

The background image is a composite of two photographs of brown algae, *Fucus serratus*. The top half shows a close-up of the algae's blade, which is a pale, yellowish-green color with a serrated edge. The bottom half shows a similar close-up, but the algae has turned a vibrant yellow, indicating a change in its physiological state. Small, brown, shell-like epibionts are visible on the surface of the algae in both sections.

Vincent Saderne

**The ecological effect of CO<sub>2</sub>  
on the brown algae *Fucus  
serratus* and its epibionts:  
From the habitat to the  
organismic scale.**



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Fucus serratus and its epibionts:  
From the habitat to the organismic scale.*

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## Summary

Carbon dioxide plays a central role in the functioning of organisms and ecosystems. For autotrophs, it is the substrate for photosynthesis while for heterotrophs it is a waste product of respiration. For two centuries Human activities, are responsible for an increase from 280 to 380  $\mu\text{atm}$  of the atmospheric  $\text{pCO}_2$ . A further increase up to 1000  $\mu\text{atm}$  is predicted for the 21<sup>th</sup> century. The ocean surface and the atmosphere are at the equilibrium for  $\text{CO}_2$ . The  $\text{CO}_2$  dissolving in seawater reduces the pH and increase of corrosiveness of water for shells and skeletons made of calcium carbonates. Thus, this process of ocean acidification is expected to have detrimental effects on calcifying organism. On the contrary, marine autotrophs are supposed to (slightly) benefit from this extra supply of  $\text{CO}_2$ .

In this thesis, we aimed at assessing the influence of  $\text{CO}_2$  on members of the nearshore macrophytes meadows of the Baltic Sea, an ecosystem naturally exposed to elevated water acidity. In a first part, we investigated the natural variations of the carbonate system in a meadow during three weeks of July, August, and September 2011 in a sheltered bay of the Western Baltic. We observed important day night dynamics together with wider scale variations (days to weeks) of magnitude exceeding future climate change predictions. We were able to explain the variations by the action of light and wind speed and direction. Light drives the uptake and release of carbon by photosynthesis and respiration of the meadow and wind influences the upwelling of offshore hypercapnic seawater. In a second part, we investigated the growth response to elevated  $\text{pCO}_2$  of one of the main primary producer of the meadows, the brown algae *Fucus serratus*, in laboratory experiments. The algae were incubated under ambient  $\text{pCO}_2$ , actual upwelling  $\text{pCO}_2$  and future upwelling  $\text{pCO}_2$ . We observed an increase of growth of 20 % at the  $\text{pCO}_2$  expected for the year 2100 and up to 50 % at  $\text{pCO}_2$  possibly occurring during future upwelling events (4000  $\mu\text{atm}$ ). However, the effect was transient and a limitation of growth by nutrients occurred after about 20 days. In the third part, we tested the effect of at the same three  $\text{pCO}_2$  on the growth and recruitment of the main members of the sessile associated communities of *Fucus serratus*: the calcifying and non-calcifying bryozoan *Electra pilosa* and *Alcyonidium hirsutum* and the calcifying tubeworm *Spirorbis spirorbis*. We tested the hypothesis of greater sensitivity of calcifiers to acidification and found a resistance of all the tested organisms to the future ambient  $\text{pCO}_2$ . In contrast, at the highest  $\text{pCO}_2$  tested (future upwelling), we observed in *Spirorbis* severe shell corrosion, reduction of growth and collapse of recruitment. The growth rates of the worm settlings were assessed at light and dark under the three experimental  $\text{pCO}_2$ . A 40 % enhancement of growth was observed at light at any  $\text{pCO}_2$ , possibly due to the algal photosynthetic reduction of  $\text{pCO}_2$  / increase of pH in the boundary layer surrounding the algal thallus. Our study illustrates the possibility of facilitation between species to resist ocean acidification.



## Zusammenfassung

Kohlenstoffdioxid hat eine zentrale Bedeutung für die Funktionsweise von Organismen und Ökosystemen. In autotrophen Organismen ist es das Substrat für die Photosynthese, während es in heterotrophen Organismen ein Abfallprodukt der Respiration darstellt. Seit zwei Jahrhunderten haben die menschlichen Aktivitäten zu einem Anstieg des atmosphärischen  $p\text{CO}_2$ s von 280 auf 380  $\mu\text{atm}$  geführt. Ein weiterer Anstieg auf 1000  $\mu\text{atm}$  wird bis zum Ende des 21. Jahrhunderts erwartet. Da das Oberflächenwasser der Ozean mit der Atmosphäre in Äquilibrium steht, kommt es zu einer vermehrten Lösung des  $\text{CO}_2$ s im Meerwasser. Dies führt zu einer Absenkung des pHs und der  $[\text{CO}_3^{2-}]$ , was zu einer höheren Korrosivität des Wasser für Schalen und Skeletten, die aus Kalziumkarbonat aufgebaut sind, führt. Deshalb geht man davon aus, dass die Ozeanversauerung negative Auswirkungen auf kalzifizierende Organismen hat. Andererseits könnten marine Autotrophe (leicht) von der erhöhten  $\text{CO}_2$  Zufuhr profitieren.

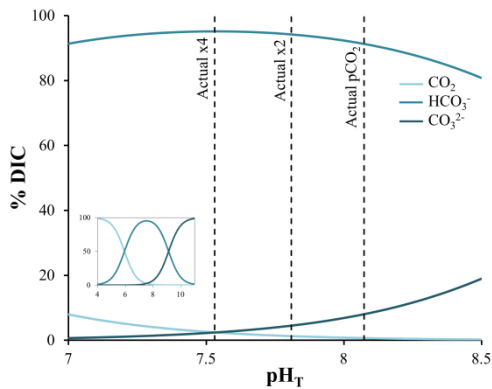
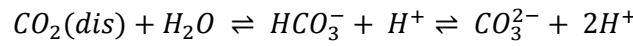
Diese Arbeit bewertet den Einfluss von  $\text{CO}_2$  auf die Lebewesen von küstennahen Makrophyten Systemen der Ostsee, einem Ökosystem das natürlicherweise einem erhöhten Säuregrad ausgesetzt ist. Im ersten Teil, haben wir in drei Wochenabschnitten im Juli, August und September die natürliche Variabilität des Karbonatsystems in einem Makrophytenbestand in einer geschützten Bucht der westlichen Ostsee untersucht. Wir haben bedeutende Tag-Nacht-Dynamiken zusammen mit längerfristiger Variabilität (Tage bis Wochen) beobachtet, die in ihrem Ausmaß die zukünftigen Klimaänderungen übertraf. Wir waren in der Lage, diese Variabilität anhand von Licht und Windgeschwindigkeit und -richtung zu erklären. Licht führte zur Aufnahme und Freisetzung von Kohlenstoff durch Photosynthese bzw Respiration des Pflanzenbestandes und Wind bewirkte Auftrieb von ablandigen  $\text{CO}_2$  reichen Wassermassen. Im zweiten Teil haben wir in einem Laborexperiment die Auswirkungen auf das Wachstum eines der wichtigsten Primärproduzenten, dem Blasentang *Fucus serratus*, unter erhöhtem  $p\text{CO}_2$  untersucht. Die Algen wurden dabei  $p\text{CO}_2$  Werten ausgesetzt, die den derzeitigen atmosphärischen entsprechen und solchen die bei heutigen und zukünftigen Auftriebsereignissen auftreten können. Wir haben einen Anstieg des Wachstums um 20 % bei  $p\text{CO}_2$  Werten entsprechend des Jahres 2100 und um bis zu 50% bei Werten die zukünftigen Auftriebsereignissen (4000  $\mu\text{atm}$ ) entsprechen, beobachtet. Allerdings waren diese Effekte nur vorübergehend und eine Nährstofflimitierung schwächte das Wachstum nach 20 Tagen ab. Im dritten Teil, haben wir die Auswirkungen der gleichen drei  $p\text{CO}_2$  Level auf das Wachstum und die Rekrutierung der drei wichtigsten Mitglieder der *Fucus serratus* Artengemeinschaft untersucht: die kalzifizierende Bryozoe *Electra pilosa*, die nicht-kalzifizierende Bryozoe *Alcyonidium hirsutum* und den kalzifizierenden Röhrenwurm *Spirorbis spirorbis*. Wir untersuchten die Hypothese, ob Kalzifizierer eine größere Sensitivität gegenüber Versauerung aufweisen und haben eine generell hohe

Resistenz gegenüber zukünftigen  $p\text{CO}_2$  Werten gefunden. Allerdings führte der höchste  $p\text{CO}_2$ , der bei zukünftigen Auftriebsereignissen auftreten kann, bei *Spirorbis* zu schweren Schalenauflösungen, Wachstumsverringerungen und Zusammenbruch der Rekrutierung. Desweiteren wurden Im Experiment die Wachstumsraten von siedelnden Würmern bei Licht und im Dunkeln, sowie bei drei verschiedenen  $p\text{CO}_2$  Werten beobachtet. Bei Licht wurde eine 40 %ige Erhöhung in allen  $p\text{CO}_2$  Behandlungen ausgemacht, die möglicherweise aus einer Verringerung des  $p\text{CO}_2$ s bzw einer pH Zunahme in der Grenzschicht der Algen durch Photosynthese resultiert. Unsere Studie verdeutlicht die Möglichkeit, dass das Zusammenspiel von Arten möglicherweise zu einer höheren Widerstandkraft gegenüber der Ozeanversauerung führen kann.



## General introduction

Carbon dioxide is a very minor constituent of the atmosphere, representing only 0.039 % (396.8  $\mu\text{atm}$ ) of the total (NOAA – ESRL, 2012). Although scarce,  $\text{CO}_2$  is necessary for life on earth. It is an essential greenhouse gas, without which mean surface temperatures would be about  $-19\text{ }^\circ\text{C}$ , and as substratum for photosynthesis, generating the primary production and the  $\text{O}_2$  essential to the heterotrophic life (IPCC, 2007). The surface ocean is at the equilibrium with the atmosphere for e.g.  $\text{CO}_2$  and  $\text{O}_2$ . Differently to this last,  $\text{CO}_2$  is an acid gas and dissociate in seawater through a double acid - base couple forming the carbonate system (ignoring  $\text{H}_2\text{CO}_3$ , cryptic specie insignificant in term of activity).



**Figure 1.1.** Bjerrum 's representation of the Carbonate system: relative concentrations of  $\text{CO}_2$  (dis),  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  in  $\mu\text{mol kg}^{-1}$  at  $\text{DIC} = 2050\ \mu\text{mol kg}^{-1}$ ,  $15\text{ }^\circ\text{C}$  and salinity 35.

The total of the three species,  $\text{CO}_2$  (dis),  $\text{HCO}_3^-$ , and  $\text{CO}_3^{2-}$  are forming the dissolved inorganic carbon or DIC. To understand the fractionation of the DIC between the three carbon species, two further concepts are necessary to introduce: total alkalinity (TA) and pH. Both are complex concept and an exhaustive explanation will be out of the scope of this thesis. Simply, the pH in seawater can be expressed under three suitable scales that are the Free, the Seawater and the Total scale.

$$\text{pH}_{Free} = -\log[\text{H}^+]_F$$

$$\text{pH}_{Total} = -\log([\text{H}^+]_F + [\text{HSO}_4^-])$$

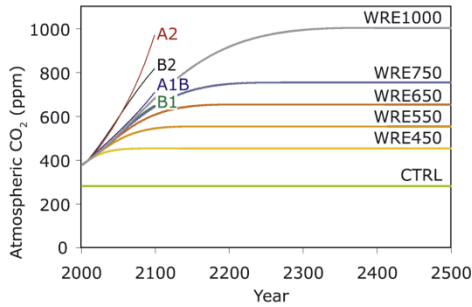
$$\text{pH}_{Seawater} = -\log([\text{H}^+]_F + [\text{HSO}_4^-] + [\text{HF}])$$

Thus, to a  $\text{pCO}_2$  of  $380\ \mu\text{atm}$  under the conditions of Fig. 1.1 corresponds:  $\text{pH}_T = 8.073$ ,  $\text{pH}_F = 8.150$  and  $\text{pH}_{\text{SWS}} = 8.061$ . The total alkalinity, as defined by Dickson (1981), is produced by the excess of proton acceptors (base formed from weak acids) over proton donors (stronger acids) in seawater.

$$\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] + [\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] + [\text{H}_3\text{SiO}_4^-] + [\text{NH}_3] \\ + [\text{HS}^-] - ([\text{H}^+]_F + [\text{HSO}_4^-] + [\text{HF}] + [\text{H}_3\text{PO}_4])$$

With in black the proton donors and in red the acceptors,  $[\text{H}^+]_F$  being free protons. Seawater is a buffered medium and any addition of acid in the system will not be followed by a proportional

decrease in pH. As consequence of the excess of proton acceptors, the  $\text{CO}_2$  in seawater do not remain as such but partly dissociates in its two associated bases, generating  $\text{H}^+$  ions. This phenomenon is often represented by a Bjerrum plot (see Fig. 1.1). Also, in the conditions of Fig. 1, under nowadays conditions of atmospheric  $\text{CO}_2$ , the  $\text{CO}_2$  (aq) fraction in seawater represents only 0.6 % of the DIC while  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  represents about 91 % and 8% respectively.

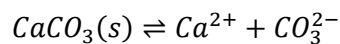


**Figure 1.2.** Atmospheric  $\text{CO}_2$  predicted for the Special Report on Emissions Scenarios (SRES) A1, A2, B1, and B2 emission pathways and allowable emissions calculated with ocean model from WRE  $\text{CO}_2$  stabilization scenarios. (Caldeira and Wickett, 2005).

Since the rise of the industrial era, fossil fuels became the main source of energy of human societies and their massive burnings since the year 1800 is already responsible for a shift of atmospheric  $\text{pCO}_2$  from  $\sim 280$  to  $397 \mu\text{atm}$  (NOAA-ESRL, 2012). By the year 2100, worst scenarios are anticipating atmospheric  $\text{pCO}_2$  up to  $1000 \mu\text{atm}$  and increasing to a maximum around  $1400 \mu\text{atm}$  in the 24<sup>th</sup> century (IPCC, 2007; Ridgwell et al., 2007) (see Fig. 1.2). The oceans have already uptaken about 30 % of the human emission of  $\text{CO}_2$  (Sabine et al., 2004).

Consequently, ocean pH already decreased of 0.1 unit since preindustrial and might drop of an extra 0.4 by the end of the century, corresponding to a shift from actual 8.2 to 7.8 (Caldeira and Wickett, 2005). This  $\text{CO}_2$  driven ocean acidification (OA) is causing an increase DIC but also, within the DIC, to a disappearance of the  $\text{CO}_3^{2-}$  fraction to the benefit of  $\text{CO}_2$  (aq) fraction. This phenomenon is presumed to have biological consequences, principally on processes of photosynthesis and calcification.

Biogenic calcium carbonate exists under two crystalline forms: aragonite and calcite, this last possibly including a certain percentage of Mg (called therefore magnesium calcite,  $\text{Mg CaCO}_3$ ). The corrosiveness of seawater to those crystals, expressed by the saturation state ( $\Omega$ ), is intrinsically depending on the carbonate system. The dissolution product of Calcium carbonate in seawater is expressed as follow:



With  $K_{sp}$  the solubility product:

$$K_{sp} = [\text{Ca}^{2+}]_{sat} * [\text{CO}_3^{2-}]_{sat}$$

Allowing to derivate the saturation state of seawater:

$$\Omega = \frac{[\text{Ca}^{2+}]_{sw} * [\text{CO}_3^{2-}]_{sw}}{K_{sp}}$$

The saturation state of seawater indicates the natural tendency of a calcium carbonate isomorph to

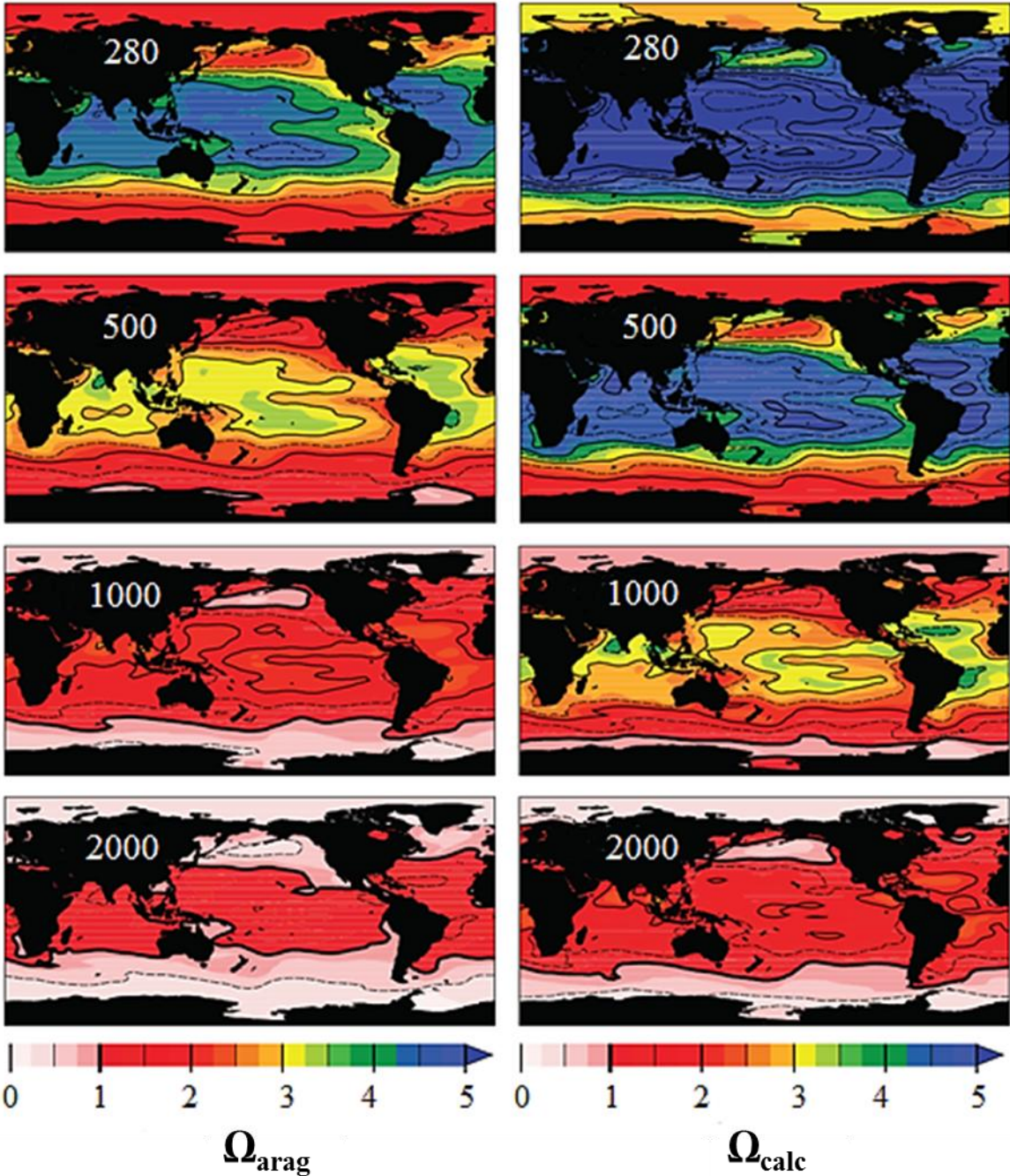
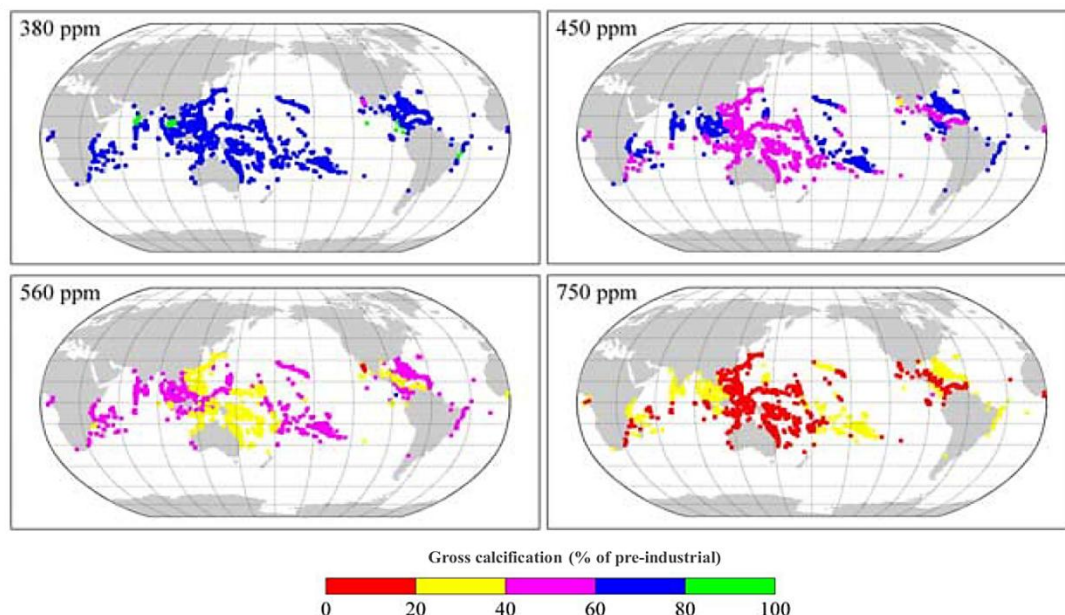


Figure 1.3. Saturation state of aragonite ( $\Omega_{arag}$ ) and calcite ( $\Omega_{calc}$ ) for different atmospheric CO<sub>2</sub> stabilization levels (ppm). By 500 ppm, aragonite will dissolve in parts of the near-surface Southern Ocean; by 1000 ppm, calcite will dissolve in most of the high latitude ocean. (redrawn from Cao and Caldeira, 2008).

dissolve ( $\Omega < 1$ ) or precipitate ( $\Omega > 1$ ). The concentration in  $\text{Ca}^{2+}$  being a function of salinity in seawater, the major variables influencing  $\Omega$  are the theoretical  $[\text{CO}_3^{2-}]_{\text{sat}}$  and the actual  $[\text{CO}_3^{2-}]_{\text{sw}}$  measured in seawater. The first is a thermodynamic variable depending of temperature and pressure but also of the type of crystal considered. Thus, high Mg-calcite is more prone to dissolution than aragonite, itself less soluble than low Mg-Calcite, while the solubility of all three increases with higher depth and latitude (colder waters). The second term is determined especially by  $[\text{CO}_2]_{\text{aq}}$  as previously explained.

