major criteria. First of all, it is mostly used for measurements of CO$_2$ which might be troublesome in water. So far no studies are based on this method conducted on submerged plants or phytoplankton (Pärnik and Keerberg, 2007; Villar et al., 1995, 1994; Yin et al., 2011; Zou et al., 2007). Secondly, low CO$_2$ levels are needed as the Laisk method is based on the interrelationship between photosynthesis and photorespiration, which is also disadvantageous in water. Thirdly, the method is only valid for C$_3$ species, since it is based on the involvement of photorespiration. In previous studies, no clear data on the occurrence of significant rates of photorespiration in $Z$. marina have been reported (but now reported in paper IV in this thesis). This together with the statement that some seagrass species have a carbon fixation mechanism with characteristics of both C$_3$ and C$_4$ plants (Beer, 1989; Borum et al., 2006; Jana and Choudhuri, 1979) made us decide against the Laisk method.

In contrast to the Laisk method, the Kok method has been well studied in aqueous media. It is also suitable for O$_2$ exchange measurements. This method is based on the relationship between the net photosynthesis and the photon flux density, where a linear relationship is usually detected at low irradiances (Kok, 1949, 1948; Ribas-Carbo et al., 2010). In the vicinity of the light compensation point, where photosynthesis and respiration rates balance each other, the linearity can often be broken; this is referred to as the Kok effect (Kok 1948). The assumption is that at very low irradiances, the respiration is inhibited. The linearity seen under the break shows the net photosynthesis plus the inhibitory rate of respiration, while above the break it is merely the net photosynthesis. When extrapolating the slope after the break, back to the y-axis, it is possible to calculate the respiration rates in light (Fig. 8). The main drawback of this method is that low light irradiances, not reflecting mid-day levels in nature, need to be used. However, the Kok method can still be used as a good indicator of the presence of $R_L$ (Bruhn et al., 2011; Heskel et al., 2013; Padmavathi and Raghavendra, 2001; Sharp et al., 1984; Yin et al., 2011), and was successfully applied in paper I.
Figure 8. The Kok method is based on the linear response of net photosynthesis (y-axis) to photosynthetic photon flux density (PPFD, x-axis). The break of the linearity normally occurring near the light compensation point, where respiration and photosynthesis are equal, is referred to as the Kok effect. This is the point where light suppression of respiration, which occurs instantly when light is applied, is saturated. After this point the inhibition does not get bigger even if higher irradiances are applied. The magnitude of respiration in light (R\textsubscript{L}) can be obtained when extrapolating the line back to the y-axis after the break and the inhibition can be calculated by dividing this value by the initial respiration rate in darkness (R\textsubscript{D}). The steeper slope before the point where the Kok effect is apparent is the net photosynthesis plus the inhibition of respiration, while above the break is merely net photosynthesis.

Photorespiratory assessment

In paper IV, the main goal was to detect and assess the influence of photorespiration on the primary production of Z. marina, R. maritima and U. intestinalis during conditions of carbon limitation. Carbon limitation can be encountered naturally in shallow vegetated bays, since the photosynthetic activity of submerged macrophytes will exhaust the carbon dioxide pool and drive the pH up towards conditions of higher carbonate content (Buapet et al., 2013). Since photosynthesis will also produce oxygen, these conditions are highly favorable for photorespiration. To be able to create these conditions in the laboratory, we were using incubations of U. intestinalis as a natural pH moderator to either drive the pH up to various levels (the higher pH,
the higher carbon limitation) by photosynthesis or down by letting the algae respire. This pH regulated water was then used in the oxygen electrode chambers to record oxygen evolution rates. To be able to detect the presence of photorespiration, the oxygen was depleted by purging of N₂ and oxygen evolution was recorded again. Similar measurements were conducted under simulated field conditions, where the photosynthetic oxygen evolution of the seagrasses were compared in sea water from open waters and bay water, where pH and oxygen levels where higher due to alterations of high photosynthetic activity, to assess the photosynthetic capacity in environments of different inorganic carbon content.

Gene expression analyses

In paper II gene expression analyses based on quantitative Real-time PCR (qRT-PCR) were used to establish how the transcript abundance of eight genes regulating different steps of oxygen consumption were altered during a diel cycle. This technique is based on normal PCR (polymerase chain reaction), however through qRT-PCR it is possible to estimate how the amplified DNA reaction progresses during the procedure and not just as a start and end value. Thus, qRT-PCR can be termed a quantitative method since an initial value of the DNA/RNA amount is obtained but there is also a possibility to follow the amplification and assess how the gene expressions change over time (Bustin, 2002, 2000).

