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# CONTENTS

LIST OF ABBREVIATIONS .....	7
1. INTRODUCTION.....	8
1.1. General background.....	8
1.2. Objectives and hypotheses.....	11
2. MATERIAL AND METHODS .....	13
2.1. Studied macrophyte species.....	13
2.2. Mesocosm experiments .....	14
2.3. Environmental variables .....	16
2.4. Laboratory experiments.....	17
2.5. Measurements of net photosynthesis.....	18
2.6. Statistical analyses.....	18
3. RESULTS .....	20
3.1. Mesocosm experiments .....	20
3.2. Laboratory experiments.....	21
3.3. Natural fluctuation of seawater $p\text{CO}_2$ and pH in shallow water macroalgal habitats .....	22
4. DISCUSSION .....	23
4.1. Interactive effects of elevated $p\text{CO}_2$ and other environmental factors on macrophytes.....	23
4.2. Sources of inorganic carbon and mechanisms of carbon acquisition for photosynthesis in macrophytes.....	25
5. CONCLUSIONS .....	27
SUMMARY IN ESTONIAN .....	28
REFERENCES.....	30
ACKNOWLEDGEMENTS .....	36
PUBLICATIONS .....	37
CURRICULUM VITAE .....	107
ELULOOKIRJELDUS.....	109

## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by Roman numerals. Original papers are reproduced by permission of the publishers Estonian Academy Publishers (I), Boreal Environment Research Publishing Board (II) and PeerJ (III).

- I. Pajusalu L, Martin G, Põllumäe A, Paalme T. 2013. Results of laboratory and field experiments of the direct effect of increasing CO<sub>2</sub> on net primary production of macroalgal species in brackish water ecosystems. *Proceedings of the Estonian Academy of Sciences* 62:148–154.
- II. Pajusalu L, Martin G, Põllumäe A, Torn K, Paalme T. 2015. Direct effects of increased CO<sub>2</sub> concentrations in seawater on the net primary production of charophytes in a shallow, coastal, brackish-water ecosystem. *Boreal Environmental Research* 20:413–422.
- III. Pajusalu L, Martin G, Paalme T, Põllumäe A. 2016. The effect of CO<sub>2</sub> enrichment on net photosynthesis of the red alga *Furcellaria lumbricalis* in a brackish water environment. *PeerJ* 4:e2505.
- IV. Pajusalu L, Martin G, Põllumäe A, Paalme T. 2016. The influence of CO<sub>2</sub> enrichment on net photosynthesis of seagrass *Zostera marina* in a brackish water environment. Submitted manuscript to *Frontiers in Marine Science*.

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Original idea			*	*
Study design	*	*	*	*
Performing the experiments	*	*	*	*
Data analyses	*	*	*	*
Manuscript preparation	*	*	*	*

## LIST OF ABBREVIATIONS

CCM	– carbon concentrating mechanism
CO <sub>2</sub>	– carbon dioxide
DIC	– dissolved inorganic carbon
DW	– dry weight
N-NO <sub>x</sub>	– nitrites+nitrates
NPP	– net primary productivity
PAR	– photosynthetically active radiation
<i>p</i> CO <sub>2</sub>	– partial pressure of carbon dioxide
pH <sub>NBS</sub>	– National Bureau of Standards scale
P-PO <sub>4</sub>	– phosphates
PSU	– practical salinity unit
TN	– total nitrogen
TP	– total phosphorus

# 1. INTRODUCTION

## 1.1. General background

Today one of the greatest global environmental issues is ocean acidification, which is directly related to our carbon dioxide (CO<sub>2</sub>) emissions. Ocean acidification is defined as a reduction in the global ocean pH, caused by the uptake of carbon dioxide from the atmosphere (Calderia and Wickett, 2003). Since the beginning of the Industrial Revolution in the 18<sup>th</sup> century, atmospheric carbon dioxide concentration has increased by ~40%, mainly due to the burning of fossil fuels and cement production emissions (Raven et al., 2005). If high CO<sub>2</sub> emissions continue, this trend will increase the CO<sub>2</sub> atmospheric concentration to ~950 ppm (Van Vuuren et al., 2011) together with the global average temperature increase of 3.2–5.4 °C by 2100 (IPCC, 2013). Since the Industrial Revolution the surface of the ocean has absorbed approximately 30% of anthropogenic CO<sub>2</sub> released from the atmosphere, resulting in a 26% increase in the acidity of the ocean (Sabine et al., 2004). However, this ocean sink buffers the effect of climate change in terrestrial systems by reducing anthropogenic CO<sub>2</sub> from the atmosphere. In fact, since the Industrial Revolution, the average surface ocean pH has already decreased by ~0.1 unit and if global emissions of CO<sub>2</sub> continue to increase, the pH may decrease an additional 0.3–0.4 units by 2100 (IPCC, 2013). The projected future changes in the pH are not uniform across the globe.

The worst-case future CO<sub>2</sub> emission scenario predicts that the surface water pH in the central Baltic Sea may decrease approximately 0.4 units by the year 2100. The low CO<sub>2</sub> emission scenario predicts a decrease in the surface water pH of about 0.26 units (Omstedt et al., 2012; Schneider et al., 2015). Moreover, the brackish water of the Baltic Sea is sensitive to increasing acidity due to its low carbonate buffering capacity compared to seawater, particularly in its northern parts (Omstedt et al., 2015). However, there is high alkalinity in the Gulf of Riga, caused by the input of large amounts of fresh water through the river runoff (Hjalmarsson et al., 2008). This makes an important change and is equivalent to an increase in the pH of 0.02–0.03 units (Schneider et al., 2015). In addition, in relatively low water temperature conditions characteristic of the Baltic Sea, CO<sub>2</sub> dissolves faster compared to warm tropical waters.

The Baltic Sea is an enclosed, brackish water sea area under a strong influence of human activities in the northern part of Europe (Fiestel et al., 2008). The main threat in the Baltic Sea is coastal eutrophication caused by over-enrichment with nutrients, first of all nitrogen and phosphorus (Pawlak et al., 2009). The complex of environmental factors characterised by wide regional and seasonal variations makes this water body a very unique and fragile environment. The biota in the Baltic Sea region consists of a mixture of marine, limnic and brackish water species (Schiewer, 2008). The carbonate system in the Baltic Sea region is affected mostly by the atmosphere–seawater gas exchange, river runoff, bottom sediments, eutrophication, hydrological processes



(upwelling), biological processes and coastal–offshore gradient (Kulinski and Pempkowiak, 2012). In the coastal areas the carbonate chemistry of seawater is also strongly affected by algal photosynthesis (increasing pH) and respiration (lowering pH) (Middelboe and Hansen, 2007). The pH and partial pressure of carbon dioxide ( $p\text{CO}_2$ ) show a remarkable amplitude of natural variability between different regions as well as a high variability on all time scales (diurnal and seasonal), especially under summer conditions (Omstedt et al., 2010; Wesslander et al., 2010).

In the shallow coastal Baltic Sea macrophytes have adapted to live in a highly variable environment. Compared to oceanic conditions, in the Baltic Sea macrophytes must cope with harsh environmental conditions such as low salinity (Larsen and Sand-Jensen, 2006), high epiphytic load (Kersen et al., 2013), high nutrient concentration and poor underwater light climate (Pawlak et al., 2009). Salinity is the main factor that controls the biomass and distribution of macrophytes all over the Baltic Sea region (Kautsky, 1988). Due to the low salinity in the north-eastern part of the Baltic Sea, the majority of the macrophytes grow at their lowest salinity limit, which may cause physiological stress for their communities. Water temperature is an important factor regulating macrophytes growth and physiological performance processes (Lobban and Harrison, 1994). In the Baltic Sea region, the water temperature is mainly associated with seasonal and annual variations (Feistel et al., 2008). Light availability controls macrophytes carbon fixation and therefore the potential productivity of coastal ecosystems where they grow (Kirk, 2011). The vertical distribution of macrophytes is mainly related to light availability, which is strongly linked to depth and water turbidity in the Baltic Sea (Kiirikki, 1996; Martin, 2000). In the Baltic Sea, the major threat to the macrophyte communities is the coastal eutrophication caused by the high nutrient supply (Cloern, 2001). Higher nutrient concentrations in seawater may cause changes in the community structure, first of all the replacement of slowly growing perennial algae with fast growing filamentous algal species (Worm and Lotze, 2006). In addition, in the north-eastern Baltic Sea the environmental conditions such as light availability and salinity are considerably different as compared to the conditions of the western Baltic Sea and therefore factors driving the dynamics of macrophyte populations are expected to be different.