Consequently, OA is expected increase the corrosiveness of seawater to Calcite and Aragonite starting by the high latitudes. By the year 2100, all surface seawater poleward of  $60^\circ$  South might become corrosive to aragonite (see Fig. 1.3) (Cao and Caldeira, 2008), threatening key calcifiers such as coccolithophores, foraminiferans or pteropods (Bijma et al., 2002; Fabry et al., 2008; Comeau et al., 2009; Beaufort et al., 2011). Unless being not expected to experience undersaturation for calcium carbonates, lower latitude ecosystems are not out of danger. In tropical areas Scleratinian coral reefs are expected to cease growing beyond  $560 \mu\text{atm CO}_2$  (Silverman et al., 2009) (see Fig. 1.4) while in temperate seas, numerous commercially important species (Cooley et al., 2011), such as mytiliids or ostriids shellfishes could be threatened (Gazeau et al., 2007; Thomsen et al., 2010). Nonetheless, some species have shown capacities of adaptation like the coccolithophores *Emiliana huxleyi* (Lohbeck et al., 2012) or acclimation as the deep water coral *Lophelia pertusa* (Form and Riebesell, 2012).



**Figure 1.4.** Global coral reef distribution and their gross calcification relative to their pre-industrial rate (280 ppm) in percent calculated employing modeled sea surface  $\Omega_{\text{arag}}$  and temperature values from Cao and Caldeira (2008) and assuming no change in live coral cover for each reef location at  $n\text{CO}_2$  stabilization levels of 380, 450, 560 and 750 ppm.



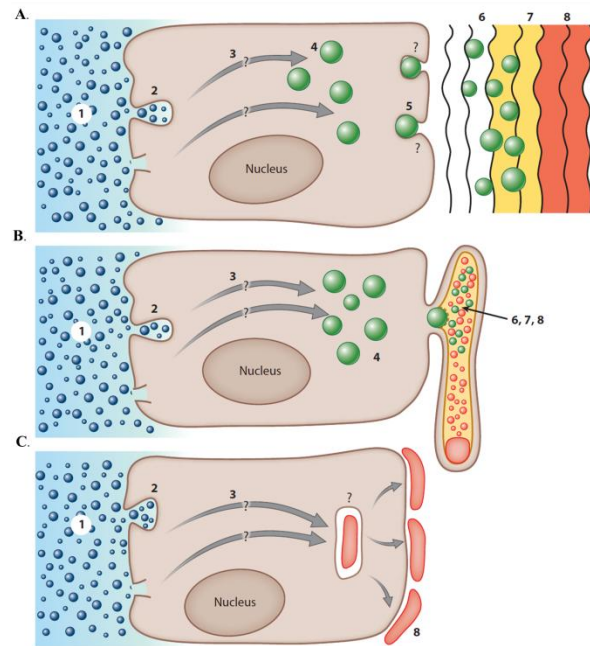
All pathways of calcification are involving the cellular uptake and concentration of  $\text{Ca}^{2+}$  and  $\text{CO}_3^{2-}$  from external seawater or body fluids before deposition and precipitation in a specialized cellular compartment or to an external nucleation site (Weiner and Addadi, 2011) (see Fig. 1.5). Thus, beside the nature of the biocrystal secreted, the sensibility of calcifiers but also of non-calcifiers to ocean acidification is linked to the ability to maintain homeostasis. The most sensitive taxa are those deprived of the capacity of buffering their body fluids (e.g. bivalves and some echinoderms).

The ionic exchanges involved in i.e. calcification or respiration can be directly influenced by the seawater pH / DIC fractionation (Bentov et al., 2009; Melzner et al., 2009). In taxa having the capacity to buffer their internal medium (e.g. cephalopods, teleosts, most of crustaceans), activities such as

calcification are less sensitive to the seawater ionic composition. However, the metabolic costs relative to the restoration of the homeostasis can still have a detrimental effect on the organisms performances (Melzner et al., 2009; Gutowska et al., 2010). Finally, In calcifiers, the presence of organic layers and tissues over the calcified structures (e.g. periostracum in bivalves) seems to play a key role in protecting shells dissolution (Hall-Spencer et al., 2008; Thomsen et al., 2010).

More than adult organisms, ocean acidification have shown to harm early life stages (Kurihara and Shirayama, 2004; Kurihara et al., 2007; Dupont et al., 2008, 2010b; Stumpp et al., 2011; Pansch et al., 2012;). In most marine animals, the exchange of gametes, fertilization and embryogenesis are occurring directly the water column and, after hatching, most animals are experiencing one or several larval stages followed by a metamorphosis to juvenile and adult. During those early stages, mortality rates up to 90 % are usual (Gosselin and Qian, 1997), probably due to the emission of a large panel of larvae adapted to different settlement conditions (Raimondi and Keough, 1990; Marshall and Keough, 2003). In such context, any further mortality caused by ocean acidification could lead to a collapse of populations.

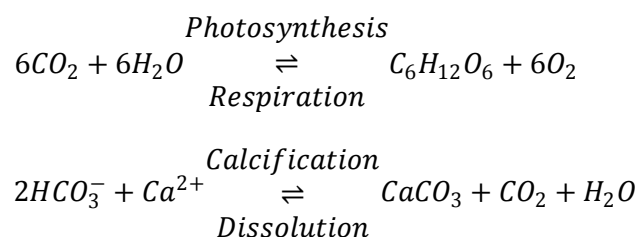
Contrarily to heterotrophs, ocean acidification is presumed to be beneficial, or at least neutral, to autotrophs.  $\text{CO}_2$  is the substrate of photosynthesis and primary production but, under actual



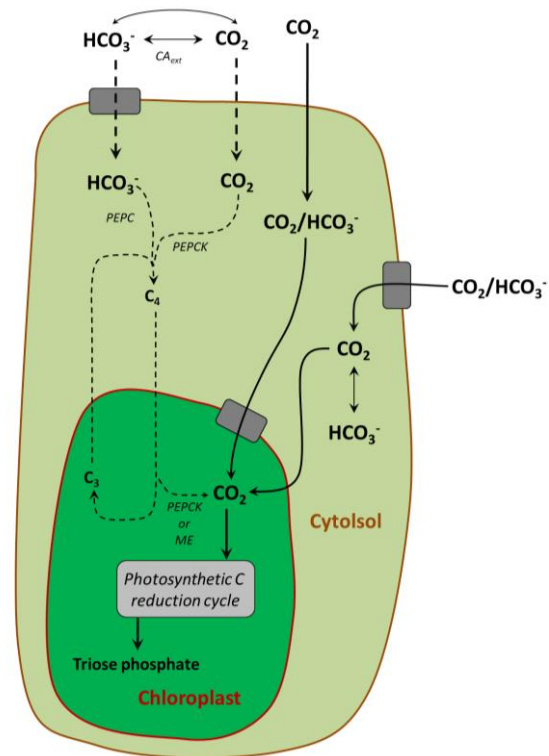
**Figure 1.5.** Crystallization pathways involving: A. an extracellular matrix, B. a vesicle-confined space (syncytium), and C. the formation of mature mineralized elements within a vesicle inside the cell. (1) The external medium (seawater or body fluids). (2) Endocytosis of seawater droplets and/or ion channels and/or transporters. (3) Transport within the cell to specialized vesicles. (4) Specialized vesicles in which the formation of the first disordered mineral phase occurs. (5) Exocytosis of the mineral-bearing vesicles. (6) Translocation of the disordered phase to the crystallization front. (7) Ordering of the crystal. (8) Mature mineralized tissue. In pathway C., the mature mineral may remain within the cell or be transported to the cell. (?) Uncertain precursor phases of the crystallization pathways. (from Weiner and Addadi, 2011).

atmospheric conditions, its concentration in seawater is low, less than  $10 \mu\text{mol kg}^{-1}$ . Furthermore, the viscosity of water limits the diffusion of gases by a factor 10,000 compared to air, generating phenomenon of diffusive boundary layer restricting the gas exchanges. In the opposite, the concentration of  $\text{HCO}_3^-$  in the ocean is typically about  $1700 \mu\text{mol kg}^{-1}$ . Therefore, algae developed strategies to exploit the external DIC and effectively saturate their photosynthetic apparatus for carbon known as carbon concentration mechanisms (CCM) (see Fig. 1.6) (Badger, 2003; Giordano et al., 2005; Hepburn et al., 2011). The most common involves the uptake of  $\text{HCO}_3^-$  as alternative source of DIC, directly or after enzymatic conversion into  $\text{CO}_2$  by carbonic anhydrases (CA). To saturate for  $\text{CO}_2$  the RubisCO, key enzyme of the photosynthetic cycle, some algae developed pathways similar to terrestrial  $\text{C}_4$  metabolisms. The uptaken DIC specie is converted into an intermediate  $\text{C}_4$  carboxylic acid. Thus, the  $\text{C}_4$  compounds are conveyed to the chloroplast and reconverted in  $\text{C}_3 + \text{CO}_2$  ready for photosynthesis. Additively in fucales, the activity of carbon uptake / carboxylation and decarboxylation / photosynthesis is uncoupled temporally similarly than in terrestrial crassulacean acid metabolism (CAM) plants (Axelsson et al., 1989; Surif and Raven, 1990; Schmid and Dring, 1996).

The Fucales in temperate nearshores are forming meadows hosting a wide variety fauna, calcifying or not, so that processes of photosynthesis, respiration, calcification and dissolution are co-occurring. Those can be simplified by the following equations:



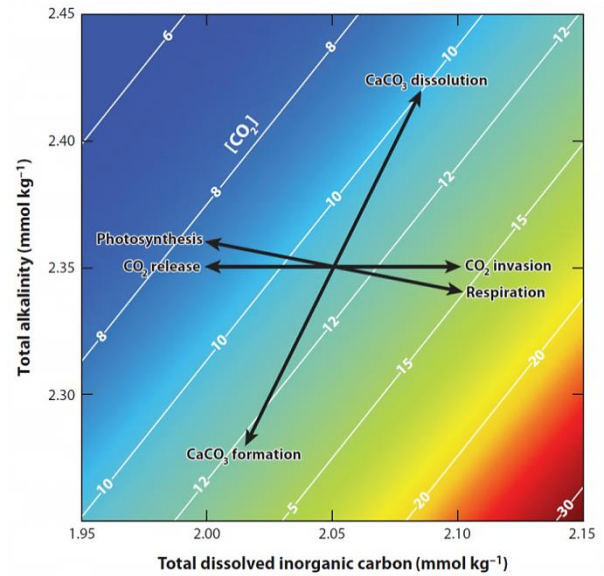
At night, respiration produces  $\text{CO}_2$  while during the day photosynthesis is consuming  $\text{CO}_2$ . Besides, heterotrophs are producing  $\text{CO}_2$  by respiration and also by calcification. In that last case, the precipitation of  $\text{Ca}^{2+}$  reduces the alkalinity of two moles per mole of  $\text{CaCO}_3$  produced. Photosynthesis



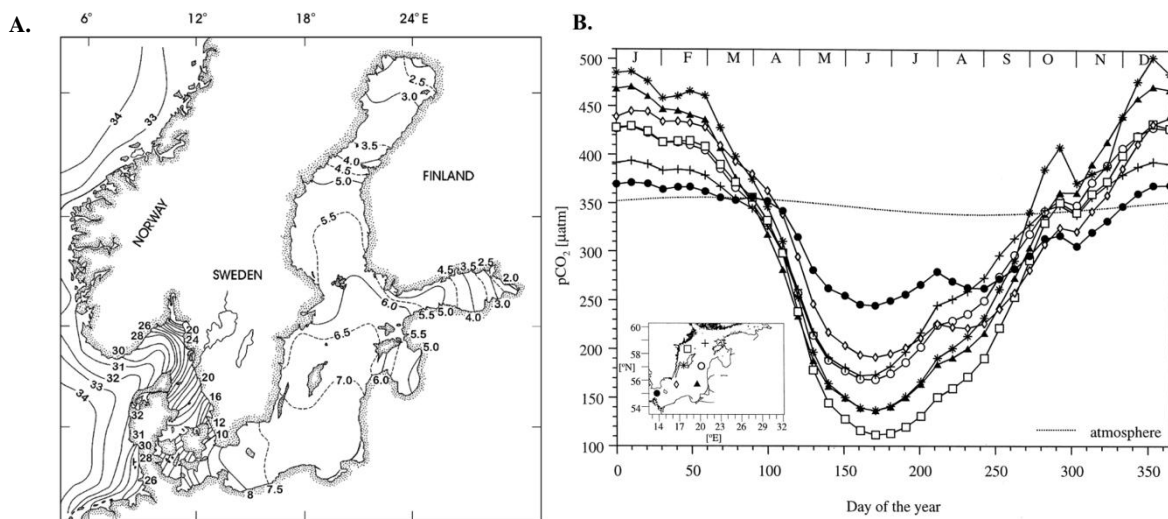
**Figure 1.6.** A schematic model for inorganic carbon transport and  $\text{CO}_2$  accumulation processes in eukaryotic algal cells. The model incorporates the possibilities for DIC transport at the plasmalemma and / or chloroplast envelope as well as a putative  $\text{C}_4$ -like mechanism.  $\text{CO}_2$  crosses membranes by diffusion, whereas active transport (shown by the shaded boxes) can be of  $\text{CO}_2$  or  $\text{HCO}_3^-$ . No attempts have been made to show the roles of the various internal CAs in the different compartments. (redrawn from Giordano et al., 2005).

and calcification therefore have synergetic effect, the first consuming the  $\text{CO}_2$  produced by the second. Oppositely, at night, the production of  $\text{CO}_2$  by respiration of the meadow can impair calcification (see Fig. 1.7). Such complex gas interactions between autotrophs and calcifying heterotrophs have been demonstrated (Drupp et al., 2011; Shamberger et al., 2011) and can lead to wide diel oscillation of the carbonate system in nearshores as shown by Hofmann et al. (2011). Coral reefs, macrophyte dominated ecosystem, estuarine and upwelling systems have shown to be the most variable ecosystems (see Fig. 1.9). The nearshores of the western Baltic Sea are fulfilling several of those characteristics.

The Baltic Sea is a brackish / estuarine-like system with salinities ranging from almost freshwater in the innermost (Gulfs of Finland and Bothnia) parts to oceanic salinity at the junction with the North Sea (see Fig 1.8 A). That low salinity and subsequent low alkalinity is the main reason for a high seasonal dynamic of  $\text{pCO}_2$  and pH. In spring and summer, the successive occurring of phytoplankton and bacterial blooms are leading to  $\text{pCO}_2$  down to  $150 \mu\text{atm}$  while in winter, the low temperature and the mixing of the water column are responsible for  $\text{pCO}_2$  up to  $500 \mu\text{atm}$  (see Fig. 1.8 B), with as consequence  $\Omega_{\text{arag}}$  and  $\Omega_{\text{calc}}$  inferior to 1 (Thomas and Schneider, 1999; Tyrrell et al., 2008; Beldowski et al., 2010; Schneider, 2011). The transition between those two dynamics occurs in late summer. At this period the collapse of the



**Figure 1.7.** Effects of various processes on the carbonate chemistry parameters at 15 °C and salinity 35. Contours indicate isolines of  $[\text{CO}_2]$  in  $\mu\text{mol kg}^{-1}$ . Invasion and release of  $\text{CO}_2$  into / from the ocean changes only the DIC, whereas photosynthesis and respiration also lightly change TA owing to nitrate uptake and release.  $\text{CaCO}_3$  formation decreases the DIC and TA in a ratio of 1:2, and increases the  $[\text{CO}_2]$  fraction of the DIC.  $\text{CaCO}_3$  dissolution has the reverse effect. (from Zeebe, 2012).



**Figure 1.8.** A. Surface water salinity of the Baltic Sea. B. The annual cycle of  $\text{pCO}_2$  in different locations of the Baltic Sea (from Hjalmarson et al., 2008 and Thomas and Schneider, 1999).



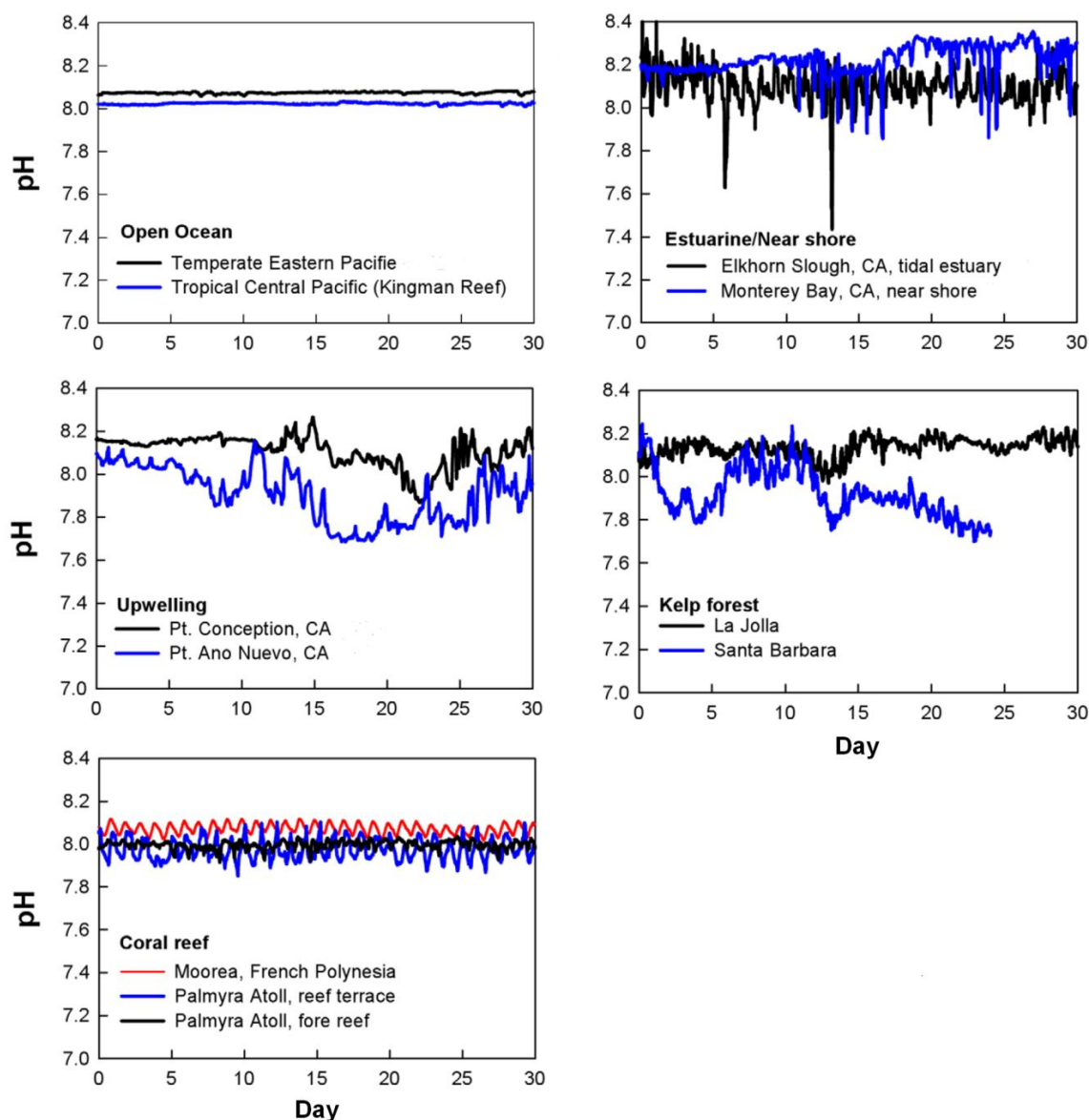


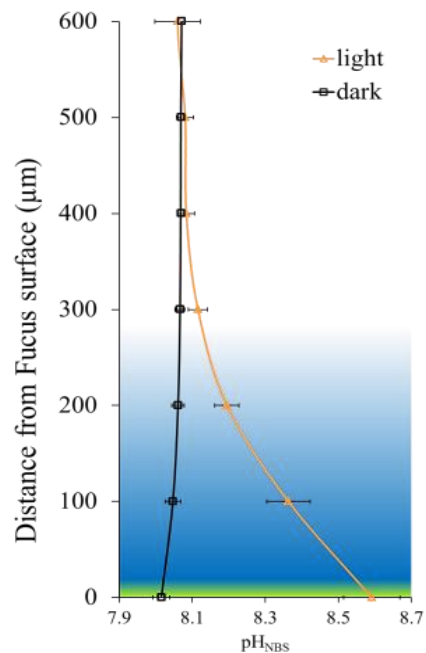
Figure 1.9. pH records from different coastal environments (redrawn from Hofmann et al., 2011).

thermic stratification together with the rise of southern / southwestern winds are leading to the upwelling of deep waters along the coastlines of the western Baltic (Myrberg and Andrejev, 2003). During those, nearshores habitats such as mussel reefs or macrophyte stands are exposed to waters with  $p\text{CO}_2$  up to  $2500 \mu\text{atm}$  (Thomsen et al., 2010). These peaks could reach up to  $5000 \mu\text{atm}$  with tripling of the atmospheric  $p\text{CO}_2$  (Melzner et al., 2012).

One of the principal Macroalgae in the nearshore meadows of the western Baltic Sea is the brown algae *Fucus serratus* (Heterokontophyta, Fucaceae) (Lüning, 1990). This dioecious perennial alga is developing dense colonies on hard bottom substratum, dominating in biomass the mid- and low-tidal area of northern Europe (Lüning, 1990). In the western Baltic, this algae grow on cobble stones together with the seagrass *Zostera marina* growing on the adjacent soft bottoms at depth ranging from 1 to 10 m (Pedersen and Snoeijs, 2001). They are a key engineering specie of the Baltic nearshores

providing spawning and nursery area, food source and substratum for macrofoulers (Hagerman, 1966; Anders and Möller, 1983; Fredriksen et al., 2005). In total, 164 macro- and microfaunal associated species, sessile and not, were inventoried as part of the *Fucus serratus* community in the western Baltic (Hagerman, 1966). A large part of this associated fauna is represented by calcifiers. Among the motile or partly-motile fauna, the most representative calcifiers are the bivalve *Mytilus edulis*, primarily settling on fucus frond but generally migrating to other substratum, the gastropods *Littorina littorea* and *Lacuna vincta* and juveniles of their predators the seastar *Asteria rubens* and the crab *Carcinus maenas*. On the other hand, the associated sessile macrobiontic community is mostly composed of the calcifying tubeworm *Spirorbis spirorbis* (formerly *S. borealis*) (Polychaeta, Serpulidae) and several species of bryozoan. Within those, the most abundant species are the calcifier *Electra pilosa* (Gymnolaemata, Electridae), that can recover up to 85 % of the *Fucus* (Hagerman, 1966) and several members of the keratinous genus *Alcyonidium* (Gymnolaemata, Alcyonidiidae).

