Effects of Ocean Acidification on Primary Production of Marine Macroalgae

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'No data can be taken out of this work without prior approval of the thesis-promoter'

DECLARATION

I hereby declare that the work presented in this thesis was conducted by me under the direct supervision of Dr. Inka Bartsch with the exception of those instances where the contribution of others has specifically been acknowledged.

None of the work presented herein has been previously submitted for any other degree.

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GLOSSARY: abbreviations and acronyms

µmol	micro mole
A_T	total alkalinity
CA	carbonic anhydrase
CCM	carbon concentrating mechanism
Ci	inorganic carbon
DIC	dissolved inorganic carbon
DMF	dimethylformamide
DW	dry weight
e.m.f.	electro-magnetic force
FW	fresh weight
g	gram
kg	kilogram
mg	milligram
^{o}C	degree centigrade
pCO_2	carbon dioxide partial pressure
ppm	parts per million
ppmv	parts per million by volume
RGR	relative growth rate
SST	sea surface temperature

ABSTRACT

Currently global warming and increase in atmospheric CO_2 levels are major concerns for our ecosystems. The ocean acidification, the consequence of rising atmospheric CO_2, is occurring in synergy with ocean temperature increase and their cumulative impacts or interactive effects may have very significant consequences for marine life and still are virtually unknown. This will not only change the ecosystem structure but very importantly the basis of the food web, namely the primary production. Marine macroalgae are important functional groups of the world's coastal ecosystems. Due to their important ecological roles, it is important to understand how the macroalgae will be affected by the changing scenarios. In this master thesis, effects of increased CO_2 concentration on *Chondrus crispus*, a common red alga of the North Atlantic, were investigated through growth experiments conducted in variable irradiance (minimal and optimal) and temperature (optimal and elevated). Productivity and performance of the algae was observed through growth rate and photosynthetic capacity. Change in the biochemical components of the algae was evaluated through C/N analysis and Chlorophyll *a* analysis. The results from this study showed increased growth of *Chondrus crispus* in elevated CO_2 concentration. The growth difference between low and high CO_2 concentration was more pronounced in the elevated temperature. However, this enhancement of growth rate was not attributed to the photosynthetic carbon assimilation of the algae, as photosynthetic rate was not increased in the high CO_2 acclimated algae. The growth enhancement in algae was most probably due to the down-regulation of energy consuming CCMs in the elevated CO_2 concentration which had been observed in some other macroalgal species as well. Final dry biomass increased significantly in the algae cultured in the high CO_2 concentration compared to the low CO_2 concentration indicating higher growth in the high CO_2 concentration. Significant decrease of Chlorophyll *a* content was also observed under the high CO_2 treatments compared to the low CO_2 treatments. Decreased net photosynthetic rates in *C. crispus* cultured under the high CO_2 concentration could be attributed to the decreased chlorophyll *a* contents in the algae cultured under this CO_2 concentration. However, both C and N contents of *Chondrus crispus* were not significantly affected by CO_2 concentration. In order to better understand the underlying physiological mechanisms of *C. crispus* leading to increased growth in elevated CO_2 concentration, further study should be focused on the functioning of CCMs in details under different CO_2 concentrations.

1 INTRODUCTION

1.1 Acidification and warming in the ocean

Currently increase in atmospheric CO_2 levels causing global warming has become a major global issue. The atmospheric CO_2 level has increased significantly from preindustrial levels of approximately 280 ppmv to nearly 384 ppmv in 2007 (Solomon et al. 2007). Atmospheric CO_2 concentration is currently increasing at a rate of ca. 3.3% yr^{-1} (Canadell et al. 2007). The rate of the present and projected increase in atmospheric CO_2 is approximately hundred times faster than in the last 650,000 years (Siegenthaler et al. 2005). A major portion of this atmospheric CO_2 is absorbed by the world's oceans. In recent decades, only half of the anthropogenic CO_2 has remained in the atmosphere while the other half has been taken up by the oceans (ca. 30%) and the terrestrial biosphere (ca. 20%) (Feely et al. 2004, Sabine et al. 2004). This oceanic uptake of CO_2 is causing pH reductions and alterations in carbonate speciation in the seawater which together are termed as ocean acidification. Ocean acidification (OA) has also been described as "the other CO_2 problem" (Henderson 2006, Turley 2005) and "global warming's evil twin" (Pelejero et al. 2010) in the scientific articles. Currently, ocean acidification is occurring at an alarming rate; since pre-industrial times, the average pH of the surface oceans has dropped by approximately 0.1 units, from approximately 8.21 to 8.10 (Royal Society 2005) and is projected to drop further by 0.3–0.4 units by the end of this century (Caldeira et al. 2007, Feely et al. 2008). Probably, the world's oceans have not experienced such magnitude of pH change in more than 20 million years of Earth's history (Feely et al. 2004). According to IPCC Scenario IS92a, the atmospheric CO_2 is expected to rise 750 by µatm by the end of this century which would triple the surface water CO_2 concentrations relative to pre-industrial values. Consequently, seawater CO_3^{2-} concentrations and pH will drop by 50% and 0.4 units respectively (Fig. 1.1, Wolf-Gladrow et al. 1999, Caldeira & Wickett 2003).

Due to the increasing atmospheric CO_2 and the resulting global atmospheric temperature increase (global warming), significant changes in the earth's climate are being forecasted. The Intergovernmental Panel on Climate Change (IPCC 2001) has found an increased likelihood of a 1–7 ^{o}C increase in mean global temperature within the next hundred years. As a consequence of rising atmospheric temperature, increase in surface ocean temperature is expected with similar trends due to the warming of the surface mixed layer (Levitus et al. 2005). During the last century, the global average sea surface temperature (SST) increased by 0.7 ^{o}C (Hulme et al. 2002). Vargas-Yánez et al. (2008) reported a warming of 0.02-0.03 ^{o}C yr^{-1} for the period 1974-2005 in the NW Mediterranean shelf. The annual mean temperature rise by a mean value of 1.67°C has been reported in the surface water at Helgoland Roads (Germany) since 1962 (Wiltshire et al. 2010). A net warming of the world's oceans has also been observed (Levitus et al. 2000, Fukasawa et al. 2004). However, coastal and shelf habitats are most likely to be especially vulnerable due to this ocean warming as observations indicate that warming has been most strongly observed in the upper 700 m of the water column (Harley et al. 2006). This warming in the ocean and the ocean acidification are being considered as two significant drivers of changes in the oceanic environment. Both of them originated as a consequence of increasing atmospheric CO_2, and occur together and their cumulative impacts or interactive effects may have significant consequences for marine life and still are mostly unknown. Such scenarios might influence marine ecosystems on all hierarchical levels from cells to communities.

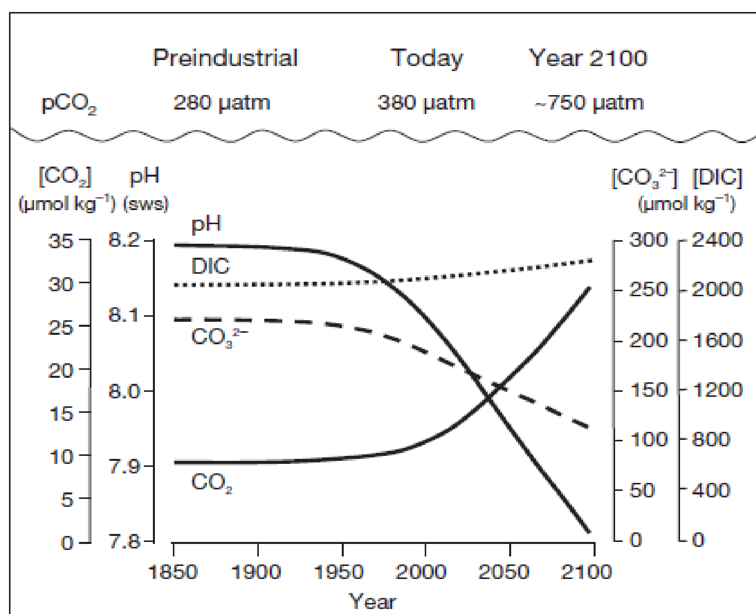
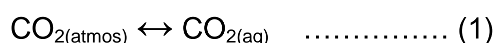


Figure 1.1 Predicted changes in the surface ocean carbonate system in response to changes in the atmospheric CO_2 assuming the IS92a Scenario. Modified after Wolf-Gladrow et al. (1999) in Rost et al. (2008).

1.2 The seawater carbonate system and the process of ocean acidification

Understanding ongoing ocean acidification processes require knowledge about seawater carbonate chemistry. Seawater carbonate chemistry is regulated by a series of chemical reactions. Atmospheric CO_2 is taken up in the seawater by air-sea flux through the sea surface. When dissolved in seawater, gaseous CO_2 reacts with water to form carbonic acid (H_2CO_3). Then this carbonic acid (H_2CO_3) dissociates into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions by releasing hydrogen ions (H^+). The processes are described by the following equations:


$CO_{2(atmos)} \leftrightarrow CO_{2(aq)}$ …………… (1)

$CO_2 + H_20 \leftrightarrow H_2CO_3$ …………. (2)*

$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$ ……….. (3)

$HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-}$ ……….. (4)

[* CO_2 as shown here refers to a hypothetical species often referred to as $CO_2{}^*{}_{(aq)}$ which is defined as the sum of the concentrations of $CO_{2(aq)}$ and $H_2CO_{3(aq)}$]

The seawater reactions are reversible and near equilibrium (Millero et al. 2002). It is in the nature of this equilibrium that any change in the concentration of one of the individual components forces the other components to readjust. For surface water with pH of ~8.1, approximately 90% of the inorganic carbon exists as bicarbonate (HCO_3^-) ions, 9% is carbonate ions (CO_3^{2-}) and only 1% is dissolved CO_2 (Doney et al. 2009). Any changes in CO_2 concentrations in the ocean surface will change the concentrations of HCO_3^-, CO_3^{2-} and H^+. With increasing CO_2 concentrations, HCO_3^- and H^+ concentrations will increase but CO_3^{2-} will

decrease because of the increasing H^+ concentrations (H^+ can then react with CO_3^{2-} to form HCO_3^-). Increased H^+ concentrations will also lower the pH in the ocean because pH= -$\log_{10}[H^+]$. These changes in the carbonate chemistry of the oceans termed as "ocean acidification" are already occurring and will intensify in the future.

Concentrations of the individual components involved in the dissolved inorganic carbon chemistry cannot be directly measured in seawater. The inorganic dissolved carbon system is described by temperature, salinity, pressure and the following four measurable parameters:

1. fugacity of CO_2 ($f\,CO_2$): it is defined as the partial pressure of CO_2 in air that is in equilibrium with seawater, taking into account that CO_2 is not an ideal gas. i.e. considering molecular interactions of CO_2 (e.g. with H_2O and other molecules),
2. dissolved inorganic carbon (DIC, also depicted as C_T or ΣCO_2): it is the sum of the concentrations of dissolved CO_2, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions,
3. pH and
4. total alkalinity (TA, also depicted as A_T): it is the total concentration of titratable weak bases in seawater relative to the reference proton condition compromising pure CO_2 in seawater
   $A_T= [HCO_3^-] + 2[CO_3^{2-}] + [OH^-] – [H^+] + (….)$
   Where (….) represents various minor acid-base species such as borate ions

If at least two of these four parameters are measured in a sample, the other two parameters can be calculated by using equilibrium constants, temperature, pressure and salinity (Lewis & Wallace 1998, Dickson et al. 2007). Which of the four parameters will be measured is determined by the research questions to be answered.

1.3 Marine macroalgae in times of ocean acidification and ocean warming

Marine macroalgae (often known as seaweeds) are important functional groups of the world's coastal ecosystems constituting up to 40% of the primary productivity of the coastal zone (Charpy-Roubaud & Sournia 1990) and about 5% of the total global oceanic production (Smith, 1981) together with seagrasses. However, their occurrence is restricted to only 0.6% of the area of the world's ocean (Smith, 1981). They, thereby also act as an important sink for anthropogenic carbon dioxide. In an assessment of the economic value of the world's ecosystems, the value of the nutrient cycling function of seagrass and macroalgal beds ranked second among the listed ecosystem values (Costanza et al. 1997). Although phytoplankton contributes substantially towards the primary productivity of the coastal area, there are certain areas along the coastal belt where production of benthic macrophytes exceeds that of phytoplankton (Buesa 1972, McRoy et al. 1977, Asmus et al. 1998). In the coral reef ecosystems, the major contribution to primary productivity is also derived from benthic communities such as sea-grasses and macroalgae rather than phytoplankton (Buesa 1972). The production per square meter of seaweeds and seagrasses is equal to or in many cases greater than that of terrestrial plant based systems. For example, sublittoral kelp communities dominated by with *Laminaria* spp. have annual productivity rates of approximately 2 kg carbon $m^{-2}y^{-1}$ and the macroalgal sea palm *Postelsia* has been estimated to produce up to 14 kg carbon $m^{-2}y^{-1}$ while the productivity of mature rainforests and intensive alfalfa crop production are generally between 1 and 2 kg carbon $m^{-2}y^{-1}$ (Kaiser et al. 2005). In addition, marine macrophytes (macroalgae and sea-grasses) have an important structural role. They serve as habitats and refuge for diverse communities of associated plants and animals,

including juvenile life stages of many fish species. Due to their important ecological role, it is of great interest to understand how marine macrophytes will respond to the changing scenarios such as ocean acidification and temperature increase. It is obvious that organisms in the surface ocean and all the photosynthetic organisms living in the euphotic zones are especially exposed to the high CO_2 environment and temperature changes. Therefore, their physiology may respond to the altered carbonate system, lowered pH and increased temperature of the seawater in this changing oceanic condition. In fact, physical, chemical and biological characteristics of the ocean are affected by such environmental change and it can affect the productivity, distribution patterns and ecology of macrophytes and many other organisms that depend on them (Short and Neckles 1999, Orth et al. 2006).

Responses of marine macroalgae (specially, non calcifying) to increased CO_2 concentrations are much less investigated so far compared to phytoplankton and higher plants. The effects of increased CO_2 concentrations on marine macroalgae mainly depend on the level of carbon limitation of algae in natural systems where they are distributed. Photosynthesis of marine macroalgae generally becomes saturated at current levels (i.e. 2.2 mM) of dissolved inorganic carbon (Ci) in seawater (Holbrook et al. 1988, Johnston et al. 1992). But their photosynthesis would be severely limited under current atmospheric conditions if it were dependent only on the diffusional entry of CO_2 from the medium to the site of fixation, considering the low availability of CO_2 in seawater. However, photosynthesis of many macroalgae is thought to be fully or nearly saturated with the current dissolved inorganic carbon composition because many of them have been found to posses carbon concentrating mechanisms (CCM) which enable them to utilize the most abundant carbon species HCO_3^- from the seawater (Beer 1994, Beer & Koch 1996, Raven 1997). There are two known pathways of utilizing HCO_3^- for photosynthesis. One mechanism is to dehydrate HCO_3^- extracellularly to form CO_2 prior to Ci uptake. This is performed with the aid of the membrane-bound enzyme carbonic anhydrase (CA). This seems to be the common way of HCO_3^- utilization. The second mechanism of HCO_3^- utilization is by direct HCO_3^- uptake which requires the presence of transport proteins to enhance its penetration through the membranes. The presence of such proteins has only been reported for three macroalgal species so far (Drechsler et al. 1993, Axelsson et al. 1995, Larsson et al. 1997). In fact, responses of marine macroalgae with CCM to increased CO_2 would depend both on the mechanism and the degree of their HCO_3^- utilization and also on the environmental conditions under which they are exposed. Therefore, their responses are expected to be heterogenous and often species specific. Generally, the prediction is that species with active CCM would show little to no response to the rising atmospheric CO_2 levels while those species exhibiting carbon limited photosynthesis (non-bicarbonate using species without CCM) might respond positively by higher carbon assimilation. Algal species could be affected by the increasing CO_2 in various ways, including photosynthetic, growth, and nutrient metabolisms and cell components. However, whether positive responses such as enhancement of photosynthesis and growth will be maintained in the long run primarily depends on the degree of acclimation to elevated CO_2 contents (Zou & Gao 2001).

A wide range of responses by macroalgae to elevated CO_2 concentrations has been observed so far. Growth of some red algal species such as *Porphyra yezoensis* (Gao et al. 1991), *Gracilaria* sp., *G. chilensis* (Gao et al. 1993) or of the brown alga *Hizikia fusiforme* (Zou & Gao 2005) increased when grown at increased CO_2 levels. These species were capable of using HCO_3^- but showed carbon limited photosynthesis in natural seawater. In case of the green seaweed *Ulva rigida*, which possesses an efficient ability to use HCO_3^-, photosynthesis was

saturated at the current Ci concentration of seawater (Björk et al. 1993, Mercado et al. 1998) but its growth was still enhanced at higher CO_2 levels (Björk et al. 1993, Gordillo et al. 2001). On the other hand, a decrease of growth rate due to elevated CO_2 has also been reported in red algal members of the same genera, namely *Gracilaria tenuistipitata* (García-Sânchez et al. 1994), *Porphyra leucostica* (Mercado et al. 1999) and *Porphyra linearis* (Israel et al. 1999). In addition to growth and photosynthesis, increasing CO_2 levels have been found to affect other physiological activities in macro-algae. Enhanced activity of nitrogen reductase (NR) in *Porphyra leucostica* (Mercado et al. 1999), *Ulva rigida* (Gordillo et al. 2001) and *Hizikia fusiforme* (Zou & Gao 2005) has been reported due to the increased CO_2. The enhanced NR would support enhanced growth rate under the high CO_2 level by providing adequate N which often is limiting and required for the metabolism. Elevated CO_2 has also been found to stimulate the uptake of NO_3^- in *Gracilaria* sp. and *G. chilensis* (Gao et al. 1993) and *Hizikia fusiforme* (Zou and Gao 2005). However, decreased uptake rate of NO_3^- at high CO_2 in *Gracilaria tenuistipitata* (García-Sânchez et al. 1994) and *G. gaditana* (Andría et al. 1999) was also reported.

Ocean acidification will be accompanied by warming in the oceans. Increasing global temperature, like the lowered temperatures in the ice ages, might lead to changes in the geographic distribution of marine macrophytes (Breeman 1988, Wiencke et al. 1994, Bischoff & Wiencke, 1995a, b). With elevated temperatures, seaweeds composition and abundance are expected to change in several cold-temperate and polar regions (Müller et al. 2009). It is possible that increased temperature together with the ocean acidification and some other local impacts will combine and have a synergistic effect on marine organisms and communities. So far, very few studies have investigated the response of marine macroalgae to the combined rise in CO_2 (i.e. ocean acidification) and temperature. Martin & Gattuso (2009) found a 50% decrease of net calcification in the crustose coralline alga *Lithophyllum cabiochae* at elevated CO_2 and temperature together while no effect was found under elevated CO_2 or elevated temperature alone. In another study, significant interactive effects of CO_2 and temperature on the physiology of the scleractinian coral *Stylophora pistillata* was found. Decrease of calcification by 50% was observed in this study when temperature and CO_2 were both elevated (Reynaud et al. 2003). However, in a recent study, a synergistic positive effect on the abundance of non-calcareous algal turfs has been observed by future predicted concentrations of CO_2 and temperature (Connell & Russell 2010). To date, knowledge of potential effects of such multiple factors especially on non calcifying marine macroalgae is scarce and more experiments are needed to assess the synergistic effects of increased CO_2 and elevated temperature. The topic of this master thesis will add one aspect to this urgent issue.

1.4 Perturbation experiments on ocean acidification

Investing the biological response to CO_2 induced ocean acidification is a recent field of research. Perturbation experiments are one of the key approaches widely used to investigate the biological responses of marine organisms to ocean acidification (Riebesell et al. 2010). CO_2 perturbation experiments with photosynthetic organisms are not straightforward because when pH of seawater is altered, the carbon speciation in the water is modified simultaneously and this modification has important implications for physiological responses of seaweeds, such as photosynthesis, respiration etc. These metabolic processes themselves also alter the pH of the seawater medium surrounding the algae. So, to keep the water chemistry constant in batch cultures containing algae is strictly not possible, but an approximation to constancy is achievable. Therefore a proper understanding, control and monitoring of carbonate chemistry

of the seawater during experiments is required as well as knowledge about the physiological processes of the experimental organisms related to carbon metabolism. Conducting CO_2 perturbation experiments require precise carbonate system manipulation of the seawater used. There are two fundamental approaches for seawater carbonate chemistry manipulation, either changing DIC at constant total alkalinity (A_T) or changing A_T at constant DIC. Theoretically, both approaches produce similar changes in all parameters of the carbonate system (Schulz et al. 2009) but recent evidence shows that there exist slight differences between the systems (Hoppe et al. 2010). Naturally occurring ocean acidification increases DIC concentration of the surface ocean due to invasion of CO_2 from the atmosphere without changing A_T. Experimental approaches to adjust seawater CO_2 by changing DIC at constant A_T involve (1) aeration with air of a known CO_2 partial pressure, (2) injections of CO_2 saturated seawater or (3) combined additions of $NaHCO_3$ or Na_2CO_3 and HCl. Change of A_T with constant DIC involves additions of either NaOH and/or HCl (Riebesell et al. 2010).

1.5 *Chondrus crispus* –a species with CCM

The macroalgal species *Chondrus crispus* Stackhouse 1797 (Fig. 1.2) was selected for the experiments to be conducted for this master thesis work. This perennial seaweed species is a common red alga (Phylum: Rhodophyta, Order: Gigartinales, Family: Gigartinaceae) of the north Atlantic. It also occurs in the northwestern Pacific along with some other species (Mikami 1965 in Chen and McLachlan 1972). This lower intertidal to shallow sub-tidal species abundantly grows along the rocky parts of the coast and in tide pools. It is commercially important because of its polysaccharide carrageenan and is used in the pharmaceutical and food industries. It is also known as Irish moss or Carrageen moss. Extreme variability of both morphological and physiological features has been observed in this species which was generally attributed to environmental factors (Newton et al. 1959 in Chen and McLachlan 1972).