Any changes in the seawater pH also cause shifts in carbonate chemistry. When  $\text{CO}_2$  dissolves in seawater, it reacts with  $\text{H}_2\text{O}$  causing the equilibrium to shift the concentration of the bicarbonate ion  $\text{HCO}_3^-$  and an increase of carbonic acid ( $\text{H}_2\text{CO}_3$ ) while the concentration of carbonate ions ( $\text{CO}_3^{2-}$ ) decreases. The relative proportions of the forms of inorganic carbon depend on the pH, salinity and water temperature. In seawater of pH  $\sim 8$  and salinity 35 PSU, about 91% of the inorganic carbon occurs as  $\text{HCO}_3^-$ , approximately 8% as  $\text{CO}_3^{2-}$  and only 1% as  $\text{CO}_2$  (Raven et al., 2005). The majority of marine macrophytes utilise the  $\text{C}_3$  photosynthetic pathway without any carbon concentrating mechanisms (CCMs) (Koch et al., 2015). Based on the external carbon source for photosynthesis there are three different types among macroalgae:  $\text{HCO}_3^-$  using

macroalgae, only  $\text{CO}_2$  using macroalgae and both  $\text{CO}_2$  and  $\text{HCO}_3^-$  using macroalgae. The vast majority of the investigated macrophytes use mainly  $\text{HCO}_3^-$  as the external  $\text{C}_i$  source for photosynthesis (Sand-Jensen and Gordon, 1984; Koch et al., 2013; Beer et al., 2014). Thus, the response of macrophytes to elevated  $p\text{CO}_2$  will depend also on which carbon source they use for photosynthesis and on the mechanisms of carbon acquisition (Van den Berg et al., 2002; Hepburn et al., 2011; Koch et al., 2013). The varying responses of different macroalgal species to elevated  $\text{CO}_2$  could be due to their different strategies for carbon use for photosynthesis, which vary significantly between macroalgal species, as well as to the habitat (Ray et al., 2003; Hepburn et al., 2011; Moulin et al., 2011; Koch et al., 2013).

Fundamental changes in the carbonate chemistry of seawater due to the rapid ocean acidification are predicted to cause extensive changes in marine ecosystems worldwide (Doney et al., 2009). The vast majority of studies have focused on the responses of calcifying organisms to the negative effects of ocean acidification, particularly corals, molluscs and calcareous micro- and macroalgae. Studies have shown mostly a decrease in calcification and enhanced dissolution in benthic calcifying macroalgae to elevated  $p\text{CO}_2$ . Therefore, a future increase in seawater acidity has been predicted to influence negatively calcifying macroalgae (e.g. Hall-Spencer et al., 2008; Jokiel et al., 2008; Kuffner et al., 2008; Martin and Gattuso, 2009; Baggin et al., 2014). For example, Kuffner et al. (2008) found a negative effect of acidification on the recruitment rate and growth of crustose coralline algae. On the other hand, studies conducted with non-calcifying macroalgae have overwhelmingly shown a positive response to  $\text{CO}_2$  enrichment; for example, resulting in enhanced photosynthesis (Porzio et al., 2011) and increased growth rate (Gao et al., 1991; Kübler et al., 1999; Eklöf et al., 2012). Thus, it has been suggested that future increasing  $\text{CO}_2$  concentrations in seawater may enhance the competitive advantages of non-calcifying over calcifying macroalgal species (Kuffner et al., 2008; Hofmann et al., 2012; Koch et al., 2013). Beside non-calcifying macroalgae, seagrass meadows are expected to benefit under a future high  $\text{CO}_2$  world. Studies have shown enhanced photosynthesis (Thom, 1996; Invers et al., 2001), increased growth rate, increased reproduction and below-ground biomass of seagrasses under elevated  $p\text{CO}_2$  (Zimmerman et al., 1997; Palacios and Zimmerman, 2007). However, several studies have reported no effect of elevated  $p\text{CO}_2$  on the photosynthesis (Cox et al., 2016) and growth of seagrasses under long-term  $\text{CO}_2$  enrichment (Campbell and Fourqurean, 2013).

The effects of the increasing  $\text{CO}_2$  concentrations on macrophytes in the brackish water Baltic Sea are still poorly studied. In recent years, there has been an increasing amount of literature on the effect of  $\text{CO}_2$  enrichment on macrophytes in brackish water conditions. Eklöf et al. (2012) investigated the seagrass *Zostera marina* and macroalgae from the Kattegat region of the Baltic and found substantial effects of warming and small positive effects of acidification on their growth. In mesocosm experiments, Graiff et al. (2015) investigated in all seasons the combined effects of elevated  $p\text{CO}_2$  and temperature on the

macroalga *Fucus vesiculosus* in the conditions of the western Baltic Sea. They found that elevated  $p\text{CO}_2$  in combination with the warming effect increases the growth of *F. vesiculosus*. A recent study from the same area focused on the single and combined effects of  $\text{CO}_2$  and warming on early life-stage *F. vesiculosus* during four seasons (Al-Janabi et al., 2016). Acidification was detected to have much weaker effects on *F. vesiculosus* compared to warming.

Ocean acidification is a progressing research area. The assessment of the impact of increasing  $\text{CO}_2$  concentrations in seawater on marine organisms can be affected by different methodological approaches. Our present understanding of the sensitivity of marine organisms to ocean acidification is based largely on short-term laboratory experiments under constant environmental conditions. In recent years, numerous long-term ocean acidification studies have been conducted near natural  $\text{CO}_2$  vents (e.g. Hall-Spencer et al., 2008; Fabricius et al., 2011; Porzio et al., 2011; Russell et al., 2013; Takahashi et al., 2015). It should be noted that these studies are observational, and it is not always clear in these studies whether responses are driven only by changes in the  $\text{CO}_2$  concentration. In situ perturbation experiments are one of the key approaches used to study the biological response to ocean acidification. These studies are manipulative and based on comparisons between elevated  $p\text{CO}_2$  treatments and control conditions (Barry et al., 2010). Additionally, one of the important questions is which techniques to use for manipulating seawater carbonate chemistry. The majority of biological experiments have used  $\text{CO}_2$  bubbling or the additions of acid (commonly  $\text{HCl}/\text{NaOH}$ ). However, it should be noted that these two techniques affect carbonate chemistry differently. The  $\text{CO}_2$  bubbling technique is more recommended because it is arguably much closer to actual ocean acidification (Hurd et al., 2009; Gattuso et al., 2010).

## 1.2. Objectives and hypotheses

*The main aim of this doctoral thesis was to describe the possible effect of elevated water  $p\text{CO}_2$  on the net photosynthesis of macrophyte species that live in a highly variable pH environment in the brackish north-eastern Baltic Sea.*

The majority of ocean acidification experiments have been conducted in laboratory under constant environmental conditions. In the shallow coastal Baltic Sea the environmental factors are characterised by large amplitude of natural variability caused by seasonality and rapid changes in meteorological conditions. However, the biological responses to elevated  $p\text{CO}_2$  in combination with these short-term natural fluctuations of environmental factors have received relatively little attention. To fill in this gap the current study focused on following the effect of elevated  $p\text{CO}_2$  on photosynthesis of macrophytes in a natural light and temperature environment.

*The second aim of this thesis was therefore to determine whether elevated  $p\text{CO}_2$  in combination with short-term natural fluctuations of environmental factors exerts an interactive effect on the net photosynthesis of macrophytes.*

Based on the above the specific hypotheses were as follows: (1) the net photosynthesis of macrophytes would benefit from elevated  $p\text{CO}_2$  levels, (2) the response would vary between species and (3) the response would vary depending on surrounding environmental conditions.

These hypotheses were tested in situ in short-term mesocosm experiments during four different experimental periods in 2011–2014 under summer conditions in the north-eastern Baltic Sea.