The sessile community is partly or totally inhibiting the diffusive boundary layer of the algae during part or all their life cycle. This layer, created by the viscosity of seawater generally have a size from tens of  $\mu\text{m}$  to 1 mm above the algal thallus at common flow velocity (Wheeler, 1980; Koch, 1994; Stevens and Hurd, 1997) but can reach up to 1.5 cm in stagnant water. (Hurd et al., 2011). At such scale, the diffusion rates of molecules are creating concentrated gradients between the mixed layer and the algal tissue, impairing the constant uptake or release in nutrients or gas linked to photosynthesis and respiration (Wheeler, 1980). Therefore, the algal DBL experiences attested diel variations of  $\text{O}_2$  and pH depending on the light intensity (Beer et al., 2008; Koch, 1994; De Beer and Larkum, 2001). At the *Fucus vesiculosus* surface, Spilling et al. (2010) have measured pH ranging from pH 9.5 at 1650  $\mu\text{E}$  light to 8 in the dark, corresponding to the pH in the mixed layer. Interestingly, no studies have shown pH depletion at dark of the same magnitude than the increase at light (Fig. 1.10). The consequence of this is that the BDL surrounding algae, but also of hard substratum bearing microalgal biofilms or encrusting algae, might offer privileged conditions for calcifiers during the day potentially sheltering those communities from ocean acidification (Hurd et al., 2011). Nevertheless, the pH measured in a DBL cannot be compared to the general seawater pH linked to  $\text{CO}_2$ . The oceanic pH measured or foreseen correspond to carbonate systems chemically equilibrated after complete relaxation (Zeebe and Wolf-Gladrow, 2001), while the DBL pH is resulting from a constant flux of DIC species, with a residency time inferior to the carbonate system relaxation time (Zeebe et al., 1999).



**Figure 1.10.** pH in the boundary layer of *Fucus serratus* under light and dark conditions in actual Baltic seawater.

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## Hypothesis

In this study, we tested:

- ❖ Chapter 1:
  - 1: The day /night variations of the carbonate system in a macrophyte meadow of the Baltic sea in summer.
  - 2: The evolution of the carbonate system at the seasonal level.
  - 3: The effect of local upwelling and photosynthesis via a statistical model involving light and wind direction and speed.
- ❖ Chapter 2:
  - The fertilizing effect of sudden DIC and pCO<sub>2</sub> elevation on the common meadow forming algae *Fucus serratus* in physiological conditions of the end of summer.
- ❖ Chapter 3:
  - High pCO<sub>2</sub> favors the non-calcifying epibionts (bryozoa *Alcyonidium hirsutum*) over the calcifying epibionts (bryozoa *Electra pilosa* and tubeworm *Spirorbis spirorbis*) with regard to growth and recruitment (*Spirorbis* only).
  - A dark / light pattern in growth of the juveniles of *Spirorbis spirorbis*, possibly indicating the effect of algal physiology on the growth of its epibionts.

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## GENERAL INTRODUCTION

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*Chapter 1 - Extreme variations of  $p\text{CO}_2$  and pH in a macrophyte meadow of the Baltic Sea in summer: evidence of the effect of photosynthesis and local upwelling.*

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## Abstract

Experimental studies of the ecological effects of acidification in nearshore waters need good field data on the mean and the expected variations in  $p\text{CO}_2$  and pH. However, in temperate areas, such data are largely missing. In this study we investigated temporal variations of the carbonate system in nearshore macrophyte meadows of the western Baltic Sea. These are key benthic ecosystems, providing spawning and nursery areas as well as food to numerous commercially important species. *In situ*  $p\text{CO}_2$ , pH (total scale), salinity and PAR irradiance were measured with a continuous recording sensor package dropped in a shallow macrophyte meadow (Eckernförde bay, western Baltic Sea) during three different weeks in July ( $p\text{CO}_2$  and Irradiance only), August and September 2011. Means  $\pm$  SD  $p\text{CO}_2$  /  $\text{pH}_{\text{tot}}$  observed were  $383 \pm 117 \mu\text{atm} / -$ ,  $239 \pm 20 \mu\text{atm} / 8.22 \pm 0.1$  and  $1082 \pm 711 \mu\text{atm} / 7.83 \pm 0.40$  in July, August and September respectively. Daily variations (difference between daily maximum and minimum) were of the same order of magnitude due to photosynthesis and respiration:  $281 \pm 88 \mu\text{atm}$ ,  $219 \pm 89 \mu\text{atm}$  and  $1488 \pm 574 \mu\text{atm}$  in July, August and September respectively. The observed variations of  $p\text{CO}_2$  were explained through a statistical model considering wind direction and speed together with PAR irradiance. At a time scale of days to weeks, local upwelling of hypercapnic water masses with offshore winds drives the variation. Within days, primary production is responsible. The results demonstrate the high variability of the carbonate system in nearshore macrophyte meadows depending on meteorology and biological activities. They highlight the need to incorporate these variations in future  $p\text{CO}_2$  scenarios and experimental designs for nearshore habitats.

## 1 Introduction

Human activities since the 19<sup>th</sup> century have led to a shift of atmospheric  $p\text{CO}_2$  from 280 to 392  $\mu\text{atm}$  (NOAA - ESRL, December 2011) and the trend is rising. Some scenarios expect an elevation of atmospheric  $p\text{CO}_2$  up to 1000  $\mu\text{atm}$  during the 21<sup>st</sup> century, peaking around 1400  $\mu\text{atm}$  in the year 2300 (IPCC, 2007; Ridgwell et al., 2007). As oceans equilibrate with the atmosphere, dissolution of  $\text{CO}_2$  in water induces a decrease in pH. Global change has already led to an average seawater “acidification” of 0.1 pH units in the global oceans (Orr et al., 2005). Once dissolved,  $\text{CO}_2$  becomes part of the carbonate system, almost entirely composed of bicarbonate- ( $\text{HCO}_3^-$ ) and carbonate ions ( $\text{CO}_3^{2-}$ ). In classical oceanic conditions,  $\text{CO}_{2(\text{aq})}$  represents less than 1 % of the overall dissolved inorganic carbon (DIC) while  $\text{HCO}_3^-$  represents ~90 % and  $\text{CO}_3^{2-}$  ~10%. The carbonate system in open oceanic environments is well known and most of the data forming the basis for the predictive models (see GLODAP database, Key et al., 2004; Feely et al., 2004; Sabine et al., 2005) are derived from the



open ocean. Those oceanic actual and future  $p\text{CO}_2$  / pH values are the ones referred to when designing ocean acidification studies (Riebesell et al., 2010). However, the biogeochemistry of nearshore ecosystems is complex, features more variations and can differ widely from the open ocean or even coastal conditions in general (Borges et al., 2005; Chen and Borges 2009). Shallow nearshore and estuarine areas are the habitat of numerous benthic calcifiers. Experimental studies on these species should take into account conditions (and their likely changes) in nearshore waters, which might be radically different from the trends identified in open oceans, in particular with respect to magnitude and temporal variation.

In general, little attention has been given to the carbonate system of nearshore habitats (Andersson and Mackenzie, 2012). Within those habitats, most of the investigations were conducted on: (1) estuaries, salt marshes, mangrove and mudflats where transfers of carbon from land are occurring (e.g. Gattuso et al., 1998; Brasse et al., 1999; Gazeau et al., 2005) and (2) reefs formed by corals or calcifying algae because of the direct effect of the carbonate system on calcification (e.g. Rees *et al.* 2007; Kleypas et al., 2011). Nearshores dominated by macrophytes (kelp forests and seagrass and / or seaweeds meadows) are poorly represented, even though they represent ~5 % of the primary production in the ocean and  $\frac{3}{4}$  of the vegetal biomass (Smith, 1981; Gattuso et al., 1997). In these habitats, the carbonate system is driven by the photosynthetic physiology of the macrophytes, taking up carbon during the day and releasing it by respiration during the night (Middelboe and Hansen, 2007; Semesi et al., 2009). Since the  $\text{CO}_2$  is a minor component of the DIC and its diffusion rate in seawater is 10,000 times lower than in air (Lobban and Harrison, 1994), most macrophytes are relying on  $\text{HCO}_3^-$  for photosynthesis (Beer and Rehnberg, 1997; Badger, 2003; Giordano et al., 2005; Hepburn et al., 2011). Macrophyte meadows are highly productive and net autotrophic (carbon sink) (Barrón et al., 2003; Duarte et al., 2010) exporting their excess of production to the neighboring ecosystems under the form of litter or dissolved organic carbon (Barrón and Duarte, 2009). Microbial degradation of exported organic carbon leads to hypercapnia and hypoxia of deeper offshore waters (Gray et al., 2002). This phenomenon is particularly important in eutrophied ecosystems like the Baltic Sea (Melzner et al., in review). In the inner bays of the western Baltic, local upwelling of hypercapnic water masses is regularly observed, increasing the surface  $p\text{CO}_2$  up to 2500  $\mu\text{atm}$ . This typically happens at the end of summer when offshore winds are upwelling deep waters after long periods of stratification (Myrberg and Andrejev, 2003; Thomsen et al., 2010; Gräwe and Burchard, 2011).

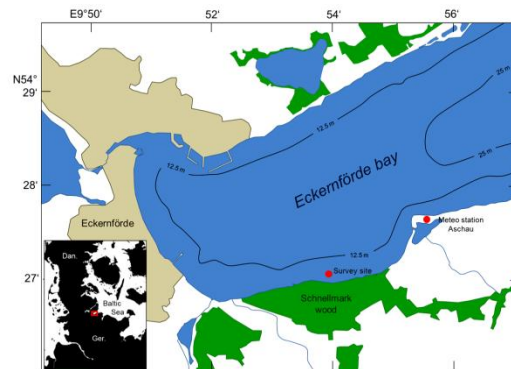
In our study conducted in the western Baltic Sea over three weeks of summer 2011, we aimed to measure (1) the day / night variations of  $p\text{CO}_2$  due to photosynthesis and respiration of a macrophyte bed, (2) the evolution of the baseline  $p\text{CO}_2$  over summer and (3) the effect of local upwelling on the carbonate system and its diel variations. Therefore, we modeled the variations of DIC measured in the macrophyte meadow with a statistical model considering wind direction and speed together with photosynthetically active radiation (PAR).

## 2 Material and Methods

### 2.1 Study site

The pCO<sub>2</sub> and PAR irradiance were recorded in less than 3m depth in Eckernförde Bay (Fig. 2. 1), western Baltic Sea, Germany, 54°27' N, 9°54' E) during 3 summer weeks in 2011: 29.06-08.07, 29.07-05.08 and 09.09-16.09. Recording systems for pH, salinity and temperature were added during the August and September deployments. Wind speed (m s<sup>-1</sup>) and direction (rad) with a resolution of 10 min were provided by the meteorological station of Aschau in Eckernförde Bay (54°27'40 N, 9°55'30 E) belonging to the division of marine technology and

research of the German Navy. The nearshore habitat of Eckernförde is a mixed bottom type dominated by macrophyte vegetation. The macrophyte vegetation covers approximately 75 % of the surface. Dominant species are the brown algae *Fucus serratus* (40-60 % of the macrophytes), growing on stones and rocks and the seagrass *Zostera marina* (< 10 % of the macrophytes) growing on sandy bottoms (Fürhaupter et al., 2008). The inner basin reaches a maximum depth of 28 m in gentle slope. The water column is stratified in summer, with a pycnocline around 15 m depth (Krey et al., 1965). September is the period of maximal stratification and hypoxia in the deep water. Hence, O<sub>2</sub> reaches minimal concentrations of about 30 µM together with peaks of pCO<sub>2</sub> up to 2500 µatm and the accumulation of dissolved methane (Bange et al., 2010; Melzner et al., in review). The benthic community in the deeper parts is restricted to deposit feeding polychaetes and bivalves (D'Andrea et al., 1996).



**Figure 2. 1.** Map of the inner Eckernförde Bay indicating the locations of the survey site and the meteorological station, urban area (brown) and forests area (green).

### 2.2 The autonomous measuring system

For the measurement of the pCO<sub>2</sub> during the deployments a HydroC™ underwater sensor was used (CONTROS Systems & Solutions, Germany). The instrument measures the CO<sub>2</sub> mole fraction in a headspace behind a membrane equilibrator by means of a single beam, two-wavelength non-dispersive infra-red detector (NDIR). A small pump within the internal gas stream continuously circulates air between the equilibrator comprising a flat silicone composite membrane, the NDIR unit and additional sensors for pressure, temperature and relative humidity necessary for the proper correction of NDIR signals as well as for the calculation of the partial pressure. As an additional drift

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correction mean beside the dual-beam configuration of the NDIR detector, the instrument regularly scrubs the CO<sub>2</sub> from the internal gas stream and thus carries out a zeroing. The zeroings are accounted during post processing of the data. A first description of the instrument can be found in (Fietzek, 2010) and deployments on various platforms are presented in (Fietzek, 2011). The instrument was calibrated by the manufacturer prior to every deployment for the range of 100-1000 µatm within a thermostatic water tank at 17 °C, 18 °C and 18 °C for the first, second and third deployment respectively. An overall accuracy of better than ± 1 % of the upper range value is assumed for post processed data. In order to achieve a constant and sufficiently short response time of the instrument, the HydroC™ is equipped with a water pump (Sea-Bird Electronics, USA) that provided a continuous water flow of approx. 35 ml sec<sup>-1</sup> to the membrane leading to a response time T<sub>63 %</sub> of 3 min and a T<sub>99 %</sub> of approximately 15 min. These response times are sufficiently fast to resolve the observed signal changes without the need for signal deconvolution. The pCO<sub>2</sub> series was corrected for drift as follows: the sensor's zero signals were regarded as nodes and linearly interpolated in time to result in a smooth signal drift correction throughout the deployment time.

Salinity (psu), voltage (analogue signal from the pH measurement) and temperature (°C) were recorded automatically every 45 min with a pH-meter / conductimeter Mettler-Toledo SG 7/8 (Mettler Toledo, Switzerland). The pH on total scale was measured as follows. Seawater TRIS pH buffers for 15 psu were made according to the SOP 6a of Dickson and Sabine (2007). A combined electrode with a solid polymer electrolyte reference, equipped with an NTC (negative temperature coefficient) thermistor was used (Inlab expert pro, Mettler Toledo, Switzerland). Calibration of a new electrode was made 24 h before each deployment. To do so, the TRIS buffer was immersed in a thermostatic bath and the voltage of the pH electrode was measured with an accuracy of ± 0.1 mV. The temperature of the buffer was varied by 1.5°C around seawater temperature. The temperature corresponding to every mV change was recorded with an accuracy better than 0.01 °C with a Fluke 5658 reference thermometer doted of a 5608 platinum resistance sensor (Fluke, USA). This process was repeated by increasing and decreasing the temperature to get an average voltage (mV) *versus* T (°C) reference curve for the electrode in the buffer. The NTC sensor of the pH sensor, of resolution 0.1 °C, was calibrated against a reference thermometer and the resulting regression was achieved with a R<sup>2</sup> > 0.99. The resulting equation was used to correct the sampled temperature. The sampled voltage and corrected temperature were converted to pH<sub>tot</sub> by making use of the initial TRIS buffer calibration of the electrode and by using the equations given in the SOP 6a. In the lab, work conducted on Certified Reference Material (CRM) (Andrew Dickson, Scripps Institution of Oceanography) together with 35 psu buffers demonstrated an accuracy of 0.003 to 0.005 pH units. Salinity was measured within 0.01 psu by a Mettler Toledo Inlab 738 conductivity probe after calibration at 25°C with KCl 0.1 mol L<sup>-1</sup> (Fischer Scientific, USA). The PAR irradiance (400 to 700 nm) was recorded every 5 min using a LI-192 quantum sensor connected to a LI-1400 data logger (Li-Cor Biosciences, USA). All series were extended to one minute interval series by linear interpolation.

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### 2.3 Calculation of the carbonate system

Samples for alkalinity were taken at the beginning, middle and end of the measurement periods of August (no replication) and September (one replicate). Alkalinity was measured with a precision of  $\pm 5 \mu\text{mol kg}^{-1}$  using an open cell potentiometric titrator as described in the SOP 3b of Dickson and Sabine (2007) (Titrand 888, Methrom, Switzerland). A regression was calculated between total alkalinity and salinity of the all the sample from August and September pooled together and the alkalinity for the entire period was estimated from the salinity series. The overall carbonate system was derived from calculated alkalinity and the measured  $\text{pCO}_2$  with the R package Seacarb (Lavigne and Gattuso, 2011) using first and second carbonate system dissociation constants for estuarine systems from Millero (2010) and the dissociations constants of HF and  $\text{HSO}_4^-$  of Perez and Fraga (1987) and Dickson (1990).

### 2.4 Statistical modeling

To understand the measured variations of DIC during the August and September deployment periods in the macrophyte meadows of Eckernförde, we developed a statistical model considering upwelling of DIC-rich deep water as a driver at a time scale of several days, and primary production and respiration as a driver at a within-day time scale. We assumed that upwelling was driven by wind and in particular by offshore-directed wind, and proceeded to fit a function of wind speed and direction to the data from which the within-day variations were filtered. This was achieved by a 24 h central running average run over the time series of  $\text{pCO}_2$  and (calculated) alkalinity. We then fit the series to the following function of wind speed weighted by wind direction:

$$WW_t = \text{wind speed} [m s^{-1}] \cdot \cos(\text{wind direction} [rad] + W_{up}),$$

With  $W_{up}$  being a parameter between 0 and  $2\pi$ , corresponding to the reference wind direction for which wind-induced upwelling is maximal. As an example, if  $W_{up} = 0$  or  $2\pi$ , maximal weights are given to northern winds, if  $W_{up} = \pi$  maximal weights are given to Southern winds. A “left-sided” running integration was performed on the weighted wind time series over a period  $k_w$  in the model:

$$Y_t = \mu + \alpha \int_{\tau=t-k_w}^{\tau=t} WW(\tau) d\tau,$$

Where  $Y_t$  is the 24h running-mean series of DIC at time t,  $WW(t)$  is the weighted wind speed at time t,  $\mu$  and  $\alpha$  are regression parameters and  $k_w$  is determined as the integration period yielding the best fit. Parameters in the regression were chosen to minimize residual variance. Formal hypothesis tests, F-statistics and regression coefficients were not considered as they would be calculated based on overestimated degree of freedoms due to the time series auto-correlations.

The within-day variations of DIC (given by the residuals after detrending the DIC series) were modeled by exponentially weighted running integration of the PAR series over a period of 12 hours with exponential decay rate  $\lambda$  ( $\text{min}^{-1}$ ) in the model:

$$Y_t = \mu + \alpha \int_{\tau=t-12h}^{\tau=t} PAR(\tau) * e^{(-\lambda(t-\tau))} d\tau$$

In a final step, the irradiance and wind sub-models were summed to obtain the final model. The standard deviation of the residuals between model and DIC observation were considered for the parameterization of  $W_{up}$ ,  $k_w$  and  $\lambda$ .

## 3 Results

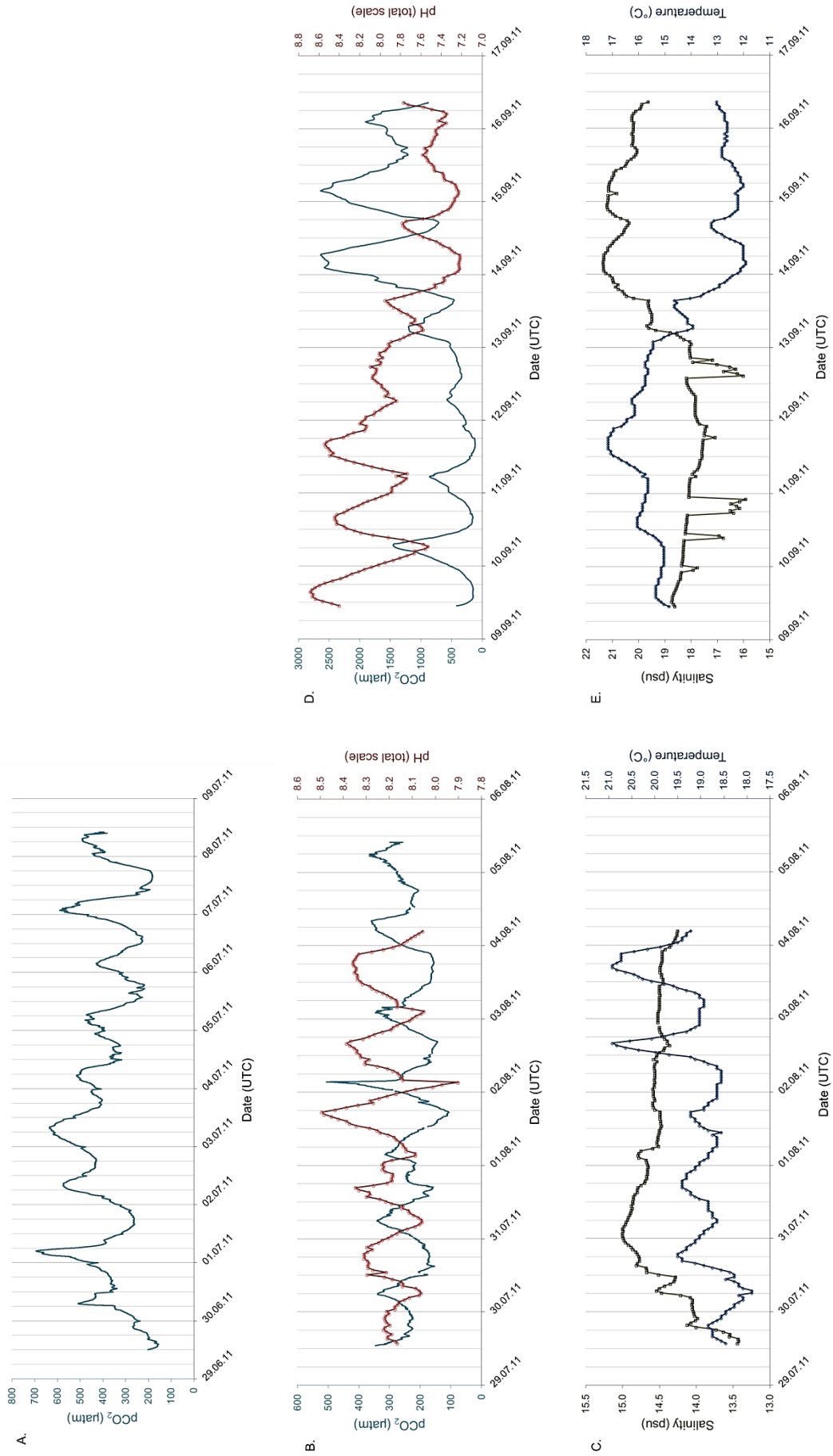
### 3.1 Measures

The three weeks revealed important day / night oscillations of pH and  $p\text{CO}_2$ . The  $p\text{CO}_2$  increased during the night and decreased during the day reaching minima and maxima at 6 o'clock in the morning and 6 o'clock in the afternoon respectively. The pH inversely mirrored these  $p\text{CO}_2$  variations.