Five genes related to the TCA cycle were chosen: pyruvate dehydrogenase alpha and beta (PDHA and PDHB) (Budde and Randall, 1990; Gemel and Randall, 1992; Tovar-Méndez et al., 2003), isocitrate dehydrogenase (IDH) (Hodges, 2002; Igamberdiev and Gardeström, 2003; Mhamdi et al., 2010), 2-oxo glutarate dehydrogenase (OGDH) (Araújo et al., 2008) and malate dehydrogenase (MDH) (Journet et al., 1981; Nunes-Nesi et al., 2005; Tomaz et al., 2010). The function of the mitochondrial electron transport chain was represented by alternative oxidase subunit 1A (AOX1A) (Clifton et al., 2006; Maxwell et al., 1999) and cytochrome C subunit 5b (COX5B) (Welchen et al., 2004, 2002). The glycine cleavage system H protein (GCSH) was chosen for its functions of the cleavage of glycine to serine in photorespiration (Douce et al., 2001; Kikuchi, 1973), thereby detecting changes in photorespiration during the experiment. Getting a more profound picture of the photorespiration would be highly interesting, hence investigating additional genes regulating this function is suggested for future studies of seagrass functionality.
Main findings and discussion

This thesis is combining different approaches attempting to define and explain oxygen consumption patterns, and to some extent oxygen evolution, in three temperate macrophyte species with the emphasis on the seagrass *Zostera marina*. As reports on mitochondrial respiration under various conditions and overall knowledge of photorespiration in temperate seagrasses have been quite sparse there were certain questions I aimed to address when starting the work of this thesis (as presented under “Objectives”). The main question of the thesis is: “Is the oxygen consumption constant, and if not what are the main motives behind the variations?”

The work has mainly been evaluating if it is relevant to assume that mitochondrial respiration is constant and whether it is appropriate to use values obtained during darkness when estimating the influence that productivity of temperate macrophytes has on coastal carbon fluxes. Also the previous assumption that photorespiration is of minor importance for the oxygen budgets has been re-evaluated. The conclusion from the studies is that the respiration is highly variable and therefore fixed values might not be as accurate as previously thought and could even be considered wrong. The input by photorespiration is also differing from what we have previously thought and should be taken into account for more accurate oxygen and carbon flux assessments.

The focus is set on revealing the effect of a certain factor (e.g. light or temperature) on a specific process (e.g. respiratory rates) in relation to yet another factor (e.g. species or age). In the studies discrepancies of oxygen consumption depending on different factors have been encountered concluding that the rates of respiration (or photosynthesis) are not constant or stable. The finding that variations are present and that various factors, i.e. light irradiiances, time of the day, temperature, tissue maturity and photorespiration, seem to influence these fluctuations is important, and should be considered when estimating carbon- and oxygen consumption patterns caused by marine macrophytes. Listed below are factors that can alter the oxygen consumption quite severely and should be considered when estimating respiratory values for seagrasses.
The respiration rates is inhibited in light

In paper I, the influence of light on the mitochondrial respiration was examined using the so called Kok method. A clear inhibitory effect on the respiration of almost 50% was seen in *Zostera marina*. This was a quite high inhibition in comparison to *Ulva intestinalis* (where the inhibition was calculated to approximately 30%) and *Ruppia maritima* (where inhibition was low and a possible Kok effect was encountered almost instantly) (Fig. 9). The method is detecting potential light suppression at low light irradiances, something that has been proposed as a drawback for the method as these low irradiances may not be encountered in natural conditions (Hurry et al., 2005; Ribas-Carbo et al., 2010). While this might be true in terrestrial systems, light availability for marine plants is generally much lower. Photosynthesis for *Z. marina* has been seen to reach saturation at irradiances as low as 35 µmol photons/m²/s⁻¹ (Zimmerman et al., 1991), thus the irradiances used in this study could be counted as relevant for natural conditions. What we do see as the Kok effect is that the inhibition of respiration is saturated, meaning that the suppression does not get larger with higher irradiances although it should still be present. However, why the suppression might not be as high as seen for low irradiances is that other properties also change with increased irradiances. For instance, the abundance of respiratory substrates (e.g. carbohydrates) will increase in response to higher photosynthetic rates, which in turn most likely elevates respiration.