*In addition we aimed to describe the short-term variability of  $p\text{CO}_2$  and pH in a natural shallow water macroalgal habitat.*

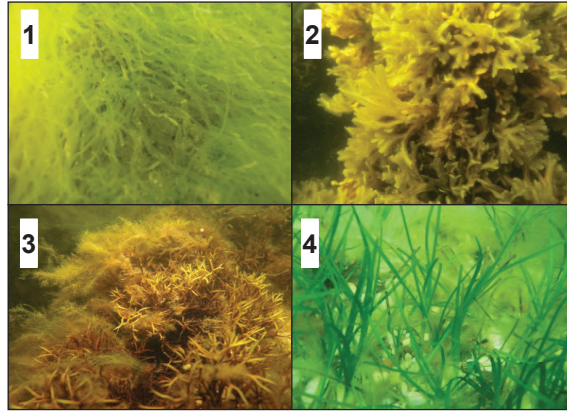
## 2. MATERIAL AND METHODS

### 2.1. Studied macrophyte species

In this thesis, macroalgae, charophytes and seagrass are collectively referred to as marine macrophytes (macro-autotrophs), which live in a highly variable pH environment in shallow coastal waters of the brackish Baltic Sea. This study focuses on three macroalgal species: *Ulva intestinalis* Linnaeus, a fast-growing green alga (I); *Fucus vesiculosus* Linnaeus, a perennial brown alga with a slow metabolism (I); and *Furcellaria lumbricalis* (Hudson) J.V. Lamouroux, a perennial red alga (I, III), three different soft bottom species of charophytes: *Chara aspera* Willd, *C. horrida* Wahlst and *C. tomentosa* L. (II) and the seagrass *Zostera marina* Linnaeus (IV). These macrophyte species play key roles in the shallow benthic ecosystem in the north-eastern part of the Baltic Sea. They have different requirements for substrates and their communities grow on different habitats throughout the Estonian coastal waters. Benthic macroalgae are dominant components of habitats in rocky shores throughout the Baltic Sea (Kontula and Haldin, 2012). Charophytes are submerged, rooted plants with well-developed complex thalli and they prefer shallow sheltered soft-bottom (especially mud) habitats (Kovtun-Kante, 2015). The seagrass *Z. marina* is one of the most common macrophytes on the species-poor sandy bottoms and is regarded as a key species of this habitat (Boström et al., 2014). Macrophyte communities are important habitats for diverse epiflora and fauna, providing food for coastal food webs, shelter and nursery areas for several species of macrozoobenthos, fish and birds. Moreover, they are the main primary producers in the shallow coastal Baltic Sea ecosystem. The studied macrophyte species are shown in Fig. 1 and Fig. 2.



**Figure 1.** Experiments were carried out with three different soft-bottom species of charophytes: *Chara horrida* (1), *Chara aspera* (2) and *Chara tomentosa* (3).



**Figure 2.** Three macroalgal species: *Ulva intestinalis* (1), *Fucus vesiculosus* (2), *Furcellaria lumbricalis* (3), and the seagrass *Zostera marina* (4) used in the experiments.

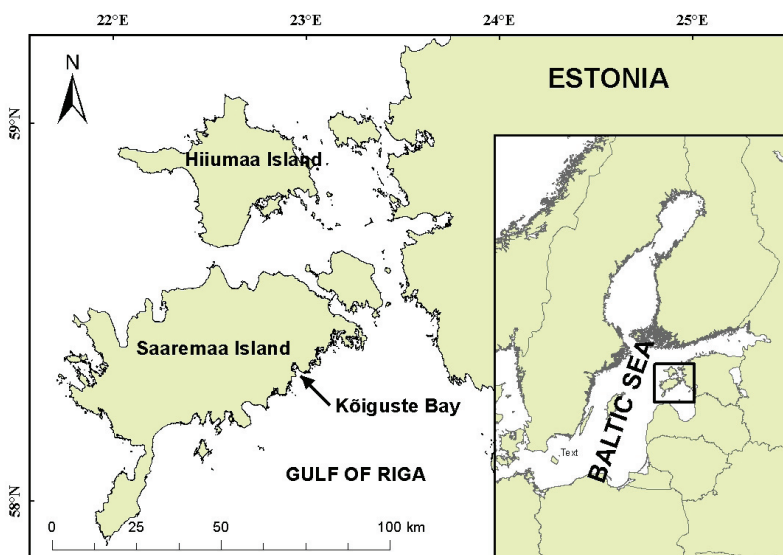
## 2.2. Mesocosm experiments

The mesocosm experiments were carried out in the shallow semi-enclosed Kõiguste Bay, Gulf of Riga, northern Baltic Sea (58.371°N, 22.980°E) (Fig. 3). The study area lacks major freshwater inflows, but is affected by nutrient inputs from the moderately eutrophic Gulf of Riga (Astok et al., 1999; Kotta et al., 2008a). The average salinity of the Gulf of Riga varies between 5.0 and 6.5 PSU (Kotta et al., 2008a). The dominant sediment types in the study area are sandy clay mixed with pebbles, boulders or gravel. The dominant macroalgal species are *Cladophora glomerata*, *Ulva intestinalis*, *Pilayella littoralis*, *Myriophyllum spicatum*, *Potamogeton perfoliatus* and *Zannichellia palustris*.

The experimental design was similar during all experimental periods in 2011–2014. In Paper **I** the experimental periods were 05 July–08 July 2011 and 19 July–22 July 2011. In Paper **II** the experimental period was 26 June–4 July 2012. In Paper **III** the experimental periods were 26 June–07 July 2012 and 18 July–27 July 2013. In Paper **IV** the experimental periods were 18 July–27 July 2013 and 16 July–26 July 2014.

Specimens of the macroalgae *F. lumbricalis*, *U. intestinalis* and *F. vesiculosus* were collected by SCUBA diving from the sea area adjacent to the mesocosm experimental sites (**I**). Charophytes *C. horrida*, *C. aspera* and *C. tomentosa* were collected by SCUBA diving from the Väike Strait (58.512°N, 23.203°E) from depths down to 2.4 m (**II**). The specimens of *F. lumbricalis* were collected by SCUBA diving in Kõiguste Bay between the depths of 1.5 and 3 m (**III**). The specimens of *Z. marina* were collected by SCUBA diving at 3.0 m depth from Küdema Bay (58.533°N, 22.238°E) (**IV**). All specimens were placed in coolers containing seawater and transported to the experimental site immediately. The collected specimens were cleaned of all macroscopic epiphytes. Specimens of the macroalgal species and charophytes (**I–III**) were acclimatised

at the experimental site for 24 hours and of the seagrass *Z. marina* (IV) for three weeks prior the start of taking net photosynthesis measurements.



**Figure 3.** Location of the study area. The arrow shows the location of the experimental site.

For all field experiments plastic bags (double wall of clear LDPE foil, 175  $\mu\text{m}$  each) externally supported by metal frames were used as mesocosms (Fig. 4). The bags were floating in the sea, fixed to the bottom by anchors at a depth of 0.8 m. The bags were open on the top, so there was free gas exchange with the atmosphere, but not with the surrounding water. In Paper I two plastic bag mesocosms, each with dimensions of 1.2 m  $\times$  1.0 m  $\times$  1.5 m and a volume of 400 l, with an elevated  $\text{CO}_2$  level were set up. The amount of the added  $\text{CO}_2$  was not actively controlled, and the concentrations fluctuated during the whole experimentation period, but always exceeded the average natural level. However, the actual  $p\text{CO}_2$  was always measured before incubations. Algae kept outside of the mesocosms were used as controls.

In Papers II–IV three plastic bag mesocosms were set up: two mesocosms with elevated  $p\text{CO}_2$  levels ca. 1000  $\mu\text{atm}$  and ca. 2000  $\mu\text{atm}$  and one with the untreated level of ca. 200  $\mu\text{atm}$  (control treatment). In the control treatment, during photosynthesis measurements the average of  $p\text{CO}_2$  was ca. 200  $\mu\text{atm}$ . The  $p\text{CO}_2$  level of 2000  $\mu\text{atm}$  is much higher than the recommended maximum  $p\text{CO}_2$  level of 1000  $\mu\text{atm}$  predicted by the year 2100 for seawater (Barry et al., 2010). The high target  $p\text{CO}_2$  level of ca. 2000  $\mu\text{atm}$  was chosen because the natural values of  $p\text{CO}_2$  in Kõiguste Bay measured prior to the start of the mesocosm experiments turned out to be well above the level of 1000  $\mu\text{atm}$  in the summer mornings. Water from the sea area adjacent to the mesocosms incubation site was sieved using a 0.25 mm mesh and used for mesocosms. The



tanks with CO<sub>2</sub> slowly bubbled food grade carbon dioxide into the water in the mesocosms. The  $p\text{CO}_2$  level was measured using an underwater (sensor) automatic CO<sub>2</sub> data logger (CONTROS™ DETECT 2.0, Germany), connected to a custom-made controller to maintain CO<sub>2</sub> in mesocosms at the required levels. However, due to the response lag of the CO<sub>2</sub> sensor used (15–20 min), the actual CO<sub>2</sub> level oscillated by 10% around the level preset by the controller. The pH<sub>NBS</sub> (National Bureau of Standards scale) values of each treatment were controlled every day before net photosynthesis measurements were taken.