The  $p\text{CO}_2$  series of July is shown in Fig. 2. 2. A. The mean  $p\text{CO}_2$  of the week was  $389.9 \mu\text{atm}$ . The mean  $\pm$  SD day / night variation was  $281.2 \pm 87.8 \mu\text{atm}$ , the mean  $\pm$  SD daily maximum and minimum values were  $557.6 \pm 92.1 \mu\text{atm}$  and  $276.0 \pm 89.1 \mu\text{atm}$ . The maximal daily amplitude observed was  $401.2 \mu\text{atm}$ .

The  $p\text{CO}_2$  in August was the lowest with a week mean of  $239.5 \mu\text{atm}$  and the most stable with a daily variation (mean  $\pm$  SD) of  $219.2 \pm 24.5 \mu\text{atm}$  (Fig. 2. 2. B.). The mean  $\pm$  SD daily maximal and minimal  $p\text{CO}_2$  values were  $374.2 \pm 66.7$  and  $155.0 \pm 31.6$  respectively. The maximal amplitude was  $415.6 \mu\text{atm}$ . The pH variation mirrored fluctuations in  $p\text{CO}_2$  (Fig. 2. 2. B.) with a weekly mean of 8.21, mean  $\pm$  SD diel amplitude of  $0.34 \pm 0.15$ , mean  $\pm$  SD daily minimum and maximum values of  $8.03 \pm 0.07$  and  $8.37 \pm 0.08$ . The maximal daily amplitude was 0.59. The average salinity and temperature during the week were  $14.5 \pm 0.3$  psu and  $19.1 \pm 0.66$  °C respectively (Fig 2. 2. C.).

The recordings of September exhibit a strong difference between the beginning and the end of the week due to an upwelling event in the middle of the week. The mean  $\pm$  SD salinity and temperature during the first three days were  $17.8 \pm 0.6$  psu and  $15.9 \pm 0.6$  °C while during the three last days they increased and decreased to  $20.7 \pm 0.5$  psu and  $12.4 \pm 0.4$  °C respectively (Fig 2. 2. E.). Similarly we observed a large discrepancy of the  $p\text{CO}_2$  / pH between the beginning and the end of the week with means of  $426.0 \mu\text{atm} / 8.14$  and  $1593.2 \mu\text{atm} / 7.46$  respectively (Fig 2. 2. D.). The second part of the



**Figure 2.** pCO<sub>2</sub> (cyan), pH (red) Salinity (black) and temperature (dark blue) recorded in (A) July, (B) (C) August and (D) (E) September. The recording frequency was one minute for pCO<sub>2</sub> and 45 minutes for the 3 other parameters. Symbols in the plots mark the recording events; linkage between the measurements every 45 min was achieved by linear interpolation.

week experienced night peaks (mean  $\pm$  SD) of  $p\text{CO}_2$  of  $2397.4 \pm 424.9 \mu\text{atm}$ , with drops of pH to (mean  $\pm$  SD)  $7.26 \pm 0.07$ . During daytime,  $p\text{CO}_2$  reached minima of (means  $\pm$  SD)  $681.3 \pm 211.4 \mu\text{atm}$  with maxima of pH of  $7.77 \pm 0.18$ . The maximal daily amplitudes recorded were  $2183.7 \mu\text{atm } p\text{CO}_2$  and 1.15 pH units.

### 3.2 Carbonate system

The carbonate system was derived from  $p\text{CO}_2$  and alkalinity for the weeks of August and September. To obtain an estimate of the alkalinity dynamics over the two measurement weeks, we performed a regression between salinity and alkalinity of discrete samples taken at different times of the weeks. This relationship was used to convert the recorded salinity time series to alkalinity. The regression was highly significant (F-statistic:  $p < 0.001$ ,  $R^2 = 0.83$ ,  $n = 9$ ): Alkalinity =  $905.17 * \text{Salinity} + 59.83$ , with both slope and intercept significant at  $p < 0.001$  and  $p < 0.01$  (t-test,  $n = 9$ ). The dynamics of DIC and the saturation state of calcium carbonates are shown in Fig. 2. 3. A. and D. for August and in Fig. 2. 3. B. and E. for September.

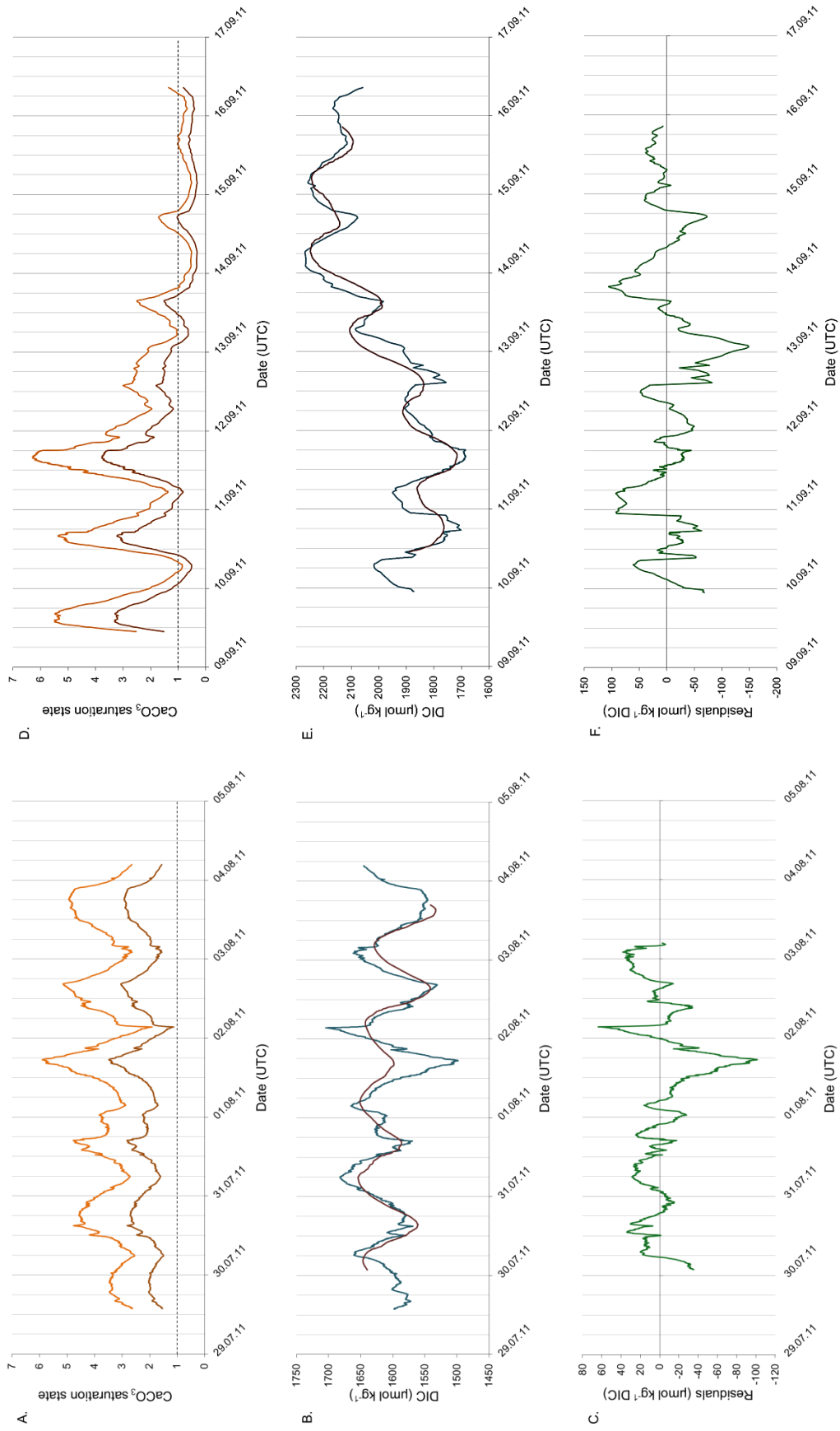
In August, means  $\pm$  SD of the week and day / night variations of DIC were  $1608.9 \pm 37.2 \mu\text{mol kg}^{-1}$  and  $130.8 \pm 45.0 \mu\text{mol kg}^{-1}$  respectively. The seawater was always supersaturated with respect to both aragonite and calcite with diel means  $\pm$  SD of  $2.2 \pm 0.2$  and  $3.7 \pm 0.3$  and means  $\pm$  SD day / night variations of  $1.4 \pm 0.6$  and  $2.4 \pm 0.9$  respectively.

In September the daily mean  $\pm$  SD DIC concentrations preceding and during the upwelling event were  $1829.3 \pm 45.1$  and  $2157.7 \pm 26.7 \mu\text{mol kg}^{-1}$  respectively. The diel variations of DIC were higher before than during the upwelling, with mean  $\pm$  SD of  $258.6 \pm 10.5 \mu\text{mol kg}^{-1}$  against  $205.1 \pm 90.7 \mu\text{mol kg}^{-1}$  respectively. Before the upwelling, mean  $\pm$  SD  $\Omega_{\text{arag}}$  and  $\Omega_{\text{calc}}$  were  $3.2 \pm 1.5$  and  $1.9 \pm 0.9$ . Minima were reached around 6 o'clock in the morning and episodes of several hours of undersaturation, mostly for aragonite, were observed: mean  $\pm$  SD  $\Omega_{\text{arag}}$ :  $0.8 \pm 0.3$  and  $\Omega_{\text{calc}}$ :  $1.4 \pm 0.5$ . Oppositely, seawater reached maxima of  $5.7 \pm 0.5$  and  $3.4 \pm 0.3$  for calcite and aragonite around 6 o'clock in the afternoon, corresponding to diel oscillation of  $4.3 \pm 0.3$  and  $2.6 \pm 0.2$  respectively. During the upwelling event the seawater was constantly undersaturated with mean  $\pm$  SD  $\Omega_{\text{calc}}$  and  $\Omega_{\text{arag}}$  of  $1 \pm 0.5$  and  $0.6 \pm 0.3$ , exception made of the hours around 18 h, where modest maxima (mean  $\pm$  SD) of  $1.9 \pm 0.6$  and  $1.1 \pm 0.4$  were reached.

### 3.3 Model

The output of the model for the weeks of August and September is presented in Fig. 2. 3.. In August, the model predicted a daily mean  $\pm$  SD of  $1610.4 \pm 15.9$  and a mean  $\pm$  SD diel variation and  $86.4 \pm 14.4 \mu\text{mol kg}^{-1}$  DIC. The differences with observations for those two last parameters are 1.5 and  $44.3 \mu\text{mol kg}^{-1}$  DIC, respectively. In September, the model predicted a daily mean  $\pm$  SD DIC of  $1852 \pm 83.5$

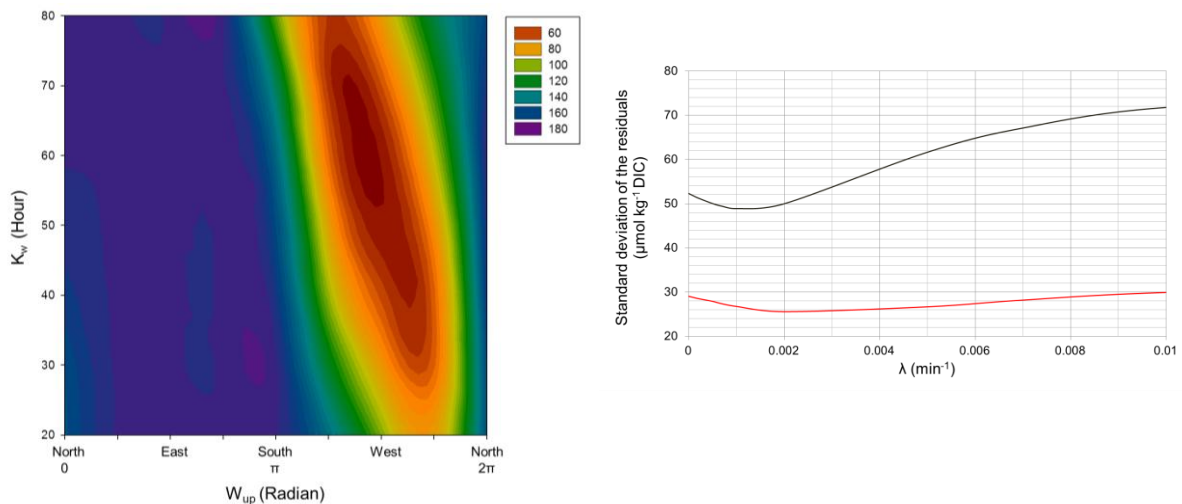




**Figure 3.** Saturation states for calcite (light brown) and aragonite (dark brown) for August (A) and September (D). Observed (cyan) and modeled (red) DIC for (B) August and (E) September. The residuals between model and observations are presented in (C) and (F) for August and September respectively.

and  $2142.7 \pm 47.3 \mu\text{mol kg}^{-1}$  preceding and during the upwelling event respectively, corresponding to absolute differences with observation of 23.2 and  $14.9 \mu\text{mol kg}^{-1}$ . In the corresponding periods, the predicted day / night amplitudes are of  $141.4 \pm 60.6$  and  $149.6 \pm 107.3 \mu\text{mol kg}^{-1}$  DIC (mean  $\pm$  SD), respectively. The differences with observations are of 117.2 and  $55.48 \mu\text{mol kg}^{-1}$  DIC before and during the upwelling respectively.

The set of parameters producing the best fitting model are  $W_{\text{up}} = 3\pi/2$ , corresponding to westerly wind, a period of integration  $k_w$  of 55 hours and  $\lambda$  of  $0.0025 \text{ min}^{-1}$  and  $0.001 \text{ min}^{-1}$  for the weeks of August and September respectively (Fig. 2. 3. B. and E.). The standard deviation of the residuals between model and observation were 25.4 and  $48.9 \mu\text{mol kg}^{-1}$  DIC, corresponding to percentages of unexplained variation of 1.6 % and 2.5 % for August and September respectively (Fig. 2. 3. C. and F.). The variation of the standard deviation of the residuals as function of the wind direction used for maximal weight ( $W_{\text{up}}$ ) and the time frame of integration ( $k_w$ ) for September is presented in Fig. 2. 4. A.. Easterly winds as maximal weights produced the worst fitting model while westerly winds the best fitting, southerly and northerly winds were intermediate. The parameter  $\lambda$  had less influence on the outcome of the model (Fig. 2. 4. B), increasing its accuracy by a maximum of 2 to  $4 \mu\text{mol kg}^{-1}$ . Despite its poor effect on the model, the presence of this parameter is justified by the consideration of the decrease of the length of the daytime between August and September (the period of integration of the irradiance being fixed to 12 h).



**Figure 2. 4.** (A) Contour plot of the standard deviation of the residuals between model and observation in  $\mu\text{mol kg}^{-1}$  DIC (color palette) for the September series as a function of  $W_{\text{up}}$ , the azimuth of reference used as maximal weight, and the  $k_w$ , the period of the running integration of the weighted wind series. (B) Evolution of the standard deviation of the residuals between model and observation in  $\mu\text{mol kg}^{-1}$  DIC in August (red) and September (black) as a function of  $\lambda$  ( $\text{min}^{-1}$ ), the instantaneous rate of increase used for the exponentially weighted running integration of the irradiance series.

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## 4 Discussion

In our study, we measured important variations of pCO<sub>2</sub> and pH on a daily and weekly basis and related those to meteorological factors. We demonstrated therefore the effect of photosynthetic physiology and local upwelling on the nearshore carbonate chemistry.

### 4.1 Measurements reliability

The accuracy of the post processed pCO<sub>2</sub> data is expected to be  $< \pm 10 \mu\text{atm}$  for values within the calibrated measuring range of 100-1000  $\mu\text{atm}$ . An additional error can be expected when the pCO<sub>2</sub> exceeds the measuring range as in this case the calibration polynomial of the instrument is extrapolated. We expect this error to be  $\pm 150 \mu\text{atm}$  at maximum for the highest pCO<sub>2</sub> recorded in September at around 2600  $\mu\text{atm}$ .

Despite our efforts, we did not achieve the pH accuracy necessary to use it as input parameter for the derivation of the carbonate system. We estimate the error in pH of the order of  $10^{-2}$  due to uncorrectable drifts during the measurement periods. In the conditions of temperature and salinity of the western Baltic, such inaccuracy in pH produces anomalies in derived alkalinity of the order of  $10^1$ - $10^2 \mu\text{mol kg}^{-1}$  at pH / pCO<sub>2</sub> inferior to 7.8 / 800  $\mu\text{atm}$  and up to  $10^3$  at higher pH / lower pCO<sub>2</sub>. For future field studies, Durafets sensors (Martz et al., 2010) or spectrophotometric sensors (Aßman et al., 2011) represent promising alternatives to glass electrodes, both capable of reaching accuracies of  $10^{-3}$  to  $10^{-4}$  pH units. We had to perform alkalinity titrations and rely on a salinity to alkalinity relationship to achieve the calculations. This method is relevant (Beldowski et al., 2010; Hjalmarsson et al., 2008) and we estimate the error on the derivated DIC to be  $< 15 \mu\text{mol kg}^{-1}$  DIC, however it ignores any changes of alkalinity at constant salinity. This phenomenon is very important in coral and shellfish reefs due to the uptake or release of Ca<sup>2+</sup> during calcification or dissolution (Drupp et al., 2011; Shamberger et al., 2011). However, in macrophyte ecosystems, we expect very marginal diel variations of alkalinity, due to photosynthesis and respiration (Chisholm and Gattuso, 1991).

### 4.2 Model

We were able to explain the DIC variations of August and September to a precision of  $\pm 50 \mu\text{mol kg}^{-1}$  with a simple statistical model, based on wind and irradiance and three parameters only. The weighing procedure of the wind speed, although simple in its geometric approach, appeared to work very well as an explanation of the multi-day trends.

Our model designates westerly winds as responsible for upwelling. This result is slightly different from previous observations and simulations in other regions of the Baltic Sea identifying

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southwesterly winds as being the most effective (Myrberg and Andrejev, 2003). This small discrepancy is certainly due to the east-west orientation of Eckernförde bay while the Baltic Sea has a general north-east / south-west orientation. The use of exponentially weighted integration of irradiance as a proxy for primary production was less effective than the similar approach used for the upwelling. Indeed, while the daily DIC means are quite accurately predicted ( $< 20 \mu\text{mol kg}^{-1}$ ), the diel amplitude is systematically underestimated by 40 to  $70 \mu\text{mol kg}^{-1}$  DIC. This variation unexplained by the model could be due to heterotrophic respiration, air-sea exchange of  $\text{CO}_2$  (Gattuso et al., 1997) or diel variation of alkalinity as discussed in 4.1. However, despite the simplistic nature of our model, it is a first step in the understanding and prediction, in a context of global change, of the carbonate system dynamics in the Baltic nearshore areas.

### *4.3 Inter-weekly dynamics*

The  $\text{pCO}_2$  of our measurement site exhibited very different weekly trends over the summer. In August, we observed an important and stable  $\text{pCO}_2$  undersaturation. This reflects the conditions of the whole Baltic at this period, generated by the succession of blooms of phytoplankton and cyanobacteria (Thomas and Schneider, 1999; Schneider, 2011). In September this stable condition is interrupted by the strengthening of the westerly winds, leading to upwelling of the water masses isolated until then below the pycnocline. The chemistry of the deep waters of Eckernförde bay are monitored since 1957 and  $\text{pCO}_2$  of about  $2500 \mu\text{atm}$ , linked to heterotrophic processes, are yearly observed in summer (Boknis Eck time series; Bange et al., 2011). Our study shows that the nearshore biota in shallow water, are effectively exposed to these high concentrations during upwelling events. The deep-water  $\text{pCO}_2$  is presumed to increase in the future by the conjunction of higher atmospheric  $\text{pCO}_2$  and reduced salinity and alkalinity of the Baltic Sea (Gräwe and Burchard, 2011). Melzner et al. (in review) are expecting this deep water body to reach  $\text{pCO}_2$ 's of 4000 to  $5000 \mu\text{atm}$  and the hypercapnic events of September to increase in duration and magnitude. Besides, the frequency and duration of those upwelling events could also increase as a reinforcement of the westerly winds in the Baltic region is expected with global warming (Neumann and Friedland, 2011).

### *4.4 Daily oscillations*

Diel oscillations, related to photosynthesis and respiration, are superimposed to the week scale dynamics. In normal summer conditions (without upwelling), the mean ( $\pm$  SD) amplitudes of the diel variations were  $243 \pm 95 \mu\text{atm CO}_2$  (July, August) and  $0.34 \pm 0.15$  pH units (August only). Such diel variations have already been observed in macrophyte stands worldwide: in seagrass beds of Australia (Smith et al., 1981), Mediterranean (Invers et al., 1997) and Zanzibar (Semese et al., 2009), in tidal rocky-shores of the northeastern Pacific (Wootton et al., 2008) and in algal meadows of the Danish islands (Middelboe and Jansen, 2007). Among nearshore ecosystems, the highest diel variations are

occurring in macrophyte dominated ecosystems estuaries, upwelling areas and estuaries (Hofmann et al., 2011). Our study site cumulates those three characteristics: brackish, weakly buffered and eutrophied ecosystem dominated by macrophytes and submitted to upwelling. We recorded diel oscillations of  $1604.9 \pm 795.7 \mu\text{atm}$  (mean  $\pm$  SD) during the upwelling event of September. To our knowledge only near-shore mangroves exhibit wider diel variations with  $3500 \mu\text{atm}$  recorded in the Bermuda (Andersson and Mackenzie, 2012).

However, the importance of these variations is relative, as the fraction of DIC present as  $\text{CO}_{2(\text{aq})}$  in high  $\text{pCO}_2$  / low pH seawater is higher than in low  $\text{pCO}_2$  / high pH seawater (see Bjerrum plot in e.g. Zeebe and Wolf-Gladrow, 2007). Thus, in September, the diel variations of  $\text{CO}_2$  are stronger during the upwelling than before but the opposite is observed for DIC (mean  $\pm$  SD DIC:  $258.6 \pm 10.5 \mu\text{mol kg}^{-1}$  against  $205.1 \pm 90.7 \mu\text{mol kg}^{-1}$ ), possibly revealing a reduction of photosynthesis during the upwelling. This could be explained by the osmotic stress engendered by the rapid increase of salinity (Ohno, 1976; Bäck et al., 1992) rather than by the hypercapnia. Indeed, any increase in DIC and  $\text{pCO}_2$  is presumed to be beneficial to marine macrophytes (Beer and Koch, 1996), even if this effect is most likely to be small (Israel and Hophy, 2002).