![Figure 9. Differences in inhibition of respiration in light. Zostera marina displayed a high inhibition rate and a clear Kok effect at the light compensation point was seen. In contrast, for R. maritima the Kok effect was not as clear and occurred at the lowest irradiance, the suppression was merely about 10% when comparing the change of the slope between the R_D (mitochondrial respiration in dark) and the R_L (mitochondrial respiration in light).](image-url)
Respiration rates are different depending on the time of day

In Paper II, our purpose was to examine if the dark respiration rates changed at different times of the day, using gas exchange measurements and gene expression analyses. Oxygen consumption rates were recorded at seven time points around the clock and at the same time seagrass leaves were collected for gene expression analyses that were carried out later. In addition, the experimental set up was kept in total darkness for three consecutive days to examine if the respiratory cues were set in accordance to a biological clock and therefore under circadian rhythmicity. In conditions mimicking natural light the highest respiration was found in the middle of the day (13:30), with high rates also encountered during night (00:00 and 03:15) (Fig. 10). The variations for respiration rates from plants in darkness showed a similar pattern as in plants grown in the light, suggesting that a circadian rhythm is involved (Fig.10). As in a previous in-situ study respiration rates varied significantly over the day (Rasmusson et al. unpublished). The transcript abundance of the genes was fluctuating greatly over the day, specifically notable in the plants kept in complete darkness. Moreover, the rates of respiration were consistently higher in complete darkness than in the light, which was unexpected as carbohydrate depletion, and therefore lack of substrates fulfilling respiratory demands, could be expected (Baysdorfer et al., 1988; Bunce, 2001; Gary et al., 2003; Journet et al., 1986).

![Figure 10. Dark respiration rates at various time points in days of natural light regimes (dotted line) and in complete darkness (solid line). Respiration rates in complete darkness were consistently higher than in light mimicking natural conditions. The trends of peaks in respiration for both treatments were at 13:30, with slightly lower rates during the two time points during night time (00:00 and 03:15).](image-url)
The age of the shoots and the leaves, together with temperature, matters

Paper III addresses whether the rates of respiration and net photosynthesis vary in different part of the leaves, in leaves of different ages and in shoots of different ages of Z. marina using both ambient (19.1°C) and elevated temperature (29.1°C). Respiratory rates were also recorded for below ground tissues of various ages for both temperatures. Moreover we calculated the Q10 effect (i.e. how much higher the rates are with an increase of 10°C in water temperature) for the different tissue types. As expected, the rates were higher in treatments with elevated temperature, no matter the age or tissue type. We did however also encounter large differences in the photosynthesis and respiration levels among the different parts of the leaves. Both respiration and photosynthesis were higher in the mid- and tip parts of the leaves compared to the basal parts (Fig. 11). These results were expected for photosynthesis, since we expected a higher amount of photosynthetically active chloroplast in the middle and top part of the leaves. The enhanced respiration rates could be related to higher carbohydrate content available as respiratory substrate. Another explanation could be that respiration is supporting maintenance of already existing tissues rather than the production of new plant material. This is suggested as the study was conducted in late August, that can be considered quite late in the growth season of Z. marina (Hansen et al., 2000).

Respiration rates were consistently higher in the younger shoots, possibly suggesting that the individual plants in a younger developing seagrass meadow might have a higher oxygen demand and carbon dioxide release than plants in an older established meadow. However, as older meadows generally contain more respiring biomass and also harbor different microbial communities this might not necessarily be the case (Barrón et al., 2004). Photosynthetic rates were constantly lower in the older leaves, possibly related to senescence or reduction of chloroplasts (Zdenek et al., 1985). Q10 values were quite stable for respiration with similar Q10 values for all parts and tissue ages of about 1.9. However, photosynthetic Q10 values where varying from 1.1 up to almost 5 and predictions on how this process will work under higher temperatures is thus more difficult than for respiration. Our results differ from previous work where the Q10 effect of respiration was higher than for photosynthesis for Z. marina (Marsh et al., 1986). However, the Q10 is depending on several factors, e.g. initial measurement temperatures (Atkin and Tjoelker, 2003; Atkin et al., 2005) and could therefore differ between studies. The average Q10 of 1.67 for the belowground tissues indicates that higher sediment anoxia might be expected in a future increase of temperature in eelgrass beds. However, as the photosynthetic rates are predicted to be even higher (average Q10 in this study =2.71) the photosynthetic oxygen production might counteract this. Thus, even though microbial respi-
ration in the sediments might be enhanced in warmer temperatures (Apple et al., 2006; Sand-Jensen et al., 2007), oxygen consumption from the seagrasses per se might not create anoxic events.
Photorespiration