Figure 1.2 *Chondrus crispus* Stackhouse, 1797 (photo: www.algaebase.org)

Due to this importance, the basic physiology of *C. crispus* is well-known and there exist many fold publications. In culture, *C. crispus* grows optimally at a broad temperature range, from 10 ^{o}C to 15 ^{o}C and growth becomes light saturated at 70 µmol photons m^{-2} s^{-2} (Fortes & Lüning 1980). However, Bird et al (1979) also reported quite similar results in unialgal culture of this species. Although *C. crispus* can grow up to 28° C, its growth starts to decline at 20 ^{o}C (Fortes & Lüning 1980). Photosynthetic rates were found to be light saturated at around 300 to 500

μmol photons m^{-2} s^{-2} in laboratory experiments (Brechignac & André 1984, Mathieson & Burns 1971). Utilization of inorganic carbon (Ci) for photosynthetic carbon fixation is special in *C. Chondrus*. It possesses carbon concentrating mechanism (CCM) facilitating its carbon uptake. It can consume HCO_3^- as a source of photosynthetic carbon and this process depends on external carbonic anhydrase (CA). HCO_3^- is dehydrated to CO_2 by CA before it can be absorbed. Then this CO_2 passively diffuses across the plasma membrane of cells. So, although HCO^{-3} is the main source of inorganic carbon, CO_2, rather than HCO^{-3} is the molecular carbon species absorbed by *C. crispus* and used for photosynthesis. Furthermore there is an intracellular CA in *Chondrus crispus* which enhances the fixation of carbon (Smith & Bidwell 1989, Brechignac & Andre 1985). As other species of Rhodophyta with a CCM such as *Gracilaria* sp., *G. chilensis* (Gao et al. 1993) and *Porphyra yezoensis* (Gao et al. 1991) or *Porphyra leucostica* (Mercado et al. 1999) showed variable reaction pattern with respect to CO_2 increase, we became interested to investigate in detail the reaction pattern of another CCM species *C. crispus* to increased CO_2 and elevated temperature.

1.6 Objectives of the study and hypotheses

In a broader aspect, this study is aimed to investigate how the increased atmospheric CO_2 induced ocean acidification would affect the primary production of marine macroalgae. Growth and photosynthesis are major proxies for the primary production and here I simulated ocean acidification through perturbation experiments in the laboratory and investigated its effects (effects of CO_2) on growth and photosynthesis in variable light intensities and temperatures. Effects of CO_2 on somethe biochemical components of *Chondrus crispus* were also investigated.

I worked with the following three hypotheses:

1. Elevated CO_2 will increase the growth and photosynthesis of *Chondrus crispus*

Although photosynthesis of marine macroalgae which can utilize HCO_3^- is thought to be fully or nearly saturated with the current level of dissolved inorganic carbon in the seawater, there is still debate on this issue. Increased growth and carbon limited photosynthesis have been observed in some macroalgal species capable of using HCO_3^- (Gao et al. 1991, Gao et al. 1993, Zou & Gao 2005). Moreover, efficient utilization of HCO_3^- in macroalgae is mostly dependent on the enzyme carbonic anhydrase (CA) which is an energy consuming process. This process, often termed as CCM (carbon concentrating mechanism), has been found to be regulated by many environmental factors such as pH, temperature, salinity, light availability, nutrients etc (Giordano et al. 2005, Beardall & Giordano 2002) and the amount of HCO_3^- concentration as well. Also the activation of a CCM needs much energy. Therefore, it is expected that elevated CO_2 levels down regulate the CCM activity, thereby save energy that may be invested into growth. Thus, a positive growth effect is expected. The effect on photosynthesis will give an insight into the processes which have taken place: either down regulation of CCM or not. Low light also down-regulate the CCM (Küberl & raven 1994). Thus, either no growth effect in different CO_2 concentrations is expected there or a slight increase under elevated CO_2 due to the higher diffusion rate of CO_2 in higher CO_2 concentration. Therefore, we expect an increase in growth and photosynthesis in *C. crispus* by elevated CO_2 concentration combined with variable light intensities.

2. There will be an interaction effect of elevated CO_2 and elevated temperature on growth and photosynthesis of *Chondrus crispus*

Although very few investigations have been conducted so far to investigate the interactive effects of elevated CO_2 and elevated temperature on macroalgae, negative effects of increased CO_2 and elevated temperature have been reported on crustose coralline algae (Martin & Gattuso 2009) and on a scleractinian coral (Reynaud et al. 2003). However, in a recent study Connell & Russell (2010) found a synergistic positive effect on the abundance of non-calcareous algal turfs by future predicted concentrations of CO_2 and temperature. Temperature is one of the very important factors controlling algal metabolisms. Therefore, an interaction effect of elevated CO_2 and elevated temperature on growth and photosynthesis of *C. crispus* is expected.

3. There will be an effect of increased CO_2 on the biochemical components of *Chondrus crispus*

Increased CO_2 levels affect not only the inorganic carbon acquisition mechanisms but also the biochemical components of algae. Fifty percent reduction of chlorophyll *a* and phycobiliproteins was observed in *Gracilaria tenuisitipitata* at high CO_2 (Mercado et al. 1999). Zou & Gao (2009) also found decreased phycobiliprotein contents in *Gracilaria lemaneiformis* with additional CO_2 in the culture at the higher growth irradiance. Usually, macroalgae exposed to variable irradiance and light quality in the natural environments try to acclimatize to such changes through decreasing pigment content in strong light or through synthesizing more pigments to enhance their efficiency in absorbing energy at low irradiances. In fact, biochemical components in algal tissues are altered when any changes occur in their physiology or metabolic activities. Therefore, I assume that increased CO_2 contents would bring changes in the biochemical contents of *Chondrus crispus* together.

2. MATERIALS AND METHODS

2.1 Algal material

Chondrus crispus (Stackhouse) was originally collected from Helgoland, Germany (a rocky island in the southern North Sea) and kept in unialgal stock- cultures (culture number: 2232) at the Alfred Wegener Institute for Polar and Marine Research (Bremerhaven, Germany). These stock-cultures have been maintained at 10 ^{o}C and an irradiance of about 40 μmol photons m^{-2} s^{-1} in a light: dark cycle of 16: 8 h with continuous aeration of natural air. These stock cultures were cultivated in natural sterile filtered seawater (pore size: 0.2 μm) nutrient enriched with Provasoli medium (Provasoli 1968) and changed every second week. Algal pieces from these stock-cultures were used for all the experiments performed for this master thesis work. Generally, algal pieces were cut to the required biomasses or sizes about 2 days before the experiments started so that the algae had time for wound healing.

2.2 Culture room and culture method

For the experiments, unialgal culture of *Chondrus crispus* (Stackhouse) was done inside a temperature controlled walk-in culture room. Algae were grown in 1L (Experiment 1) and 5L (Experiment 2 and 3) glass bottles (Schott, Germany) filled with sterile and filtered (pore size: 0.2 μm), unbuffered Provasoli (See Table 2.1) enriched seawater (5 ml Provasoli L^{-1} SW). Light was provided by white fluorescent lamps (Osram, L36W/954; Germany). Light intensities in different experiments were adjusted by changing the number of lamps and the distances of the lamps from the culture bottles. For creating irradiances of 10 and 70 μmol photons m^{-2} s^{-1}, 2 and 5 lamps were used respectively. A photo meter (LI-COR Inc. LI-185B, USA) was used for measuring light intensity in the cultures. All the experiments were carried out in a light: dark cycle of 16:8 h. The bottles with algal materials in the culture room are shown in the Fig. 2.1.

Figure 2.1 Culture of *Chondrus crispus* in the temperature controlled culture room.

2.3 Experimental set-up

2.3.1 Experimental designs

In order to test the hypotheses set for this master thesis work, three multi-factorial experiments were conducted. The overviews of the experimental designs are presented by the schematic diagrams (See Fig. 2.2, Fig. 2.3 and Fig. 2.4 in the next pages).

Table 2.1 Nutrient medium after Provasoli (1968)

Stock Solution	
6000 ml	Aquadest
23.4 g	Hepes –Na-Salt (for Co2 exp. No buffer)
1500 ml	PII solution
1500 ml	Fe solution
21 g	NaNo3
4.6 ml	Na-glycerophosphate(50%)
16 mg	Potassium iodide
Sterilized in steamer for 1 hour at 110 °C	

Vitamins were added after cooling down the nutrient solution at <60 °C

30 ml	Vitamin B12(5 mg/250 ml aquadest)
30 ml	Thiamin (250 mg/250 ml aquadest)
30 ml	Biotin (5 mg/250 ml aquadest)
pH= 7.6-8.0	

P II solution		Fe- solution	
5000 ml	Aquadest	5000 ml	Aquadest
5 g	Titriplex III (Na_2 EDTA)	3.3 g	Titriplex III(Na_2 EDTA)
5.7 g	Boric acid	3.51 g	$Fe(NH_4)_2(SO_4)_2.6\ H_2O$ equivalent to 0.1 mg Fe+++/ml
0.82 g	$MnSo_4.\ 4\ H_20$		
0.11 g	$ZnSo_4.\ 7\ H_2O$		
0.024 g	$CoSo_4.\ 7H_2O$		
0.245 g	$FeCl_3.6\ H_2O$ (stock solution was fixed at 2.45 g/100 ml and 10 ml portion was freezed up)		

Experiment 1

Constant condition: temperature (15 °C), irradiance (70 μmol photons m^{-2} s^{-1}), L: D= 16: 8 (light-dark cycle), nutrients

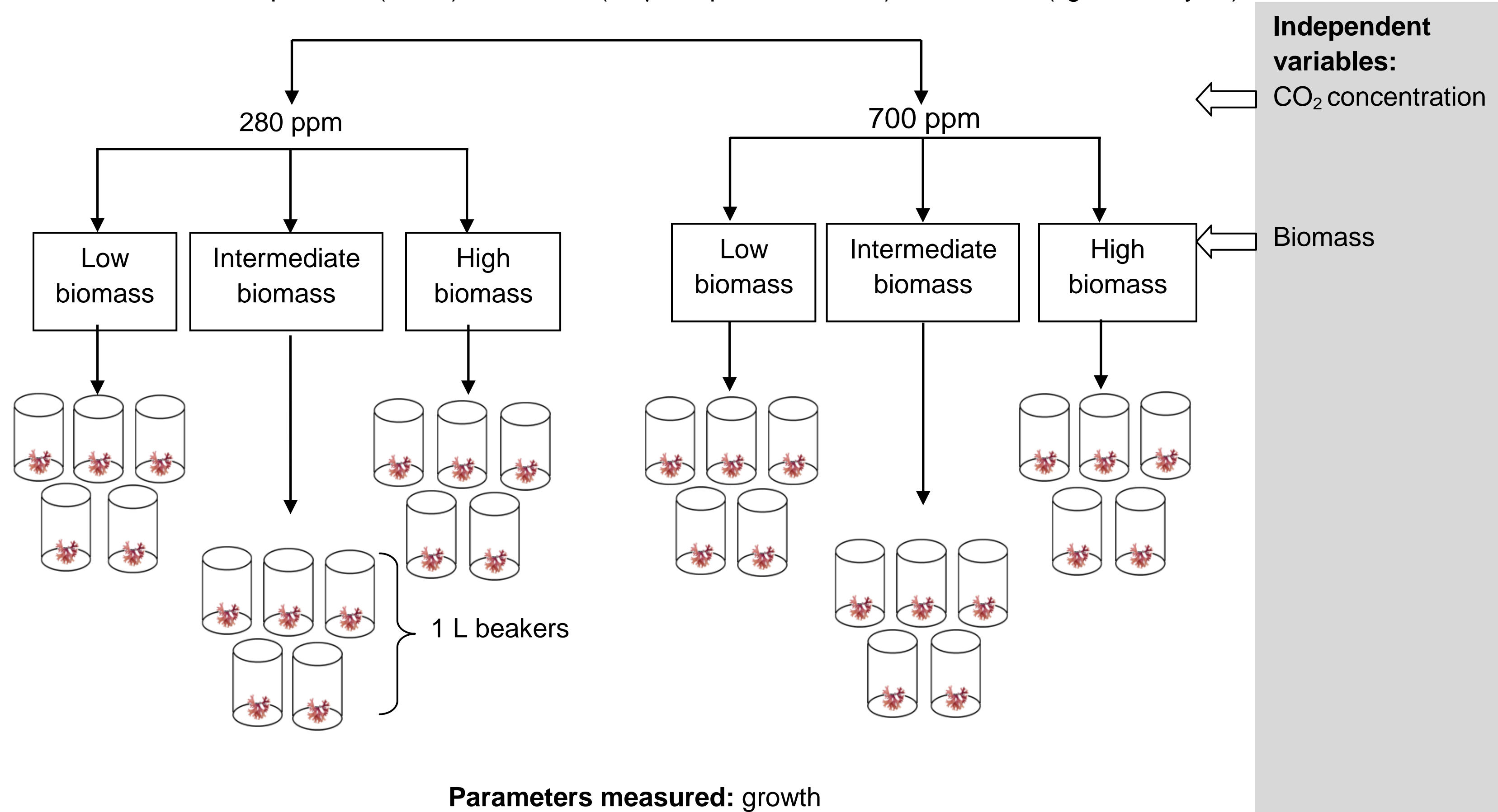
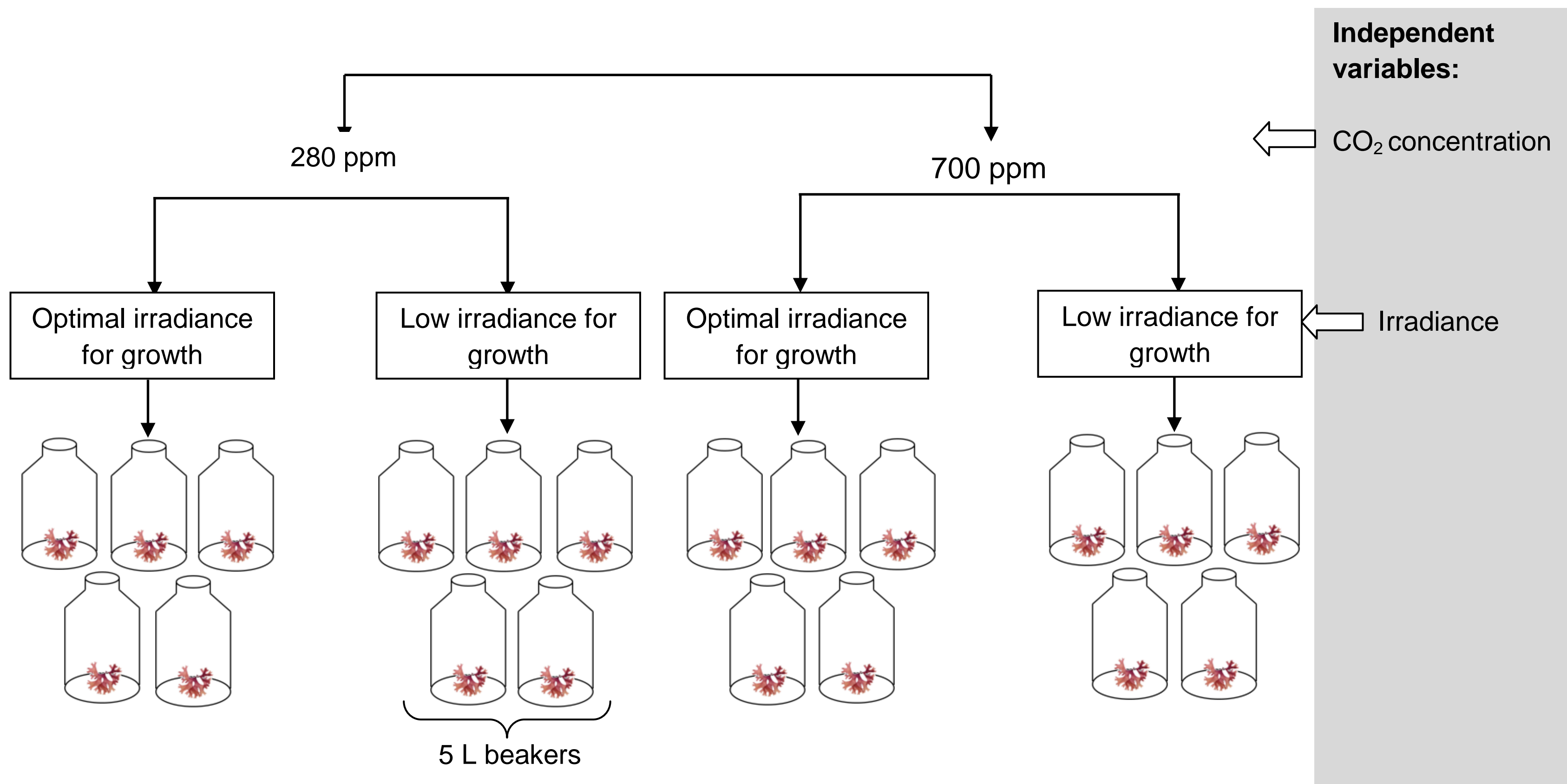


Figure 2.2 Schematic diagram of Experiment 1: Testing effects of algal biomass on water chemistry

Experiment 2

Constant condition: temperature (15 °C), L: D= 16: 8 (light-dark cycle), nutrients

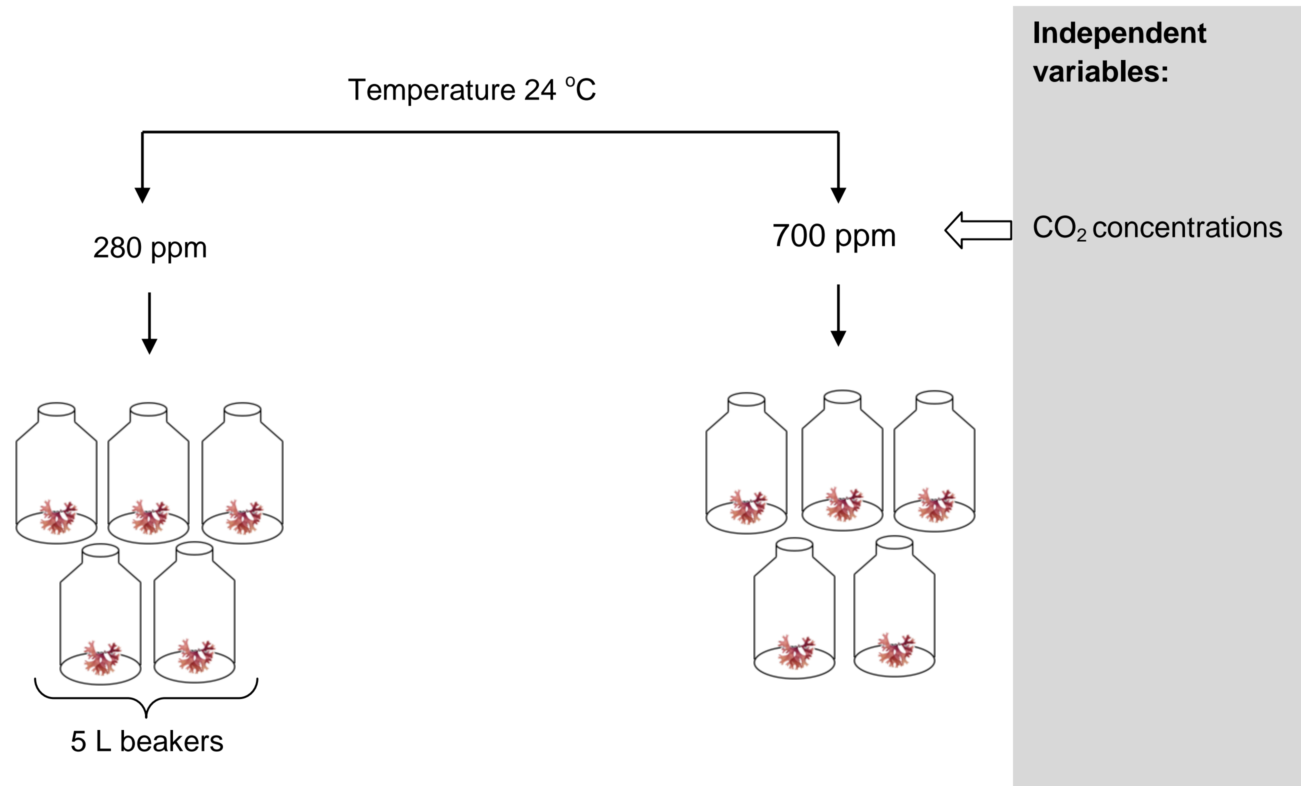


Parameters measured: growth, photosynthesis at low, high and saturating irradiances, dry weight, C/N, Chl *a*

Figure 2.3 Schematic diagram of Experiment 2: Testing effects of CO_2 concentration and cultivating light intensity on *C. crispus*

Experiment 3

Constant condition: irradiance (70 µmol photons m^{-2} s^{-1}), L: D= 16: 8 (light-dark cycle), nutrients



Parameters measured: growth, photosynthesis at low, high and saturating irradiances, dry weight, C/N, Chl *a*

Figure 2.4 Schematic diagram of Experiment 3: Testing effects of CO_2 concentration and temperature on *C. crispus*

Exp 1: Testing effects of algal biomass on water chemistry
This experiment was conducted primarily in the purpose of methodological development of the other experiments. In this experiment, algal pieces of three different biomasses: high biomass (116.8 ± 5.1 mg), intermediate biomass (47.2 ± 1.8 mg) and low biomass (20.6 ± 1.1 mg) were cultured in 1 L glass beakers (Schott, Germany) under two different CO_2 concentrations: 280 ppm and 700 ppm at 15 ^{o}C temperature (optimum temperature for growth of *Chondrus crispus*) and an irradiance of 70 μmol photons m^{-2} s^{-1} (optimum irradiance for growth of *C. crispus*) (Fig. 2.2). So, there were 6 different treatments each with 5 replicates (in total 30 experimental units). Experimental duration was 7 days and during this period no water change was done.