#### 4.5 Effect on fauna:

The daily oscillations of  $\text{pCO}_2$  generated by photosynthesis could be of prime importance for calcifiers, creating at daytime periods of high saturation states favourable to  $\text{CaCO}_3$  precipitation. Such coupling between photosynthesis and calcification has already been observed in a Hawaiian coral reef by Drupp et al., 2011 and Shamberger et al., 2011 where calcification is maximal at midday when the  $\text{pCO}_2$  is minimal due to planktonic photosynthesis. In that reef, the intensity of the photosynthesis is modulated by wind driven inputs of nutrients from the flume of a neighboring estuary. A coupling between photosynthesis and calcification has also been observed at the microhabitat level, between brown macroalgae and calcifying epibionts. At daytime, photosynthesis induces a drastic increase of pH within a boundary layer of few hundreds of micrometers above the algal frond favouring the calcification of fouling tubeworms *Spirorbis spirorbis* (Saderne and Wahl, in review).

More generally, first investigations tend to show that animals, calcifying or not, inhabiting highly variable ecosystems with regards to  $\text{pCO}_2$  are resilient to ocean acidification (Thomsen and Melzner, 2010). Also, in the Baltic Sea, the mussels *Mytilus edulis* experiences reduced growth and dissolution of the shell only when  $\Omega_{\text{arag}} \leq 0.15$  corresponding to a  $\text{pCO}_2$  of  $\sim 4000 \mu\text{atm}$  (Thomsen et al., 2010). Despite this weakening of their shell, the predation by seastars *Asterias rubens* and crabs *Carcinus maenas* maintained at similar  $\text{pCO}_2$  are reduced by 56 % and 41 % respectively (Appelhans et al., in press). Besides, the growth of the brackish barnacle *Amphibalanus improvisus*, competitor of *Mytilus* for space, remains unaffected at both larval and adult stage at  $\text{pCO}_2 > 3000 \mu\text{atm}$  (Pansch et al., 2012). Macrophyte meadows are also transient habitats, sheltering early life stages of numerous animal

species. Those might exhibit more robustness to ocean acidification as well. As example, the spawns of Baltic herrings *Clupea harengus*, deposited on macrophyte beds (Aneer et al., 1989), are not affected in their embryonic development by high pCO<sub>2</sub> / low pH (Franke and Clemmensen, 2011).

## 5. Conclusion

Our study represents one of the first attempts of high resolution continuous measurement of the carbonate system in the highly variable environment that is the Baltic Sea's nearshore. The three weeks showed quite different results related to the dynamics of the whole Baltic carbonate system, to the meteorological condition and to very local processes of photosynthesis and respiration. This study highlights the importance of the natural variations of pCO<sub>2</sub> and pH and emphasizes the consideration of these in ocean acidification studies on nearshore organisms. The variations and extremes presented here lie far above future acidification levels predicted for the year 2100 and let hope for the potential resilience of nearshore ecosystems to future ocean acidification.

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*Chapter 2 - Elevated pCO<sub>2</sub> from late summer coastal upwellings in the western Baltic Sea enhances vegetative growth in the brown algae *Fucus serratus*: a laboratory assessment.*

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## Abstract

The brown algae *Fucus serratus* is one of the major meadow forming algae of the Western Baltic Sea nearshore ecosystem. At the end of summer, those meadows are exposed to local upwelling suddenly increasing the pCO<sub>2</sub> and DIC up to 2500 μatm and 2250 μmol kg<sup>-1</sup> resp., for period of days to weeks. This study investigates the growth response of summer's vegetative *Fucus serratus* to elevated pCO<sub>2</sub> (1350 and 4080 μatm) during a 40 days laboratory incubation. After 10 days, increases of growth rates of 20 % and 47 % of the control were observed in the 1350 and 4080 μatm pCO<sub>2</sub> treatments respectively. Beyond 20 days, the growth rates collapsed in all treatments due to nutrients shortage, as demonstrated by high C:N ratios (95:1) and low N tissue content (0.04 % of dry weight). The collapse occurs faster at higher pCO<sub>2</sub>. On day 30, growth rates were reduced by 40 % and 100 % relative to the control at 1350 and 4080 μatm respectively. These results are consistent with a fertilizing effect of elevated pCO<sub>2</sub> on *Fucus serratus* presumably linked to the transition from active HCO<sub>3</sub><sup>-</sup> to passive CO<sub>2(aq)</sub> uptake. This positive effect is limited by nutrients resources, low seawater dissolved inorganic N and P and shortage of the nutrients reserves accumulated over the previous autumn and winter.

## 1 Introduction

Carbon dioxide is an essential substratum to the production of organic matter by photosynthesis. In the marine ecosystem the access to this resource is restricted by boundary layer restrictions due to the viscosity of water (Lobban and Harrison, 1994; Wheeler, 1980) and to the reactive nature of CO<sub>2</sub>. CO<sub>2</sub> is an acid gas dissociating in solution in two acid base couples, and three species: CO<sub>2(aq)</sub>, HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>. At current atmospheric CO<sub>2</sub> partial pressure of 380 μatm, only ~1 % of the dissolved inorganic carbon (DIC) pool in seawater remains under the form of CO<sub>2(aq)</sub>, 90 % being under the form of HCO<sub>3</sub><sup>-</sup>, the rest as CO<sub>3</sub><sup>2-</sup>. To insure the carbon saturation of their photosynthetic apparatus, macroalgae developed carbon concentration mechanisms (CCM) involving the direct uptake of HCO<sub>3</sub><sup>-</sup> and / or its conversion to CO<sub>2</sub> through the action of carbonic anhydrase (Badger, 2003; Giordano et al., 2005; Hepburn et al., 2011). These mechanisms go with an additional enzymatic cost compared to passive assimilation of CO<sub>2(aq)</sub>. A decrease of the carbonic anhydrase activity at high CO<sub>2</sub> concentrations has been observed in algae (Hurd et al., 2011; Johnston and Raven, 1990; Surif and Raven, 1989; Zou and Gao, 2003). Thus, increased CO<sub>2</sub> could lead to increased algal production (Cornwall et al., 2012). Both CCM and HCO<sub>3</sub><sup>-</sup> uptake have been observed in the seaweed *Fucus serratus* (Surif and Raven, 1989), major meadow forming algae of nearshores of the western Baltic

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Sea. In such meadows, algal photosynthesis on a normal summer day imposes pCO<sub>2</sub> and DIC variations between day and night of 200 to 400 μatm and 100 to 150 μmol kg<sup>-1</sup> respectively (Saderne et al., in prep). As the summer ends, the collapse of the thermal stratification of the water column in coincidence with the arise of westerly winds engenders the upwelling of the deeper waters of the bays to the macrophytes meadows, briskly elevating pCO<sub>2</sub> and DIC to 1500 μatm and 2164 μmol kg<sup>-1</sup>, respectively, for periods of several days (Melzner et al., 2012; Saderne et al., in prep). During such periods, nightly maximum of pCO<sub>2</sub> / DIC of 2500 μatm / 2250 μmol kg<sup>-1</sup> can be reached. Therefore, not only the overall DIC increase but also the CO<sub>2(aq)</sub> fraction, reaching 5 % of the DIC due to the non-linearity of the DIC to pCO<sub>2</sub> relationship and to a drop in the water temperature of 4 to 5 °C (Zeebe and Wolf-Gladrow, 2001). This five-fold increase in the metabolically “cheaper” carbon source might enhance growth rates of the alga.

Thus, we aimed in this study to test the fertilizing effect of sudden DIC and pCO<sub>2</sub> elevation on the common meadow forming algae *Fucus serratus* in the physiological and physicochemical condition at the end of summer.

## 2 Material and Methods

### 2.1 Sampling, incubation and seawater chemistry

*Fucus serratus* were sampled in 1 - 2 m depth in Eckernförde bay (Western Baltic Sea, Germany, 54°27' N, 9°53' E) the 9<sup>th</sup> of August 2011. Vegetative apices of *Fucus* from a large pool of individuals were cut to areas of 32.4 ± 1.3 (mean ± SD) and randomly grouped per twelve. Each group was allocated to one polystyrene bottle of 4.65 L for tissue culture (roller bottles: length 53.16 cm, diameter 11.73 cm, 1750 cm<sup>2</sup>, Corning Life Science, USA). Five bottles, each representing one treatment replicate, were allocated to each pCO<sub>2</sub> treatment. The treatments were aerated either with ambient air (490 μatm), or with pre-mixed air containing 1350 and 4080 μatm pCO<sub>2</sub> respectively. The bubbling treatments were maintained for 40 days in a 15°C thermostatic room. Three neon tubes (Biolux, Osram, Germany) generated 270 μE m<sup>-2</sup> s<sup>-1</sup> light on a 12h day : night cycle, which is well above the compensation value for *F. serratus* (~10 μE m<sup>-2</sup> s<sup>-1</sup>) (King and Schramm, 1976). The incubation medium was natural seawater from the Kiel Bay (Western Baltic Sea, Germany, 54°19' N, 10°08') filtrated successively through 1 μm and 0.2 μm (Polycap 36 AS and 75 AS, Whatman, UK) and finally UV sterilized (UV 5 clarifier, Tetra, Germany). The seawater was adjusted to a salinity of 15 psu and stored in a 200 L tank aerated with a protein skimmer (miniflotor, Aquamedic, Germany). On the day preceding water exchanges, the seawater was pre-conditioned to the three experimental pCO<sub>2</sub> conditions into 60 L tanks. The seawater in the experimental bottles was exchanged every fifth day. At the same occasion, the three stock tanks and two random bottles per treatment were sampled

for nitrogen, phosphorus and silicon concentrations and for  $\text{pH}_{\text{tot}}$  and DIC according to the Standard operating procedure (SOP) 1 of the Guide to Best Practices for Ocean  $\text{CO}_2$  Measurements (Dickson et al., 2007). The  $\text{pH}_{\text{tot}}$  was measured by potentiometry according to Saderne and Wahl (in prep.). The DIC was measured by the principle of infrared measurement of  $\text{CO}_{2(\text{g})}$  purged out of an acidified seawater sample (SOP 2 in Dickson et al., 2007) (AIRICA, Marianda, Germany). The carbonate system was derived from  $\text{pH}_{\text{tot}}$  and DIC with the R package Seacarb (Lavigne and Gattuso, 2010) using dissociation constants of Millero (2010), Perez and Fraga (1987) and Dickson (1990) for carbonates,  $\text{HF}^-$  and  $\text{HSO}_4^-$ , respectively. The dissolved inorganic phosphorus and nitrogen in seawater (DIP and DIN) were assessed by standards spectrometric methods (Grasshoff et al., 1999) with an Autoanalyzer 3 (SEAL analytical, UK).

## 2.2 Measurements

The growth of fucoids is known to be apical (Diouris and Floc'h, 1984), therefore we estimated the fertilizing effect of  $\text{pCO}_2$  by the area increment of *Fucus* apices over time. This technique is preferable for its non-destructive nature and low sensitivity to measurement error (notably compared to organic carbon measurement or dry / wet weight measurement), considering that all fucus individual, harvested at the same emplacement, had similar surface / volume and surface / carbon content ratios, (see Nielsen, 1990 and Markager and Sand-jensen, 1996). Pictures of *Fucus* apices were taken at start and every tenth day of incubation. The area was measured by image analysis using the software imageJ (National Institutes of Health, USA).

Cumulative growth (CG) in % was measured as follow:

$$CG_t = \frac{Area_t - Area_{t_0}}{Area_{t_0}} \cdot 100$$

Relative growth rate (RGR) in one replicate in  $\% \text{ d}^{-1}$  was measured as follow:

$$RGR_t = \frac{\ln\left(\frac{Area_t}{Area_{t-1}}\right)}{\Delta t} \cdot 100$$

With  $Area_t$ , the sum of the areas of twelve apices of a replicate at  $t$ .

The photosynthetic quantum yield of the photosystem II (PSII) was measured by pulsed amplitude modulated (PAM) fluorimetry as an estimation of the health state of the algae (Maxwell and Johnson, 2000; Schreiber et al., 1995) using a Diving-PAM (Walz, Germany). Apices were adapted to darkness for a period of 5 min prior to the application of a saturating flash of 1s. The quantum yield was measured at start and every tenth days of the 40 days of incubation in nine apices per bottle.



## 2.3 Carbon and nitrogen content

The apices of each bottle were pooled, desiccated and ground using a ball mill. The total carbon and nitrogen content in % dry weight (DW) were analyzed with a CN elemental analyzer (Carlo Erba, Italy).

## 2.4 Statistical analysis

Significant differences between treatments were tested by repeated measure analysis of variance (RM ANOVA) for the cumulative growth, growth rates and photosynthetic quantum yield. The carbon and nitrogen and C:N ratio were compared by one-way ANOVA. The normality of the datasets were tested by Shapiro-Wilk's tests and abnormal datasets transformed. Prior to the one-way ANOVA, the homoscedasticity of the data was tested by a Levene's test. Concerning the RM ANOVA, the assumption of sphericity of the data was tested by a Mauchley's test. In case of violation of that last, the F and p values were corrected using the Greenhouse-Geissner's method. Analyses were performed with the software Statistica 7 (Statsoft, USA).

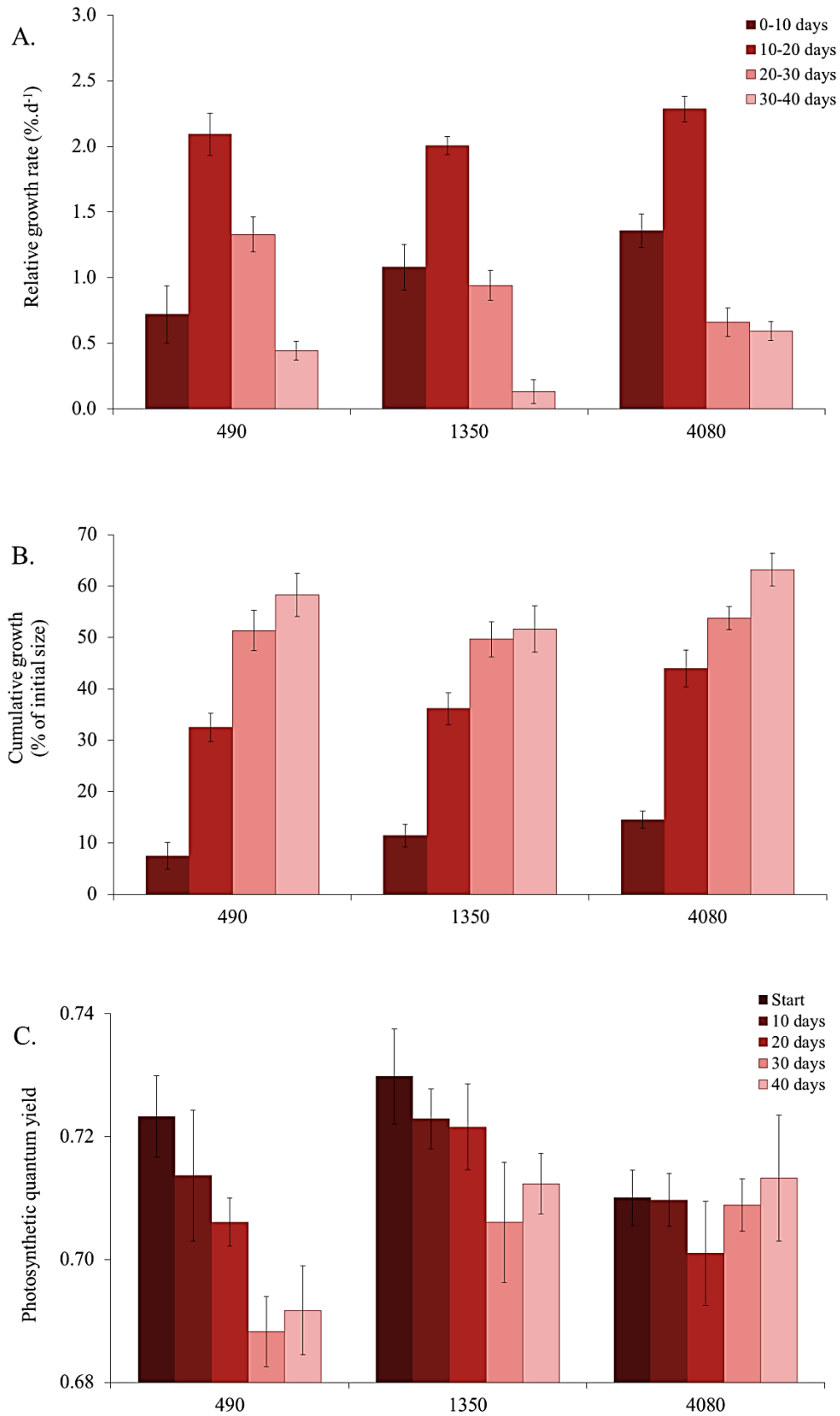
# 3 Results

## 3.1 Chemistry

Natural seawater was used during the 40 days of the experiment. A summary of the main chemical parameters is presented in Table 2.1. The pCO<sub>2</sub> during the 40 days incubations was 486 ± 81, 1355 ± 230 and 4078 ± 338 μatm (mean ± SD), respectively. The DIC was 1879 ± 28, 1996 ± 24 and 2162 ± 25 μmol kg<sup>-1</sup> (mean ± SD) in the 490, 1355 and 4080 μatm treatments, respectively. In those last treatments, the average percentage of DIC represented by the dissolved CO<sub>2</sub> fraction was 1.1 ± 0.2, 2.8 ± 0.5 and 7.8 ± 0.7 μmol kg<sup>-1</sup> respectively (mean ± SD over the 30 days incubations). The dissolved inorganic nitrogen and phosphorus means were 1.9 and 0.2 μmol kg<sup>-1</sup> respectively in the 490 and 1120 μatm treatments. In the 4080 μatm treatment DIN and DIP were 1.4 and 0.17 μmol kg<sup>-1</sup>, respectively.

**Table 2.1.** Summary of the chemical parameters in the experimental bottles. The data are the mean ± SD of the chemistry of two bottles sampled every five days over the 40 days of incubation (n = 8). DIN and DIP: means ± SD over the 40 days of incubation of the averaged values of two bottles before and prior to water exchanges (n = 8).

Treatment	T (°C)	Salinity	pH <sub>tot</sub>	pCO <sub>2</sub> (μatm)	DIC (μmol kg <sup>-1</sup> )	Alkalinity (μmol kg <sup>-1</sup> )	CO <sub>2</sub> (μmol kg <sup>-1</sup> ) (% DIC)	HCO <sub>3</sub> <sup>-</sup> (μmol kg <sup>-1</sup> ) (% DIC)	CO <sub>3</sub> <sup>2-</sup> (μmol kg <sup>-1</sup> ) (% DIC)	DIN (μmol kg <sup>-1</sup> )	DIP (μmol kg <sup>-1</sup> )
Control	15.4 ± 0.3	15.5 ± 0.5	7.99 ± 0.07	486 ± 81	1879 ± 28	1963 ± 37	20 ± 3	1780 ± 27	80 ± 13	1.9 ± 1.1	0.2 ± 0.09
490 μatm							1.1 ± 0.2	94.7 ± 0.5	4.3 ± 0.7		
1350 μatm	14.9 ± 0.3	15.2 ± 0.4	7.57 ± 0.05	1355 ± 230	1996 ± 24	1989 ± 33	56 ± 9	1907 ± 24	33 ± 5	1.9 ± 1.3	0.2 ± 0.1
							2.8 ± 0.5	95.6 ± 0.2	1.6 ± 0.3		
4080 μatm	15.3 ± 0.4	15.4 ± 0.3	7.11 ± 0.07	4078 ± 338	2162 ± 25	2009 ± 34	168 ± 15	1982 ± 32	12 ± 1	1.4 ± 0.6	0.17 ± 0.1
							7.8 ± 0.7	91.7 ± 0.7	0.5 ± 0.1		



**Figure 2.1.** Means  $\pm$  SD in 5 bottles per treatment of (A) the relative growth rates (% d<sup>-1</sup>), (B) the cumulative growth rates (% of initial size) and (C) the photosynthesis quantum yield of vegetative apices of *Fucus serratus* exposed to 490, 1350 and 4080  $\mu\text{atm}$  pCO<sub>2</sub> over the 40 day duration of the incubation.

### 3.2 Growth

pCO<sub>2</sub> and time had a significant ( $p \leq 0.05$  and 0.001, respectively) and interactive effects ( $p < 0.001$ ; Table 2.2. A. and B.) on cumulative growth and relative growth rates (Fig. 2.1. A. and Fig. 2.1. B). The interaction between the 2 factors is due to a large part to the fact that the pCO<sub>2</sub> effect on growth changed over time. Growth in the first 10 days was by  $0.72 \pm 0.22$ ,  $1.08 \pm 0.17$  and  $1.36 \pm 0.13$  % d<sup>-1</sup> (mean  $\pm$  SD) in the 490, 1350 and 4080  $\mu$ atm pCO<sub>2</sub> treatments, respectively. Between days 10 and 20, the maximal growth rates were  $2.09 \pm 0.16$  at 490,  $2.01 \pm 0.07$  at 1350 and  $2.29 \pm 0.10$  % d<sup>-1</sup> at 4080  $\mu$ atm. After 20 days, the growth rates collapsed in all treatments albeit at faster rates with elevated pCO<sub>2</sub>  $0.66 \pm 0.10$ ,  $0.94 \pm 0.11$  and  $1.33 \pm 0.13$  % d<sup>-1</sup> (mean  $\pm$  SD) on day 30 in the 4080, 1350 and 490  $\mu$ atm treatments respectively. By the end of the experiment, the growth almost stopped in the 1350  $\mu$ atm treatment ( $0.13 \pm 0.09$  % d<sup>-1</sup>) and was notably reduced in the 490  $\mu$ atm treatment ( $0.44 \pm 0.07$  % d<sup>-1</sup>) and the 4080  $\mu$ atm treatment ( $0.59 \pm 0.07$  % d<sup>-1</sup>).