**Figure 4.** Three plastic bag mesocosms were set up in the Kõiguste Bay: two mesocosms with elevated  $p\text{CO}_2$  levels ca. 1000  $\mu\text{atm}$  and ca. 2000  $\mu\text{atm}$  and one with the untreated level of ca. 200  $\mu\text{atm}$ .

### 2.3. Environmental variables

In the mesocosm experiments water temperature, oxygen saturation, pH<sub>NBS</sub> and salinity were measured continuously using a YSI 6600V2 environmental multiprobe (pH electrode YSI 6589FR). Measurements were performed during a full 24-hour cycle with a frequency of 30 seconds. The irradiance at the incubation depths was measured as photosynthetically active radiation (PAR) using a light meter LI-COR 250 (I) and a spherical light intensity sensor (Alec Electronics Co Ltd.) (II–IV). Carbonate parameters  $p\text{CO}_2$  and pH<sub>NBS</sub> and water salinity and temperature were used to calculate total dissolved inorganic carbon (DIC), total alkalinity,  $\text{CO}_3^{2-}$  and  $\text{HCO}_3^-$  using the CO2SYS software (Lewis and Wallace, 1998), with carbonate system dissociation constants for estuarine studies (Cai and Wan, 1998). Additionally, in parallel with the photosynthesis measurements, the diurnal fluctuations of water pH,  $p\text{CO}_2$  and oxygen saturation were measured outside the mesocosms at a depth of ca. 0.5 m in a natural shallow water macroalgal habitat. Water samples were taken from the surface in each mesocosm and outside each mesocosm once a day using the standard



method ISO 5667-9. The volume of one sample was 250 ml. Samples were frozen immediately in a deep freezer until further laboratory analyses using the standard method EN ISO 5667-3. Nutrient concentrations: total nitrogen (TN), total phosphorus (TP), phosphates (P-PO<sub>4</sub>) and nitrites+nitrates (N-NO<sub>x</sub>) were measured in a laboratory with a continuous flow automated wet chemistry analyser Skalar SAN<sup>plus</sup> (Skalar Analytic B.V., De Breda, The Netherlands) using the standard methods EN ISO 11905-1, EN ISO 15681-2 and EN ISO 13395.

## 2.4. Laboratory experiments

In Paper I the aim of the laboratory experiments was to develop the necessary techniques and experience for the following field experiments. The laboratory experiments were carried out using the laboratory facilities of the Estonian Marine Institute, University of Tartu. The experiments were conducted using specimens of *F. lumbricalis* collected from Kakumäe Bay (at 3.8 m depth) on 28.04.2011. In the laboratory, specimens were acclimated in 54-litre aquariums for 14 days (28.04–11.05.2011) before incubation experiments were carried out with manipulated pH for 9 days (12.05–20.05.2011). Macroalgae were acclimated at pH ~ 8.0 and the temperature was enhanced every second day degree by degree up to 15 °C. The pH sensors were connected to a multi-channel pH controller, which automatically streamed CO<sub>2</sub> through the inlets into the aquarium according to the predetermined pH, namely, 6.5, 7.0 and control ~ 8.0. The salinity was kept at the natural level of ~ 5.0, water temperature at 15 °C; the steady temperature was acquired through an active temperature controller. The light–dark cycle was 12 : 12 h and during the light cycle the photosynthetically active radiation (PAR) was ~ 200 µmol m<sup>-2</sup> s<sup>-1</sup>. The net photosynthetic rate of macroalgae was measured using the oxygen method, described in detail below.

In Paper III the aim of laboratory experiments was to determine the optimal temperature for photosynthesis in *F. lumbricalis*. The experiments were carried out using the laboratory facilities of the Kõiguste field station of the Estonian Marine Institute, University of Tartu. Specimens of *F. lumbricalis* were acclimated at different water temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C) in 54-litre aquariums (filled with filtered sea water, *p*CO<sub>2</sub> ca. 200 µatm) for 7 days before measurements of net photosynthesis. The net photosynthetic rate of macroalgae was measured using the oxygen method, described in detail below. The steady temperature was maintained through an active temperature controller (±0.1 °C, AquaMedic cooling units Titan 1500). The light–dark cycle was 12 : 12 h and during the light cycle under luminophore light the PAR was ca. 200 µmol m<sup>-2</sup> s<sup>-1</sup>. An ODYSSEY PAR Logger sensor was used to measure PAR.

## 2.5. Measurements of net photosynthesis

In all experiments (I–IV) the photosynthetic rate of macrophytes at different  $p\text{CO}_2$  levels (mesocosms) was measured once a day using the oxygen method. For this procedure about 0.1 g (I–III) and 0.5 g (IV) (dry weight, dw) of plant material was incubated in 600 ml glass bottles. For field experiments, the glass bottles were filled with water from inside the mesocosm and placed horizontally on special transparent trays hanging outside at a depth of 0.1 m (I) and 0.5 m (II–IV). For laboratory experiments, the glass bottles with algal material were filled with water from inside aquariums and placed horizontally at the bottom (I, III). All incubations with macroalgae and charophytes were performed in triplicate per treatment and with the seagrass *Z. marina* in six replicates per treatment. Bottles without plant material (in triplicate per treatment) served as controls. The dry weight of the plant material was determined after drying at 60 °C for 48 h. Net primary productivity (NPP) (given as  $\text{mg O}_2 \text{gdw}^{-1} \text{h}^{-1}$ ) was calculated from the differences in dissolved oxygen concentrations in incubation bottles with and without algal material, measured over the incubation period (ca. 1 h) (Paalme, 2005). The dissolved oxygen concentrations were measured with a Marvet Junior dissolved oxygen meter (MJ2000, Elke Sensor, Estonia) using the standard method EN ISO 5814. Measurements were carried out between 10 am and 4 pm.

## 2.6. Statistical analyses

In Paper I the results of laboratory experiments were statistically analysed using one-way analysis of variance (ANOVA): pH as the independent variable with three levels and net photosynthetic rate as the dependent variable. Analysis of covariance (ANCOVA) was used to evaluate separate and interactive effects of  $\text{CO}_2$ , PAR (covariates) and species (categorical predictor) on the NPP rate in the mesocosm experiments. We used square transformed  $\text{CO}_2$  and PAR values as covariates to meet the assumption of homogeneity of variances (Cochran's test). Effects were considered to be statistically significant if the  $p$ -value was  $<0.05$ . When significant differences among main factors or their interactions were found, Bonferroni tests were used as *post hoc* comparisons to contrast specific means.

In Paper II the results of the field experiments were statistically analysed using the factorial ANOVA:  $p\text{CO}_2$  with three levels, species, experimental days and their combinations as the independent variables and NPP as the dependent variable. Bartlett's test was used to test for homoscedasticity of the data before ANOVA. Effects were considered to be statistically significant at  $p < 0.05$ . When significant differences among main factors or their interactions were found, subsequent multiple comparisons of the means were done using Tukey's HSD *post hoc* test.

In Paper **III** the effect of water temperature on the net photosynthetic rate of *F. lumbricalis* in the laboratory experiment was assessed using ANOVA: temperature as the independent factor with five levels. Tukey's HSD *post hoc* test was used to find means that were significantly different from one another. In Papers **III** and **IV** one-factor permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations was used to statistically test single and interactive treatment effects on net photosynthetic rates of macrophytes:  $p\text{CO}_2$  was used as the fixed factor with three levels (**III**, **IV**) and four levels in 2014 (**IV**); PAR and water temperature were treated as covariates. Significant effects were explored when necessary with pairwise *post hoc* tests (with 9999 permutations). Statistical analyses were performed using PERMANOVA (PRIMER, PRIMER-E Ltd, Plymouth, UK) and STATISTICA 7. For all statistical tests, a probability of 0.05 was used to determine statistical significance.

### 3. RESULTS

#### 3.1. Mesocosm experiments

##### Macroalgae

The response of macroalgal photosynthesis to CO<sub>2</sub> enrichment was species specific (**I**). The highest response to elevated  $p\text{CO}_2$  levels was measured for the fast-growing filamentous alga *Ulva intestinalis*. The higher water CO<sub>2</sub> concentrations also showed an increased net photosynthesis of the perennial red alga *Furcellaria lumbricalis* while the brown alga *Fucus vesiculosus* with a slow metabolism did not respond to elevated  $p\text{CO}_2$  levels on short-term basis. ANCOVA results indicated that the rate of net primary productivity was dependent on species and was interactively affected by the increase of  $p\text{CO}_2$  and the amount of PAR. Based on the Bonferroni *post hoc* test significant differences ( $p < 0.01$ ) between the NPP rates of all tested algal species (i.e. *U. intestinalis*, *F. vesiculosus* and *F. lumbricalis*) were found (**I**).