Exp 2: Testing effects of CO_2 concentration and light intensity on *Chondrus crispus*
To test the effects of CO_2 concentration and irradiance, two algal pieces (159.7 ± 32.4 mg in total) were cultured in the sea water (5 L glass bottles; Schott, Germany) under combinations of two different CO_2 concentrations (280 ppm vs. 700 ppm) and two different irradiances (70 vs. 10 μmol photons m^{-2} s^{-1}, optimal and minimal irradiances growth of *C. crispus* respectively) in a crossed design. Temperature (15 ^{o}C, optimum temperature for growth of *C. crispus*) and other conditions (photoperiod, nutrients) were constant. There were 20 experimental units with 4 different treatments each with 5 replicates (Fig. 2.3). Experiment duration was 9 days and culture water was changed on every 3rd day (Day 3 and Day 6) with new Provasoli enriched seawater pre-incubated with CO_2 for 2 days

Exp 3: Testing effects of CO_2 concentration and temperature on *Chondrus crispus*
To test the effects of CO_2 and temperature, two algal pieces (141.3 ± 5.6 mg in total) were cultured in the sea water (5 L glass bottles; Schott, Germany) under two different CO_2 concentrations (280 ppm vs. 700 ppm) at 24 ^{o}C (elevated temperature for growth of *C. crispus*) in an experiment conducted for 9 days. Irradiance (70 μmol photons m^{-2} s^{-1}, optimum light for growth of *C. crispus*) and other conditions (photoperiod, nutrients) were constant. So, there were 10 experimental units with 2 different kinds of treatments each with 5 replications (Fig. 2.4). Water change of the cultures was done as in Exp 2. To reduce efforts needed for the experiment, we applied only one temperature (24 ^{o}C) in this experiment as we already had data of another temperature level (15 ^{O}C) from Experiment 2. We assumed that the algal materials used in both the experiments were homogenous as those were from the same stock cultures. Also the experimental conditions were alike as mentioned above. Therefore, we analyzed the data of Experiment 3 together with some data from the Experiment 2 to observe temperature effects.
The responses of *Chondrus crispus* to experimental conditions were evaluated using the response variables: growth, photosynthesis (measured at low, high and saturating irradiance) dry weight, C and N contents and Chl *a* contents.
2.3.2 Controlling seawater carbonate chemistry

In all our experiments, pCO_2 of seawater in the glass bottles was adjusted by manipulating DIC through aeration with CO_2 enriched artificial air (80 % nitrogen, 20 % oxygen; gas-mixer: HTK Hamburg GmbH) at target pCO_2 levels while keeping the A_T constant. Seawater in the culture bottles were continuously sparged with the CO_2 enriched artificial air by using bubbling stones

fixed with glass sticks and special non gas leaking PVC tubes connected with the main gas mixture line. Aeration in the bottles was started at least 2 days before the start of the experiments so that the water of the culture bottles could reach the required pCO_2 values when the experiments started.

2.4 Analytical methods for water chemistry monitoring

Measuring and reporting of the carbonate system of the culture waters prior to, at the end and ideally during the experiments is very important to ensure constant conditions or reveal any possible shift. Therefore, water chemistry parameters such as water temperature, salinity and pH (NBS scale) were measured in the culture bottles at the start of the experiment and on every 3rd day of the experiments (Day 0, Day 3, Day 6 and Day 9) by using a pH meter (WTW Series 720) and a salinometer (WTW LF197-S). Calibration of the pH meter (2-point calibration) was done on a daily basis before measurements by using the technical buffer (TRACE, WTW-D-82362; Weilheim). Additionally, around 250 ml water samples from the culture bottles were collected in brown bottles during the time period 11 am – 1 pm for measuring total alkalinity (A_T). Bottles were filled in completely so that no air remains in the headspaces. If the analysis of the water samples were not done on the same day, samples in the black bottles were stored at 4°C for analysis within the next few days. Water sampling and storage of the samples for A_T measurements were done with care so that any mishandling did not compromise the measurements. However, sampling for A_T measurements is not critically influenced by changes in DIC or pH related to gas exchange (Riebesell et al. 2010). But care was taken so that there was no evaporation and the salinity remained the same in the samples. A_T was determined by potentiometric titration of the samples using an automated titration system (TW alpha plus, SI Analytics) controlled by a computer running the program TitriSoft 2.60 In this procedure, sea water samples are titrated with hydrochloric acid (HCl) and titration is monitored by reading the electro-magnetic force (e.m.f.) of a glass electrode. The detection of the titration end point from the titration results requires mathematical procedures and involves linearization of the titration curve by a Gran plot (Gran, 1952). We calculated total alkalinity (A_T) from the linear Gran Plots (Gran, 1952) prepared in Microsoft Office Excel 2007 from the titration data obtained directly from the computer connected with the automated titration system. The carbonate systems of the waters (concentrations of HCO_3^-, CO_3^{2-} , pCO_2 etc) were calculated using the program CO2SYS by Lewis and Wallace (1998). For the calculation, input parameters in this program were temperature, salinity, pH and total alkalinity (A_T).

2.5 Growth measurement

In order to estimate growth rate, fresh weight biomass (g) of the algae in each culture was measured at the beginning of the experiments, in regular intervals (on every 3rd day of the experiments) during the experiments and finally at the end of the experiments using a balance (Sartorius, LA310S; Germany). Before fresh weight determination surface moisture on the algae was removed by blotting gently on tissue paper. Growth rates were calculated as relative growth rate (RGR) which is expressed as percentage increase in fresh weight (FW) biomass per day (% d^{-1}).

The relative growth rate was determined according to the formula

$RGR = [(lnW_t - lnW_o)/t] \times 100$

where W_o represents the initial and W_t the final fresh weight of the algae, and t is the culture period in days.

2.6 Measurement of photosynthesis and dark respiration

Generally, photosynthesis and respiration of plants are measured by measuring oxygen fluxes (i.e. oxygen production during photosynthesis and uptake during respiration) in incubation. In our experiments, photosynthetic and respiration rates were measured as oxygen production and oxygen depletion respectively by using oxygen micro-optodes (fiber-optic oxygen meter, Microx TX3 with needle type oxygen microsensors of 140 µm tip diameter; PreSens, Germany). The whole setup was connected with a laptop computer and controlled by proprietary software (OxyView TX3- V6.20) (Fig. 2.5). This optical oxygen microsensor (optode) has very fast response time and does not require correction for consumption effects as it does not consume oxygen. It is the latest generation of equipment capable of measuring small scale fluxes of oxygen with high spatial resolution and ideally suited for the examination of very small sample volumes. In it, light of a specific wave length is conducted via fine glass fiber to the measuring tip. The tip contains fluorescent dye which fluoresces at a different wavelength to the exciting light. The intensity of fluorescence depends on the concentration of the substance being measured, i.e. oxygen. Before measurement, optodes were calibrated on a daily basis. Calibration was performed using a conventional two-point calibration in oxygen-free water (cal 0) and water-vapor saturated air (cal 100). Calibration solution 0 (oxygen-free water) was prepared by dissolving 1 g of sodium sulfite (Na_2SO_3) in 100 ml water in a vessel. Calibration standard 100 (water vapor-saturated air) was prepared by placing wet cotton wools in a vessel.

For the measurement, algal pieces were weighed and introduced into the incubation chamber (volume= 0.017 L) placed in a temperature controlled room and filled with filtered natural seawater which was magnetically stirred continuously during the whole measurement periods. At first, the incubation chamber was made completely dark with a black cover and dark respiration was measured for about 15 minutes. Then the photosynthetic rate was measured for 15-20 minutes at three irradiance levels: 10 µmol photons m^{-2} s^{-1} (suboptimal irradiance for growth, Fortes & Lüning 1980, Bird et al. 1979), 70 µmol photons m^{-2} s^{-1} (optimal irradiance for growth; Fortes & Lüning 1980, Bird et al. 1979) and 500 µmol photons m^{-2} s^{-1} (saturating irradiance for photosynthesis; Brechignac & André 1984, Mathieson & Burns 1971). Light intensities were adjusted by using black nets above the incubation chamber. For creating saturating irradiance halogen lamps (HALOPOINT-VARIO42358, 12V max. 50W; OSRAM, Germany) were used. Raw data of the measurements were obtained as ASCII text files which were subsequently transferred to the program Microsoft Office Excel 2007 and recalculated to get the photosynthetic and respiration rates in desired unit. Corrections for air pressure, salinity, temperature, and oxygen saturation were also done during calculation using a macro in Microsoft Excel. Net photosynthesis was obtained from the measured gross photosynthesis as net photosynthesis is equal to gross photosynthesis minus respiration. Water used for photosynthesis and respiration measurements was natural filtered seawater and not aerated or

CO_2 enriched. Photosynthesis was measured only in Experiment 1 and 2. Measurements were done before the start of the experiments (day 0), on the 4th and the 8th day of the experiments.

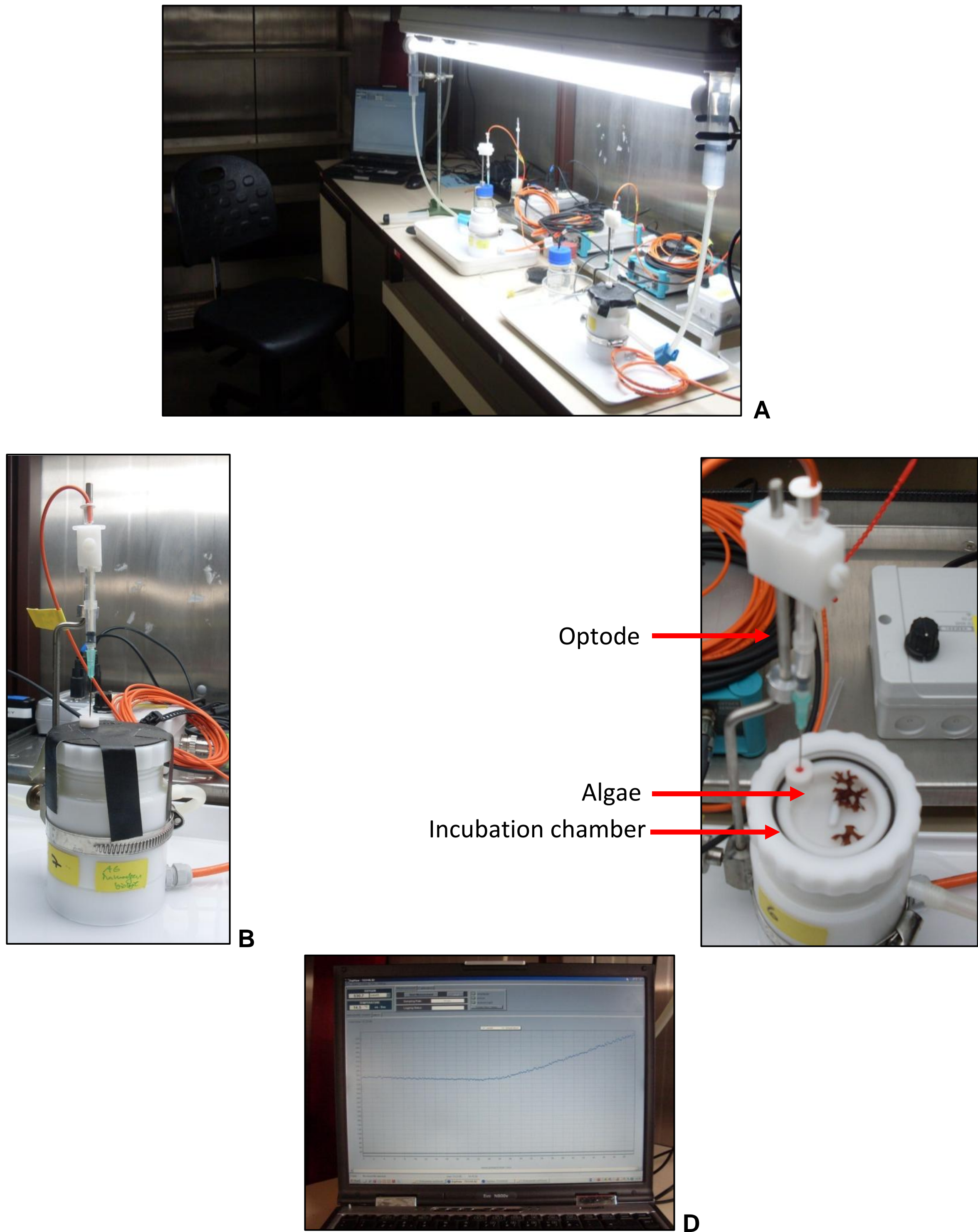


Figure 2.5 Measurement of photosynthesis and respiration by optode. A: The whole setup connected with a laptop, B: measuring respiration, C: measuring photosynthesis, D: graphical representation of the raw data in the laptop during measurement.

2.7 Determination of dry weight

At the end of the experiments, one of the two algal pieces cultured were weighed separately and then dried in the oven (Heraeus) at 60° C for 24 h. After drying, the samples were kept at normal temperature until they cool down and then stored in a desiccator to keep the samples dry. Finally, dry weights of the samples were measured using a balance (Sartorius, LA310S; Germany). Wet: dry biomass ratios were calculated from the wet (fresh) weight and dry weight of the algal samples.

2.8 Determination of C and N contents

For C and N content analysis, previously dried (at 60° C for 24 h) samples were finely pulverized with liquid nitrogen using mortars and pestles cleaned properly with acetone. Then, between 1.0 and 2.0 mg sub-samples weighed on a Sartorius super-micro balance were put into tin cups and assayed for total C and N with a CHN analyser (EuroVector, EuroEA 3000) calibrated with acetanilide. C and N values were expressed as percentage dry weight (% DW) and as C: N calculated from their values. C/N analysis was done with the help of the Eco-physiology research group at AWI.

2.9 Chl *a* analysis

Chlorophyll *a* (Chl *a*) was extracted from the sub-samples frozen with liquid nitrogen and kept in – 80^oC. Extraction was done for 5 days in 5ml 100% *N, N*- dimethylformamide (DMF) in darkness at 4 ^oC. Then the extinction of the DMF solution was measured spectrophotometrically at 664.5 nm against a DMF blank in a Spectrophotometer (U-3310; HITACHI, Japan). The Chl *a* content was calculated using the formula: Chl *a* (mg/g FW) = 12.7 $E_{664.5}$ after Inskeep and Bloom (1985).

2.10 Data treatments and statistical analysis

Mean values and standard deviations were calculated from the different replicates per treatments using the program Microsoft Office Excel 2007. Data were tested for homogeneity of variance (Levene’s test) before performing 2 factorial ANOVA. Statistical significance of the data with homogenous variances was tested using analysis of variance (ANOVA). In case of parameters growth, wet: dry biomass ratios, C/N contents, C: N ratios and chl *a*, Factorial ANOVA was performed. Photosynthetic rates and respiration rates were analyzed with Repeated Measures ANOVA (RMANOVA) with Tukey’s HSD *post hoc* test. Corresponding transformations were done to the heteroskedastic (differing variance) and non-normal data before performing ANOVA. Differences were considered to be statistically significant if the p value was ≤ 0.05 (5% significance level). The program STATISTICA 7.0 (StatSoft. Inc. USA) was used for statistical analysis.

3. RESULTS

3.1 Growth response

The relative growth rate (RGR) of *Chondrus crispus* based on fresh biomass was positive in all the experiments performed. In Experiment 1, growth in different starting algal biomasses and CO_2 concentrations but constant temperature and irradiance was compared (Fig. 3.1), the mean growth rates varied from 3.93 ±0.65 to 6.47±0.71 % day $^{-1}$. In general, the different start biomasses had no obvious effect on growth rate with respect to CO_2 concentration ($F = 0.844$, $p>0.44$), but the overall pooled growth rate was significantly affected by CO_2 concentration ($F = 29.13$, $p<0.0001$) with higher growth rates in 700 ppm CO_2 concentration than in 280 ppm (Fig. 3.1 b). The intermediate start biomass also showed a significant increase of RGR in elevated CO_2 concentrations (700 ppm).

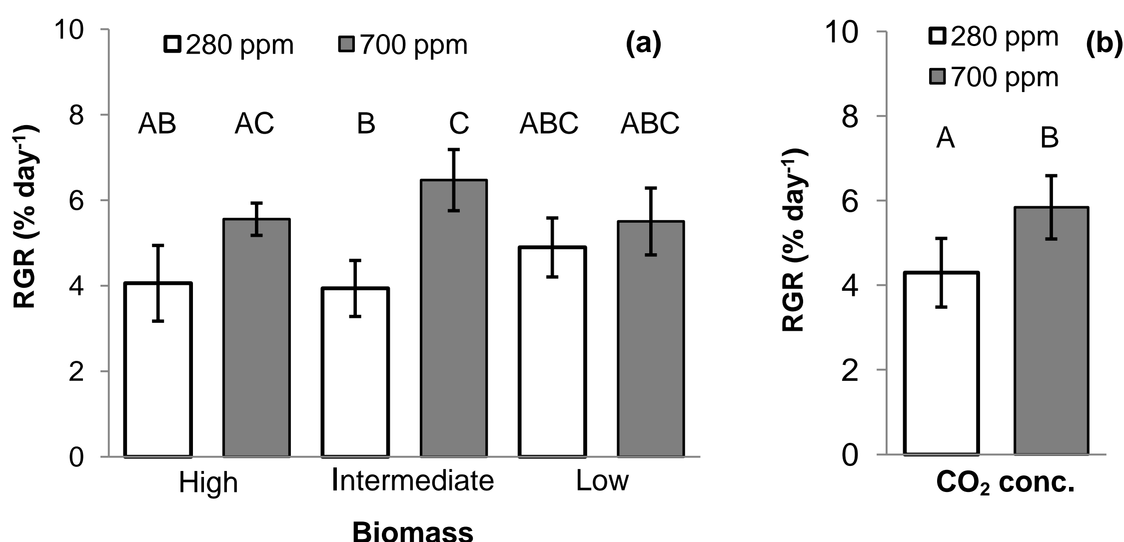


Figure 3.1 Relative growth rate (RGR) of *Chondrus crispus* after 7 days under 280 ppm and 700 ppm CO_2 concentrations in Seawater and 70 μmol photons m^{-2} s^{-1} irradiance (optimal for growth) at 15 °C (optimal for growth). High, intermediate and low biomasses refer to significantly different ($p<0.001$) starting biomasses of 116.8 ± 5.1, 47.2 ± 1.8 and 20.6 ± 1.1 mg respectively. Data are means ± SD (n=4). Graph (b) is formed by pooling all the RGR data from different biomasses. Different letters on graphs refer to significant differences between values ($p<0.05$).

In Experiment 2, effect of cultivating irradiance and CO_2 concentration on the growth rate of *Chondrus crispus* was investigated. Here, the mean growth rates in different treatments varied from 2.20 ± 0.55 to 8.17 ± 0.32 % day $^{-1}$ (Fig. 3.2). As expected, growth rate was significantly higher ($F = 503.96$, $p<0.0001$) in optimal irradiance for growth (70 μmol photons m^{-2} s^{-1}) than in minimal irradiance (10 μmol photons m^{-2} s^{-1}), but no effect of CO_2 concentration became visible in both irradiance conditions ($F = 2.77$, $p>0.12$). Also there was no interactive effect of CO_2 and light intensity ($F = 1.22$, $p>0.29$) on growth.

When growth in different temperatures and CO_2 concentrations but constant optimal irradiance was compared (Fig. 3.3) both CO_2 and temperature showed significant positive and negative effects on growth, respectively ($F = 17.47$, $p<0.0007$and $F = 5.52$, $p<0.03$ respectively). Here, mean growth rates varied from 6.33 ± 0.38 to 8.19 ± 0.28 % day^{-1} in the different treatments. An overall significantly higher growth rate was observed in the 700 ppm CO_2 concentration compared to the 280 ppm CO_2 concentration when all data were pooled, but also in 24°C

alone (Fig. 3.3). As expected growth rate was significantly lower in the elevated temperature (24 °C) compared to the optimal temperature (15 °C). No interactive effect of CO_2 and temperature was found in the treatments (F = 3.42, p>0.08).

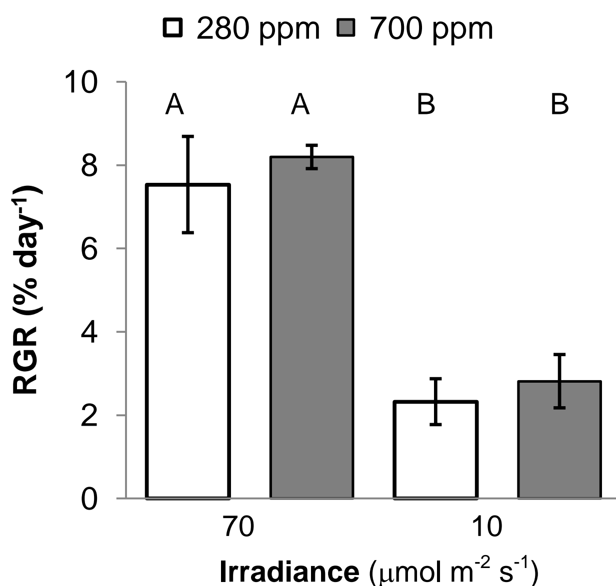


Figure 3.2 Relative growth rate (RGR) of *Chondrus crispus* after 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and 70 and 10 μmol photons m^{-2} s^{-1} irradiance (optimal and minimal for growth respectively) at 15 °C temperature (optimal for growth). Data are means ± SD (n=4). Different letters on graphs refer to significant differences between treatments (p<0.05).