### 3.3 Photosynthetic quantum yield

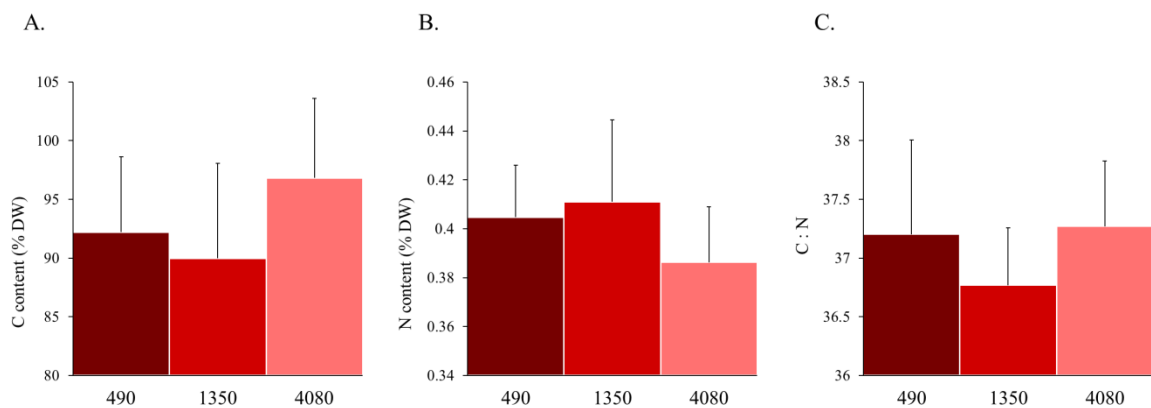
pCO<sub>2</sub>, time and the interaction between the two factors are significant (RM ANOVA:  $p < 0.05$ ,  $p < 0.001$  and  $p < 0.01$  respectively) (Fig. 2.1. C, Table 2.2. C). Initial yield values were highest in the 490 and 1350  $\mu$ atm pCO<sub>2</sub> treatments but decreased over time, especially so in the low pCO<sub>2</sub> treatment. In contrast, the yield was moderately high but stable over time in the highest pCO<sub>2</sub> treatment.

**Table 2.2** Repeated measures ANOVA results of the *Fucus serratus* thallii for (A) the relative growth rates, (B) the cumulative growth and (C) the photosynthetic quantum yield over the 40 days of incubations (n = 5). \*: p-values corrected using the Greenhouse-Geisser's method in case of violation of the assumption of sphericity.

A. Relative growth rate						C. Photosynthetic quantum yield					
	d.f.	SS	MS	F	p		d.f.	SS	MS	F	p
pCO <sub>2</sub>	2	1.3	0.7	11.8	<b>0.001</b>	pCO <sub>2</sub>	2	2.6x10 <sup>-3</sup>	1.3x10 <sup>-4</sup>	10.5	<b>0.023</b>
Days	3	59.4	19.8	301.2	<b>&lt;0.001*</b>	Days	4	3.7x10 <sup>-3</sup>	9.2x10 <sup>-4</sup>	9.7	<b>&lt;0.001</b>
pCO <sub>2</sub> x Days	6	8.5	1.4	21.6	<b>&lt;0.001*</b>	pCO <sub>2</sub> x Days	6	2.8x10 <sup>-3</sup>	3.4x10 <sup>-4</sup>	3.6	<b>0.002</b>
B. Cumulative growth											
	d.f.	SS	MS	F	p						
pCO <sub>2</sub>	2	8.3x10 <sup>7</sup>	4.1x10 <sup>7</sup>	4.84	<b>0.029</b>						
Days	3	2.7x10 <sup>9</sup>	9.2x10 <sup>8</sup>	886.55	<b>&lt;0.001*</b>						
pCO <sub>2</sub> x Days	6	6.5x10 <sup>7</sup>	1.1x10 <sup>7</sup>	10.43	<b>&lt;0.001*</b>						

### 3.4 C:N ratio content

The carbon and nitrogen and C:N ratio did not differ significantly among the treatments (one-way ANOVA,  $p > 0.05$ , Fig.2).



**Figure 2.2** means  $\pm$  SD in 5 bottles per treatment of (A) the nitrogen content (% DW) (B) the carbon content (% DW) and (C) the C:N ratio of vegetative apices of *Fucus serratus* exposed to 490, 1350 and 4080  $\mu\text{atm}$  pCO<sub>2</sub> after 40 days.

## 4 Discussion

In all treatments, the dynamic of the growth rates could be decomposed in two phases. During phase 1, growth rates increased until day 20 possibly due to a progressive acclimation of the algae to the incubation conditions. In phase 2, growth rates decreased possibly due to intensifying nutrients shortage. Notwithstanding this common trend, we observed higher initial growth rates and more severe growth reduction with higher pCO<sub>2</sub>. Thus, both acclimation and collapse by nutrient deficiency were faster under high pCO<sub>2</sub> conditions. This result supports the hypothesis of a positive effect of pCO<sub>2</sub> on growth of the algae *Fucus serratus*.

The increase of the growth rates during the first 20 days could be explained by a process of acclimation to the experimental condition of maintenance, most likely to light. *Fucus serratus* light harvesting mechanisms are acclimated to the light conditions (King and Schramm, 1976). The algae were transferred from 14h:10h day : night cycles with light intensity exceeding 1500  $\mu\text{E m}^{-2} \text{s}^{-1}$  at noon at the collection site (mean daily light dose: 15.8  $\text{E m}^{-2} \text{d}^{-1}$ ) (Saderne, unpublished data) to a 12h:12h day : night cycle with constant 270  $\mu\text{E m}^{-2} \text{s}^{-1}$  in the experiment (daily light dose: 11.7  $\text{E m}^{-2} \text{d}^{-1}$ ). The reduced irradiance might have required an acclimation of the photosynthetic apparatus through *e.g.* the increase of pigments (Fortes and Lüning, 1980). In brown algae, carboxylic acids are non-photosynthetically stored during the night and enzymatically transformed to CO<sub>2</sub> readily usable for photosynthesis during the day. This latter process is partially induced by an internal clock mechanism (Axelsson et al., 1989; Schmid and Dring, 1996). The transition to a 12h:12h day: night cycle could have disturbed the inner circadian cycle of the algae for growth and DIC uptake (Schmid and Dring, 1996; Strömberg and Nielsen, 1986). Despite the need for an acclimation period, the photosynthetic quantum yield of the algae remained relatively constant during the incubation in all treatments. Such yields of about 0.7 are commonly documented in fucoids in the field, corroborating

the absence of major stressor affecting the algae's photosynthetic apparatus in our experiment (Collén and Davison, 2001; Magnusson, 1997; Svahn et al., 2012; Viñeola et al., 2006). A CO<sub>2</sub> deficiency is known to increase photo-damage of the PSII resulting in a decrease of the photosynthetic yield in algae (Murata et al., 2007; Takahashi and Murata, 2008). This effect was not observed even in the lowest pCO<sub>2</sub> treatment (control 490 µatm), demonstrating the carbon saturation of the photosynthetic apparatus. The photosynthetic yield of *Fucus* was also unaffected by the nutrient deprivation, similarly to what observed by Young et al., (2009).

During the first 10 days we observed a doubling of the growth rates between the 490 and 4080 µatm pCO<sub>2</sub> treatments. Such positive effect of pCO<sub>2</sub> on growth rates have already been observed in the rhodophyte algae *Porphyra yezoensis*, *Gracilaria* sp. and *Hypnea musciformis* (Gao et al., 1991, 1993; Israel and Hophy, 2002) and in the chlorophyte *Ulva rigida* (Gordillo et al., 2001). In contrast, Israel and Hophy (2002) did not find any fertilizing effect of elevated pCO<sub>2</sub> (750 µatm) in 14 algae of the three major divisions. In *Fucus serratus*, our result could be explained by the reduction of the enzymatic cost of growth relative to a switch from active to passive carbon incorporation, as observed by Cornwall et al. (2012) in the red algae *Corallina officinalis* and two green algae of the genus *Ulva*. The photosynthesis of *Fucus serratus* is saturated for carbon at CO<sub>2(aq)</sub> concentration ~17 µmol kg<sup>-1</sup> (Mercado and Gordillo, 2011; Middelboe and Hansen, 2007) and the mechanisms of active uptake of HCO<sub>3</sub><sup>-</sup> by external carbonic anhydrase activated at pH<sub>NBS</sub> above 7 (Haglund et al., 1992), corresponding to pH<sub>tot</sub> of ~8 (pH units non-convertible, see Zeebe and Wolf-Gladrow, 2007 and Riebesell et al., 2010). The control treatment (pH<sub>tot</sub> = 7.99; [CO<sub>2</sub>]<sub>aq</sub> = 20 µmol kg<sup>-1</sup>) is presumably close to the activation threshold of the CCM while the two other treatments might be more relying on passive entry of CO<sub>2(aq)</sub> for carbon supply. Nevertheless, this explanation remains unsatisfactory with regard to the literature and should be subject to further investigations.

The maximum growth rates of about 2 % d<sup>-1</sup> were reached after 20 days of incubation in all three treatments. These rates correspond to the in situ growth observed on *Fucus serratus* individuals from the North Sea (Fortes and Lüning, 1980) and the south-west coast of England (Nielsen and Nielsen, 2010; Nielsen et al., 2003) and similar to those observed in August - September on experimental *Fucus serratus* communities of the Isle of Man (Creed et al., 1998).

Beyond 20 days of incubation, the growth rates decreased in all treatments albeit with a steeper slope at high pCO<sub>2</sub>. The collapses of growth might be caused by a deficiency for the nutrients supporting the production of organic compounds. Due to the naturally low DIN / DIP concentration in the seawater used during the incubation, the nutrient supply was apparently ensured by internal reserves of N, as revealed by the high C:N ratio and low N % DW at the end of the incubation. The unequal slopes of decrease in the pCO<sub>2</sub> treatments may hint at a faster exhaustion of these reserves due to the initial fertilizing effect of pCO<sub>2</sub>, leading to faster growth at higher pCO<sub>2</sub> concentration during

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the first 10 day period. As medium we used unmodified Baltic seawater sampled all along the experiment in August-September. This period corresponds to the yearly minimal concentrations of DIN and DIP in the *Fucus* stands of the Kiel bight, concentrations similar to those measured in our incubation medium ( $1 < \text{DIN} < 5 \mu\text{mol L}^{-1}$  and  $0.5 < \text{DIP} < 1 \mu\text{mol L}^{-1}$ ; Schramm et al., 1988; Bocknis Eck series, Bange, unpublished data). The collapse of the DIN and DIP concentration lies in early spring, caused by phytoplankton blooms (Vahtera et al., 2007). To insure growth and reproduction in spring and summer, when light is abundant but nutrients are low, *Fucus* relies on internal reserves of nutrients accumulated during winter, when dissolve nutrient concentrations are more than ten time higher but light low (Lehvo et al., 2001; Schramm et al., 1988; Young et al., 2007). Consequently, the C:N ratio and nitrogen content of the algal tissue vary over the year, driven by external DIN fluctuations and the physiological dynamics of the internal reserves. In *Fucus serratus* of the Irish sea, while the carbon content remains stable at ~35 % DW over the year, the nitrogen content varies between ~3.5 % DW in marsh and ~2 % DW in late September, leading to a variation of the C:N ratio from 5 to 25 (Young et al., 2007). In our study we found substantially higher C:N ratios, ranging from 90 to 97. This difference was due to remarkably low N content (about 0.4 % DW) while the C content was similar (~37 % DW) to the one observed in the Irish Sea. More comparable C:N ratios and N content (up to 50 and 0.5 % DW respectively) have been reported for *Fucus vesiculosus* in the Baltic Sea in September, (Ilvessalo and Tuomi, 1989; Lehvo et al., 2001). However, the ratios found at the end of our incubation were substantially higher than any found in the field, strengthening the assumption that progressively depleted DIN and DIP supply caused a deceleration of growth when nitrogen in the algal tissues approached 0.4 % DW.

The effect of elevated  $\text{pCO}_2$  on marine macrophytes has been poorly investigated compared to their terrestrial counterparts (according to Körner, 2006: “several thousand scientific articles and approx. 120 reviews”). In terrestrial plants, an increase of  $\text{pCO}_2$  from 350  $\mu\text{atm}$  to 700  $\mu\text{atm}$  leads to increased photosynthetic rates of 30 % and growth rates of about 10 % in average (Kirschbaum, 2011). Nevertheless, up-scaling from monoculture to ecosystem is not straightforward since biotic interactions or abiotic limitations such as for nutrients or light can interfere. The beneficial effect of  $\text{CO}_2$  can therefore be only transient until a new equilibrium is reached (Körner, 2006; Loehle, 1995).

Our study supports the transient nature of the fertilization effect by elevated  $\text{pCO}_2$  due to nutrient limitation. In our study, the dissolved nutrient depletion was partly enhanced by our experimental design (bottle volume and frequency of water exchanges). *In-situ* it may occur during plankton blooms or in dense algal stands. In contrast, during upwelling periods, deep waters with concentrations in DIP and DIN approximately 15 and 5 fold higher than surface waters, respectively, may replenish the macrophytes beds (Bocknis Eck time series, Bange, unpublished data). The coincidence of high nutrient concentration and high  $\text{pCO}_2$ , due to the upwelling, can enhance the algae growth rates. In algae the enzymatic reduction of nitrates to nitrites is a major rate limiting process to nitrogen

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assimilation (Campbell, 1999). The nitrogen reductase, principal enzyme involved have an improved activity under elevated pCO<sub>2</sub> (Gordillo et al., 2001; Lartigue and Sherman, 2005; Mercado et al., 2000; Zou, 2005). Thus, the co-occurrence of peaks of CO<sub>2</sub> and of nutrients enhances the uptake rate and faster the constitution of nitrogen reserves, insuring primary production during and after the upwelling (Fujita et al., 1989; Lartigue and Sherman, 2005). Ephemeral (Ulvales) and epiphytic algae have incorporation rates of N ~6 fold more elevated than perennial algae (Fucales, Laminariales), conferring them a competitive advantage for the exploitation of upwelling events (Pedersen and Borum, 1997; Pérez-Mayorga et al., 2011; Worm and Sommer, 2000). The elevated growth rates of ephemerals request important nutrient supplies rendering them less tolerant to prolonged periods of oligotrophy (Bokn et al., 2003; Fujita et al., 1989). Their elevated minimum threshold of nitrogen content in tissues compared to perennial algae is commonly limiting their performances (Duarte, 1992; Fujita et al., 1989). Consequently, an increased frequency of upwelling of water bodies rich in nutrients and CO<sub>2</sub> might favor green algae mats over perennial brown algae meadows. In the western Baltic, increased storminess and warming as predicted for the future will enhance the formation of such water bodies and their upwelling (Gräwe and Burchard, 2011; Schrum, 2001). Furthermore, with future CO<sub>2</sub> driven ocean acidification, the pCO<sub>2</sub> of the upwelling waters could reach up to 4500 µatm, corresponding to the consumption of all the dissolved oxygen in the deep waters by heterotrophic processes (Melzner et al., 2012).

In the western Baltic the lower depth limit of *Fucus* decreased from 10 to 2 m since the 50's due to eutrophication and the strengthening of phytoplankton blooms (Rohde et al., 2008; Vogt and Schramm, 1991). Climate change could help in the recovery of the *Fucus* population, at the condition they are not outcompeted by ephemeral algae, seagrasses or phytoplankton. An exhaustive answer to this question will need further investigations, combining elevated pCO<sub>2</sub> to temperature and nutrients, at the species specific and inter-specific level.

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*Chapter 3 - Effect of Ocean acidification on growth and recruitment of calcifying and non-calcifying epibionts of brown algae.*

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## Abstract

Seaweeds are key species of the Baltic Sea benthic ecosystems. They are the substratum of several fouling epibionts like bryozoan and tubeworms. Several of these species are bearing calcified structures and could be impacted by the future CO<sub>2</sub> induced phenomenon of ocean acidification. Consequently, this could cause a shift of the fouling communities toward fleshy species. The aim of the present study was to test the sensitivity of selected seaweed macrofoulers to higher pCO<sub>2</sub> concentrations in their natural microenvironment, i.e. the boundary layer at the thallus surface. Fragments of the macroalga *Fucus serratus* bearing together the calcifiers *Spirorbis spirorbis* (Annelida) and *Electra pilosa* (Bryozoa) and the non-calcifier *Alcyonidium hirsutum* (Bryozoa) were maintained for 30 days under three pCO<sub>2</sub> conditions: natural  $460 \pm 59$   $\mu\text{atm}$  and enriched  $1193 \pm 166$   $\mu\text{atm}$  and  $3150 \pm 446$   $\mu\text{atm}$ . Our study showed a significant reduction of growth rates and recruitment of *Spirorbis* individuals only at the highest pCO<sub>2</sub>. Tubeworm recruits exhibited enhanced calcification of 40 % during irradiation hours compared to dark hours, possibly reflecting an acidification – modulating effect algal photosynthesis as opposed to an acidification-enhancing effect of algal respiration. *Electra* colonies showed significantly increased growth rates at 1193  $\mu\text{atm}$ . No effect on *Alcyonidium* colonies growth rates was observed. The results suggest a remarkable resistance of the algal macro-epibionts to acidification levels foreseen in year 2100 for open ocean conditions (~1000  $\mu\text{atm}$ ).

## 1 Introduction

Since the pre-industrial time, human activities led to a shift in the atmospheric pCO<sub>2</sub> from 280 to 396  $\mu\text{atm}$  (NOAA-ESRL, 2012). CO<sub>2</sub> is an acid gas and when dissolving in seawater, leads to the reduction of seawater pH. This phenomenon of ocean acidification (OA) has already caused a drop of 0.1 pH units since the year 1800. The most severe scenarios are predicting atmospheric pCO<sub>2</sub> up to of 1000  $\mu\text{atm}$  to occur during the 21<sup>st</sup> century, decreasing the pH by further 0.4 (Caldeira and Wickett, 2005). This shift will increase the corrosiveness of seawater to calcium carbonate, the major component of most shells and skeletons of marine species. Consequently, in the course of ocean acidification, non-calcified organisms may outcompete calcifying organisms (Fabry et al., 2008) as observed between corals and seaweeds (Diaz-Pulido et al., 2011) or among seagrasses epiphytes in the Mediterranean Sea (Kuffner et al., 2007; Martin et al., 2008).

The Baltic Sea is prone to acidification due to its low salinity, alkalinity and temperature such that undersaturation for calcium carbonates ( $\Omega_{\text{arag}}$  and  $\Omega_{\text{calc}} < 1$ ) occurs naturally in winter (Thomas

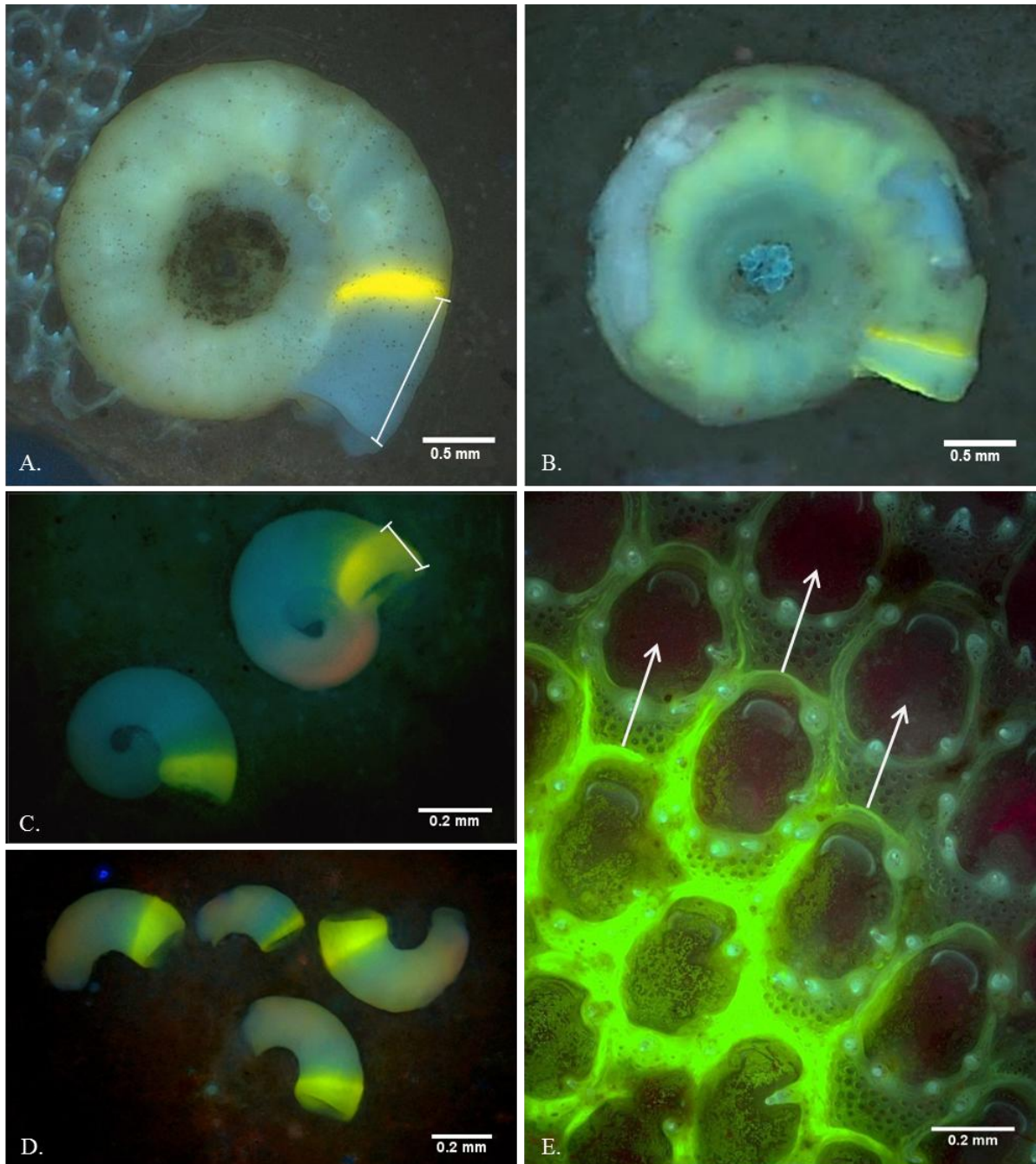
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and Schneider, 1999; Tyrell et al., 2008). In spring and summer, the photosynthetic and respiration activities of phytoplankton and benthic macrophytes are imposing to the nearshore day / night variations of pCO<sub>2</sub> of 200 to 400 µatm, causing fluctuation of Ω from 1.5 to 2.5 (Thomas and Schneider, 1999; Saderne et al., in prep). In September, the intensification of westerly winds together with the collapse of the thermocline leads to the upwelling events of hypercapnic bottom waters to the nearshore habitats (Thomsen et al., 2010). During such events, daily means of pCO<sub>2</sub> of 1600 µatm with night peaks up to 2600 µatm are measured in macrophyte stands (Saderne et al., in prep.). Thus, conditions in coastal habitats may be undersaturated for aragonite and calcite for several days despite the intermittent CO<sub>2</sub> uptake by the macrophytes for photosynthesis.