The PERMANOVA analyses (conducted separately with the data obtained in 2012 and 2013) indicated that the net photosynthetic rate of *F. lumbricalis* varied significantly between treatments at different  $p\text{CO}_2$  levels (PERMANOVA:  $p < 0.05$ ) (**III**). According to a PERMANOVA pair-wise *post hoc* test, the differences in the NPP rates of *F. lumbricalis* at the  $p\text{CO}_2$  levels of 200  $\mu\text{atm}$  and 1000  $\mu\text{atm}$  were slight but at 2000  $\mu\text{atm}$  a significantly higher photosynthetic rate was measured compared to the lower levels in 2012. In 2013 the differences in the NPP rates of *F. lumbricalis* at the  $p\text{CO}_2$  levels of 1000  $\mu\text{atm}$  and 2000  $\mu\text{atm}$  were slight, but at of 200  $\mu\text{atm}$  a significantly lower average photosynthetic rate was measured (PERMANOVA pair-wise *post hoc* test:  $p < 0.05$ ). In 2012 the NPP rates of *F. lumbricalis* were affected by PAR and the interactive effect of  $p\text{CO}_2$  and PAR as well  $p\text{CO}_2$  and the water temperature (PERMANOVA:  $p < 0.05$ ). In 2013, besides the effect of elevated  $p\text{CO}_2$ , the NPP rates of *F. lumbricalis* were also affected by water temperature (PERMANOVA:  $p < 0.05$ ). The higher NPP rates of *F. lumbricalis* were measured at the lower water temperatures in 2013 compared to the values of 2012.

##### Charophytes

Our results showed that NPP rates of charophytes varied both between species and treatments at different  $p\text{CO}_2$  levels (**II**). The highest average rate of net photosynthesis was measured for *C. tomentosa*. The response of *C. horrida* to the elevated CO<sub>2</sub> levels was less pronounced as compared with that of *C. tomentosa*, while *C. aspera* showed a slight response during the experimental period (Tukey's HSD:  $p < 0.05$ ). The differences in the NPP rates of *C. horrida* at the  $p\text{CO}_2$  levels of 200  $\mu\text{atm}$  and 1000  $\mu\text{atm}$  were slight but at 2000  $\mu\text{atm}$  a significantly higher photosynthetic rate was measured than at the lower levels (Tukey's HSD:  $p < 0.05$ ). Based on a *post hoc* test (Tukey's HSD) the elevated  $p\text{CO}_2$  levels had no significant effect on the NPP rates of *C. aspera* ( $p > 0.05$ ).

Among the tested charophyte species, the photosynthetic rate of neither *C. tomentosa* nor *C. horrida* differed significantly at the control treatment and at the highest  $p\text{CO}_2$  level while at the intermediate  $p\text{CO}_2$  level the NPP rate of *C. tomentosa* was significantly higher. The NPP rates measured for *C. aspera* were significantly lower as compared with those of the other studied charophyte species at all three  $p\text{CO}_2$  levels (Tukey's HSD:  $p < 0.05$ ). Factorial ANOVA indicated that all tested factors, i.e.  $p\text{CO}_2$ , species, experimental days and their combinations, significantly affected the photosynthetic rate of the three tested charophyte species.

### Seagrass

The PERMANOVA analyses (conducted separately with the data obtained in 2013 and 2014) indicated that the photosynthetic rate of the seagrass *Z. marina* varied significantly between treatments at different  $p\text{CO}_2$  levels (PERMANOVA:  $p < 0.05$ ) (IV). In 2013 the variations of NPP rates were also affected by one of the tested environmental variables, namely water temperature, while the effect of the PAR was dependent on the level of  $p\text{CO}_2$  and water temperature (PERMANOVA:  $p < 0.05$ ). In 2014 the NPP rates of *Z. marina* were affected by interactions of the PAR with water temperature and with the level of  $p\text{CO}_2$  (PERMANOVA:  $p < 0.05$ ). There were remarkable differences in the NPP rates of *Z. marina* between the two experimental periods: the average NPP rate was  $4.08 \text{ mgO}_2\text{g}_{\text{DW}}^{-1}\text{h}^{-1}$  in 2013 and  $5.19 \text{ mgO}_2\text{g}_{\text{DW}}^{-1}\text{h}^{-1}$  in 2014. The highest NPP rates for *Z. marina* were measured in the untreated water (control conditions), while at the intermediate  $p\text{CO}_2$  level the seagrass had lower rates than at the high  $p\text{CO}_2$  levels. Based on a PERMANOVA pairwise *post hoc* test, the differences in the average NPP rates of *Z. marina* at the  $p\text{CO}_2$  levels between  $1000 \mu\text{atm}$  and  $2000 \mu\text{atm}$  were significant ( $p < 0.01$ ) in 2013. At the same time, NPP rates of *Z. marina* at the  $p\text{CO}_2$  levels between  $200 \mu\text{atm}$  and  $2000 \mu\text{atm}$  were not significant, while at the  $p\text{CO}_2$  level of  $200 \mu\text{atm}$  a significantly higher average NPP rate was measured compared to the elevated  $p\text{CO}_2$  level of  $1000 \mu\text{atm}$  ( $p < 0.01$ ) in 2013. In 2014 the differences in the NPP rates of *Z. marina* at the  $p\text{CO}_2$  levels of  $1000 \mu\text{atm}$  and  $2000 \mu\text{atm}$  were not significant but at the  $p\text{CO}_2$  level of  $200 \mu\text{atm}$  a significantly higher average photosynthetic rate compared to the elevated  $p\text{CO}_2$  levels was measured (PERMANOVA pairwise *post hoc* test:  $p < 0.05$ ). In the natural conditions outside of the mesocosm the average photosynthetic rates of *Z. marina* were significantly lower (PERMANOVA pairwise *post hoc* test:  $p = 0.0001$ ) compared to the NPP values measured in mesocosms in 2014.

## 3.2. Laboratory experiments

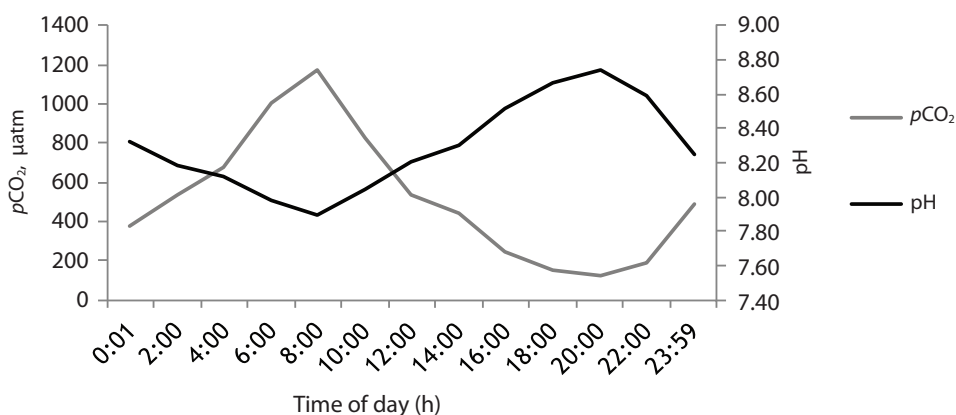
In case of the laboratory experiments the macroalga *F. lumbricalis* demonstrated increasing photosynthetic rates with decreasing pH due to increased acidification (I). Our results showed that the highest average NPP rate of

*F. lumbricalis* was measured at pH 6.5 compared to NPP rates at pH 7.0, while the lowest average NPP values were measured at pH ca. 8.0. Based on the Bonferroni *post hoc* test significant differences ( $p < 0.01$ ) were found between the *F. lumbricalis* photosynthetic rates and three different pH levels.

The water temperature showed a significant effect on the NPP rates of *F. lumbricalis* in the laboratory experiments (one-way ANOVA:  $F = 23.11$ ,  $p < 0.05$ ) (III). The significantly higher average photosynthetic rate of *F. lumbricalis* was measured at 10 °C compared to NPP rates at 5 °C, 15 °C, 20 °C and 25 °C. At the same time, based on Tukey's HSD *post hoc* test, there were no significant differences in the NPP rates measured at 5 °C, 15 °C and 20 °C ( $p > 0.9$ ) while a significantly lower average NPP rate of *F. lumbricalis* was measured at 25 °C compared to the above-mentioned temperatures (Tukey's HSD *post hoc* test:  $p < 0.05$ ).