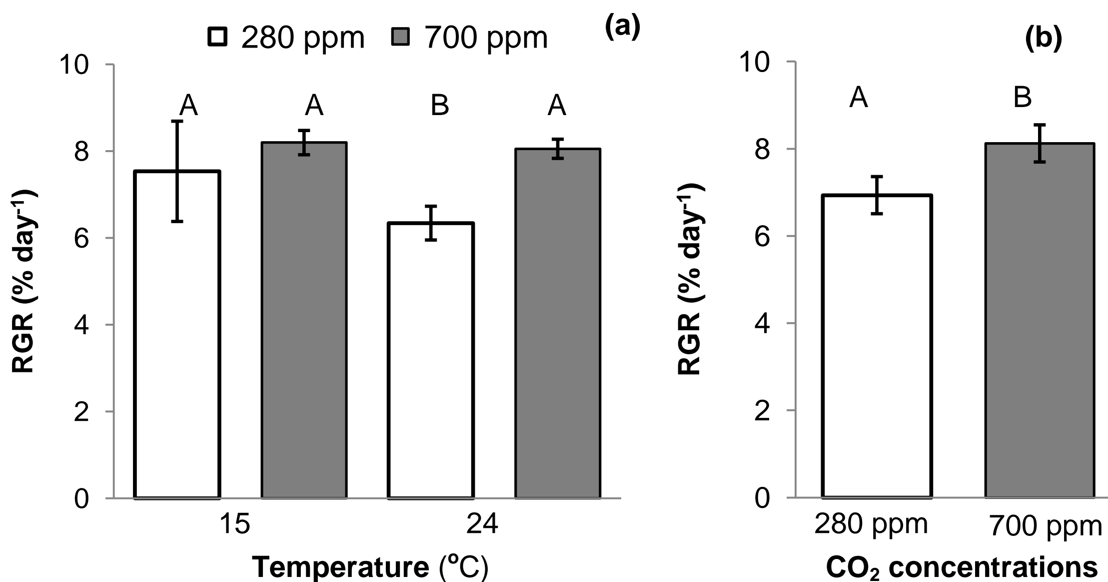


Figure 3.3 Graph (a): Relative growth rate (RGR) of *Chondrus crispus* after 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and 15 °C and 24 °C (optimal and elevated for growth) at optimal irradiance of 70 μmol photons m^{-2} s^{-1}. Data are means ± SD (n=5). Graph (b) is formed by pooling all the RGR data from the two CO_2 treatments. Different letters on graphs refer to significant differences between treatments (p<0.05).

3.2 Net Photosynthesis

Net photosynthetic rates of *Chondrus crispus* cultured in different irradiances and CO_2 concentrations (Experiment 2) are shown in Figure 3.4. Photosynthesis was measured at low, high and saturating irradiances (10, 70 and 500 μmol photons m^{-2} s^{-1}respectively). The mean net photosynthetic rates measured at low, high and saturating irradiances varied from 8.48 ± 2.50 to 19.27 ± 6.97, 36.74 ± 3.55 to 49.95 ± 5.94 and 63.44 ± 7.62 to 75.50 ± 8.79 μmol O_2 g^{-1} FW h^{-1}, respectively.

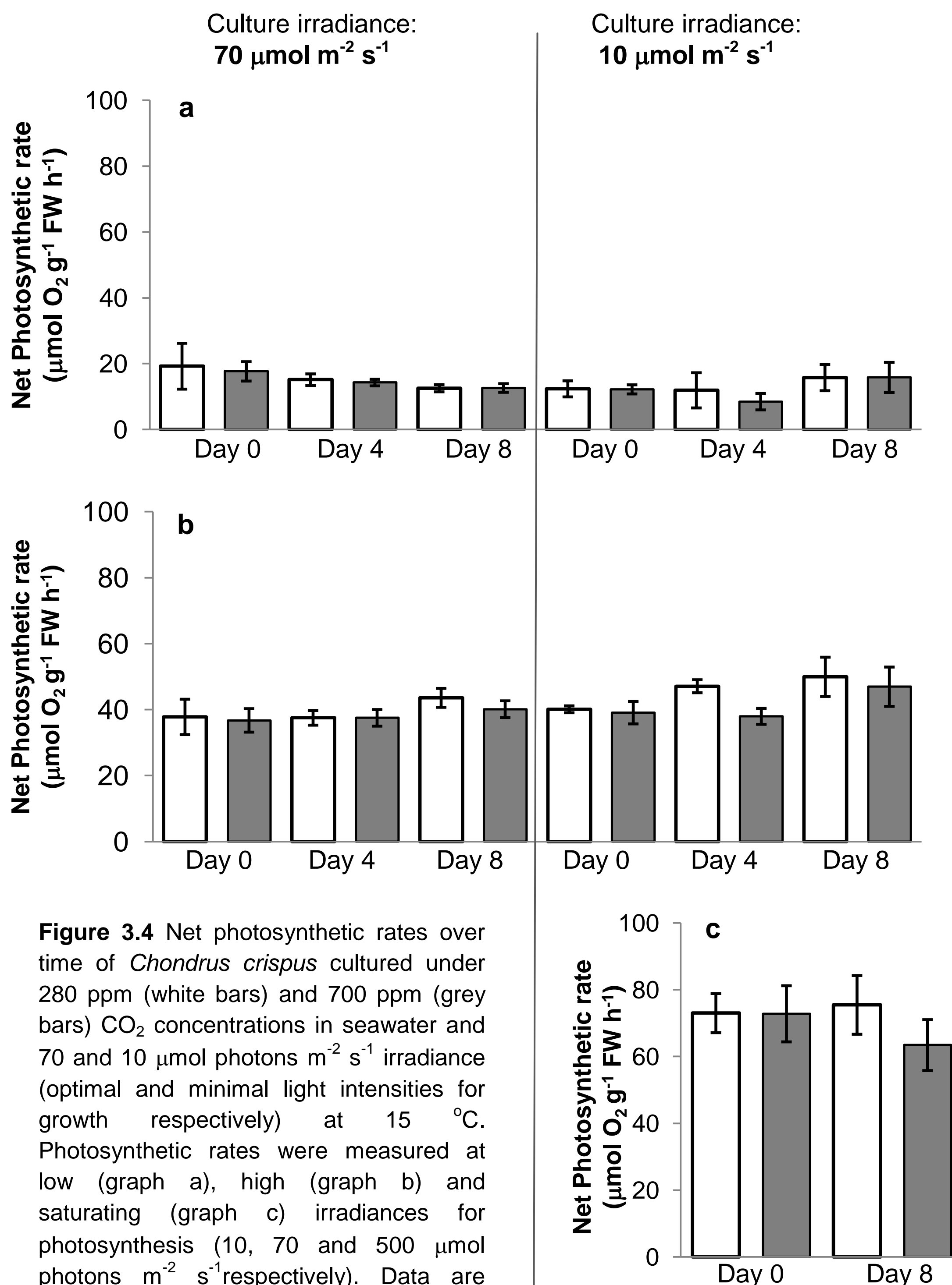


Figure 3.4 Net photosynthetic rates over time of *Chondrus crispus* cultured under 280 ppm (white bars) and 700 ppm (grey bars) CO_2 concentrations in seawater and 70 and 10 μmol photons m^{-2} s^{-1} irradiance (optimal and minimal light intensities for growth respectively) at 15 ^{o}C. Photosynthetic rates were measured at low (graph a), high (graph b) and saturating (graph c) irradiances for photosynthesis (10, 70 and 500 μmol photons m^{-2} s^{-1}respectively). Data are means ± SD (n=5). For statistical analysis refer to Table 3.1.

CO_2 concentrations and measuring light significantly affected net photosynthesis. In contrast to growth rates (Fig. 3.2), net photosynthetic rates were significantly lower in the high CO_2 concentration (700 ppm) than in the low CO_2 concentration (280 ppm) (F =10.25, p<0.0031). As expected high measuring irradiance significantly increased net photosynthesis compared to the low measuring irradiance (F = 22159.47, p<0.0001). Net photosynthesis was also significantly affected by time (F =133.20, p<0.0002) showing higher rates on day 8 compared to day 0 and day 4. However, cultivating irradiance did not affect net photosynthetic rates significantly (F =3.07, p>0.003). Net photosynthetic rates measured at saturating irradiance (500 μmol photons m^{-2} s^{-1}) were analyzed separately. No effect of CO_2 concentration and time was observed here (F =2.10, p>0.18; F =1.94, p>0.20)

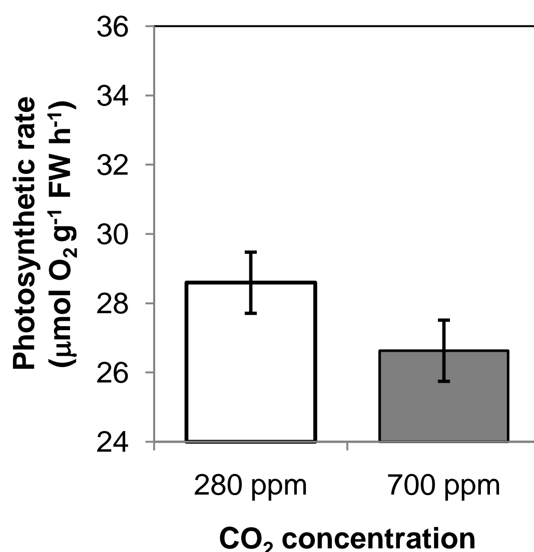


Figure 3.5 Net photosynthetic rates of *Chondrus crispus* cultured under 280 ppm and 700 ppm CO_2 concentrations in seawater and 70 and 10 μmol photons m^{-2} s^{-1} irradiance (optimal and minimal light intensities for growth respectively) at 15 °C. This graph shows pooled data of 280 ppm and 700 ppm CO_2 concentration conditions from all treatments showing the overall effect of CO_2 concentration on net photosynthetic rates (measured in low PAR and high PAR). See Table 3.1 for statistical analysis.

Table 3.1 Repeated measurement (RM)-ANOVA. Effects of CO_2 concentrations (280 ppm versus 700 ppm), culture light intensities (10 versus 70 μmol photons m^{-2} s^{-1}) and measuring lights (low PAR i.e. 10 μmol photons m^{-2} s^{-1} versus high PAR i.e. 70 μmol photons m^{-2} s^{-1}) on net photosynthetic rates in Experiment 2.

Variable	**Source**	**df**	**MS**	**F**	**p**
Net photosynthetic rates[1]	(1) CO_2 concentrations	1	115.75	10.250	**0.003084***
	(2) Culture light	1	34.75	3.077	0.088986
	(3) Measuring light	1	22159.47	1962.320	**0.000000***
	CO_2 concentrations x Culture light	1	20.28	1.795	0.189701
	CO_2 concentrations x Measuring light	1	28.81	2.551	0.120024
	Culture light x Measuring light	1	381.88	33.817	**0.000002***
	CO_2 concentrations x Culture light x Measuring light	1	11.66	1.033	0.317182
	Error	32	11.29		
	(4) Time	2	133.20	9.969	**0.000170***
	Time x CO_2 concentrations	2	15.53	1.162	0.319261
	Time x Culture light	2	121.70	9.108	**0.000330***
	Time x Measuring light	2	157.12	11.759	**0.000045***
	Time x CO_2 concentration x Culture light	2	33.57	2.512	0.089041
	Time x CO_2 concentration x Measuring light	2	6.45	0.483	0.619165
	Time x Culture light x Measuring light	2	27.08	2.027	0.140105
	4 x1 x 2 x 3	2	8.11	0.607	0.548015
	Error	64	13.36		
Net photosynthetic rates[2]	CO_2 concentrations	1	188.4	2.109	0.184464
	Error	8	89.3		
	Time	1	59.9	1.940	0.201143
	Time x CO_2 concentrations	1	175.4	5.682	**0.044295***
	Error	8	30.9		

1: Net photosynthetic rates measured at low PAR & high PAR; 2: Net photosynthetic rates measured at saturating PAR; Asterisk (*) indicates significant.

In Figure 3.6, net photosynthetic rates of *Chondrus crispus* in relation to different CO_2 and temperature treatments are presented. Photosynthetic rates were measured at low, high and saturating irradiance for photosynthesis of *C. crispus* (10, 70 and 500 μmol photons m^{-2} s^{-1}respectively) as in Experiment 2. Mean net photosynthesis measured at low irradiance, high irradiance and saturating irradiance varied from 12.58 ± 1.10 to 24.58 ± 1.71, 36.74 ± 3.55 to 66.42 ± 3.92 and 61.07 ± 5.08 to 66.37 ± 5.95 μmol O_2 g^{-1} FW h^{-1}, respectively. CO_2 concentration and culture temperature were found to significantly affect the net photosynthetic rates negatively and positively, respectively. As in experiment 2, significantly lower photosynthetic rate were observed in high CO_2 concentrations (700 ppm) compared to low CO_2 concentrations (280 ppm) (F =4.52, $p<0.042$) again contrasting the positive growth effects in high CO_2 shown above in Fig 3.3. Although growth rate was lower in 24°C than in 15°C, net photosynthesis was significantly higher in elevated temperatures (24 °C) compared to optimal growth temperatures (15 °C) (F = 204.98, $p<0.0001$). As expected net photosynthesis was also significantly affected by measuring irradiance (F =1421.88, $p<0.0001$) showing higher photosynthetic rates in the high irradiance compared to the low irradiance. However, unlike Experiment 2, net photosynthetic rates were found to be negatively affected by time (F =16.43, $p<0.0001$) with significantly lowered rates on day 8 compared to day 0 and day 4. Net photosynthetic rates measured at saturating irradiance (500 μmol photons m^{-2} s^{-1}) were analyzed separately again and in this case, no effect of CO_2 concentration and time was observed (F =0.02, $p>0.87$; F =1.66, $p>0.23$).

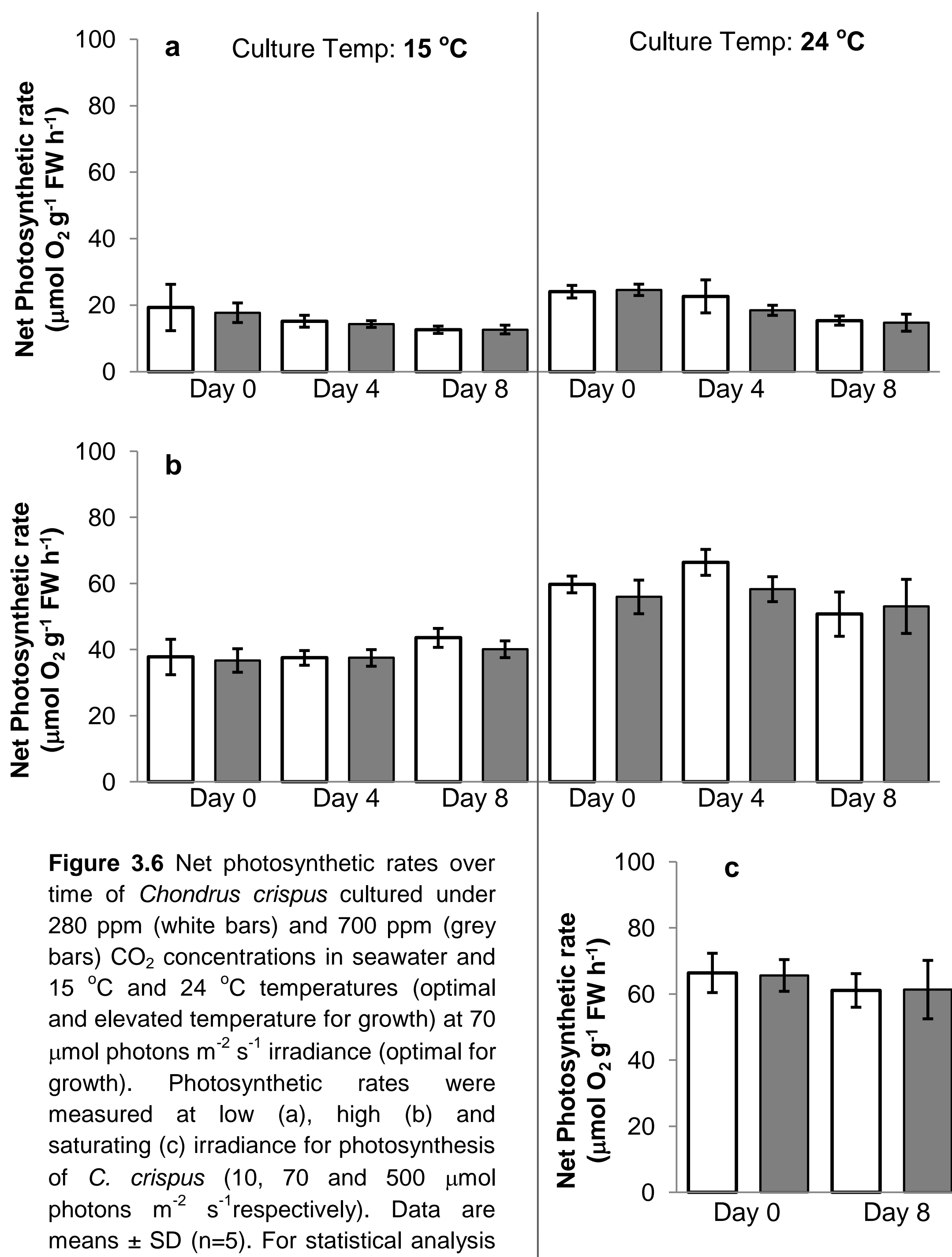


Figure 3.6 Net photosynthetic rates over time of *Chondrus crispus* cultured under 280 ppm (white bars) and 700 ppm (grey bars) CO_2 concentrations in seawater and 15 ^{o}C and 24 ^{o}C temperatures (optimal and elevated temperature for growth) at 70 µmol photons m^{-2} s^{-1} irradiance (optimal for growth). Photosynthetic rates were measured at low (a), high (b) and saturating (c) irradiance for photosynthesis of *C. crispus* (10, 70 and 500 µmol photons m^{-2} s^{-1}respectively). Data are means ± SD (n=5). For statistical analysis refer to Table 3.2.

Table 3.2 Repeated measurements ANOVA. Effects of CO_2 concentrations (280 ppm versus 700 ppm), culture temperatures (15 versus 24 °C) and measuring irradiance (low irradiance i.e. 10 μmol photons m^{-2} s^{-1} versus high irradiance i.e. 70 μmol photons m^{-2} s^{-1}) on net photosynthetic rates.

Variable	Source	df	MS	F	p
Net photosynthetic rates[1]	(1) CO_2 concentrations	1	89.0	4.526	**0.041188***
	(2) Culture temperature	1	4029.9	204.989	**0.000000***
	(3) Measuring light	1	27952.7	1421.881	**0.000000***
	CO_2 concentrations x Culture temperature	1	10.1	0.513	0.478905
	CO_2 concentrations x Measuring light	1	11.4	0.580	0.452023
	Culture temperature x Measuring light	1	1434.3	72.958	**0.000000***
	CO_2 concentrations x Culture temperature x Measuring light	1	2.0	0.103	0.750392
	Error	32	19.7		
	(4) Time	2	195.1	16.432	**0.000002***
	Time x CO_2 concentrations	2	21.2	1.782	0.176584
	Time x Culture temperature	2	226.1	19.045	**0.000000***
	Time x Measuring light	2	143.9	12.123	**0.000034***
	Time x CO_2 concentration x Culture temperature	2	44.2	3.719	**0.029647***
	Time x CO_2 concentration x Measuring light	2	1.9	0.157	0.854799
	Time x Culture temperature x Measuring light	2	82.4	6.942	**0.001868***
	4 x1 x 2 x 3	2	26.6	2.239	0.114885
	Error	64	11.9		
Net photosynthetic rates[2]	CO_2 concentrations	1	0.32	0.026	0.875075
	Error	8	12.05		
	Time	1	114.69	1.660	0.233637
	Time x CO_2 concentrations	1	1.30	0.019	0.894477
	Error	8	69.10		

1: Net photosynthetic rates measured at low PAR & high PAR; 2: Net photosynthetic rates measured at saturating PAR; Asterisk (*) indicates significant.

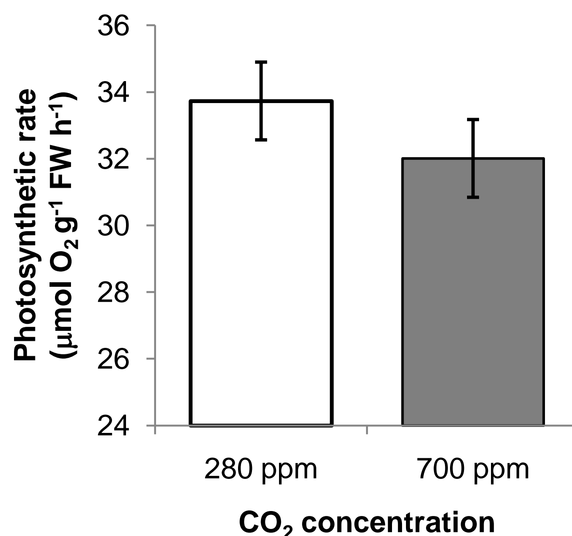


Figure 3.7 Overall net photosynthetic rates of *Chondrus crispus* cultured under 280 ppm and 700 ppm CO_2 concentrations in seawater and 15 ^{o}C and 24 ^{o}C temperatures (optimal and elevated temperature for growth) at 70 μmol photons m^{-2} s^{-1} irradiance (optimal for growth). This graph exhibits pooled data of the 280 ppm and 700 ppm CO_2 concentration to show the overall effect of CO_2 concentration on net photosynthetic rates (measured in low and high irradiance). See Table 3.2 for statistical analysis.

3.3 Dark respiration

Dark respiration rates of *Chondrus crispus* in different irradiance and CO_2 conditions (Experiment 2) are presented in Figure 3.8. Mean dark respiration rates varied from -3.61 ± 0.78 to 2.21 ± 1.49 μmol O_2 g^{-1} FW h^{-1}. Unlike photosynthesis, no significant effect of CO_2 concentration on respiration was found (F =0.001, p>0.99). Culture light intensity however had a significant positive effect on respiration rates (F =195.29, p<0.0001) showing higher respiration rates in the algae grown in high irradiance (70 μmol photons m^{-2} s^{-1}) compared to the algae grown in low irradiance (10 μmol photons m^{-2} s^{-1}). The respiration rates did not differ significantly with time (F =0.02, p>0.86).

In Figure 3.9, dark respiration rates in different temperature and CO_2 conditions are shown. The mean respiration rates varied from -2.86 ± 0.75 to 10.46 ± 2.37 μmol O_2 g^{-1} FW h^{-1}. Unlike photosynthesis, CO_2 concentration had no significant effect on respiration (F =0.06, p>0.79) as in Experiment 2. Temperature significantly affected respiration rates (F =171.19, p<0.0001) showing about 173% increase of respiration rates in 24 ^{o}C compared to 15 ^{o}C. Respiration rates did not significantly differ with time here also (F =3.71, p>0.07) as in Experiment 2.