In paper IV, we evaluated the assumption that photorespiration is of minor importance in marine macrophytes (Beer, 1989; Frost-Christensen and Sand-Jensen, 1992). We used the seagrasses *Z. marina* and *R. maritima* and the green algae *U. intestinalis* and studied whether photorespiration was present during conditions of natural carbon limitation stress caused by photosynthesis. High photosynthetic rates have been seen to cause an increase of the pH and oxygen content in shallow temperate bays (Buapet et al., 2013; Frankignoulle and Bouquegneau, 1990), conditions that are favorable for photorespiration (Lorimer, 1981; Ogren, 1984; Tolbert, 1997), and might therefore stimulate photorespiratory oxygen consumption. The results showed that the photosynthetic rates were negatively affected by increased pH. The two seagrasses were more sensitive than the macroalgae, something that could be related to more efficient carbon concentrating mechanism of *U. intestinalis*. This species has been seen to have highly efficient bicarbonate utilization mechanisms (Björk et al., 2004), which is an advantage when competing with other photosynthesising organisms under low carbon dioxide conditions. When creating experimental conditions of low oxygen, thus suppressing photorespiration, the gross photosynthesis was elevated for the two seagrasses, although no effect was detected for the algae (Fig. 12). We concluded that photorespiration will most likely occur in seagrasses under natural conditions. The process might lead to enhanced carbon dioxide release and oxygen consumption into the surrounding water when the investigated plants grow in shallow waters. As a consequence, the primary production might be lower which could have an ecological significance.
Figure 12. Gross photosynthetic rates as a function of pH for Z. marina (A), R. maritima (B) and U. intestinalis (C) under normal and O₂ depleted conditions. The higher gross photosynthetic rates for the two seagrasses in depleted O₂ indicates the presence of photorespiration, which seems absent for U. intestinalis.
Conclusions

The results in this thesis show clearly that oxygen consumption rates of Zostera marina are not constant but vary depending on both abiotic factors and on the developmental stage of the plant tissue as well as with the time of day. These findings may have substantial influence on the estimations of the respiratory quota determining gross photosynthesis and the overall estimations of oxygen and carbon fluxes in vegetated coastal areas of the northern hemisphere where Z. marina is one of the fundamental habitat building species. To sum up the four papers in this thesis I conclude that:

- Mitochondrial respiration is suppressed by light. This means that during the hours of light, the carbon dioxide release from the macrophytes Z. marina, and U. intestinalis (and possibly also R. maritima) might be lower than previously estimated.

- Respiratory values obtained during darkness are variable during a diel cycle, and might be controlled by circadian rhythmicity.

- The rates of respiration and photosynthesis differed with tissue age and part of the leaves measured. Shoot age was of importance for respiratory rates whilst photosynthesis changed in accordance of leaf age.

- The average Q10 for respiration and photosynthesis was 1.94 and 2.71, respectively, suggesting a slight skewing pattern between these processes in a future warmer world. However, the respiratory Q10 was steady (i.e. the Q10 did not depend on age of tissue or part of leaf), while that of photosynthesis was highly unstable (the rates fluctuated from 1.10 to 4.82).

- Photorespiration might have a much greater impact on seagrass primary productivity than previously thought during carbon limitation, events that occur naturally due to high photosynthetic activity in shallow bays.

Overall the findings of this thesis suggest a re-evaluation of our common knowledge of the function and impact of mitochondrial respiration and photorespiration of Z. marina and to some extent also of R. maritima and U. intestinalis.
Future perspectives

The work in this thesis has revealed factors that are altering the oxygen consumption of the two most common seagrasses in Swedish waters and one very common macroalga. The methods used are general and could be applied for other species in temperate areas as well as for other systems.

The study has revealed that common abiotic factors such as light, temperature and diurnal fluctuations greatly affects the rates of respiration. In nature, these factors will be acting together, thus investigating respiratory responses to a combination of abiotic factors simultaneously would add to the ecological relevance.

Elevated carbon dioxide levels have been thought to have minor impact on respiration, but this should be examined for seagrasses as well, to be able to predict the influence of ocean acidification on this important process.