The brown algae *Fucus serratus* is one of the most widespread brown macroalga in the Baltic, providing the typical primary producer services, spawning and nursery areas, food source and substratum for macrofoulers (Boaden et al., 1975; Kautsky et al., 1992). In total, 164 macro- and micro-faunal species have been inventoried as associated with *F. serratus* in the Baltic (Hagerman, 1966). Most prevalent sessile species epibiotic on *Fucus serratus* are the calcifying tubeworm *Spirorbis spirorbis*, the calcifying bryozoan *Electra pilosa* and the non-calcifying one *Alcyonidium hirsutum* (Hagerman, 1966). Those species are playing an important role in seaweed ecology. *Spirorbis spirorbis* can overgrow fucoid blades presumably causing shading and increases in weight and brittleness (O'Connor and Lamont, 1978; Saderne, pers. obs.). Similarly, shading by *Electra pilosa* and *Alcyonidium hirsutum* can reduce the photosynthetic activity of *Fucus serratus* by up to 85% (Oswald et al., 1984) and their physical presence may contribute to the degeneration of the underlying thallus (Dixon, 1981; Krumhansl and Scheibling, 2011). In extreme case, the overgrowth by bryozoan can lead to extensive defoliation of the algal meadows (Saier and Chapman, 2004), affecting the entire ecosystem and ultimately the stocks of commercially important species (Wharton and Mann, 1981; Scheibling et al., 1999). On the other hand, bryozoan are directly supplying the host algae with ammonium (Hurd et al., 1994) and dissolved inorganic carbon (DIC) (Munoz et al., 1991) and are a food source for grazers such as nudibranchs and urchins (Seed, 1976; Harvell, 1984; Nestler and Harris, 1994).

The epibiotic species spend at least the initial part of their life cycle within the diffusive boundary layer (DBL) surrounding the *Fucus* thallus. This layer typically is 50 µm to 1 mm thick depending of flow velocity (Wheeler, 1980; Hurd et al., 2011). It is characterized by the slow diffusion of molecules, creating steep concentration gradients of compounds produced or consumed by the alga and its micro- and macroepibionts (Wheeler, 1980; Koch, 1994; Stevens and Hurd 1997; Hurd, 2000, Wahl et al. 2012). Thus, as an example, in the boundary layer on the thallus of *Fucus vesiculosus* pH may vary between 8 in the dark to 9.2 at 785 µE light (Spilling et al. 2010). Therefore, we would expect this space to shelter sessile calcifiers from ocean acidification during daylight hours (Hurd et al. 2011) but possibly enhance acidification stress during the night.



**Figure 4. 1.** Illustrative examples of *Spirorbis spirorbis* and *Electra pilosa*. (A, B) Adult *Spirorbis spirorbis* tubes after 30 day incubation at 460  $\mu\text{atm}$  and 3150  $\mu\text{atm}$   $\text{CO}_2$  respectively. (C, D) Juveniles *Spirorbis spirorbis* tubes after 30 day incubation at 460  $\mu\text{atm}$  and 3150  $\mu\text{atm}$   $\text{CO}_2$  respectively. Note the important dissolution of the shell in B. and the disappearance of part of the tube in (D). (E) *Electra pilosa* colony. All photos were taken under epifluorescence microscope, yellow/green: calcein staining. The white lines are showing the considered distances for the growth measurements of spirorbis tubes. Arrows indicate the direction of the growth subsequent to the calcein staining in *Electra*.

In the present study, we tested the hypothesis whether ocean acidification (i) impacts epibiotic species and (ii) disadvantages the calcifying sessile fauna of *Fucus serratus* over the non-calcifying one. The calcifying tubeworm *Spirorbis spirorbis* and two bryozoan species, the calcifying cheilostome *Electra pilosa* and the keratinous ctenostome *Alcyonidium hirsutum* were investigated regarding their growth, and recruitment (*Spirorbis* only) during 30 days of incubation under three pCO<sub>2</sub> conditions. In addition, we tested at the same pCO<sub>2</sub> conditions the effect of light on the growth of the *Spirorbis* recruits as a first indicator of a potential protection of calcification in the DBL by the host algae metabolism.

## 2 Materials and Methods

### 2.1 Animal collection

*Fucus serratus* individuals bearing *Spirorbis spirorbis*, *Electra pilosa* and *Alcyonidium hirsutum* together on the same thallii were collected in less than 2 m depth in Eckernförde Bay (Western Baltic Sea, Germany, 54°27' N, 9°53' E) on the 1<sup>st</sup> of February 2011, transported in coolbox and stored in aquarium at 15 °C.

### 2.2 staining

Two days after sampling, equivalently fouled sections of *Fucus* thallus were cut to an average ( $\pm$  SE) weight of  $1 \pm 0.02$  g. Each section was bearing all three epibionts with in average ( $\pm$  SE)  $26.8 \pm 5.3$  *Spirorbis* individuals,  $6.2 \pm 1.9$  colonies of *Electra pilosa* and  $1.9 \pm 0.6$  colonies of *Alcyonidium hirsutum*. Animals were stained for 5 days with calcein / seawater at  $50 \text{ mg L}^{-1}$  under ambient pCO<sub>2</sub> and in the condition of light and temperature of the future incubation. The incorporation of the dye into the newly grown tubes and cystids (bryozoan skeletons) served a double purpose: it quantified growth rates for each individual or colony under standard conditions and it marked a starting point for growth under the following incubation conditions (see Fig. 4. 1.) (Smith et al., 2001; Comeau et al., 2009; Dissard et al., 2009). During this staining period, the animals were fed *ad libitum* with the microalgae *Rhodomonas sp.* (Hermansen et al., 2001; Lisbjerg and Petersen, 2001). The expansion of the tubes and colonies as well as the important filtrating activity of the worms and polypids attested of the adequate maintenance conditions.

### 2.3 Experimental design

Each *Fucus* section bearing the three epibionts was independently incubated in a 650 mL culture flask (Sarstedt, Germany). Ten flasks were assigned to each pCO<sub>2</sub> treatment. Flasks were constantly

aerated with premixed air containing either 460  $\mu\text{atm CO}_2$  (ambient), 1200  $\mu\text{atm CO}_2$  or 3000  $\mu\text{atm CO}_2$  using an automatic system mixing  $\text{CO}_2$  with ambient air (Linde gas and HTK Hamburg, Germany) (see Thomsen et al., 2010 for details). The vigorous gas bubbling assured a continuous convective movement of water the flasks. Temperature was maintained at 15°C and light was provided by three Biolux neon tubes (Osram, Germany), delivering a total of 300  $\mu\text{E}$  under 12 / 12 h day / night cycles. The seawater of the flasks was renewed every third day and the animals fed thereafter with *Rhodomonas sp.* to a final concentration of 10,000 cells  $\text{mL}^{-1}$ . The concentrations of the stock cultures were assessed with a Coulter Counter (Beckman Coulter, USA).

Natural Baltic seawater from the Kiel Bight (Western Baltic Sea, Germany, 54°19' N, 10°08' E) was used in the experiment after storage in a 300 L tank aerated with ambient air and sterilized by a Microfloat 1 floating UV lamp (Aqua Concept Karlsruhe, Germany). 24 h before use, the seawater was equilibrated with the three experimental  $\text{pCO}_2$  in three separate tanks.

After 30 days of incubation, algal fragments were conserved in borax-formaldehyde-seawater solutions suitable for preservation of calcareous structures (see Maybury and Gwynn, 1993, for the detailed recipe) prior to the measurement of growth rates.

#### 2.4 Measurement of relative growth rates (RGR) of the bryozoan

Colonies were photographed by overlapping fields of vision under epifluorescence microscope (Axio Scope A1, Carl Zeiss, Germany) at the end of the experiment. The partial pictures (as the one presented 1. E) were reassembled into complete colony pictures. The initial colony surface (SI) (pre-staining) and the final total colony surface (SF) (after 30 days incubation) were measured by image analysis (ImageJ, U. S. National Institutes of Health). In each flask, SI and SF of all the colonies were summed for each species so that in each flask (replicate) the relative growth rate in percent (RGR) was:

$$RGR = \ln\left(\frac{\sum_{j=1}^n SF_j}{\sum_{j=1}^n SI_j}\right) \cdot 100$$

With  $n$  the number of colonies in one flask. The logarithmic growth pattern of bryozoan colonies has been assessed by Hermansen et al. (2001). *Alcyonidium hirsutum* colonies (non-stainable), were photographed prior and after incubation. The areas were measured and the RGR calculated as for *Electra pilosa*.

## 2.5 Measurement of growth and recruitment of *Spirorbis spirorbis*

For *Spirorbis*, the absolute tube growth in mm was estimated as the length of the external arc of the coil comprised between the staining front and the tube edge (see Fig. 4. 1. A.). In each flask, the new tube length increment of all worms was averaged as the flask represents one replicate with a treatment level. *Spirorbis* recruitment was quantified for each flask as the number of juveniles found on algae sections at the end of the experiment divided by the number of adults.

## 2.6 Effect of light on the growth of *Spirorbis spirorbis* juveniles

To test the effect of photosynthetic activity and pCO<sub>2</sub> on the growth of juvenile worms, calcein was added to all the flasks (final concentration: 20 mg L<sup>-1</sup>) during the last 24 h hours of the experiment. Subsequently, half of the flasks were kept in constant light, the other half was darkened by wrapping in aluminum foil. Growth was measured as the size of the stained newly formed tube (see Fig.1. C.).

## 2.7 Seawater chemistry

Seawater processing for carbonate system measurements was made according to the standard operating procedure (SOP) 1 of Dickson and Sabine (2007). Three random flasks in each treatment and the three mixing tanks were sampled prior to each water exchange. The temperature of the flasks and the tanks were measured with 0.01 °C precision for future in-situ pH recalculation (see below). Samples were analyzed in the laboratory for DIC and pH<sub>T</sub>. DIC was measured with an AIRICA (Marianda, Germany), the measurement principle is based on the Infrared measurement of CO<sub>2,g</sub> purged out of an acidified sample. The system was calibrated on every measurement day with Certified Reference Material (CRM) (Andrew Dickson, Scripps Institution of Oceanography). The pH on total scale (pH<sub>T</sub>) was measured as follows. Seawater TRIS pH buffers for 15 psu were made according to the SOP 6a of Dickson and Sabine (2007). A combined reference/measurement electrode Metrohm Ecotrode (Metrohm, Switzerland) was used together with a pH-meter/conductimeter Mettler-Toledo SG 7/8 (Mettler Toledo, Switzerland). For calibration, the TRIS buffer was immersed in a thermostatic bath and the voltage ( $\pm 0.1$  mV) of the pH electrode was measured. The temperature of the buffer was modulated on a range of 1.5°C. The temperature corresponding to every mV change was recorded with  $> 0.01$  °C accuracy with a Fluke 5658 reference thermometer equipped with a 5608 platinum resistance sensor (Fluke, USA). The procedure was repeated by increasing and decreasing the temperature to get an average voltage (mV) versus T (°C) reference curve for the electrode in the buffer. The fitting of the electrode voltage to the curve was assessed on every measurement day to check for the natural depolarization of the electrode. This calibration protocol was repeated at least once a week. The voltage and temperature of the sample was measured at the temperature corresponding to the calibration range. The sample voltage, sample temperature and the theoretical



TRIS buffer voltage (corresponding through the calibration curve to the sample temperature) were computed through the equation of the SOP 6a to obtain the laboratory pH on total scale of the sample. The temperature of the sample measured the climate room at the time of sampling was used to recalculate the in-situ pH. Preparative work conducted on CRM together with 35 psu buffers demonstrated an accuracy of this method of 0.003 to 0.005 pH units. Salinity of the samples was measured to an accuracy of 0.01 psu with a Mettler Toledo Inlab 738 conductivity probe after calibration at 25°C with KCl 0.1 mol L<sup>-1</sup> (Fischer Scientific, USA). The overall in situ-pH and carbonate system was recalculated with the R package Seacarb (Lavigne and Gattuso, 2011) using first and second carbonate system dissociation constants for estuarine systems from Millero (2010) and the dissociations constants of HF and HSO<sub>4</sub><sup>-</sup> of Perez and Fraga (1987) and Dickson (1990).

## 2.8 Statistical analysis

Statistical analyses were conducted with Statistica 7 (Statsoft, USA.). Treatments were compared using one way ANOVAs and Tukey's HSD tests. Assumption of normality and homoscedasticity were tested with Shapiro-Wilk's and Levene's tests. In case of abnormality a transformation by natural logarithm was done.

## 3 Results

### 3.1 Chemistry

A summary of the variables of the carbonate system measured in the flasks during the 30 days duration of the experiment derived from pH<sub>T</sub> and DIC are presented in Table 1. Average ( $\pm$  SD) pCO<sub>2</sub> were 460  $\pm$  59  $\mu$ atm, 1193  $\pm$  166  $\mu$ atm and 3150  $\pm$  445  $\mu$ atm respectively for the control and the two elevated treatments. Saturation states (mean  $\pm$  SD) below one, for aragonite (0.82  $\pm$  0.14) and for calcite (0.66  $\pm$  0.14) were reached in the 1200  $\mu$ atm and in the 3150  $\mu$ atm pCO<sub>2</sub> treatments, respectively.

**Table 1.** Seawater carbonate system in the experimental flasks, derived from DIC and pH<sub>T</sub>, data are the means  $\pm$  SD of three flasks sampled every three days before water exchange, along the 30 days duration of the experiment. (n = 10).

Treatment	T° (°C)	Salinity (psu)	pH <sub>T</sub>	pCO <sub>2</sub> ( $\mu$ atm)	DIC ( $\mu$ mol kg <sup>-1</sup> )	A <sub>T</sub> ( $\mu$ mol kg <sup>-1</sup> )	$\Omega_{\text{arag}}$	$\Omega_{\text{calc}}$
Control	16.1	16.7	8.105	460.56	1966.4	2089.2	1.80	3.02
	$\pm 0.7$	$\pm 0.4$	$\pm 0.031$	$\pm 59.42$	$\pm 40.4$	$\pm 52.5$	$\pm 0.22$	$\pm 0.34$
1200 $\mu$ atm	15.7	16.7	7.726	1193.36	2115.2	2135.0	0.82	1.38
	$\pm 0.5$	$\pm 0.4$	$\pm 0.076$	$\pm 166.38$	$\pm 62.4$	$\pm 71.0$	$\pm 0.14$	$\pm 0.23$
3150 $\mu$ atm	15.9	16.8	7.334	3150.40	2306.8	2298.9	0.40	0.66
	$\pm 0.8$	$\pm 0.4$	$\pm 0.079$	$\pm 445.73$	$\pm 251.8$	$\pm 57.5$	$\pm 0.09$	$\pm 0.14$

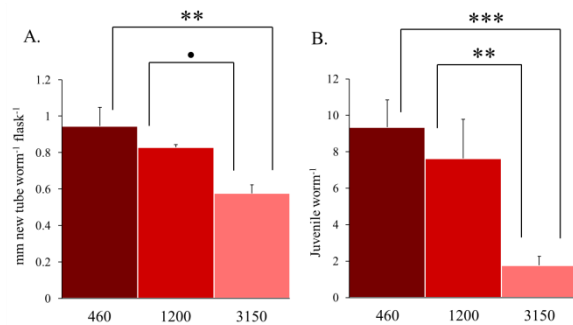


### 3.2 *Spirorbis spirorbis*

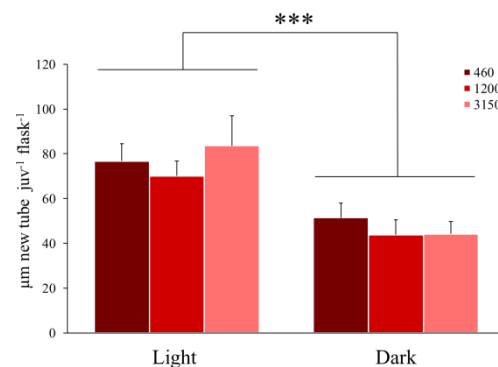
The growth of *Spirorbis* tubes was significantly affected by pCO<sub>2</sub> (one way ANOVA,  $p \leq 0.01$ , Fig. 2. A). Reductions of growth rates were found at 3150  $\mu\text{atm}$  compared to the 460  $\mu\text{atm}$  (Tukey's HSD,  $p \leq 0.01$ ) and, as a trend, relative to 1200  $\mu\text{atm}$  (Tukey's HSD,  $p \leq 0.1$ ). While no signs of shell injuries were visible at 460  $\mu\text{atm}$  and 1200  $\mu\text{atm}$ , all *Spirorbis* tubes exhibited substantial shell dissolution at 3150  $\mu\text{atm}$  (see Fig. 4. 1. A. and B.). This loss of integrity of the outer spiral of the tube exposed the worm soft bodies and the embryo bags to the external seawater. Recruitment of *Spirorbis* was significantly affected by pCO<sub>2</sub> (one way ANOVA,  $p \leq 0.001$ , Fig. 2. B.). The number of settled juveniles per adults were significantly lower at 3150 than at 1200  $\mu\text{atm}$  (Tukey's HSD,  $p \leq 0.01$ ) or in the control (Tukey's HSD,  $p \leq 0.001$ ). Juvenile tubes growth was faster by about 40 % at light compared to dark conditions (Factorial ANOVA,  $p \leq 0.001$ ; Fig. 3). The pCO<sub>2</sub> treatments did not significantly affect juvenile growth rates but important damages on the early tubes were observed in the 3150  $\mu\text{atm}$  treatment (see Fig. 1. C. and D.).

### 3.3 Bryozoan

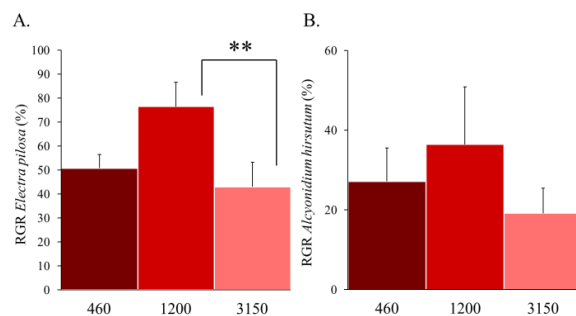
Growth rates of *Electra pilosa* was enhanced at the intermediate acidification level, 1200  $\mu\text{atm}$ , relative to the highest or lowest levels. However, only the difference between the 3150  $\mu\text{atm}$  treatment and the 1200  $\mu\text{atm}$  was significant (Tukey's HSD,  $p \leq 0.01$ ) (Fig. 4. 4.. A.). In the highest pCO<sub>2</sub> treatment, the skeleton of uninhabited zooids dissolved completely, resulting



**Figure 4. 2.** (A) Growth of the tubes of *Spirorbis spirorbis* during the 30 days incubations at 460  $\mu\text{atm}$ , 1200  $\mu\text{atm}$  and 3150  $\mu\text{atm}$  CO<sub>2</sub>, data are the mean  $\pm$  SE of newly formed tubes sections in mm per worm per flask. (B) Recruitment of *Spirorbis spirorbis* during the 30 days incubations at 460  $\mu\text{atm}$ , 1200  $\mu\text{atm}$  and 3150  $\mu\text{atm}$  CO<sub>2</sub>, data are the mean  $\pm$  SE of the number of juveniles settled per adult between flasks. One-way ANOVAs,  $n = 10$ , statistical significance: \*\*\*:  $p \leq 0.001$ , \*\*:  $p \leq 0.01$ , \*:  $p \leq 0.05$ . •:  $p < 0.1$ .



**Fig. 4. 3.** Growth in  $\mu\text{m}$  of the tubes of the juveniles of *Spirorbis spirorbis* during 24 h in light and dark condition exposed to pCO<sub>2</sub> of 460  $\mu\text{atm}$ , 1200  $\mu\text{atm}$  and 3150  $\mu\text{atm}$ . Data are the mean  $\pm$  SE newly formed tubes sections in  $\mu\text{m}$  per worm per flask Two-way ANOVA,  $n = 5$ , statistical significance: \*\*\*:  $p \leq 0.001$ .



**Fig. 4. 4.** Relative growth rate (RGR) of (A) *Electra pilosa*, (B) *Alcyonidium hirsutum* colonies during the 30 days incubations at 460  $\mu\text{atm}$ , 1200  $\mu\text{atm}$  and 3150  $\mu\text{atm}$  CO<sub>2</sub>. Data are the mean  $\pm$  SE of the growth rates in % in  $n=10$  flasks. ANOVA, statistical significance: \*\*:  $p \leq 0.01$ .

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in a “ghost” organic matrix of the exact same shape. The growth rates of *Alcyonidium* were similar under all pCO<sub>2</sub> levels (ANOVA,  $p > 0.1$ ; Fig. 4 4.. B.).

## 4 Discussion

At the pCO<sub>2</sub> predicted for the year 2100 (1200  $\mu\text{atm}$  treatment) none of our experimental species was negatively impacted in growth rates or recruitment (investigated only for the polychaete). At very high pCO<sub>2</sub> level, 3150  $\mu\text{atm}$ , the bryozoan remained unaffected while the tubeworms were severely impacted. Since we worked with only three species, two of them calcifiers, one not, we cannot speculate on the acceptance or rejection of the hypothesis that the calcifiers will be the “losers” of ocean acidification to the benefit of non-calcifiers. It remains to be noted that one of the calcifiers showed some sensitivity to extreme acidification, while the non-calcifier did not, and that generally the impact of acidification on all three species was surprisingly low.

Both the skeleton of *Electra pilosa* and the tube of spirorbis are calcitic with low percentage of MgCO<sub>3</sub> (7 to 15 %) (Rucker and Carver, 1969; Bornhold and Milliman, 1973; Smith et al., 2006; Taylor et al., 2009). However, the bryozoan skeletons are collated to live tissues and protected by a chitinous outer organic cover (periostracum) (Tavener-Smith and Williams, 1972) while the tubes of *Spirorbis* are not protected by any tissue or organic matrix (Ippolitov and Rzhavsky, 2008). We observed in the calcifying tubeworm *Spirorbis spirorbis* a reduction of growth in adults and important shell damages in adults and juveniles at 3150  $\mu\text{atm}$  and  $\Omega_{\text{calc}} \leq 1$  while at this same pCO<sub>2</sub> the growth of *Electra* colonies was unaffected and only the empty cystids were decalcified, similarly to what was observed on the bryozoan *Myriapora truncata* in the CO<sub>2</sub> vents of Ischia (Italy) (Rodolfo-Metalpa et al., 2010). Therefore, for extreme pCO<sub>2</sub> level which may be reached in coastal habitats (Melzner et al. 2012), our study suggests that *Spirorbis spirorbis* may be impacted more severely than calcifying and non-calcifying bryozoan co-occurring in this epibiotic microhabitat. This difference seems to corroborate the importance of the protection conferred by living tissues and organic layers to calcifiers against ocean acidification (Hall-Spencer et al., 2008; Thomsen et al., 2010, Rodolfo-Metalpa et al., 2011).