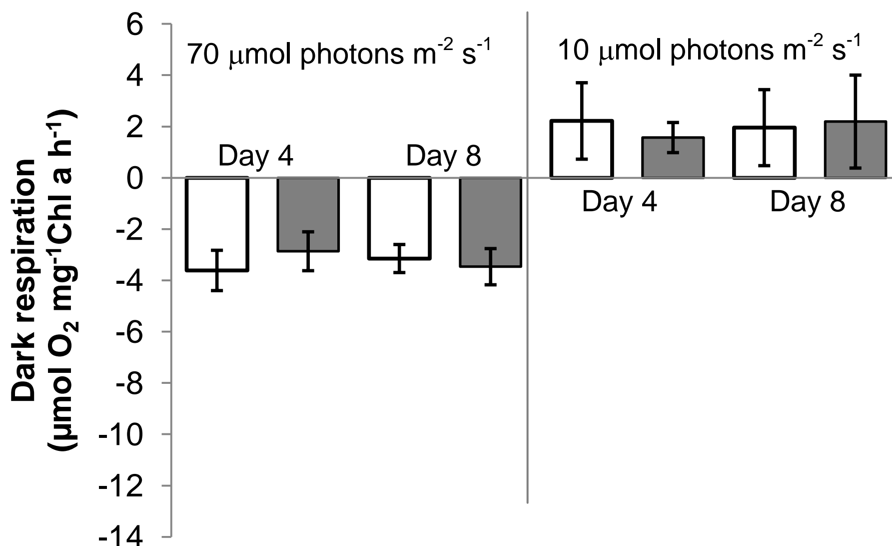


Figure 3.8 Dark respiration rates over time of *Chondrus crispus* cultured under 280 ppm (white bars) and 700 ppm (grey bars) CO_2 concentrations in seawater and irradiances of 70 and 10 μmol photons m^{-2} s^{-1} (optimal and minimal for growth respectively) at 15 ^{o}C. Data are means ± SD (n=5). For statistical analysis refer to Table 3.3.

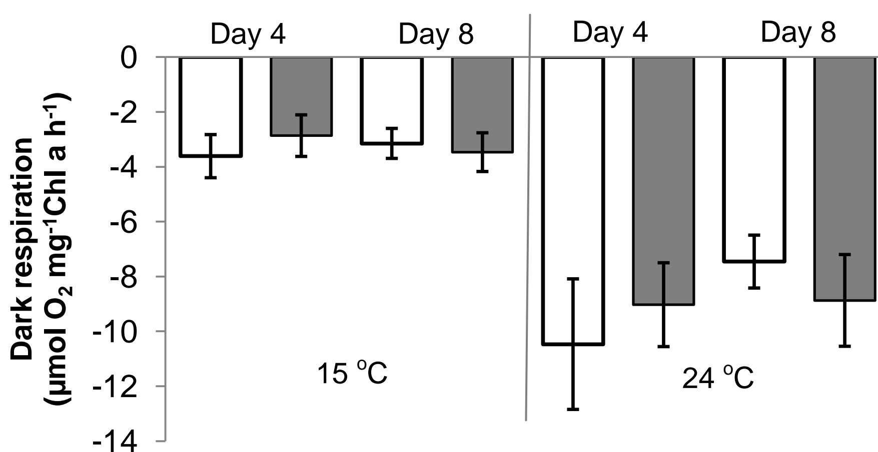


Figure 3.9 Dark respiration rates over time of *Chondrus crispus* cultured under 280 ppm (white bars) and 700 ppm (grey bars) CO_2 concentrations in seawater and 15 ^{o}C and 24 ^{o}C (optimal and elevated temperature for growth) at an irradiance of 70 μmol photons m^{-2} s^{-1} (optimal for growth). Data are means ± SD (n=5). For statistical analysis refer to Table 3.4

Table 3.3 RM-ANOVA. Effects of CO_2 concentrations (280 ppm versus 700 ppm) and culture light intensities (10 versus 70 μmol photons m^{-2} s^{-1}) on dark respiration rates in Experiment 1.

Variable	Source	df	MS	F	p
Dark respiration rates	CO_2 concentrations	1	0.0002	0.0002	0.990078
	Culture light	1	276.1765	**195.2970**	**0.000000***
	CO_2 concentrations x Culture light	1	0.4408	0.3117	0.584366
	Error	16	1.4141		
	Time	1	0.0313	0.0286	0.867801
	Time x CO_2 concentrations	1	0.0207	0.0190	0.892191
	Time x Culture light	1	0.1559	0.1425	0.710803
	Time x CO_2 concentration x Culture light	1	2.3771	2.1725	0.159897
	Error	16	1.0941		

Asterisk (*) indicates significant.

Table 3.4 RM-ANOVA. Effects of CO_2 concentrations (280 ppm versus 700 ppm) and culture temperatures (15 versus 24 ^oC) on dark respiration rates in CO_2 versus temperature treatments.

Variable	Source	df	MS	F	p
Dark respiration rates	CO_2 concentrations	1	0.128	0.0680	0.797646
	Culture temperature	1	322.772	171.1992	**0.000000***
	CO_2 concentrations x Culture temperature	1	0.103	0.0546	0.818136
	Error	16	1.885		
	Time	1	5.727	3.7178	0.071774
	Time x CO_2 concentrations	1	9.598	6.2303	**0.023862***
	Time x Culture temperature	1	6.818	4.4256	0.051574
	Time x CO_2 concentration x Culture temp.	1	1.995	1.2947	0.271940
	Error	16	1.541		

Asterisk (*) indicates significant.

3.4 Biochemical parameters

3.4.1 Wet: dry biomass ratio

Wet to dry biomass ratios of *Chondrus crispus* grown in different irradiance and CO_2 concentrations (Experiment 2) are presented in the Figure 3.10 Here wet to dry biomass ratios varied from 4.92± 0.11 to 5.24 ± 0.29. Algae grown at high CO_2 concentrations (700 ppm) had significantly higher dry matter than those grown at low CO_2 concentrations (280 ppm) (F =6.51, p<0.022) irrespective of the amount of available irradiance during culture (Fig. 3.10; F = 1.56, p>0.22). No interactive effect of CO_2 concentration and irradiance was observed (F = 1.88, p>0.18).

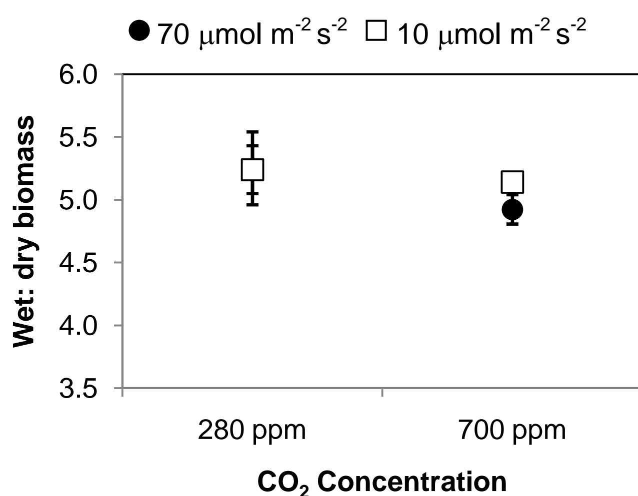


Figure 3.10 Wet to dry biomass ratios of *Chondrus crispus* cultured for 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and irradiances of 70 and 10 μmol photons m^{-2} s^{-1} (optimal and minimal for growth respectively) at 15 ^oC. Data are means ± SD (n=5).

Wet to dry biomass ratios of *C. crispus* grown in different temperatures and CO_2 concentrations varied from 4.10± 0.12 to 5.24± 0.29. Both, CO_2 concentration and temperature had significant positive effects on the final wet to dry biomass ratios of *Chondrus crispus* (F =7.28, p<0.016 and F = 111.36, p<0.0001 respectively). The ratios were significantly higher at optimal temperatures (15 ^oC) than at elevated temperatures (24 ^oC) and also higher at low CO_2 concentrations (280 ppm) compared to high CO_2 concentrations (700 ppm). This means that dry matter content was higher in the algae grown at elevated CO_2 concentrations (700 ppm) and temperatures (24 ^oC) (Fig. 3.11).

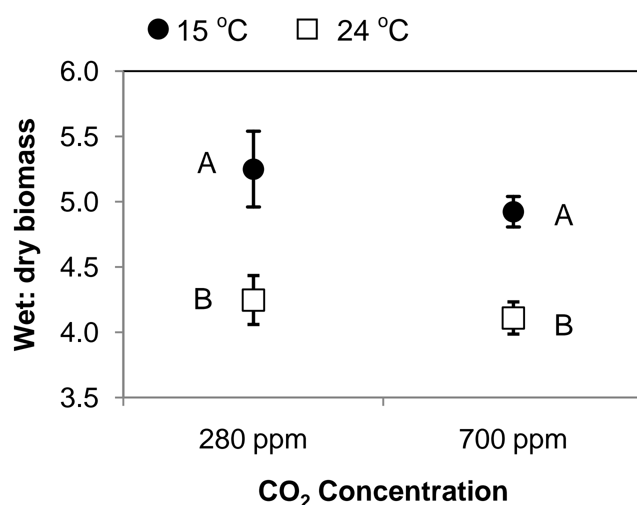


Figure 3.11 Wet to dry biomass ratios of *Chondrus crispus* cultured for 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and 15 ^oC and 24 ^oC temperatures (optimal and elevated for growth respectively) at an irradiance of 70 μmol photons m^{-2} s^{-1} (optimal for growth). Data are means ± SD (n=5). Different letters on graphs refer to significant differences between treatments (p<0.05).

3.4.2 Carbon (C) and Nitrogen (N) contents

Carbon (C) and nitrogen (N) contents of *Chondrus crispus* at the end of experiments are shown in Figure 3.12 and 3.13 In *C. crispus* grown in different irradiances and CO_2 concentrations, mean %C and %N contents of dry biomass varied from 26.00 ± 2.63 and 2.70 ± 0.14 to 28.37 ± 1.32 and 3.19 ± 0.14, respectively (Fig. 3.12). The C contents were not significantly affected by both CO_2 concentration and light intensity (F =0.36, p>0.55 and F =0.59, p>0.45 respectively) in all combinations of CO_2 and light intensity (Fig. 3.12a) while N content was significantly affected by light intensity (F =0.36, p<0.0038) but not by CO_2 concentration (F =1.24, p>0.28) (Fig. 3.12b). Higher N content was present in minimal light intensities (10 μmol photons m^{-2} s^{-2}) compared to optimal light intensities (70 μmol photons m^{-2} s^{-2}) irrespective of the CO_2 concentrations.

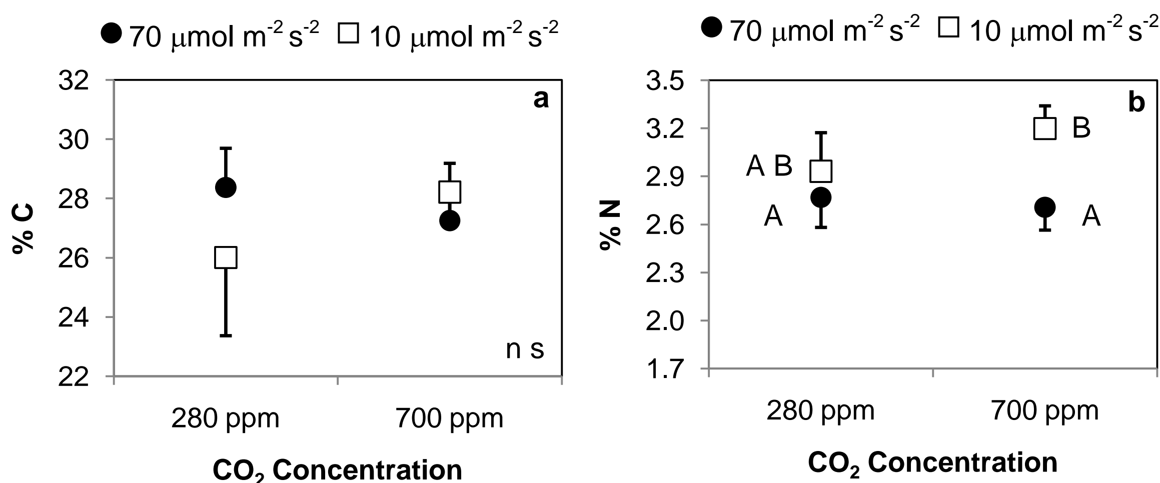


Figure 3.12 Percentage C (a) and N (b) contents of *Chondrus crispus* cultured for 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and irradiances of 70 and 10 μmol photons m^{-2} s^{-1}, (optimal and minimal light intensities for growth respectively) at 15 °C. Data are means ± SD (n=4). Different letters on graphs refer to significant differences between values (p<0.05); ns: not significant.

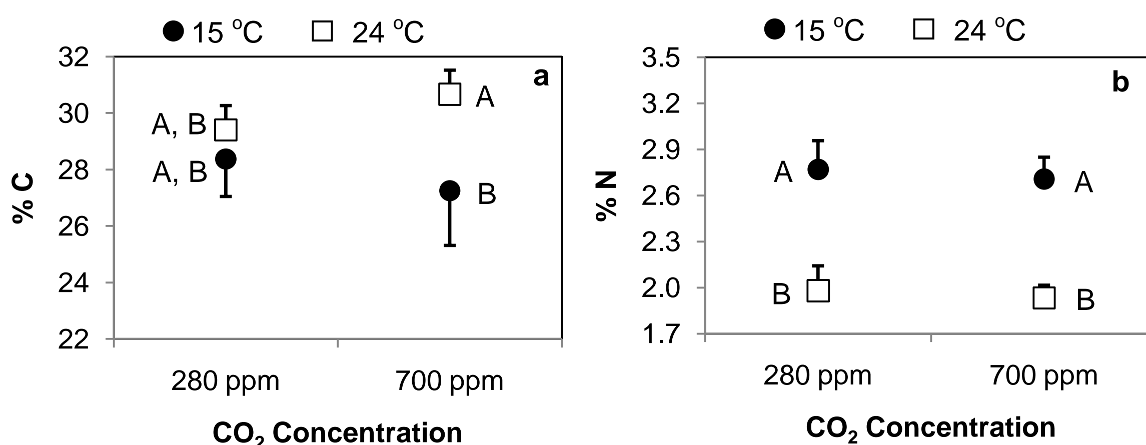


Figure 3.13 Percentage C and N contents of *Chondrus crispus* cultured for 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and 15 °C and 24 °C (optimal and elevated for growth respectively) at an irradiance of 70 μmol photons m^{-2} s^{-1} (optimal for growth). Data are means ± SD (n=4). Different letters on graphs refer to significant differences between values (p<0.05).

In the different CO_2 and temperature combinations (Fig. 3.14), mean %C and %N contents of dry biomass varied from 27.25 ± 1.93 and 1.93 ± 0.08 to 30.66 ± 0.85 and 2.76 ± 0.18, respectively. Both C (Fig. 3.14a) and N (Fig. 3.14b) contents were significantly affected by temperature (F=11.32, p<0.006 and F =110.99, p<0.0001 respectively) but not by CO_2 concentration (F =0.01, p>0.91 and F =0.53, p>0.47). The C content only increased slightly while the N content decreased by approx. 40% in 24 °C compared to 15 °C.

C:N ratios from the different experimental treatments are presented in Figure 3.14 and 3.15. In CO_2 versus light intensity treatments and CO_2 versus temperature treatments, C: N ratios varied from 10.29 ± 0.17 to 11.97 ± 0.48 and 11.73 ± 0.26 to 18.53 ± 0.80 respectively. C:N ratio was significantly affected by culture irradiance (F =91.41, p<0.0001) but CO_2 concentration (F =0.73, p>0.41) and the interaction of CO_2 and light intensity (F =0.37, p>0.55) had no effect. Thus, there was a higher C:N ratio in optimal light intensity for growth (70 µmol photons m^{-2} s^{-2}) compared to the minimal light intensity (10 µmol photons m^{-2} s^{-2}) irrespective of the CO_2 concentrations (Fig. 3.14). Similarly, the C:N ratio was not significantly affected by CO_2 concentrations (F =0.01, p>0.91) in the CO_2 vs temperature treatments (Fig. 3.15). However, temperature itself affected the C:N ratio significantly (F =195.29, p<0.0001) showing a higher C:N ratio in 24 °C compared to 15 °C. There was no interaction of CO_2 and temperature (F =2.38, p>0.148) in the treatments.

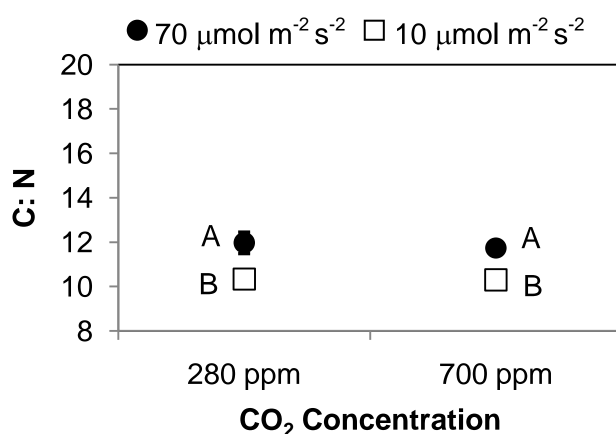


Figure 3.14 C:N ratios of *Chondrus crispus* cultured for 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and irradiances of 70 and 10 µmol photons m^{-2} s^{-1} (optimal and minimal for growth respectively) at 15 °C. Data are means ± SD (n=4). Different letters on graphs refer to significant differences between treatments (p<0.05).

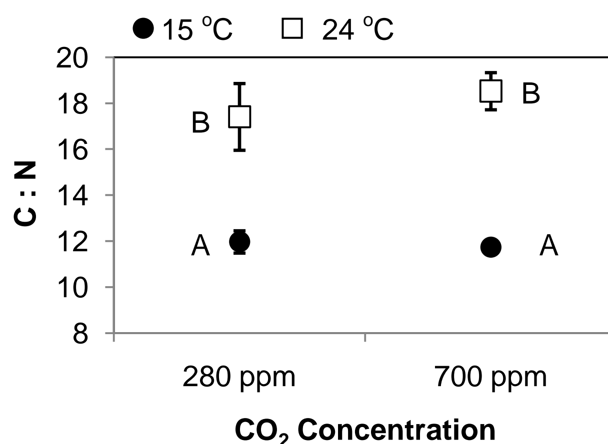


Figure 3.15 C:N ratios of *Chondrus crispus* cultured for 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and 15 °C and 24 °C temperatures (optimal and elevated for growth respectively) at an irradiance of 70 µmol photons m^{-2} s^{-1} (optimal for growth). Data are means ± SD (n=4). Different letters on graphs refer to significant differences between treatments (p<0.05).

3.4.3 Chl a contents

Chl *a* contents in the different experimental treatments are shown in Figure 3.16 and 3.17. The mean Chl *a* contents varied from 0.20 ± 0.08 to 0.29 ± 0.02 mg g^{-1} FW in the CO_2 vs light intensity treatments (Fig. 3.16) while it varied from 0.15 ± 0.01 to 0.22 ± 0.08 mg g^{-1} FW in the CO_2 vs temperature treatments (Fig. 3.17). In low irradiance (10 μmol photons m^{-2} s^{-1}) and 15 °C the Chl *a* content was significantly higher at low CO_2 concentrations (280 ppm) than at high CO_2 concentrations (700 ppm) (F =7.39, $p<0.027$) corresponding to the results of the net photosynthetic rate (see chapter 3.2). This CO_2 effect was not present at optimal irradiance (70 μmol photons m^{-2} s^{-1}) in 15 °C (F =0.259, $p>0.62$) (Fig. 3.16) but in 24 °C: here the Chl *a* content (F =10.83, $p<0.011$) was again significantly higher at low CO_2 concentrations (280 ppm) than at high CO_2 concentration (700 ppm) (Fig. 3.17).

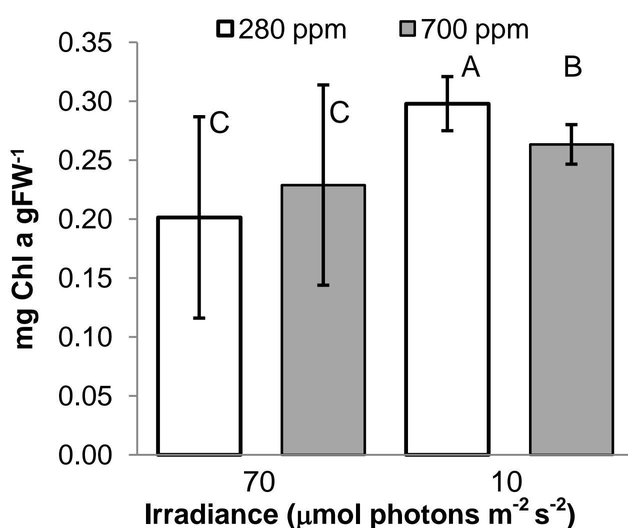


Figure 3.16 Chlorophyll *a* contents of *Chondrus crispus* cultured for 9 days culture under 280 ppm and 700 ppm CO_2 concentrations in seawater and irradiances of 70 and 10 μmol photons m^{-2} s^{-1} (optimal and minimal for growth respectively) at 15 °C (optimal for growth). Data are means ± SD (n=5). Different letters on graphs refer to significant differences between treatments ($p<0.05$).