Since this work has been based on the measurement of leaf segments in the laboratory it gives good indications on general plant functions. However, to obtain more ecologically relevant results, in situ studies would be a good complement to get more profound evidence on the influence of these macrophytes on coastal carbon budget estimations.
Sammanfattning

Våra kustzoner består av en mängd olika miljöer som berikar den biologiska mångfalden i havet. Levnadsförhållanden i dessa områden varierar stort, och bestäms av flera olika icke-biologiska faktorer såsom strömmar, temperatur, salthalt och ljustillgång, men också av tillgång till näringsämnen, syre och oorganiskt kol. Marina organismer kan själva också ha stor inverkan på dessa faktorer och på så vis påverka sina egna levnadsvillkor. Havets fotosyntetiserande organismer tar upp koldioxid ur vattnet och släpper tillbaka syre genom sin fotosyntes. För att sen tillgodogöra sig energin ur de sockerarter som bildas genom fotosyntesen och ur andra organisiska molekyler så sker respiration konstant, en funktion där syre istället konsumeras och koldioxid släpps ut. En annan syrgaskonsumerande och koldioxidergende funktion är fotosyntesens kolfixeringsmekanism och sänker därför produktiviteten hos växten. Dessa tre processer har tillsammans en stor inverkan på balansen av syre och kol i havet och studier av dessa är av avgörande betydelse att förstå kolets kretslopp och möjliggöra beräkningar av tillförlitliga kolbudgetar. I grunda kustnära områden täcks botten i många fall av makrofyter, det vill säga sjögräs och makroalger, och det är respirationens roll i hur dessa organismers primärproduktion påverkar kol- och syrgasfluktuationer som denna avhandling handlar om.

Sjögräs finns längs nästan alla kuster runtom vår planet. Utbredningen har dessvärre minskat drastiskt på senare tid på grund av bland annat övergödning, ökad avrinning från terrestra system och konkurrens om plats med byggnationer och liknande. Sjögrässängar är viktiga komponenter i den marina miljön sett ur både ekonomisk och ekologisk synvinkel. Många djurarter lever hela eller delar av sina livscykler bland sjögräsen. I och med sina rötter kan sjögräsen hålla kvar sediment och motverka erosion, skotten i sig kan fungera som vågbrytare och bladen har filtrerande egenskaper som på så vis kan öka vattenkvalitén. Dessutom kan den höga fotosynteshastigheten bidra till att sjögräsekosystem fungerar som naturliga koldioxidfilter. En stor del av kolet kan dessutom bindas nere i sedimenten under långa tidsperioder vilket gör att sjögräs kan ses som effektiva marina kolsänkor. För att utreda hur väl dessa undervattensväxter fungerar som koldioxidfilter behövs också mått på deras utsläpp av koldioxid, det vill säga respiration och fotosyntes, något som har utretts inom detta avhandlingsarbete.
Fokus har varit på om, och i sådant fall hur, respirationen hos ålgräs (Zostera marina), som är den vanligaste sjögräsarten i svenska vatten, och i viss mån sjögräset hårnating (Ruppia maritima) och grönalgen tarmalg (Ulva intestinalis), ändras beroende på olika faktorer såsom ljustillgång, tid på dygnet, temperatur och ålder på den vävnad som undersöks. Anledningen till att detta har utretts är att respirationsprocesser hos havsväxter inte är så väldokumenterad, vare sig för dessa arter eller för marina makrofyter generellt. Traditionellt har värden använts som man fått genom att mäta respiration i mörker och dessa värden har sedan antagits vara konstanta över dygnet. Anledningen till detta är att respirationsen är komplicerad att mäta i ljus då andra processer som fotosyntes, fotorespiration och Mehler reaktionen sker samtidigt. Fotorespirationen har inte heller uppmärksammat i någon större utsträckning hos sjögräs, och de få undersökningar som finns noterar denna funktion som relativt obetydlig. Det har däremot inte gjorts några försök på hur fotorespirationen fungerar under förhållanden med hög syrgashalt och låga halter av oorganiskt kol, dvs förhållanden som är fördelaktiga för denna process. Sådana förhållanden kan vara vanliga i vikar med stora sjögräsbeständ där produktiviteten kan bli mycket hög.