Our highest pCO<sub>2</sub> treatment reduced settling of about 80 % relative to the control in *Spirorbis spirorbis*. In this species, the embryos are bred within the parental tube for a period of 10 to 25 days until the emission of a fully formed larvae settling within few hours (Knight-Jones, 1951; Daly, 1978). The drastic reduction of settlings at 3150  $\mu\text{atm}$  could be the outcome one or several accident having happened during the most critical life stages that are fertilization, cleavage (embryogenesis), planktonic larva, settlement and metamorphosis (Kurihara, 2008). Exchange of sperm and fertilization of eggs might not have occurred during the experiment since sperm stored during the previous summer

in a specific organ of the head could have been used to fertilize the eggs (Potswald, 1967; Daly and Golding, 1977). Likewise, some embryos in dormancy at the beginning of the experiment could have seen their development reactivated. A more probable explanation for our results would be a delaying or interruption of the embryogenesis due to the exposure of the embryo bags to external acidified seawater after dissolution of the parental tubes. Such embryogenesis accidents have been noted for gastropods, bivalves and echinoderms (Desrosiers et al., 1996; Kurihara and Shiryama, 2004; Ellis et al., 2009). The planktonic stage is another sensitive life stage for invertebrates on which ocean acidification can be deleterious (Dupont et al., 2008; Kurihara, 2008). In *Spirorbis*, however, the shortness of the pelagic phase (a few minutes to 3 h) and the non-calcifying nature of the larvae may reduce the impact of acidified seawater. *Spirorbis spirorbis* specifically settles on brown algae and this preference is most likely chemically mediated (De Silva, 1962; Al-Ogily, 1985; Qian, 1999). The necessary sensitivity to cues could be affected by seawater pH and making the larvae incapable of recognizing their hosts. This has been found in clown fish (*Amphiprion percula*) larvae, becoming “blind” to their host anemones cues under high pCO<sub>2</sub> (Munday et al., 2009).

As a novel aspect of epibiotic associations, our study suggests that encrusting organisms inhabiting the DBL on an algal thallus could benefit from the host’s photosynthesis. The thickness of the DBL is mostly depending on the flow velocity, ranging from almost 2 mm in stagnant water exponentially decreasing to a threshold of 50 to 150  $\mu\text{m}$  at flow velocity above 10  $\text{cm s}^{-1}$  (Hurd, 2000; Koch, 1994; Stevens and Hurd, 1997; Wheeler, 1980). In the experimental flasks a vigorous and turbulent water motion was insured by the continuous gas bubbling which, however, was not measured. The water movement served to ensure homogeneous conditions throughout the water column, to optimize feeding conditions for the animals (Hermansen et al., 2001), and to warrant exchange of nutrients and gases at the thallus surface. While water movement per se was strong it should be noted that the shear velocity at the thallus surface was presumably moderate. We could expect therefore the thickness of the DBL to be of some intermediate strength, maybe between 100 and 500  $\mu\text{m}$ , such as only *Spirorbis* juveniles might fully experience its effects. The conditions within this boundary layer is strongly influenced by the physiological activity of the host algae and the micro- and macroepibiont species living in it (e.g. Wahl et al. 2012 and references therein). For instance, during alternating cycles of algal respiration (“night”) and photosynthesis (“day”), oxygen concentration and pH at 100  $\mu\text{m}$  above the thallus surface can vary between 200 and 750  $\mu\text{mol O}_2 \text{ L}^{-1}$  and between 8.0 and 9.2 pH units, resp. (data extracted from plots in Spilling et al. 2010). Thus, although this has never been investigated to date, one would expect that OA effects would be dampened in the DBL during daytime at least. Supporting this, we found growth rates of the juveniles about 40 % faster under light than in the dark. A contrasting night response (enhancement of OA effects by algal respiration) was not found. However, the day / night rhythm of calcification observed could reflect some inherent activity pattern of the worm, or be stress engendered by e.g. the variations of O<sub>2</sub> more than by the variations of  $\Omega$ .

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The carbonate system in the DBL of non-calcifying algae has not yet been properly assessed. Only pH and O<sub>2</sub> have been so far measured in DBL of algae but the diffusion speed DIC species, of similar scale than their dissociation rates, added to the complexity of the algal DIC exchanges impairs to clearly link or compare the DBL pH to any pCO<sub>2</sub> or pH measured in the open ocean. The chemistry in the boundary layer is the consequence of a flux of molecules, such that residency time of DIC species in the DBL is of the order of seconds to minutes. As an example, the time for a molecule of CO<sub>2</sub> to diffuse through 100 μm (at 20 °C) would be of 6 s (calculated from Zeebe, 2011) while the relaxation time of the carbonate system is of approx. 90 s (Zeebe et al. 1999). In such conditions, the carbonate system cannot be derived from two of its parameters as the system is not in a fully relaxed equilibrium. In that context, the complexity of the exchanges between algae and seawater could have unpredictable effects on the DBL carbonate chemistry. The main processes to consider are the active HCO<sub>3</sub><sup>-</sup> uptake (involving OH<sup>-</sup> antiport or H<sup>+</sup> symport, Beardall and Raven 1981), the enzymatic conversion in CO<sub>2</sub> in the DBL (Badger 2003; Badger and Price 1994), the diel uncoupling between CO<sub>2</sub> and O<sub>2</sub> release and uptake (CAM-like metabolism, Axelsson et al. 1989; Schmid and Dring 1996; Surif and Raven 1990) and the emission of a vast amount of DOC species possibly affecting the DBL alkalinity (Grosser et al., 2012). Thus, assessing i.e. Ω<sub>arag</sub> and Ω<sub>calc</sub> requires direct measurement of CO<sub>3</sub><sup>2-</sup> via microsensors (De Beer et al. 2008), technology not available commercially yet.

In our experiment, epibionts of *Fucus serratus* resisted future pCO<sub>2</sub> expected in open ocean by the year 2100 and part of this resistance may have been caused by the alga's photosynthetic activity. Only at 3150 μatm we observed detrimental effect on growth and settlement for the tubeworm *Spirorbis spirorbis*. With climate change, the pCO<sub>2</sub> in the Baltic Sea is predicted to increase, however the amount of this increase remains unknown (Schneider, 2011). This incertitude is caused by the concomitant shifts of different parameters: increase of mean sea surface temperature by 2 to 3.5 °C, increase of the river inputs in dissolved organic carbon inputs, strengthening of westerly wind and decrease of sea surface salinity (Schrum, 2001; Gräwe and Burchard, 2011). Those are expected to generate extended periods of upwelling with pCO<sub>2</sub> up to 5000 μatm in the algal beds (Melzner et al., 2012). Consequently, even epibionts of macroalgae may ultimately be affected by global change directly - and also indirectly by shifts in the physiology of their host algae. This study illustrates, (i) that calcifiers may not be “losers” in the future high pCO<sub>2</sub> ocean if their shells are protected by organic layers or living tissues and (ii) that small scale processes at the habitat or microhabitat scale could mitigate the impact of acidification on species and communities.

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## General discussion

The aim of this thesis was to study the interactions between biota and seawater carbonate chemistry in a temperate macrophyte meadow, a characteristic habitat of the Western Baltic Sea nearshores. In chapter 1, we demonstrated the important variations of the carbonate system in the meadows and explained these by the interactions between biotic and abiotic forcings. In chapter 2, we addressed the question of feedback of the observed variations on the growth of the meadow forming alga *Fucus serratus* and have shown a transient fertilizing effect of pCO<sub>2</sub>. In chapter 3 we investigated the effect of pCO<sub>2</sub> on the growth and settlement of the main macrofoulers associated with *F. serratus*: the calcifying tubeworm *Spirorbis spirorbis*, the calcifying bryozoa *Electra pilosa* and the non-calcifying bryozoa *Alcyonidium hirsutum*. We showed a resistance of the macrofoulers to the highest pCO<sub>2</sub> predicted for the year 2100. At higher pCO<sub>2</sub>, the tubeworm suffered growth reduction, shell injuries, and drastic reduction of settlement.

In chapter 1, we observed mean seawater pCO<sub>2</sub> in equilibrium with the atmosphere in July (390 µatm), undersaturated in August (240 µatm) and supersaturated in September during the upwelling events (1590 µatm). During all weeks of measurement, the daily DIC variations were of the range of 100 to 300 µmol kg<sup>-1</sup>. We explained those variations by two abiotic forcings acting at the regional scale, PAR irradiance and wind driven upwelling of deep waters. However, we did not investigate the feedback of the nearshore meadows at the regional scale. *F. serratus* meadows export about 70 % of their gross primary production (GPP) under the form of dissolved organic carbon (DOC) (Barrón et al., 2003). This DOC is exported offshore and remineralized by heterotrophic processes, causing hypoxia and hypercapnia in the bottom water of the Baltic (Barrón and Duarte, 2009; Melzner et al., 2012). A part of this carbon sequestered in deep waters is returned to the meadows by upwelling events during the summer, supplying photosynthesis for DIC and / or out-gassing as CO<sub>2</sub> in the atmosphere (Borges et al., 2005). I roughly estimate the GPP of our study meadow ranging from 0.8 to 1.2 gC m<sup>-2</sup> d<sup>-1</sup>, corresponding to what observed in the intertidal *Fucus* meadows of Norway (Barrón et al., 2003). That estimation is based on the diel variations of DIC measured, the production to respiration ratios for *F. serratus* (Barrón et al., 2003) and *Zostera marina* (Duarte et al., 2010) and their relative coverage at the study site (Karez, 2008). The DOC exported by the meadows would range from 0.5 to 0.9 gC m<sup>-2</sup> d<sup>-1</sup>.

In chapter 2, we showed a transient increase of growth rates in *F. serratus*, up to 200 % when exposed to 4080 µatm pCO<sub>2</sub> (compared to ambient air). Up-scaling this result, we would expect wider daily variation of DIC during an upwelling in the meadow, a consequence of increased algal production. Contrarily, we observed in chapter 1 a slight decrease of the DIC variation during the

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upwelling in September. As an explanation for this discrepancy, we proposed the physiological stress generated by the rapid decrease of temperature and increase of salinity co-occurring with elevated pCO<sub>2</sub> during the upwelling. Beyond this initial stress, we would possibly observe the expected increases of DIC consumption. This might be true as long as the upwelling conditions persist, as expected with climate change in the Baltic Sea. Like in the global ocean, Baltic Sea pCO<sub>2</sub> is predicted to increase, however the amount of this increase remains unknown (Schneider, 2011). This incertitude is caused by the simultaneous but sometimes opposite shift of different parameters: increase of mean sea surface temperature by 2 to 3.5 °C, decrease of sea surface salinity and increase of the riverine DOC inputs (Schrum, 2001; Gräwe and Burchard, 2011). Some of these shifts are caused by a future increase in frequency and strength of westerly winds, the same winds responsible for the nearshore upwelling events. The resulting extension of the hypercapnic events could enhance algal growth rates. Consequently, the meadows production and export of DOC could increase (Riebesell et al., 2007). Melzner et al., (2012) calculated that the Western Baltic bottom water could reach up to 5000 µatm with a tripling of the atmospheric pCO<sub>2</sub>. This value is based on the sum of the atmospheric CO<sub>2</sub> and the CO<sub>2</sub> generated from the consumption of all the subpycnoclinal oxygen by the heterotrophic degradation of organic matter. A future increase of DOC export by the coastal algal meadows could strengthen further hypercapnia / hypoxia of the sub-basins waters. In contrast, the increase in DOC export could lead to increase microbial activity and water turbidity, restricting the depth distribution of macrophytes by shading.

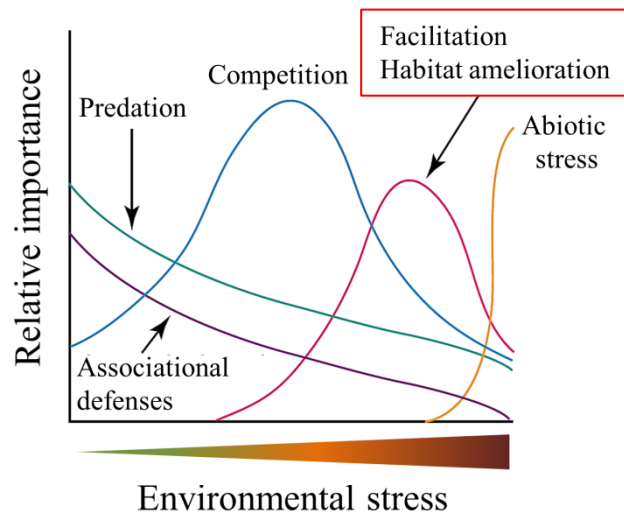
As a general rule, hypercapnia and hypoxia are intrinsically related in coastal ecosystems (Cai et al., 2011). Nevertheless, the response of organisms to this co-occurrence of these multiple factors remain understudied (Melzner et al., 2012). In heterotrophs, reduced O<sub>2</sub> impairs the respiratory metabolism, causing in a major threat to the biodiversity of coastal area (Vaquer-Sunyer and Duarte, 2008). On the contrary, in autotrophs oxygen depletion is generally beneficial to photosynthesis. In the carbon fixation process, O<sub>2</sub> and CO<sub>2</sub> compete at the site of the RuBisCO and both hypercapnia and hypoxia are presumed to favor photosynthesis over photorespiration. (Dromgoole, 1978; Holbrook et al., 1988; Touchette and Burkholder, 2000; Raven et al., 2005). This will not affect all macrophyte taxa equally but rather benefit the poor competitors for carbon acquisition, entraining ecological shifts. In terrestrial ecosystems, this phenomenon is expected to create wide-scale shifts from grasslands to forests (Collatz et al., 1998). In marine ecosystems, the possibilities of such transitions are not studied. So far, correlations have been observed between the vertical zonation of algal species and their ability to extract DIC (Murru and Sandgren, 2004). The algae with the highest affinity for DIC (green and fucales) inhabiting the intertidal and nearest shore while the lesser (kelps) the furthest, where diel variations of DIC are less drastic (Surif and Raven, 1989; Mercado et al., 1998; Murru and Sandgren, 2004). Unlike algae, seagrasses are limited by CO<sub>2</sub> (aq) (in actual conditions) as their mechanism of DIC uptake is not efficient enough to saturate their photosynthesis for carbon (Beer and Koch, 1996;

Invers et al., 1997; Janssen and Bremer, 2004). With future ocean acidification, this competitive disadvantage is likely to be reduced (Zimmerman et al., 1997; Fabricius et al., 2011) and seagrasses might get the upper hand over brown algae in mixed macrophyte assemblages. Beside the concentration of CO<sub>2</sub>, upwelling events bring waters that are 5 - 15 times more concentrated in N and P. Those nutrient pulses are more favorable for ephemeral and epiphytic algae, nutrient limited in normal conditions, over perennial brown algae and seagrasses, better competitors in oligotrophic waters (Fujita et al., 1989; Bokn et al., 2003; Burkholder et al., 2007). Nonetheless, the harshest competitors of perennial macrophytes in the Baltic seem to be the bacterio- and phytoplankton. The eutrophication of the Baltic water in the second half of the 20<sup>th</sup> century resulted in increased phytoplankton blooms, causing a reduction of the euphotic zone limiting the vertical distribution of macrophytes (Vogt and Schramm, 1991; Rohde et al., 2008).

In contrast to the previous paragraph, important ecological shifts from calcifier- to non-calcifier-dominated ecosystems are expected with ocean acidification. The scleratinian coral reefs are presumed to disappear at the benefit of seagrass or seaweed meadows at pCO<sub>2</sub> above 1000 µatm, as observed in the natural CO<sub>2</sub> vents of Papua New Guinea (Solomon Sea) (Diaz-Pulido et al., 2011; Fabricius et al., 2011). Similarly, in the vents of Ischia (Mediterranean Sea), a shift from calcifying to non-calcifying algae has been observed, along with a disappearance of the calcifying epibionts of seagrasses (Hall-Spencer et al., 2008; Martin et al., 2008). In chapter 3 we tested whether this theory is also valid for common epibionts of the brown algae *F. serratus* in the Baltic Sea. The growth and recruitment (*Spirorbis* only) of the calcifying tubeworm *Spirorbis spirorbis*, the calcifying bryozoa *Electra pilosa* and the gelatinous bryozoa *Alcyonidium hirsutum* was investigated at ambient, 1200 and 3150 µatm pCO<sub>2</sub>. All exhibited a resistance to the most elevated pCO<sub>2</sub> expected in the year 2100 (1200 µatm, 1.38 ± 0.23 treatment). The epibiotic community of *Fucus* in the Baltic demonstrated a better resistance to ocean acidification than the epibiotic community of the seagrasses of Mediterranean. Partly composed of comparable species (e.g. *Electra posidonia*, *Spirorbis marioni*), all disappeared from the shoots at pCO<sub>2</sub> of ~1500 and Ω<sub>calc</sub> > 1 (Martin et al., 2008; Cigliano et al., 2010). These differences could be the consequence of the selective pressure exerted on populations by the natural acidified conditions occurring in the Baltic, promoting the rise of tolerant genotypes (Marshall et al., 2010). Similar resistance of populations of calcifiers in the Baltic compared to populations from other locations have been observed for *Mytilus edulis* (Thomsen et al., 2010) and *Amphibalanus improvisus* (Pansch et al., in prep). However, at 3150 µatm and Ω<sub>calc</sub> < 1, spirorbid worms showed a strong reduction of growth, serious dissolution of the tubes and an 80 % collapse of larval recruitment. This could be due to the lack of organic lining protecting the tube of spirorbis such as the periostracum of bryozoans and mussels. Nowadays, events of undersaturation for calcite in the macrophyte meadows during upwelling events are not likely to be of enough duration and intensity to cause the detrimental effect observed. However, possible intensification of upwelling events in the future could extend the

periods of calcite undersaturation from days to weeks. Therefore, the “larval bottleneck” effect identified by Dupont et al., 2010 could happen to *Spirorbis* populations in the Baltic sea.

In the other hand, *Spirorbis* juveniles could be protected by the effects of algal photosynthesis during the day. In chapter 3, 40 % increased growth was recorded in the *Spirorbis* juveniles in light conditions compared to dark conditions, even at the highest pCO<sub>2</sub> tested. Further experiments are necessary to determine whether this is caused by CO<sub>2</sub> and pH, as we expect, or by other factors such as O<sub>2</sub> or the emission of algal metabolites (Grosser et al., 2012). As



**Figure 5.1** The Menge–Sutherland model with facilitation. Models predict the relative importance of predation, competition, abiotic stress and two types of facilitation (amelioration of abiotic stress and associational defenses). (Bruno et al., 2003)

previously mentioned, theories on ocean acidification so far almost only consider competition between groups of organisms that according to theory are either, “OA losers” or “OA winners”: autotrophs vs. heterotrophs, non-calcifiers vs. calcifiers, protected shells vs. non protected, physiologically tolerant animals vs. non tolerant, adults vs. early stages, etc. (Fabry et al., 2008; Melzner et al., 2009; Dupont et al., 2010; Thomsen et al., 2010). The results presented in this thesis agree with some of those theories: increase of growth of *Fucus serratus*, advantage of organic protection of shells, larval bottleneck. However, our results also suggest the possibility of collaboration between potential “losers” and “winners” to resist OA. In chapter 1, the photosynthesis of marine autotrophs in the nearshore is creating very favorable conditions for calcification during the day. In chapter 3, we assume that this phenomenon explains the day night difference of growth of the *Spirorbis* juveniles. On the contrary, the process of calcification and respiration during the day could supply autotrophs photosynthesis in carbon and enhance their growth rates, as shown in chapter 2. Such positive interactions have been recognized as major drivers of ecosystems, notably in nearshores (Bertness and Callaway, 1994; Bruno et al., 2003). Bertness and Callaway (1994) theorized that the increase of physical stress, such as OA, reduces interspecific competition and thus leads to facilitation. The primary space-holders buffer the neighbors from stresses, maintaining the biodiversity in the ecosystem (see Fig. 1.5). This effect is presumed to act until a certain threshold of stress above which biodiversity is expected to collapse (Bruno et al., 2003). So far, such “collaboration” between autotrophs and calcifiers have been shown between corals and macroalgae (Kleypas et al., 2011), corals and microalgae (Drupp et al., 2011; Shamberger et al., 2011) and seagrasses and coralline algae (Semese et al., 2009).

Recently, Alexandridis et al., (2012) synthesized all current knowledge on biotic and abiotic



interactions around the alga *Fucus vesiculosus*. They highlighted the negative impact of phytoplankton-induced shading and epibionts on the growth and lower depth limit of *Fucus*. The development and extension of such models is necessary to assess the effect of climate change and its main components (salinity, temperature and pH / pCO<sub>2</sub>) on the macrophytic habitats. To do so, important efforts of research on the cross effects of multiple stressors at the community level will be needed.

### *Conclusions and outlooks*

This thesis investigated the interactions between seawater pCO<sub>2</sub>, nearshore macrophyte meadows, the meadow forming algae *Fucus serratus* and its epibiontic sessile fauna.

In chapter 1, we demonstrated the strong effect of photosynthesis and respiration of the nearshore meadows on the carbonate chemistry of the surrounding water masses at daily and monthly levels. We assessed the need to perform high resolution and long term carbonate chemistry records in autotroph and calcifiers dominated ecosystems. For this, creation of high resolution and accuracy recording systems will be necessary to implement from newly available technologies such as Durafet or spectrophotometric sensors for pH and infrared gas sensor for CO<sub>2</sub> (Aßmann et al., 2011; Fietzek and Körtzinger, 2010; Martz et al., 2010). A major emphasis should be placed on the consideration of the day / night or seasonal variations of the carbonate system in studies on ocean acidification, particularly to study the couplings photosynthesis / respiration and calcification / dissolution. In chapter 2, we showed an increase of growth of the brown algae *Fucus serratus* at elevated pCO<sub>2</sub>, possibly explained by a reduction of the enzymatic costs of carbon assimilation. Further studies on the physiology of algae would be necessary to prove this theory. Moreover, this work should be extended to other abiotic parameters such as salinity, temperature and nutrients that are expected to vary with climate change and that could have interactive effects with CO<sub>2</sub> on algal primary production. The experiment conducted on the fouling community of *Fucus serratus* demonstrated a great resistance of the animals, calcifying or not, to elevated CO<sub>2</sub>. This work verifies some of the major theories about ocean acidification such as the role of organic tissues and lining in the protection of skeleton and shells and the high sensitivity of the early life stage. In addition, we bring a first observation of a possible protection of epiphytes by the activity of the algal host in the boundary layer. This protection is yet of unknown nature and more studies will be necessary to assess whether this protection is due to by abiotic (O<sub>2</sub>, CO<sub>2</sub>) or biotic parameters (infochemicals). The work presented in this thesis is a first step to the creation of mechanistic models forecasting the evolutions of the meadows in the context of ocean acidification.

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## GENERAL DISCUSSION

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Hiermit versichere ich, dass ich die Hausarbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe, alle Ausführungen, die anderen Schriften wörtlich oder sinngemäß entnommen wurden, kenntlich gemacht sind und die Arbeit in gleicher oder ähnlicher Fassung noch nicht Bestandteil einer Studien- oder Prüfungsleistung war.

Kiel, den 13.09.2012

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