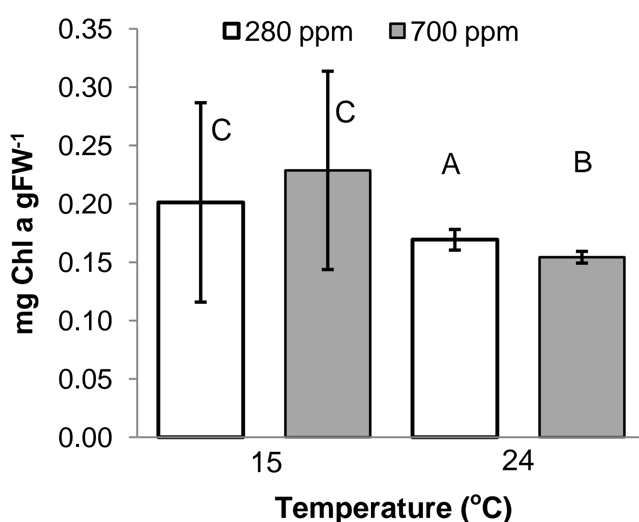


Figure 3.17 Chlorophyll *a* contents of *Chondrus crispus* cultured for 9 days under 280 ppm and 700 ppm CO_2 concentrations in seawater and 15 °C and 24 °C temperatures (optimal and elevated for growth) at an irradiance of 70 μmol photons m^{-2} s^{-1} light intensity (optimal for growth). Data are means ± SD (n=5). Different letters on graphs refer to significant differences between treatments ($p<0.05$).

3.5 Water Chemistry

Statistical analysis of the water chemistry data was not performed because many data sets were not with homogenous variances even after trying with different types of transformations. Therefore, the data are presented by graphs to show their general trends.

3.5.1 Salinity

In Figure 3.18, initial (day 0) and final salinities (day 7) of the culture water from the biomass experiment (Experiment 1) are shown. There was much fluctuation in salinity in this experiment ranging from initial values of 32.4 ± 0.2 to 33.0 ± 0.7 to final salinities of 34.0 ± 0.4 to 35.7 ± 0.7.

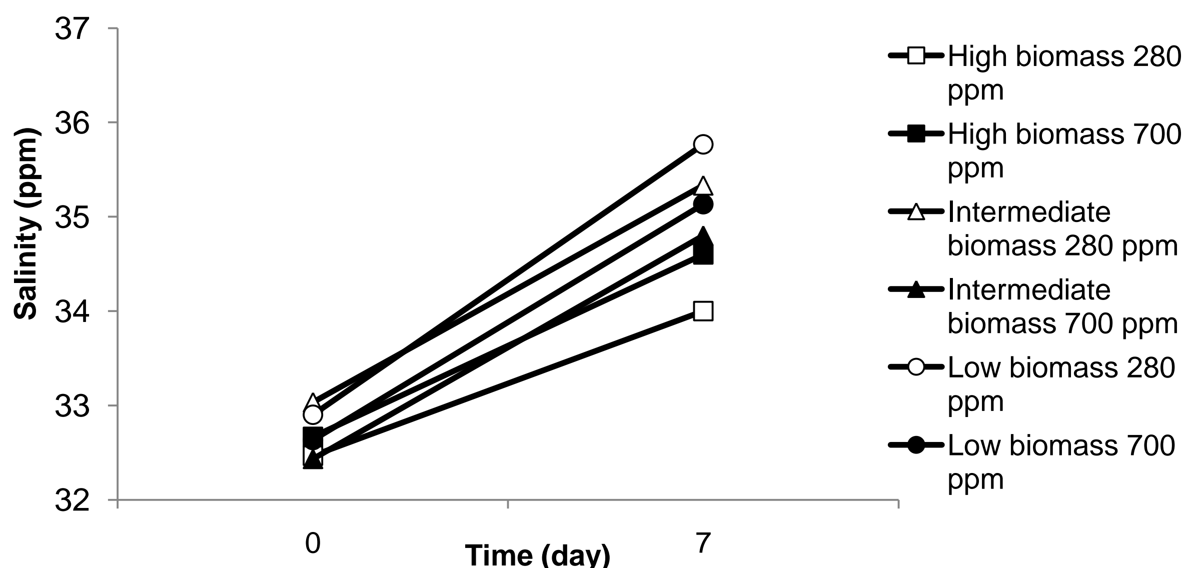


Figure 3.18 Salinities of the water over time (initial and final) in 7 days culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in Seawater at an irradiance of 70 μmol photons m^{-2} s^{-1} (optimal for growth) and at 15 °C (optimal temperature for growth). High, intermediate and low biomasses refer to (significantly different, $p<0.05$) starting biomasses of 116.8 ± 5.1, 47.2 ± 1.8 and 20.6 ± 1.1 mg respectively. Data are means (n=3). SDs are not exhibited for reasons of clarity.

Salinities of the culture water measured over time in Experiment 2 are shown in Figure 3.19. As this experiment had been performed in 5 L bottles compared to 1L glasses used in experiment 1, salinities varied only slightly between 31.7 ± 0.1 and 32.3 ± 0.05 psu. Between Day6 and Day 9 there was an increase in salinity by 1 in the 280 ppm and 700ppm condition in irradiance of 70 μmol photons m^{-2} s^{-1}.

In Figure 3.20, salinities of the culture water over time in Experiment 3 are presented. Salinities in this experiment increased by 0.5 on the 3rd day staying moe or less constant afterwards. Mean salinities only varied between 32.2 ± 0.05 and 32.4 ± 0.05 in exp. 3.

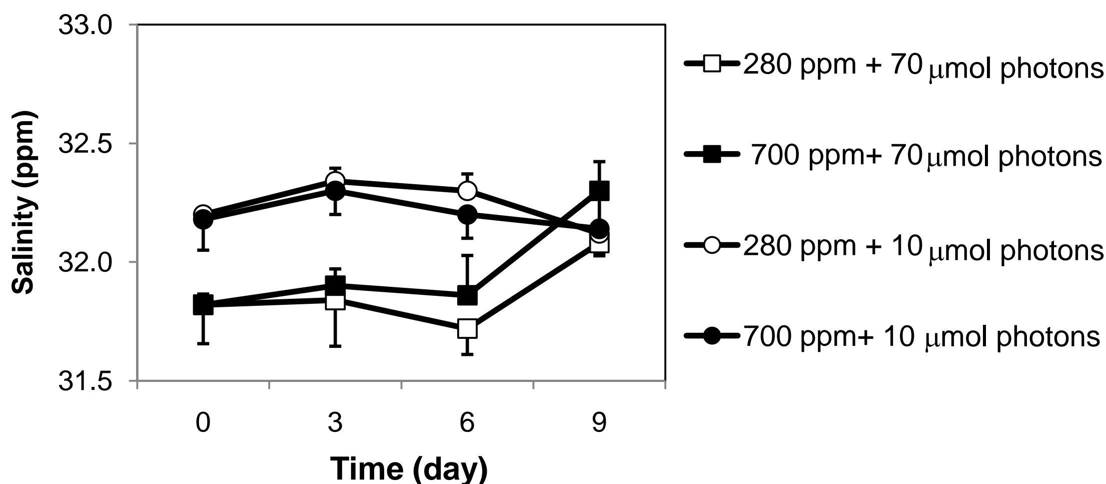


Figure 3.19 Salinities of the culture water over 9 days of culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater and 70 and 10 µmol photons m^{-2} s^{-1} light intensities (optimal and minimal for growth respectively) at 15 °C. Data are means ± SD (n=5). Value of day 0 is the salinity of new water before introducing the algal materials. Salinity measurements on day 3 and 6 were done before changing water and salinity on day 9 is the salinity at the end of the experiment.

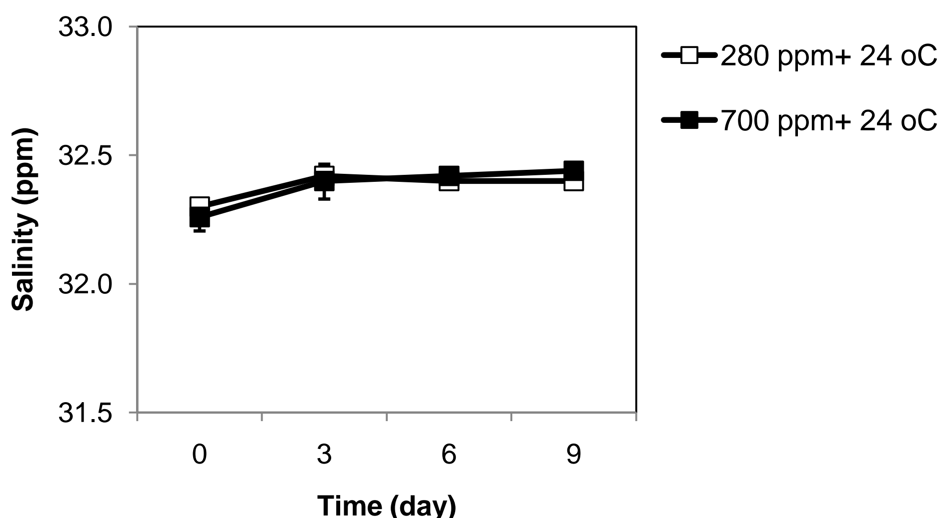


Figure 3.20 Salinity of the water over time in the 9 days culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater at 70 µmol photons m^{-2} s^{-1} light intensity (optimal for growth) and at 24 °C. Data are means ± SD (n=5). Value of day 0 is the salinity of new water before introducing the algal materials. Salinity measurements on day 3 and 6 were done before changing water and salinity on day 9 is the salinity at the end of the experiment.

3.5.2 pH

In Figure 3.21 initial and final pH values of the culture water from different treatments of Experiment 1 are presented. There was a general slight decrease by approx. 0.11 units between day 0 and 7. The mean pH values in the treatments with 280 ppm CO_2 concentration varied from 8.03 ± 0.02 to 8.18 ± 0.04 whereas in the treatments with 700 ppm CO_2 concentration, the values varied from 7.66 ± 0.02 to 7.86 ± 0.02.

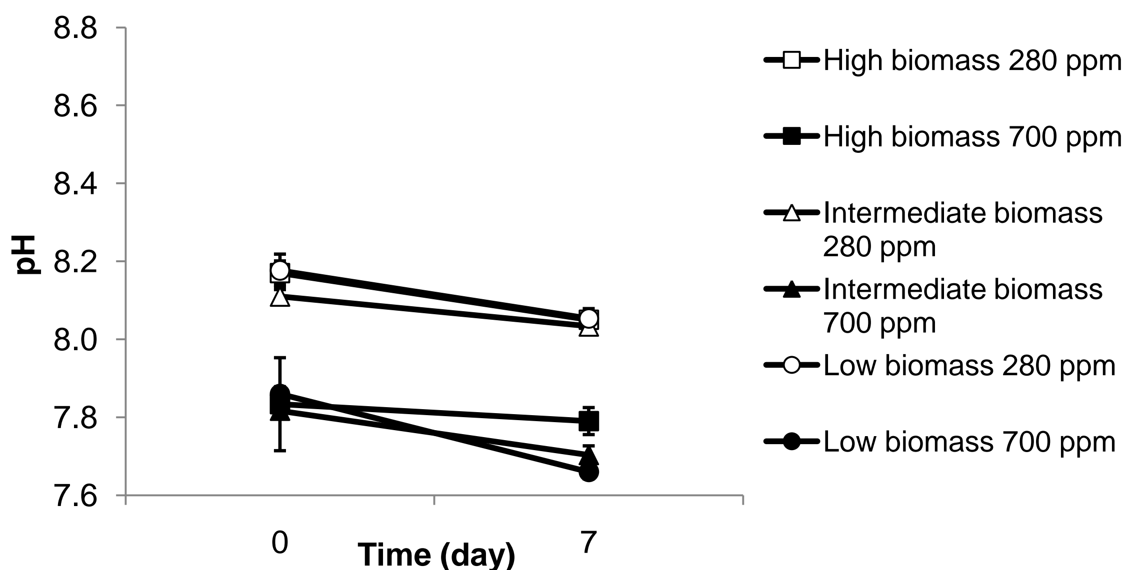


Figure 3.21 pH of the culture water over time (initial and final) in 7 days culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater at 70 μmol photons m^{-2} s^{-1} light intensity (optimal for growth) and at 15 °C (optimal temperature for growth). High, intermediate and low biomasses refer to (significantly different, $p<0.05$) starting biomasses of 116.8 ± 5.1, 47.2 ± 1.8 and 20.6 ± 1.1 mg respectively. Data are means ± SD (n=3). pH measurements were done between 11 am and 1 pm.

pH of the culture water over time in the 9 days culture of *Chondrus crispus* in Experiment 2 is shown in Figure 3.22. The pH values fluctuated by around 0.1 units after the 3rd day of the experiment and particularly in the treatments with 280 ppm CO_2 concentration. The mean pH values in the treatments with 280 ppm and 700 ppm CO_2 concentrations varied from 8.16 ± 0.02 to 8.41 ± 0.08 and 7.89 ± 0.01 to 7.99 ± 0.01 respectively.

In the Figure 3.23, change of pH in the culture water over time of Experiment 3 is shown. In this experiment pH values fluctuated by about 0.1 to 0.2 units in the treatments with 280 ppm CO_2 concentration while the pH in the treatments with 700 ppm CO_2 concentration were quite constant. The mean pH values in the treatments with 280 ppm and 700 ppm CO_2 concentrations variedy between 8.36 ± 0.03 and 8.05 ± 0.01 to 8.55 ± 0.03 and 8.07 ± 0.01 respectively.

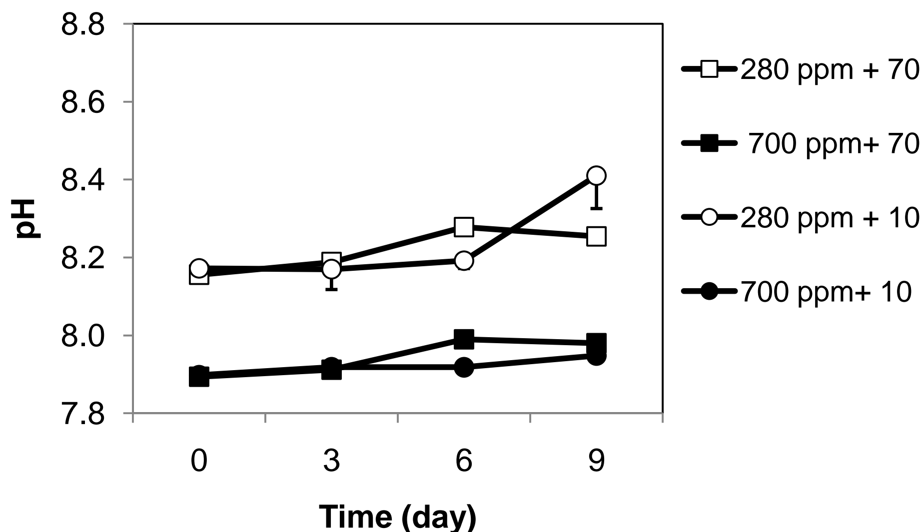


Figure 3.22 Change of pH of the culture water over time during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater and irradiances of 70 and 10 μmol photons m^{-2} s^{-1} (optimal and minimal for growth respectively) at 15 ^{o}C. Data are means ± SD (n=5). pH measurements were done between 11 am and 1 pm. Value of day 0 is the pH of new water before introducing the algal materials. pH measurements on day 3 and 6 were done before changing water and pH on day 9 is the pH at the end of the experiment.

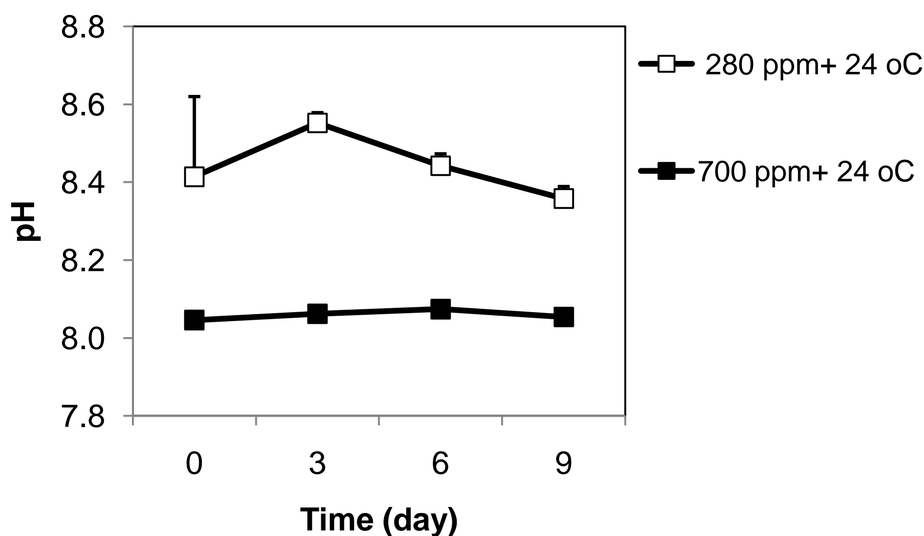


Figure 3.23 pH of the culture water over time during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in Seawater at an irradiance of 70 μmol photons m^{-2} s^{-1} (optimal for growth) and at 24 ^{o}C. Data are means ± SD (n=5). pH measurements were done between 11 am and 1 pm. Value of day 0 is the pH of new water before introducing the algal materials. pH measurements on day 3 and 6 were done before changing water and pH on day 9 is the pH at the end of the experiment.

3.5.3 Total alkalinity (A_T)

Initial and final total alkalinities of the culture water of Experiment 1 are shown in Figure 3.24. Total alkalinities changed by around 200 units in the 7 days culture period of this experiment. The mean total alkalinities in the treatments with 280 ppm and 700 ppm CO_2 concentration varied from 2487.33 ± 11.85 and 2468 ± 29.10 to 2753.67 ± 79.91 and 2734.33 ± 65.61 μmol kg^{-1} respectively.

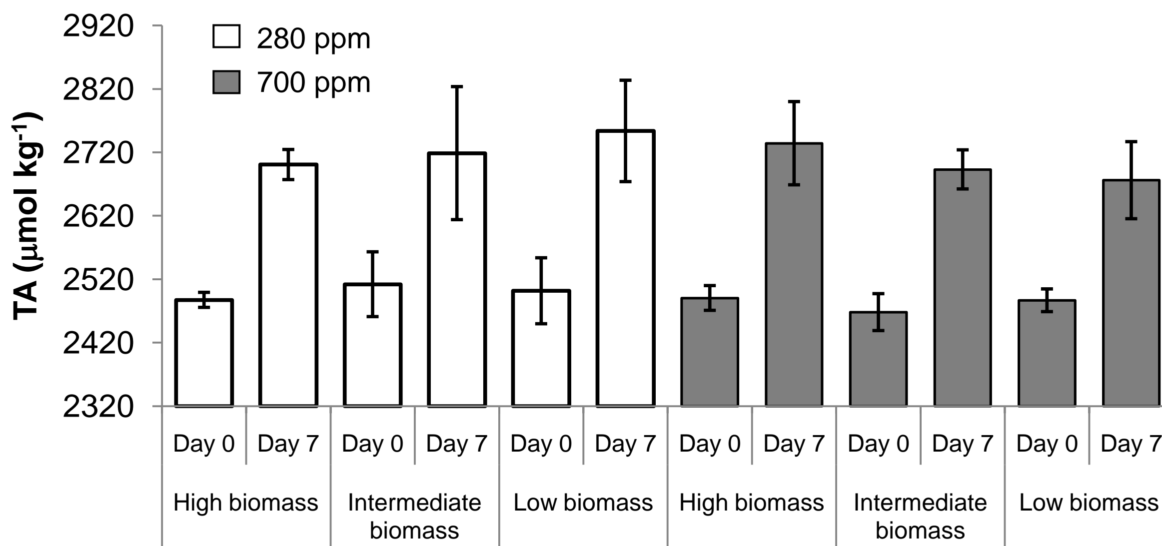


Figure 3.24 Total alkalinity of the culture water over time (initial and final) durings culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater at an irradiance of 70 μmol photons m^{-2} s^{-1} (optimal for growth) and at 15 °C (optimal temperature for growth). High, intermediate and low biomasses refer to (significantly different, $p<0.05$) starting biomasses of 116.8 ± 5.1, 47.2 ± 1.8 and 20.6 ± 1.1 mg respectively. Data are means ± SD (n=3). Water samplings were done between 11 am and 1 pm.

In Figure 3.25 total alkalinities of the culture water over time in the 9 days culture of *Chondrus crispus* in Experiment 2 are shown. Total alkalinities were much lower in the treatments with 10 μmol photons m^{-2} s^{-1} light intensities compared to that in the treatments with 70 μmol photons m^{-2} s^{-1} light intensities. However, total alkalinities were quite constant in the treatments with 10 μmol photons m^{-2} s^{-1} light intensities. In this experiment, the mean total alkalinities in the treatments with 280 ppm and 700 ppm CO_2 concentrations varied from 2370.60 ± 4.72 and 2368.80 ± 5.36 to 2430.00 ± 5.96 and 2432.20 ± 10.73 μmol kg^{-1} respectively.

Total alkalinities of the culture water over time in Experiment 3 have been presented in Figure 3.26. In this experiment, total alkalinities of the water in the treatments with 280 ppm and 700 ppm CO_2 concentrations varied from 2367.00 ± 2.24 and 2374.00 ± 3.39 to 2394.40 ± 10.69 and 2399.40 ± 35.93 μmol kg^{-1} respectively.