En viktig upptäckt i denna avhandling är att syrgaskonsumtionen hos ålgräs inte är konstant utan ändras ganska markant beroende på olika faktorer. Undersökningarna är till största del utförda på laboratorium och baserade främst på gasutbytesmekaniker.

I artikel I undersökte ljusets potentiella inhibering av respiration med den så kallade Kok-metoden, inte bara hos ålgräs utan även hos hårnating och tarmalg. Hos samtliga tre arter upptäcktes en inhibering av respirationen, där störst påverkan observerades hos ålgräset som visade en nedgång med nästan hälften av vad som sågs i mörker. Detta innebär att dessa växter kan släppa ut mindre koldioxid än tidigare beräknat under dygnets ljusa timmar.

För att undersöka om respiration i mörker varierar under dygnet hos ålgräs och om dess respiration styrs av en biologisk klocka så mättes styrekonsumtionshastighet vid åtta tidpunkter under åtta dagar (artikel II). Under de första fem dagarna så hade sjögräsen, som var planterade i akvarium, tillgång till ljus av intensiteter som efterliknade vad som kan finnas i naturen. Under de efterföljande tre dagarna så befann sig växterna i konstant mörker. Resultaten från gasutbytesmätningarna visar att respirationen ändras under dygnet, och liknande mönster kunde skönas mellan de dagar som var i ljus och konstant mörker, något som kan tyda på involvering av en biologisk klocka. Dessa resultat kompletterades med genomförda analyser där reglering av åtta gener viktiga för respirationsfunktion analyserades. Resultatet av denna analyser visade att respirationen ändrades nämnvärt under dygnet, framför allt i mörker.

I artikel III undersökte om åldern på skott och blad, men också vilken del av bladet man mäter i, har någon inverkan på fotosyntesen och respirationen hos ålgräs. Även syrgaskonsumtionen hos de underjordiska delarna, det vill
säga rötterna och rhizomen, av olika ålder mätttes. Stora skillnader i syrgaskonsumtion upptäcktes hos skott av olika ålder och i olika delar av bladet, vilket indikerar betydelse av ålder hos sjögräsäng när man uppskattar kol- och syrgasfluktuationer. Åldern hos bladen verkade dock vara av mindre betydelse för respirationen men spelade stor roll för fotosyntesen. Mätningarna utföres i rådande havsvattentemperatur (19.1°C) och i förhöjt temperatur (29.1°C). Detta för att se hur fotosyntesen och respirationen påverkades av temperaturhöjningar men också för att kunna beräkna Q10-effekten. Detta är en temperaturkoefficient som används för att kunna förutse hur olika processer svarar på en temperaturökning på 10°C. Respirationen hade ett lägre (dock stabilare) Q10-värde än fotosyntesen, något som kan innebära att det blir skev balans mellan dessa processer vid högre vattentemperaturer då fotosyntesen är högre. 

Förekomsten av fotorespiration hos ålgräs, hårnating och tarmalger utsatta för stress i form av brist på oorganiskt kol undersöktes i artikel IV. Fotorespiration visade sig förekomma hos båda sjögräsarterna, och påverkade deras produktivitet negativt. Tarmalgen däremot visade inga tecken på fotorespiration något som troligen beror på en mer effektiv kolupptagsmekanism.

Sammanfattningsvis så visar denna avhandling tydliga indikationer på att vanligt förekommande sjögräsarter i tempererad miljö har en tydligt varierande respiration, beroende av faktorer såsom ljus, temperatur, tid på dygnet och vävnadsålder. Tidigare antaganden att syrgaskonsumtionen är konstant över dagen kan därför ses som direkt felaktiga. Vidare konstaterades förekomsten av fotorespiration som vanligt förekommande i situationer av låga halter av oorganiskt kol. Upptäckterna i avhandlingen är inte bara viktiga för att förstå de grundläggande funktionerna hos dessa marina växter, utan också av stor betydelse när kol- och syrgasberäkningar ska utföras i marina kustnära system.
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The love pages

I kind of did the same evolutionary journey as the seagrasses once did although in a much shorter time perspective. I started with my master’s thesis in the ocean examining macroalgal stress responses. Felt super stressed myself and decided to move my way from submersion to terrestrial ecosystems to see if it suited me better just to realize that it was below the water surface where I belonged. None of this would have been possible without my great supervisor Mats Björk to whom I will be forever grateful for giving me the chance to get back into his research group and the wonderful world of science. Thank you for being an inspiring mentor and for believing in me more than I usually do myself.


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