### 3.3. Natural fluctuation of seawater $p\text{CO}_2$ and pH in shallow water macroalgal habitats

The pH and  $p\text{CO}_2$  show a substantial amplitude of natural variability under summer conditions. The daily pH fluctuations may be larger than 1 unit. For example, fluctuation between 8 and 9 are common in shallow water macroalgal habitats in the north-eastern Baltic in summer conditions. In the early morning  $p\text{CO}_2$  values turned out to be well above the  $p\text{CO}_2$  of 1000  $\mu\text{atm}$ . However, during the day when inorganic carbon was used for photosynthesis they declined to  $\sim 150$   $\mu\text{atm}$  (Fig. 5).



**Figure 5.** Natural fluctuation of seawater  $p\text{CO}_2$  and pH levels in shallow water macroalgal habitats in Kõiguste Bay. An example of measurement results from 28.07.2014 (continuous recordings).

## 4. DISCUSSION

In the current thesis, we focused on the most common benthic macrophytes in the north-eastern Baltic Sea that play key roles in the local ecosystem. Due to different requirements concerning abiotic factors such as light, temperature, salinity, bottom substrate, depth and exposure, their communities grow in different habitats throughout the Estonian coastal waters. Our results suggest that future increasing CO<sub>2</sub> concentrations are expected to enhance photosynthesis of at least some macrophyte species in the north-eastern Baltic Sea under summer conditions (**I, II, III**) (Table 1). However, this effect was highly species specific, most probably due to differences in the photosynthetic biochemistry, eco-physiology, life strategy and life stage of the species (**I, II**). Similarly, several studies conducted outside of the Baltic Sea have shown that the effect of elevated *p*CO<sub>2</sub> on macrophytes is highly species specific (e.g. Invers et al., 1997; Ow et al., 2015; Porzio et al., 2011; Koch et al., 2013). Additionally, the species specific response of macrophytes to the pH suggests that pH is one of the most important environmental factors in regulating primary production in shallow coastal ecosystems.

**Table 1.** The effect of CO<sub>2</sub> enrichment on the photosynthetic rate of the studied macrophyte species.

Macrophyte species	Responses to elevated <i>p</i> CO <sub>2</sub>
<b>Macroalgae</b>	
<i>Fucus vesiculosus</i>	None
<i>Furcellaria lumbricalis</i>	Positive
<i>Ulva intestinalis</i>	Positive
<b>Charophytes</b>	
<i>Chara aspera</i>	None
<i>Chara horrida</i>	Positive
<i>Chara tomentosa</i>	Positive
<b>Seagrass</b>	
<i>Zostera marina</i>	None

### 4.1. Interactive effects of elevated *p*CO<sub>2</sub> and other environmental factors on macrophytes

Numerous studies have shown that macrophytes' responses to ocean acidification depend on other limiting environmental factors such as nutrients content, light availability (e.g. Celis-Plá et al., 2015), water temperature and interactions of various factors (e.g. Graiff et al., 2015; Al-Janabi et al., 2016). Moreover, different macrophyte species have different requirements for abiotic factors for photosynthesis and growth. For example, the net photosynthesis of *C. aspera*

showed a significantly weaker response to elevated  $p\text{CO}_2$  levels than the other tested charophytes (II). As compared with other charophyte species in Estonian coastal waters, *C. aspera* is considered to be the most tolerant species to the variation of environmental factors such as light, salinity, bottom substrate, depth and exposure, while habitat requirements of *C. tomentosa* and *C. horrida* are stricter (Torn et al., 2004; Torn, 2008; Torn et al., 2015).

Considering that in the shallow coastal Baltic Sea the environmental factors have turbulent dynamics caused by seasonality and rapid changes in meteorological conditions (Voipio, 1981; Feistel et al., 2008) the mesocosm experiments with *F. lumbricalis* (III) and *Z. marina* (IV) were carried out during two experimental periods (years). In both experiments, some differences in the NPP between the two experimental periods were found. The dissimilarity in NPP rates between the two experimental periods could be explained first of all by the differences in the water temperature. For example, in our experiment the higher NPP values of *Z. marina* were measured under the higher water temperatures in 2014 (average water temperature 21.9 °C) compared to the values of 2013 (average water temperature 13.5 °C) (IV). In contrast, the experimental results showed that higher NPP values of *F. lumbricalis* were measured under the lower water temperatures in 2013 (average water temperature 13 °C) as compared to values of 2012 (average water temperature 18 °C) (III). Based on our findings it could be suggested that a future increase in water temperatures under climate change may reduce the photosynthetic rate of *F. lumbricalis* while the photosynthesis of *Z. marina* may benefit under the same temperature conditions to  $\text{CO}_2$  enrichment. The photosynthetic response of these species to elevated  $p\text{CO}_2$  levels appears to be the result of interactions with water temperature. In the Baltic Sea region where the water temperature is characterised by high seasonal and annual variations, the effects of increasing  $\text{CO}_2$  and water temperature should be observed together.

In addition, several studies have found that the benefits of elevated  $p\text{CO}_2$  for macroalgae (Celis-Plá et al., 2015) and seagrasses (Martínez-Crego et al., 2015) are more pronounced when combined with increased nutrients. In the nutrient-rich (eutrophicated) Baltic Sea, under the future  $\text{CO}_2$  increase scenario, the natural level of nutrients will probably not become a limiting factor for the photosynthesis of macrophytes. There is high seasonal variation in nitrogen and phosphorus concentrations: during summer and autumn their concentrations are relatively low in shallow coastal conditions (Pawlak et al., 2009). Perennial macroalgae, including *F. lumbricalis*, are capable to store nutrient reserves in their thallus from seawater for periods of low nutrients availability (Indergaard and Knutsen, 1990). Therefore, the interaction of an increasing  $\text{CO}_2$  concentration with a high nutrients availability in a brackish water may enhance the photosynthesis of *F. lumbricalis* (I, III). On the other hand, several studies in the Baltic Sea have shown that the decrease of *Z. marina* distribution is caused by eutrophication-related poor underwater light climate (Boström et al., 2014 and references therein). In the Estonian coastal waters, the main distribution depth of *Z. marina* is between 2 and 4 m, which is probably due to the poor



underwater light conditions (Möller et al., 2014). Our results indicated that NPP rates of macrophytes were affected by the combined effects of elevated  $p\text{CO}_2$  and light (**I**, **III**, **IV**). Thus, macrophyte responses to elevated  $p\text{CO}_2$  will depend also on the light availability in the north-eastern Baltic Sea.

Additionally, in Paper **I** it was demonstrated that under elevated  $\text{CO}_2$  concentrations the filamentous fast-growing green alga *U. intestinalis* had significantly higher photosynthetic rates than the perennial algae *F. lumbricalis* and *F. vesiculosus*. Thus, it could be theorised that future increasing  $\text{CO}_2$  concentrations may cause shifts in the macroalgal species composition, first of all towards the replacement of slowly growing perennial algae with fast growing filamentous algal species. A similar response pattern of macroalgae was predicted by climate change (Kotta et al., 2000, 2008b; Paalme et al., 2002). Moreover, mass occurrence of filamentous macroalgae is considered to be one of the main effects of eutrophication in shallow coastal Baltic Sea waters. Thus, future increasing  $\text{CO}_2$  concentrations in seawater may indirectly enhance the eutrophication effect by accelerating the growth of ephemeral filamentous macroalgae in the coastal Baltic Sea.

## **4.2. Sources of inorganic carbon and mechanisms of carbon acquisition for photosynthesis in macrophytes**

The response of macrophytes to elevated  $p\text{CO}_2$  depends also on which carbon source they use for photosynthesis (Van den Berg et al., 2002; Hepburn et al., 2011; Koch et al., 2013). The vast majority of the investigated seagrasses (Sand-Jensen and Gordon, 1984; Beer and Rehnberg, 1997; Koch et al., 2013), macroalgae (Raven, 2010; Koch et al., 2013; Beer et al., 2014) and charophytes (Moore, 1986) use mainly  $\text{HCO}_3^-$  as the external  $\text{C}_i$  source for photosynthesis, which will become slightly more available with the expected increasing  $\text{CO}_2$  content in seawater (Raven et al., 2005). However, several studies have shown that macroalgae prefer  $\text{CO}_2$  over  $\text{HCO}_3^-$  for photosynthesis, and  $\text{HCO}_3^-$  use can be facultative, i.e. at high  $\text{CO}_2$  concentrations  $\text{HCO}_3^-$  use is downregulated (Sand-Jensen and Gordon, 1984; Hepburn et al., 2011; Cornwall et al., 2012). Raven et al. (2011) pointed out that this facultative ability of macroalgae to alter the dependence of photosynthesis on  $\text{HCO}_3^-$  to  $\text{CO}_2$  may provide a competitive advantage at future increasing  $\text{CO}_2$  concentrations because of reduced energy requirements for carbon acquisition.