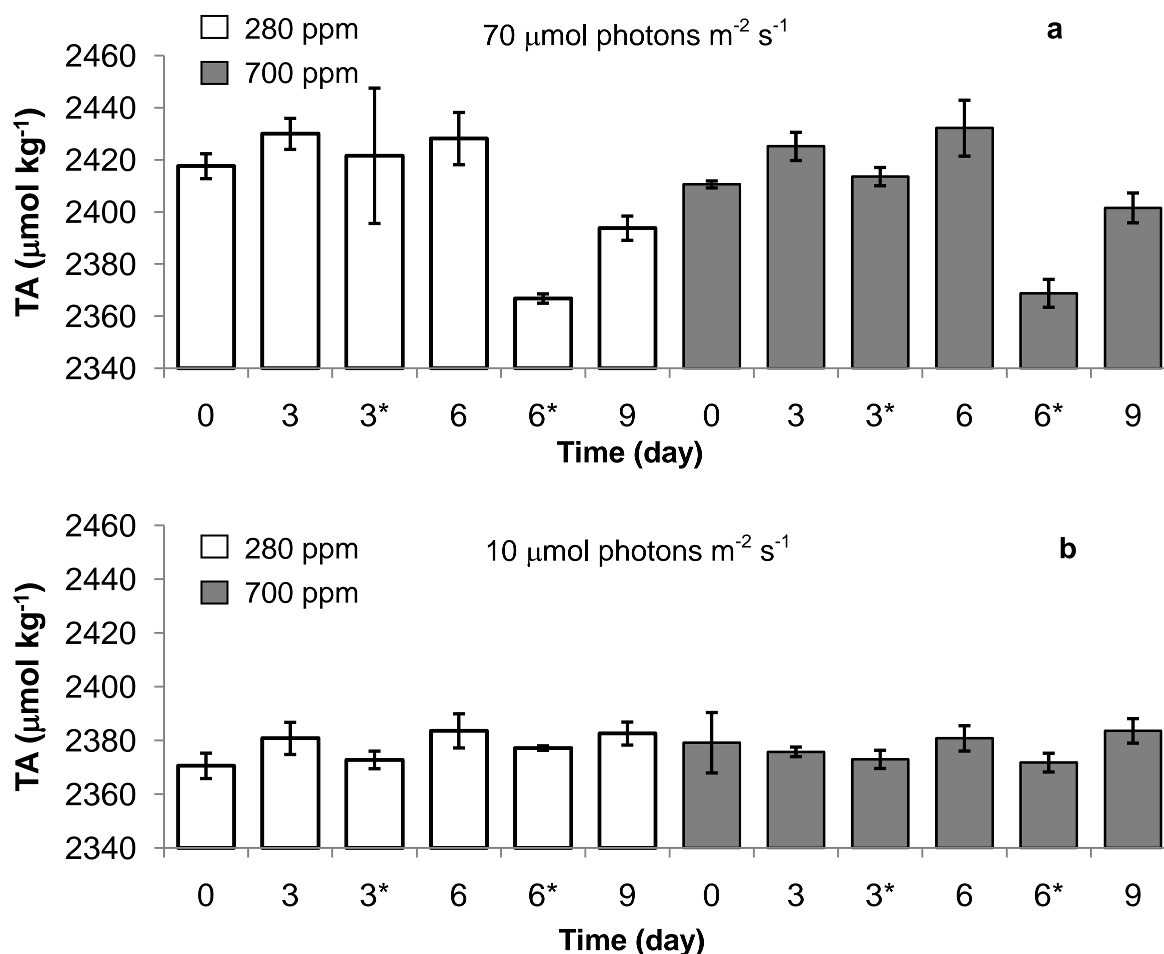


Figure 3.25 Total alkalinity of the culture water over time during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater and 70 (a) and 10 (b) µmol photons m^{-2} s^{-1} irradiances (optimal and minimal for growth respectively) at 15 °C temperature. Data are means ± SD (n=5). * indicates values for the new water on the respective days. Water samplings were done between 11 am and 1 pm. Value of day 0 is the TA of new water before introducing the algal materials. Water samplings on day 3 and 6 were done before changing water and value on day 9 is the TA at the end of the experiment.

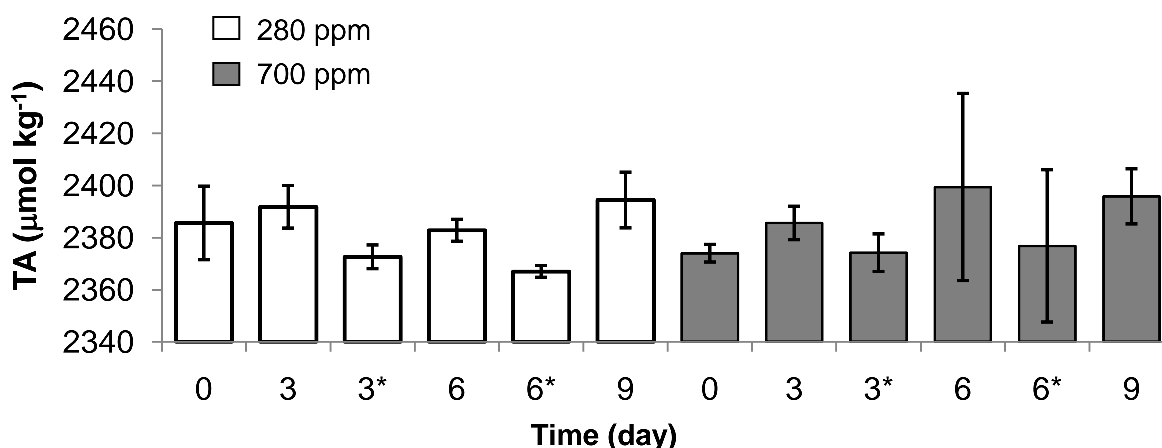


Figure 3.26 Total alkalinity of the water over time during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater at an irradiance of 70 μmol photons m^{-2} s^{-1} light intensity (optimal for growth) and at 24 °C temperature. Data are means ± SD (n=5). * indicates values for the new water on the respective days. Water samplings were done between 11 am and 1 pm. Value of day 0 is the TA of new water before introducing the algal materials. Water samplings on day 3 and 6 were done before changing water and value on day 9 is the TA at the end of the experiment.

3.5.4 pCO_2

The initial and final mean pCO_2 of the culture water from different treatments in the 7 days culture of *Chondrus crispus* in Experiment 1 have been shown in Figure 3.27. The mean pCO_2 in the 280 ppm CO_2 concentration treatments varied from 360.75 ± 35.96 to 577.50 ± 41.51 μatm respectively with the overall mean 480.42 μatm. In case of 700 ppm CO_2 concentration treatments, the mean pCO_2 varied from 904.31 ± 251.50 to 1525.49 ± 55.4 μatm with the overall mean value of 1121.19 μatm .

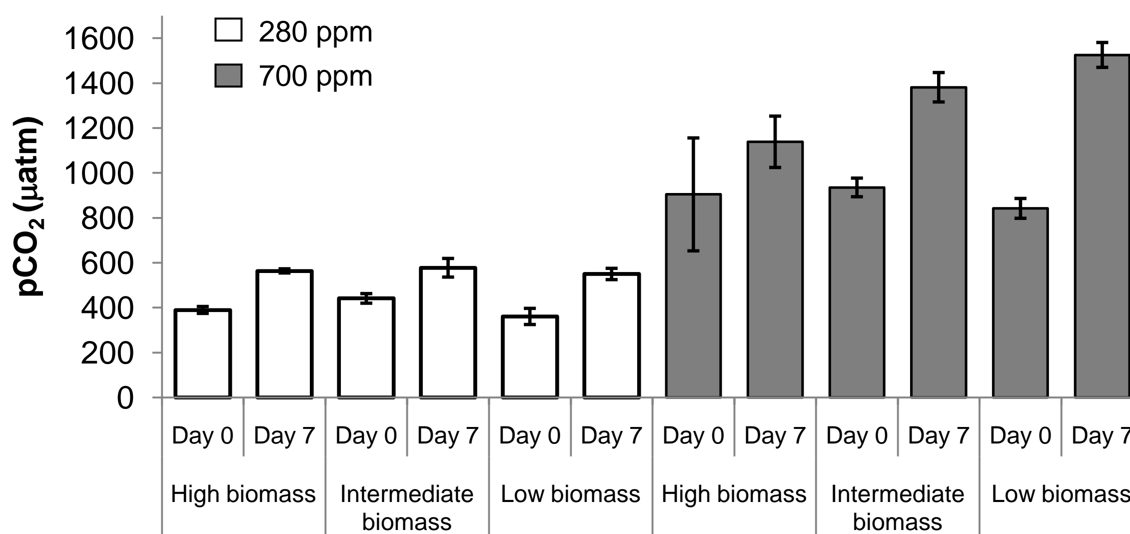


Figure 3.27 pCO_2 of the culture water over time (initial and final) during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater at an irradiance of 70 μmol photons m^{-2} s^{-1} light intensity (optimal for growth) and at 15 °C (optimal temperature for growth). High, intermediate and low biomasses refer to (significantly different, $p<0.05$) starting biomasses of 116.8 ± 5.1, 47.2 ± 1.8 and 20.6 ± 1.1 mg respectively. Data are means ± SD (n=3). Water samplings were done between 11 am and 1 pm.

In the Figure 3.28, mean pCO_2 of the culture water over time in the 9 days culture of *Chondrus crispus* in Experiment 2 are shown. pCO_2 of the water in all the treatments in this experiment were much higher at the beginning days of the experiments which eventually dropped over time in the next days. The mean pCO_2 in the 280 ppm CO_2 concentration treatments varied from 205.87 ± 55.50 to 495.12 ± 15.20 μatm with the overall mean value of 402.42 μatm whereas it varied from 711.02 ± 21.24 to 974.51 ± 12.47 μatm in the 700 ppm CO_2 concentration treatments with the overall mean values of 809.86 μatm.

The mean pCO_2 of the culture water in Experiment 3 have been presented in Figure 3.29. On the 3rd day of this experiment, a sudden drop of pCO_2 of the culture water was observed in all the treatments but later on a continuous increase in all the treatments was observed in the next days. In this experiment, the mean pCO_2 in the 280 ppm CO_2 concentration treatments varied from 136.48 ± 12.30 to 309.83 ± 11.76 μatm with the overall mean value of 232.73 μatm whereas it varied from 542.80 ± 62.40 to 691.91 ± 16.87 μatm in the 700 ppm CO_2 concentration treatments with the overall mean values of 642.98 μatm.

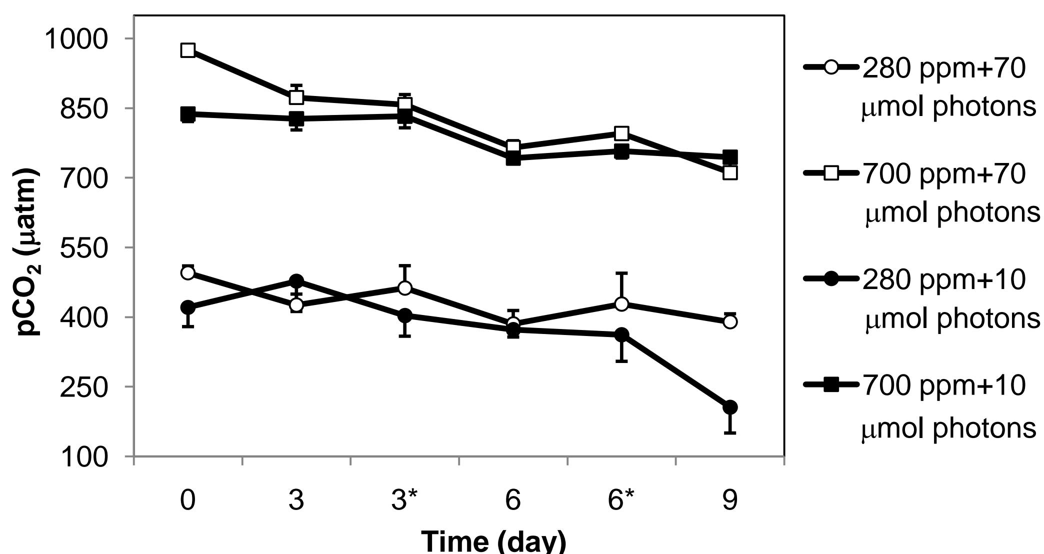


Figure 3.28 pCO_2 of the culture water over time during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater and 70 and 10 μmol photons m^{-2} s^{-1} irradiances (optimal and minimal for growth respectively) at 15 °C. Data are means ± SD (n=5). * indicates values for the new water on the respective days. Water samplings were done between 11 am and 1 pm. Value of day 0 is the pCO_2 of new water before introducing the algal materials. Water samplings on day 3 and 6 were done before changing water and value on day 9 is the pCO_2 at the end of the experiment.

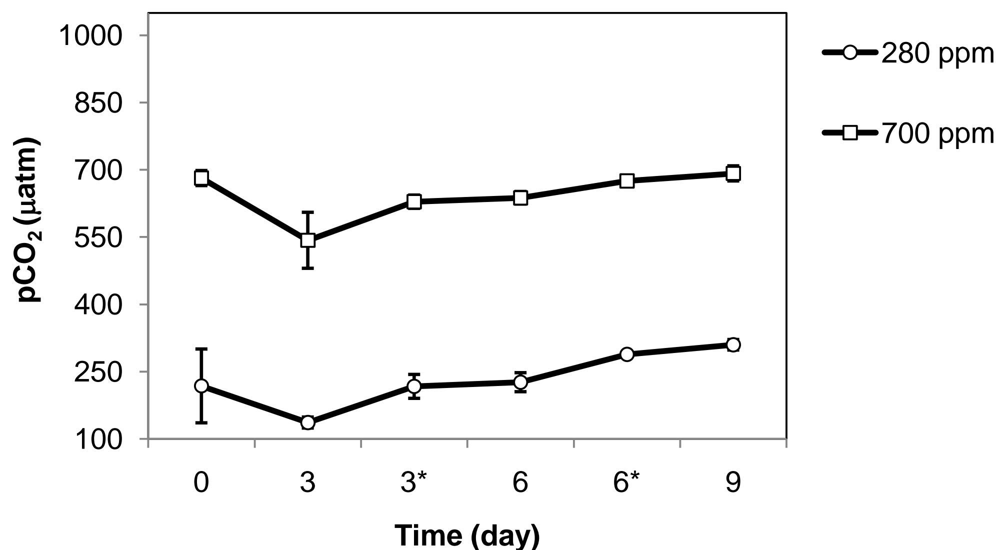


Figure 3.29 pH of the culture water over time during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater at an irradiance of 70 µmol photons m^{-2} s^{-1} (optimal for growth) and at 24 °C. Data are means ± SD (n=5). * indicates values for the new water on the respective days. Water samplings were done between 11 am and 1 pm. Value of day 0 is the pCO_2 of new water before introducing the algal materials. Water samplings on day 3 and 6 were done before changing water and value on day 9 is the pCO_2 at the end of the experiment.

3.5.5 Dissolved inorganic carbon contents

The initial and final dissolved inorganic carbon contents of the culture water in the different treatments of Experiment 1 are shown in Figure 3.30. The mean HCO_3^-, CO_2 and CO_3^- contents in the 280 ppm CO_2 concentration treatments varied from 2034.65 ± 36.40, 13.62 ± 1.38 and 162.33 ± 2.74 to 2331.02 ± 40.23, 21.51 ± 1.40 and 192.66 ± 17.40 µmol kg^{-1} respectively with the overall mean value of 2191.95, 18.02 and 173.57 µmol kg^{-1} respectively. In 700 ppm CO_2 concentration treatments, the mean HCO_3^-, CO_2 and CO_3^- contents varied from 2245.12 ± 15.47, 31.84 ± 1.68 and 71.84 ± 4.87 µmol kg^{-1} to 2504.74 ± 72.43, 56.90 ± 2.08 and 99.56 ± 4.97 µmol kg^{-1}, respectively with the overall mean value of 2376.30, 42.09 and 88.94 µmol kg^{-1}, respectively.

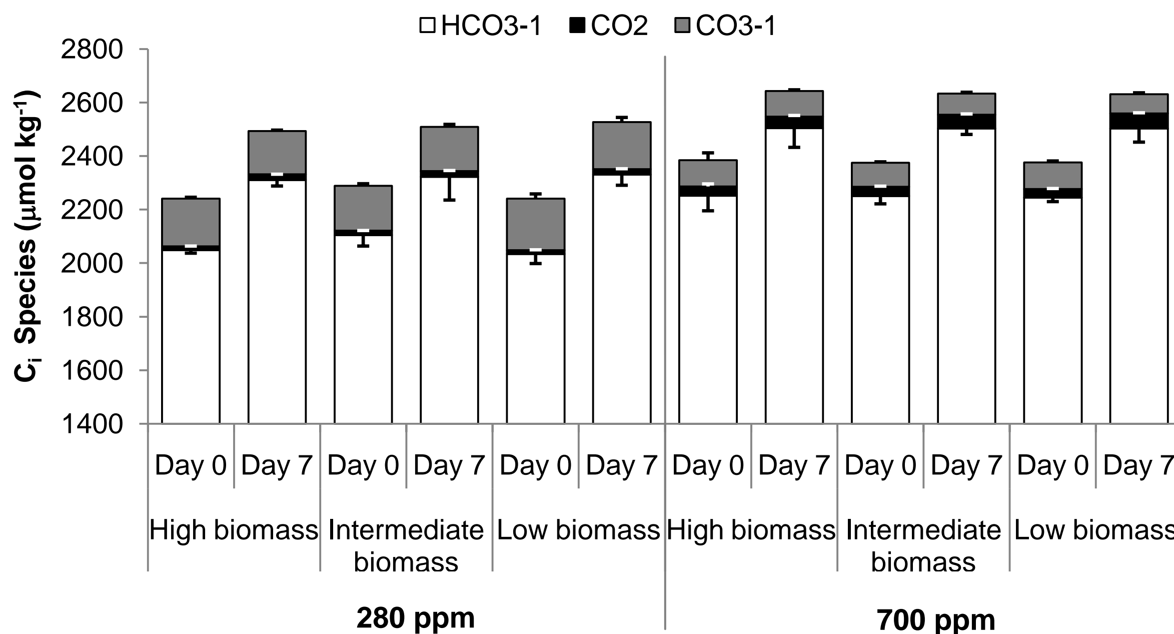


Figure 3.30 Dissolved inorganic carbon species (Ci) of the culture water over time (initial and final) during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater at an irradiance of 70 μmol photons m^{-2} s^{-1} (optimal for growth) and at 15 ^{o}C (optimal temperature for growth). High, intermediate and low biomasses refer to (significantly different, $p<0.05$) starting biomasses of 116.8 ± 5.1, 47.2 ± 1.8 and 20.6 ± 1.1 mg respectively. Data are means ± SD (n=3). Water samplings were done between 11 am and 1 pm.

In the Figure 3.31, dissolved inorganic carbon contents of the water in the different treatments of Experiments 2 have been presented. The mean HCO_3^-, CO_2 and CO_3^- contents in the 280 ppm CO_2 concentration treatments varied from 1946.10 ± 41.35, 7.68 ± 2.07 and 144.25 ± 2.94 to 2067.15 ± 9.09, 18.54 ± 0.55 and 259.46 ± 39.03 μmol kg^{-1}, respectively with the overall mean value of 1980.53, 15.04 and 169.85 μmol kg^{-1}, respectively. In the 700 ppm CO_2 concentration treatments, the mean HCO_3^-, CO_2 and CO_3^- contents were found to vary from 2127.81 ± 2.07, 26.12 ± 0.82 and 83.83 ± 1.19 to 2206.78 ± 4.15, 36.38 ± 0.57 and 110.21 ± 2.86 μmol kg^{-1}, respectively with the mean value of 2156.76, 30.23 and 97.03 μmol kg^{-1}, respectively. The HCO_3^-, CO_2 and CO_3^- contents were quite constant in the treatments with 700 ppm CO_2 concentration + 10 μmol photons m^{-2} s^{-1} light intensity but most fluctuating in the treatments with 280 ppm CO_2 concentration + 10 μmol photons m^{-2} s^{-1} light intensity.

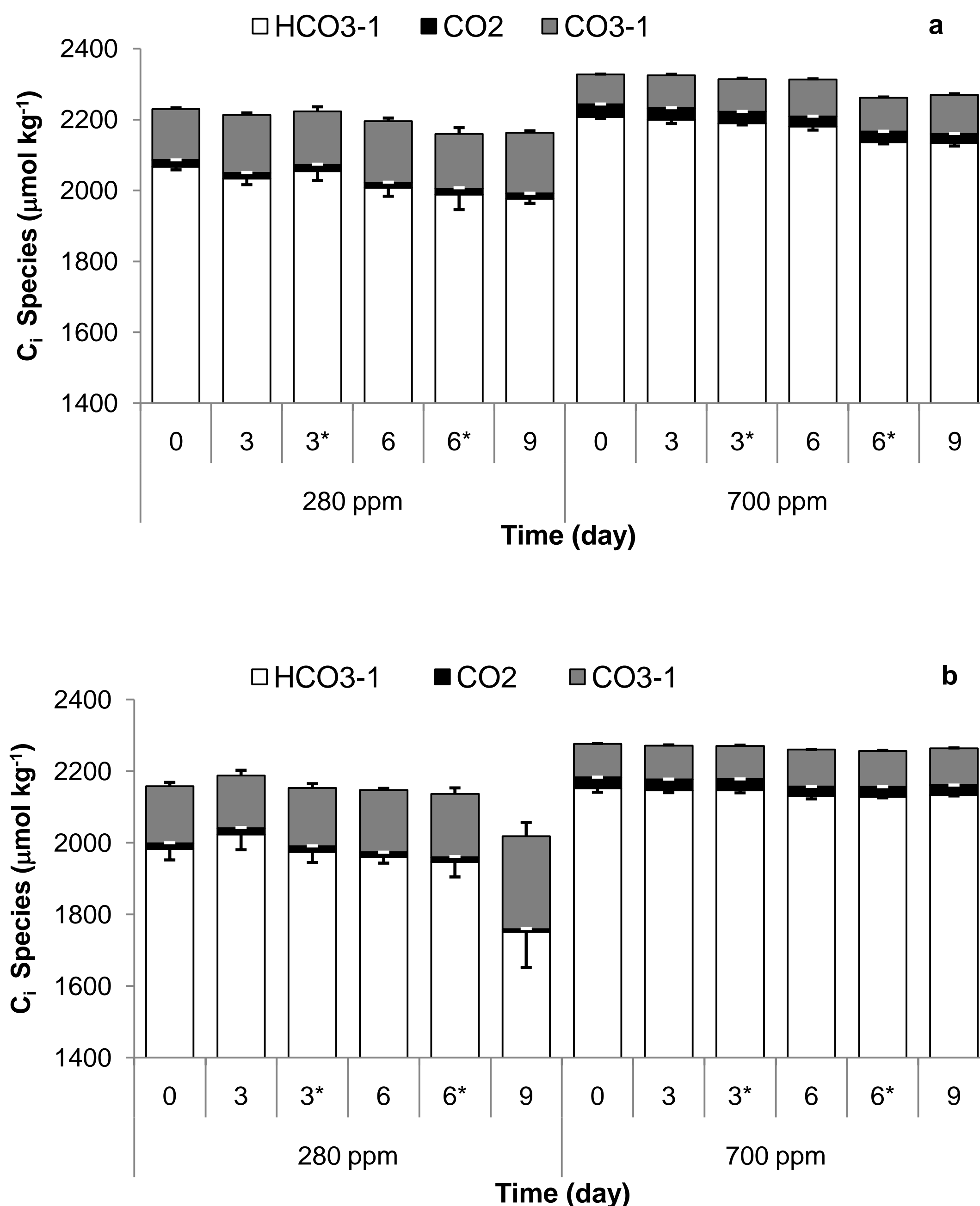


Figure 3.31 Dissolved inorganic carbon species (Ci) of the culture water over time during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater and 70 (a) and 10 (b) μmol photons m^{-2} s^{-1} irradiances (optimal and minimal for growth respectively) at 15 ^{o}C temperature. Data are means ± SD (n=5). * indicates values for the new water on the respective days. Water samplings were done between 11 am and 1 pm. Values of day 0 are the Ci species of new water before introducing the algal materials. Water samplings on day 3 and 6 were done before changing water and values on day 9 are the Ci at the end of the experiment.