Additionally, the response of macrophytes to elevated  $p\text{CO}_2$  depends on which mechanisms of carbon acquisition they use for photosynthesis. Several studies have shown that *Z. marina* can use different mechanisms of carbon acquisition for photosynthesis in different environmental conditions (e.g. Beer and Rehnberg, 1996; Hellblom et al., 2001). It could be speculated that in our experiment under high-pH treatment in the enclosed conditions  $\text{HCO}_3^-$  transport was more efficient for *Z. marina* (**IV**). However, this statement needs further verification. Van den Berg et al. (2002) demonstrated that *C. aspera* is a better

competitor for  $\text{HCO}_3^-$  than *Potamogeton pectinatus* and will be the ‘winner’ of this interspecific competition under future ocean acidification. In Paper **II** our results also suggest that the species specific response of charophytes to elevated  $p\text{CO}_2$  levels may have implications for interspecific competition from increased carbon availability under future ocean acidification.

One of the key questions is whether or not the photosynthesis of macrophytes is saturated by the seawater DIC under present-day conditions. Several studies have found that photosynthesis and growth in most species are not saturated under present-day  $\text{CO}_2$  concentrations (e.g. Israel and Hophy, 2002; Wu et al., 2008). It should be noted that future increasing  $\text{CO}_2$  concentrations in seawater are expected to influence species without carbon-concentrating mechanisms (CCMs) more than those possessing CCMs. The reason is that species with CCMs have shown photosynthesis close to saturation by the seawater DIC under today’s conditions (Beer et al., 2014). Kübler et al. (1999) indicated that macroalgae that rely exclusively on  $\text{CO}_2$  diffusion may be carbon limited under the present-day environmental conditions due to the lower concentrations of  $\text{CO}_2$  compared to  $\text{HCO}_3^-$ . On the other hand, numerous studies have indicated that the photosynthesis of *Z. marina* may be carbon limited by the low seawater DIC under present conditions, even with their capacity to utilise  $\text{HCO}_3^-$  for photosynthesis (Beer and Koch, 1996; Beer and Rehnberg, 1997; Zimmerman et al., 1997; Hellblom et al., 2001; Invers et al., 2001; Palacios and Zimmerman, 2007; Buapet et al., 2013; Koch et al., 2013). As previously mentioned, our results showed that the net photosynthetic rate of macrophytes varied significantly between different  $p\text{CO}_2$  levels. In our experiments (**I**, **II**, **III**) the vast majority of studied macrophytes demonstrated lower photosynthetic rates in the untreated water (control conditions) as compared to elevated  $p\text{CO}_2$  treatments. This suggests that the photosynthesis of macrophytes ( $\text{HCO}_3^-$  users) is limited by the current water  $\text{CO}_2$  concentration of shallow coastal waters in the north-eastern Baltic Sea during their active vegetation period.

Furthermore, various studies have found seagrass meadows to be globally significant as carbonate reservoirs (e.g. Daurte et al., 2010; Hendriks et al., 2014; Mazarrasa et al., 2015). Photosynthetic processes are likely to buffer ocean acidification in seagrass meadows, but the magnitude of buffering depends on metabolic parameters and hydrodynamic processes of each system and varies regionally as well as seasonally (Hendriks et al., 2014). As previously mentioned, our results showed that the photosynthesis of several macrophyte species may be carbon limited at the current summer conditions. Therefore, we can conclude that also in the Baltic Sea conditions macrophyte communities may have a buffering role under the conditions of elevated  $p\text{CO}_2$  in seawater.

Ocean acidification is a relatively new research field. As to future research, it is important to conduct long-term mesocosm and in situ field experiments to study evolutionary adaptation. It is critical to measure local variability in the pH values within shallow coastal ecosystems as these natural pH fluctuations could interact with the effects of ocean acidification. Future studies should also focus on the effect of elevated  $p\text{CO}_2$  with multiple stressors on community level.

## 5. CONCLUSIONS

- The elevated  $p\text{CO}_2$  levels in brackish water are expected to enhance photosynthesis of macrophytes in the north-eastern Baltic Sea under summer conditions. However, this effect is species specific, most probably due to differences in the ecophysiology, life strategy and life stage of the species.
- The magnitude of the effect of elevated  $p\text{CO}_2$  levels on macrophytes is affected by various environmental factors, mainly by changes in water temperature and light availability.
- The natural concentrations of inorganic carbon in brackish water most likely limit the net photosynthesis of macrophytes, especially in shallow water macrophyte habitats during the active vegetation period.
- During the active vegetation period macrophyte communities will have a buffering role under future increasing  $\text{CO}_2$  concentrations in the north-eastern Baltic Sea conditions.
- Increased  $\text{CO}_2$  concentrations may cause shifts in the species composition of macrophyte communities, first of all the replacement of slowly growing perennial algae with fast growing filamentous algal species. These shifts could have implications for interspecific competition and community structure in a future high  $\text{CO}_2$  world.
- By accelerating the growth of filamentous fast growing macroalgae an increase of water  $\text{CO}_2$  levels may enhance indirectly the phenomena usually attributed to eutrophication in the Baltic Sea.

## SUMMARY IN ESTONIAN

### Merevee hapestumise mõju bentiliste makrofüütide fotosünteesile riimveelistes tingimustes

Ookeanide hapestumine on kiiresti kasvav üleilmne keskkonnaprobleem, mis on otseselt seotud inimtekkelise süsihappegaasi ( $\text{CO}_2$ ) emissiooniga atmosfääri. Hapestumise all mõistetakse ookeanide pH-taseme langust, mida peamiselt põhjustab suurenenud atmosfäärse süsinikdioksiidi neeldumine pinnavees. Alates tööstusrevolutsioonist 18. sajandi lõpul on umbes kolmandik inimtegevuse tõttu atmosfääri eraldunud  $\text{CO}_2$ -st talletunud maailma ookeanides, põhjustades ookeanide pinnavee pH-taseme languse umbes 0,1 ühiku võrra. Hinnanguliselt neeldub ookeanides 24 miljonit tonni süsinikdioksiidi päevas. Kui  $\text{CO}_2$ -emissiooni ei reguleerita, võib ookeanivee pH-tase järgmise sajandi alguseks langeda 0,3–0,4 ühiku võrra, mis tähendaks merevee happesuse kolmekordistumist. Sarnaseid muutusi ennustatakse ka Läänemere piirkonnas. Igasugune merevee pH-taseme muutus, muudab omakorda kogu merekeemia tasakaalu. See aga võib endaga kaasa tuua laiaulatuslikke muutusi mereökosüsteemides.

Merevee hapestumise mõju mereorganismidele ei ole suures osas veel täpselt teada. Märkimisväärne osa hapestumise teemalistest teadusuuringutest keskendub lubiskeletsete organismide negatiivsele mõjule (näiteks: korallid, karbid, kokolitofoorid). Samal ajal on suurem osa uuringuid näidanud, et  $\text{CO}_2$ -sisalduse suurenemine merevees mõjutab positiivselt mitte-lubiskeletsete makrovetikate ja meriheina füsioloogilisi protsesse.

Antud doktoritöö peamine eesmärk oli selgitada välja, kuidas mõjutab  $\text{CO}_2$ -emissioonist põhjustatud merevee happesuse suurenemine Eesti rannikumeres laialt levinud bentiliste makrofüütide fotosünteesi riimveelistes tingimustes. Teine eesmärk oli uurida temperatuuri ja valguse lühiajalise loodusliku varieeruvuse ja suurenenud  $\text{CO}_2$ -sisalduse koosmõju makrofüütide fotosünteesile. Lisaks mõõdeti süsinikdioksiidisüsteemi iseloomustamiseks looduslikes tingimustes merevee pH-d ja süsiniku partsiaalarõhku ( $p\text{CO}_2$ ), eesmärgiga koguda taustaandmeid produktsioonikatsete korraldamiseks erinevatel  $p\text{CO}_2$ - ja pH-tasemetel ning ühtlasi selgitada välja nende parameetrite ööpäevane kõikumine madalas rannikumeres tihedas vetikakoosluses.

Laboritingimustes korraldatud esimeste katsete peamine eesmärk oli arendada meetodeid ja leida tehnilisi lahendusi in situ mesokosmikatsete jaoks. Aastatel 2011 kuni 2014 vahemikus juuni-juuli viidi Saaremaa lõunarannikul Kõiguste lahes läbi mesokosmikatsed. Vaatluse all olevaid liike inkubeeriti lühiajalistes katsetes, mille käigus manipuleeriti erinevate  $\text{CO}_2$ -tasemetega: kõrgendatud  $p\text{CO}_2$  tase ca 1000  $\mu\text{atm}$  ja ca 2000  $\mu\text{atm}$  ning kontroll tingimused ca 200  $\mu\text{atm}$ . Erinevate  $p\text{CO}_2$ -tasemetega juures mõõdeti makrofüütide fotosünteesi hapniku meetodil. Eksperimentide käigus monitooriti keskkonnaparametreid salvestavate automaatsete mõõteriistadega ka erinevaid keskkonnategureid:

soolsust, valgust ja hapniku. Lisaks mõõdeti erinevate toitainete: üldlämmastiku, üldfosfori, fosfaatide ja nitraatide sisaldust automaatanalüsaatoril.

Antud töö põhitulemused on:

- Uurimistulemused näitasid, et bentilised makrofüüdid on võimelised fotosünteesima kõrgete  $p\text{CO}_2$ -sisalduste juures.  $\text{CO}_2$  tõusust tingitud pH-taseme languse mõju on liigispetsiifiline ja selle mõju ulatus sõltub koosmõjust teiste keskkonnateguritega, peamiselt vee temperatuuri ja valguse kättesaadavusega
- $\text{CO}_2$  looduslik sisaldus merevees limiteerib makrofütide fotosünteesi vegetatsiooniperioodi jooksul.
- Makrofütide kooslused võivad tulevikus vegetatsiooni perioodi jooksul puhverdada merevee hapestumise negatiivset mõju Läänemere kirdeosa tingimustes.
- Üks peamisi merevee happesuse suurenemise tagajärgi tulevikus on muutused põhjakoosluste liigilises koosseisus.  $\text{CO}_2$ -sisalduse suurenemine merevees võib süvendada kaudselt Läänemere ühe suurema probleemi, eutrofeerumise mõjusid, kuna soodustab eelkõige üheaastaste niitjate suurvetikate vohamist.

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## **PUBLICATIONS**

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2013–... University of Tartu, Faculty of Science and Technology, Estonian Marine Institute, Department of Marine Biology; Junior Research Fellow in Marine Biology

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1. The effect of elevated  $p\text{CO}_2$  on macrophytes in a brackish water environment.
2. Restoration of seagrass *Zostera marina* beds in a brackish water environment.
3. Macroalgae cultivation in the northern Baltic Sea.

### Publications:

Pajusalu L, Martin G, Põllumäe A, Paalme T. 2013. Results of laboratory and field experiments of the direct effect of increasing  $\text{CO}_2$  on net primary production of macroalgal species in brackish water ecosystems. *Proceedings of the Estonian Academy of Sciences* 62:148–154.

Pajusalu L, Martin G, Põllumäe A, Torn K, Paalme T. 2015. Direct effects of increased  $\text{CO}_2$  concentrations in seawater on the net primary production of

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- Pajusalu L, Martin G, Põllumäe A, Paalme T, Põllumäe A, Oganjan K. 2015. Ocean acidification is one of the biggest threats to the marine ecosystem. *Estonian Nature* 66: 8–14. (Popular science article.)
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### Conference presentations:

- Pajusalu L, Martin G. The effect of marine acidification on the benthic vegetation in the conditions of NE Baltic Sea. 8th Baltic Sea Science Congress. St. Petersburg, Russia, 22–26 August 2011. (Poster presentation. Best poster award.)
- Pajusalu L, Martin G, Põllumäe A, Paalme T, Torn K. The direct effects of increasing CO<sub>2</sub> concentration in seawater on net primary production of charophytes in the shallow coastal brackish water ecosystem. 9th International Carbon Dioxide Conference. Beijing, China, 3–7 June 2013. (Oral presentation.)
- Pajusalu L, Martin G, Põllumäe A, Paalme T. Effects of increased CO<sub>2</sub> concentration in seawater on net primary production of seagrass *Zostera marina* L. in the brackish water ecosystem. Ocean Sciences Meeting 2014. Honolulu, Hawaii, USA, February 23–28, 2014. (Poster presentation.)
- Pajusalu L, Martin G, Põllumäe A, Paalme T, Torn K. Response of photosynthetic activity of macrophytes to increased CO<sub>2</sub> in a brackish-water ecosystem. Aquatic Sciences Meeting. Granada, Spain, 22–27 February 2015. (Oral presentation.)
- Paula JR, Pajusalu L, Keys M, Pablo L. Effects of ocean acidification and ocean warming on a macroalga-associated ecosystem. Workshop—Chemical, biological, and statistical considerations for ocean acidification experiments. Hobart, Tasmania, Australia. 29 April–2 May 2016. (Oral presentation.)

### Awards and scholarships:

- 2011 Best poster award in 8th Baltic Sea Science Congress in St. Petersburg
- 2013 ESF DoRa T6 scholarship, A semester abroad for Doctoral students, SA Archimedes

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2008 MSc, Eesti Maaülikool (rakendushüdrobioloogia)

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### Teenistuskäik:

2013–... Tartu Ülikool, Loodus- ja tehnoloogiateaduskond, Tartu Ülikooli Eesti Mereinstituut, merebioloogia nooremteadur

2013 November–2014 Aprill Otago Ülikool, Mereteaduste osakond, Uus-Mere-maa, Külalisüliõpilane

2006–2012 Tartu Ülikool, Loodus- ja tehnoloogiateaduskond, Tartu Ülikooli Eesti Mereinstituut, laborant

### Teadustöö põhisuunad:

1. Merevee hapestumise mõju makrofüütidele
2. Meriheina *Zostera marina* elupaikade taastamine
3. Makrovetikate kultiveerimise võimalused Läänemere tingimustes

### Publikatsioonid:

- Pajusalu L, Martin G, Põllumäe A, Paalme T. 2013. Results of laboratory and field experiments of the direct effect of increasing CO<sub>2</sub> on net primary production of macroalgal species in brackish water ecosystems. *Proceedings of the Estonian Academy of Sciences* 62:148–154.
- Pajusalu L, Martin G, Põllumäe A, Torn K, Paalme T. 2015. Direct effects of increased CO<sub>2</sub> concentrations in seawater on the net primary production of charophytes in a shallow, coastal, brackish-water ecosystem. *Boreal Environmental Research* 20:413–422.



- Pajusalu L, Martin G, Põllumäe A, Paalme T, Põllumäe A, Oganjan K. 2015. Hapestuv maailmamerevesi on suur oht kogu mereökosüsteemile. *Eesti Loodus* 66:8–14. (populaarteaduslik artikkel)
- Pajusalu L, Martin G, Paalme T, Põllumäe A. 2016. The effect of CO<sub>2</sub> enrichment on net photosynthesis of the red alga *Furcellaria lumbricalis* in a brackish water environment. *PeerJ* 4:e2505.
- Pajusalu L, Martin G, Põllumäe A, Paalme T. 2016. The influence of CO<sub>2</sub> enrichment on net photosynthesis of seagrass *Zostera marina* in a brackish water environment. Submitted manuscript to *Frontiers in Marine Science*.

### **Saadud uurimistoetused ja tunnustus:**

2011 Parima postri auhind konverentsil 8th Baltic Sea Science Congress.

2013 ESF DoRa T6 stipendium, Doktorantide semester välismaal, SA Archimedes

### **Konverentsiettekanded:**

- Pajusalu L, Martin G. The effect of marine acidification on the benthic vegetation in the conditions of NE Baltic Sea. 8th Baltic Sea Science Congress. St. Petersburg, Russia, 22–26 august 2011. (stendiettekanne, parima postri auhind)
- Pajusalu L, Martin G, Põllumäe A, Paalme T, Torn K. The direct effects of increasing CO<sub>2</sub> concentration in seawater on net primary production of charophytes in the shallow coastal brackish water ecosystem. 9th International Carbon Dioxide Conference. Beijing, China, 3–7 juuni 2013. (suuline ettekanne)
- Pajusalu L, Martin G, Põllumäe A, Paalme T. Effects of increased CO<sub>2</sub> concentration in seawater on net primary production of seagrass *Zostera marina* L. in the brackish water ecosystem. Ocean Sciences Meeting 2014. Honolulu, Hawaii, USA, 23–28. veebruar 2014. (stendiettekanne)
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- Paula JR, Pajusalu L, Keys M, Pablo L. Effects of ocean acidification and ocean warming on a macroalga-associated ecosystem. Workshop—Chemical, biological, and statistical considerations for ocean acidification experiments. Hobart, Tasmania, Austraalia. 29 aprill – 2 Mai 2016. (suuline ettekanne)

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