The dissolved inorganic carbon contents of the water in the different treatments of Experiments 3 are shown in Figure 3.32. In this experiment, HCO_3^-, CO_2 and CO_3^- contents in the 280 ppm CO_2 concentration treatments were very much fluctuating over the times. The mean HCO_3^-, CO_2 and CO_3^- contents in the 280 ppm conditions varied from 1462.31 ± 38.38, 4.03 ± 0.34 and 254.56 ± 1.94 to 1762.31 ± 36.19, 8.60 ± 0.91 and 382.62 ± 12.73 μmol kg^{-1}, respectively with the overall mean value of 1660.54, 7.10 and 297.48 μmol kg^{-1}, respectively. In the 700 ppm CO_2 concentration treatments, the mean HCO_3^-, CO_2 and CO_3^- contents varied from 1959.39 ± 32.91, 15.74 ± 1.65 and 143.96 ± 1.43 to 2038.00 ± 12.99, 20.19 ± 0.34 and 176.09 ± 12.02 μmol kg^{-1}, respectively with the overall mean value of 2010.09, 18.81 and 154.62 μmol kg^{-1}, respectively.

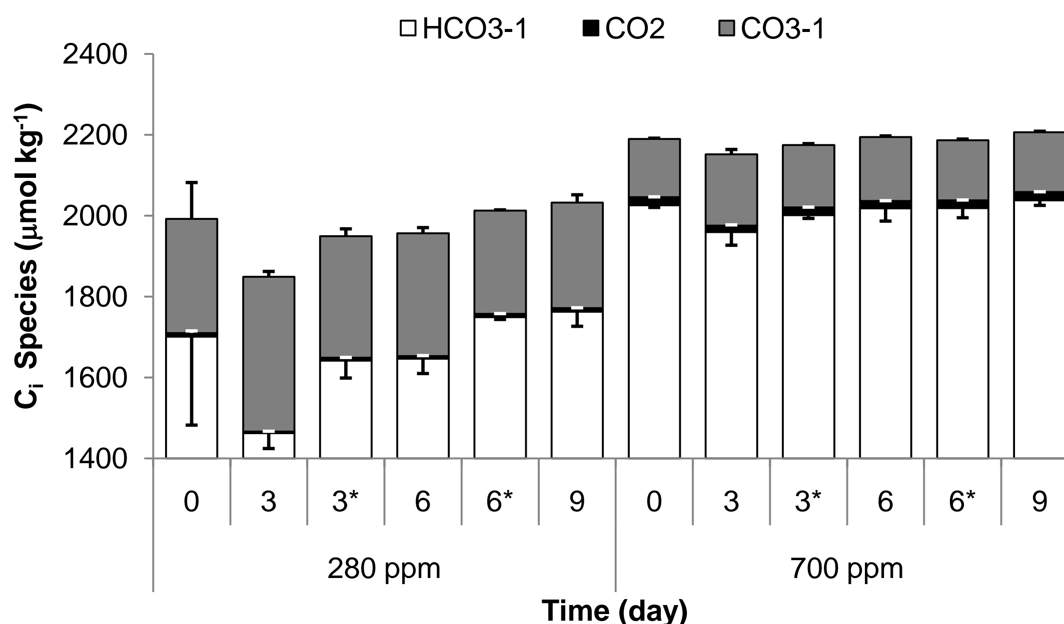


Figure 3.32 Dissolved inorganic carbon species (Ci) of the culture water over time during culture of *Chondrus crispus* under 280 ppm and 700 ppm CO_2 concentrations in seawater at an irradiance of 70 μmol photons m^{-2} s^{-1} (optimal for growth) and at 24 ^{o}C temperature. Data are means ± SD (n=5). * indicates values for the new water on the respective days. Water samplings were done between 11 am and 1 pm. Values of day 0 are the Ci species of new water before introducing the algal materials. Water samplings on day 3 and 6 were done before changing water and values on day 9 are the Ci at the end of the experiment.

4 DISCUSSION

4.1 Water chemistry

Manipulating seawater carbonate chemistry at target levels as well as maintaining the constant condition for the whole experimental periods is very important and the most challenging aspect of conducting perturbation experiments in the ocean acidification research. In all our experiments, seawater carbonate chemistry was manipulated by bubbling the seawater with CO_2 enriched air. This method is a very efficient way of manipulating seawater carbonate chemistry and it exactly mimics the carbonate chemistry changes that is naturally occurring in the ocean (increase in pCO_2 and DIC; decrease in pH without changing of A_T). In order to monitor the complete carbonate system of the culture water, pH and total alkalinity (A_T) were measured regularly together with the other two parameters temperature and salinity, which were eventually used in the calculation of the program CO2SYS in order to calculate the carbonate species (Lewis & Wallace 1998).

Experiment 1 was mainly conducted for methodological purposes i.e. to know the effects of algal biomass on water chemistry. Biological processes such as photosynthesis, respiration, nutrient uptake and release etc. can strongly modify the pH and significantly alter the target carbonate chemistry in culture water by changing DIC and A_T when experiments are conducted with high biomass (Rost et al. 2008). In the Experiment 1, three different low starting biomasses of algae (116.8 ± 5.1, 47.2 ± 1.8 and 20.6 ± 1.1 mg) were cultured in 1 L glass beakers (filled with 900 ml water). After 7 days of culture, drastic changes in salinity and in other parameters, especially in total alkalinity were observed in all treatments, even those with the lowest biomass. The overall mean initial salinity of all the treatments was 32.7 ± 0.2 which increased to 34.9 ± 0.6 after 7 days. This drastic change in the salinity occurred due to the evaporation of water which intensified due to the continuous air bubbling in the beakers and because of the low water volume as well. Also, pH values were found to decrease by 0.04 to 0.2 units. Total alkalinity, rather than being more or less constant, increased by around 200 to 250 units after 7 days of culture. Drastic changes in all these parameters led to a high variation in pCO_2 values in the culture water at the end of the experiment. The target pCO_2 values for the experiments were 280 and 700 μatm. However, in Experiment 1, the mean initial pCO_2 values of the 280 ppm and 700 ppm treatments were 397.19 ± 40.72 and 893.86 ± 47.54 μatm, respectively which increased to 563.65 ± 13.82 and 1348.52 ± 195.55 μatm, respectively after 7 days. The initial pCO_2 values were also much higher than the target value, probably because of the fluctuating gas flow rate of the gas mixing device. We had no flow meter for continuous monitoring of the gas flow rate. Visual control of the flow rate was done just by checking the display screen of the gas mixer where the gas flow rates were displayed. In fact, standard procedures for controlling the gas flow rate in the experimental treatments were not well developed at the beginning of this study. The much higher calculated values of pCO_2 could also be due to a measuring error of pH. The used pH meter and pH electrode (gel electrode) for this experiment were not precise enough and the calibration of the pH meter was also problematic. In fact, the pH value of the water greatly affects the calculated output value of pCO_2 in the CO2SYS program. It has been observed that a pH change of 0.1 units leads to a change in the calculated pCO_2 value by about 100 μatm. The high value of pCO_2 in the treatments also led to the comparatively higher amounts of inorganic carbon contents in the water (Fig. 3.24). From this experiment, it was learnt that algal biomass can have a significant effect on water chemistry if the culture water volume is rather small. As similar volumes have been used in many physiological algal culture experiments without obvious effects on the

algae (e.g. Wiencke & tom Dieck 1989, Roleda et al. 2004), this reaction had not been expected at the start of the experiment. In order to overcome the problem, we decided to use higher water volumes (5 L bottle) for algal cultures with similar biomasses and change the culture water regularly (on every 3rd day) in Experiment 2 and 3.

This decision had been right as in Experiment 2 and 3 all water chemistry parameters were rather constant. Salinities in the different treatments were more or less constant over time with variations of only up to 0.5 units which were within the reasonable range and much better compared to the variations in Experiment 1. It improved because of using high water volume for algal cultures and precise regulation of air bubbling speed in the water, thereby reducing the evaporation rate of the water. The pH in the treatments of Experiment 2 and 3 was quite constant except the dramatic increase on day 9 in the 280 ppm CO_2 + 10 μmol photons m^{-2} s^{-1} irradiance treatment of Experiment 2 and on day 3 in the 280 ppm CO_2 + 24 °C treatment of Experiment 3. These fluctuations of pH probably were mainly due to fluctuating gas flow rates of the gas mixing device. It happened that sometimes the gas mixing device was not working precisely, particularly when its flow rate exceeded an upper or lower limit. It happened mainly at times when too many gas outlets were in use at the same time in the different culture rooms and also when very few gas outlets were in use. Thus, it became obvious that control of low rates is crucial in CO_2 perturbation experiments.

In order to control the water chemistry system (Dickson et al. 2007) fluctuating values of total alkalinities were observed in the treatments as well. However, total alkalinity was quite constant in the 10 μmol photons m^{-2} s^{-1} light intensity treatments of Experiment 2, although the values were comparatively low. The consistent and much lower values of total alkalinity may be attributable to less biological activities of the algae in the minimal light intensity (10 μmol photons m^{-2} s^{-1}). The mean pCO_2 values in the 280 and 700 ppm treatments of Experiment 2 were 402.24 ± 71.27 and 809.86 ± 69.79 μatm respectively while in case of Experiment 3, the values were 232.73 ± 55.98 and 642.98 ± 50.32 μatm for 280 and 700 ppm treatments, respectively. All pCO_2 values were comparatively lower in Experiment 3 than that in Experiment 2. Moreover, the actual pCO_2 values deviated much from the target values in both experiments, although the differences between the 280 ppm treatments and 700 ppm treatments were always approx. 400 μatm. This difference was in accordance with the requirement of the experimental design, and thereby should lead to comparable results. The comparatively lower pCO_2 values partially observed in the treatments of Experiment 3 were induced by a sudden drop of the gas flow rate during the experiment. The low pCO_2 values were also reflected in the relatively lower concentration of dissolved inorganic carbon contents of the water (Fig. 3.26) compared to Experiment 1 and 2.

4.2 Responses in growth and photosynthesis

Both light and temperature are significant factors controlling growth and photosynthesis of macroalgae. In our experiments, the relative growth rate (RGR) of *Chondrus crispus* varied between 2.20 and 8.19 % day^{-1} depending on the experimental conditions. This range is comparable to the growth rates determined in some previous studies (Bird et al. 1979, Fortes & Lüning 1980). As expected, growth rate was significantly lower in the low irradiance (10 μmol photons m^{-2} s^{-1}) than in the optimal irradiance (70 μmol photons m^{-2} s^{-1}). Also the growth rate was significantly lower in the elevated temperature (24 °C) in comparison to optimal temperature (15 °C). This is in accordance with previous studies, where maximum growth rate

of *Chondrus crispus* was reported at 15 °C with decreasing values at further increasing temperatures (Bird et al. 1979, Chen & McLachlan 1972). Although *C. crispus* is known to be a species with a carbon concentrating mechanism (Smith and Bidwell 1989, Brechignac and Andre 1985) and thereby being partially independent from CO_2 values (Holbrook et al. 1988, Johnston et al. 1992, Beer 1994, Raven et al. 1995, Beer & Koch 1996, Giordano et al. 2005), the overall relative growth rate of *Chondrus crispus* was significantly higher in the high CO_2 concentration (700 ppm) than in the low CO_2 concentration treatment. This became evident in two experiments when (1) the three different biomasses of algae were cultured in optimal irradiance and temperature and (2) in experiment 3 where *C. crispus* had been cultured at elevated temperatures and CO_2 treatments in optimal irradiance. In this case, the differences in the growth rate between the two CO_2 treatments became more pronounced in elevated temperature. In experiment 2 where the differences in cultivating irradiance had been in the foreground, CO_2 concentrations did not show significant differences in growth but the same trend. So far, increased growth rates in response to high CO_2 treatments have been reported for many algal species such as the red algae *Gracilaria lemaneiformis* (Xu et al. 2010) *Porphyra yezoensis* (Gao et al. 1991), *Gracilaria* sp and *G. chilensis* (Gao et al. 1993), the brown alga *Hizikia fusiforme* (Zou 2005) or the green alga *Ulva rigida* (Bjork et al. 1993, Gordillo et al. 2001). All of these species are capable of using HCO_3^- as *Chondrus crispus*.

In contrast to this growth effect, net photosynthesis showed the opposite effect in the CO_2 perturbation experiments. Net photosynthetic rates measured in normal seawater significantly decreased in the algae cultured in the high CO_2 concentration compared to the algae cultured in the low CO_2 concentration. This decreasing rate of net photosynthesis in high CO_2 concentration is not in accordance with the higher growth rate observed in the high CO_2 concentration. The enhancement of growth in response to higher CO_2 concentration generally occurs only when nitrogen assimilation is also increased. In higher plants, the enhancement of growth by increased CO_2 treatments is usually a transitory effect caused by an enhanced photosynthesis. Generally it is believed that increased CO_2 concentrations can stimulate growth of many microalgal species, especially those without CCM or with less ability of CCM. The enhanced growth rate could be achieved through two ways: (1) when the photosynthesis is limited by the present availability of dissolved inorganic carbon in water, elevated CO_2 concentration could increase the DIC in water and thus promotes the photosynthesis; (2) the enriched CO_2 in water can lower the requirement of light energy for CCMs or HCO_3^- transportation, this may stimulate the growth under light-limited conditions (Chen & Gao 2004). In *Chondrus crispus* photosynthesis was not enhanced at high CO_2 concentration in the present study. In fact, CO_2 enrichment can regulate not only the photosynthetic performance but also some other physiological functions such as light-capture strategy and energy partitioning finally determining the growth efficiency. CCM has been found to be regulated by many environmental factors (Giordano et al. 2005, Beardall & Giordano 2002). High CO_2 concentration can also reduce the activity of CCM-related enzymes, such as carbonic anhydrase. Therefore, it is expected to down-regulate the capacity of CCMs if cells become acclimated to the high CO_2 concentration (Israel & Hophy 2002). For example, *Fucus serratus* cultured under high CO_2 for three weeks still had the ability to use HCO_3^- as a source of inorganic carbon but with a reduced capacity (Johnston & Raven 1990). In fact, CCMs are energy consuming processes. When exposed to CO_2 enriched conditions, the expression and activity of CCMs are generally repressed (Björk et al. 1993, Gordillo et al. 2001) and the energy normally being invested in such carbon concentrating process is made available to other processes (Raven 1991). Decrease in the capacity to use HCO_3^- or CA activity had been

reported also in *Ulva* spp. (Björk et al. 1993), *Gracilaria tenuistipitata* (García-Sânchez et al. 1994), *Porphyra leucostica* (Mercado et al. 1999), *G. gaditana* (Andria et al. 1999) and *Hizikia fusiforme* (Zou et al. 2003). In our study we assume that when exposed to high CO_2 concentration, *Chondrus crispus* was relieved from the energy consuming CCM due to down-regulation of this mechanism. Thus, high CO_2 concentration brought a change in the energy capture and partitioning process of *Chondrus crispus*, leading to a more efficient use of the energy for its higher growth. Such non-photosynthetic enhancement of growth was reported also in the green macroalga *Ulva rigida* which was related to an increase in nitrate reductase activity (Gordillo et al. 2001). The enhanced nitrate reductase (NR) supports enhanced growth rate by providing N required for the metabolism of the algae under high CO_2 condition. Larsson et al. (1985) also reported higher growth rate in the green alga *Scenedesmus* through CO_2 stimulation which is correlated with high nitrate uptake rates.

The decreased net photosynthetic rates of the algae grown in high CO_2 concentration can be explained by its down-regulation or deactivation of CCM in the high CO_2 concentration. Photosynthesis was measured in natural air equilibrated seawater and therefore, algae grown in the high CO_2 concentration showed decreased photosynthetic rates in this natural seawater (with less Ci) due to its down-regulation of CCM. On the other hand, algae grown in the low CO_2 concentration had an advantage because of its operative CCM and showed higher rates of net photosynthesis.

In the present study, net photosynthetic rates measured in low, high and saturating irradiances differed significantly in both experiments. Photosynthetic rates were lowest in the low irradiance while the highest rates were observed at saturating irradiance which is a general trend for photosynthetic plants. Also the net photosynthetic rates significantly increased in the elevated temperature (24 ^{o}C) compared to the optimal temperature (15 ^{o}C) when the algae were grown at optimal light intensity. Mathieson & Burns (1971) found an increasing rate of apparent photosynthesis in *Chondrus crispus* up to 20 ^{o}C but above this temperature the rate decreased. In contrast to this observation, in our CO_2 perturbation experiments, higher net photosynthetic rates were observed above 20 ^{o}C irrespective of CO_2 concentration.

Dark respiration rates were significantly higher in the algae cultured at optimal irradiance in comparison to the algae cultured at minimal irradiance. Like photosynthesis, respiration was also significantly higher in 24 ^{o}C compared to 15 ^{o}C. This is in accordance with Mathieson & Burns (1971) who observed a substantial increase in respiration rate for *Chondrus crispus* above 20 ^{o}C. In our study, dark respiration rates of the algae cultured in 15 ^{o}C and 70 μmol photons m^{-2} s^{-1} light intensity treatments were comparable to the respiration rates (4 -6 μmol O_2 g^{-1} FW h^{-1}) reported by (Furbank & Rebeille, 1986) for the same species. But the dark respiration rates of the algae cultivated in 15 ^{o}C and 10 μmol photons m^{-2} s^{-1} light intensity got positive values, probably because of their very low oxygen uptake rate and the concomitant measuring error. However, unlike photosynthesis, respiration rates of the algae cultivated in the high and the low CO_2 concentration did not differ significantly in the present study. Dark respiration in *H. fusiforme* was also reported to remain unchanged by the CO_2 levels in culture when measured at the normal Ci concentration in natural seawater (Zou 2005). Similar results have also been described for *Porphyra leucostica* (Mercado et al. 1999). In another recent study, Zou and Gao (2010) reported that dark respiration of the brown seaweed *Endarachne binghamiae* was not affected by CO_2 concentration. Also in some terrestrial plant species similar results were found (Roberntz & Stockfors 1998, Tjoelker et al. 1999).

4.3 Response in biochemical contents

In the present study, final dry biomass increased significantly in the algae cultured at the high CO_2 concentration compared to the low CO_2 concentration irrespective of the available irradiance for growth. The higher dry matter found in the high CO_2 concentration indicates higher growth in the algae which is in accordance with the higher growth rates observed in the high CO_2 concentrations. Similar result was found in *Lomentaria articulate* where cultures grown at higher CO_2 had relatively higher dry matter than those grown at low CO_2 (Kübler et al. 1999).

If HCO_3^- is involved in inorganic carbon acquisition through any of the existing mechanisms, we may expect little sensitivity of the elemental composition to variable CO_2 concentration in the bulk medium. In our study as well, both the C and N contents of *Chondrus crispus* were not changed significantly by CO_2 concentration. Also in *Porphyra leucosticte,* an another red algal species, total C content did not change significantly but total N content was reported to decrease in high Ci grown discs compared to current Ci grown discs (Mercado et al. 1999). However, changes in the elemental composition in response to variable CO_2 concentrations had been observed in the marine diatom *Skeletonema costatum* (Burkhardt & Riebesell 1997) and in some other marine phytoplankton species (Burkhardt et al. 1999). Also in higher plants, high CO_2 was found to decrease levels of nitrogen and soluble protein (Cave et al. 1981, Petterson & McDonald 1992) and increase of soluble carbohydrate (Sheen 1990). In macroalgae, changes in C and N contents with CO_2 enrichments were reported for the red alga *Lomentaria articulate* (Kübler et al. 1999) and also for *Gracilaria tenuistipitata* (García-Sánchez et al. 1994) where the algae grown at high Ci concentration had higher C/N ratios.

In the present study, C contents were only affected by temperature. Higher C contents were found in the algae cultured at 24 ^{o}C in comparison to the algae cultured at 15 ^{o}C. C and N contents are usually used as indicators of growth and photosynthesis in macroalgae (Chapman and Craigie 1978, Gagne et al. 1982). C content is linked to tissue organic molecules. The minimum value of C can occur when growth rates occur very fast and exceed assimilation rates. On the other hand, the C reserves in algal tissues could increase if high photosynthesis occurs and carbon assimilation exceeds carbon utilization. In the present study, higher growth rates and lower photosynthetic rates were observed at 15 ^{o}C which could be the reason for being low C contents in the algae cultured at this temperature. On the other hand, higher photosynthetic rates but comparatively lower growth rates were found at 24 ^{o}C which could lead to the higher C contents at this temperature. The N contents were significantly higher in the algae cultured in the low irradiance than in the optimal irradiance but it decreased significantly in the algae cultivated at 24 ^{o}C compared to the algae cultivated at 15 ^{o}C. Low N contents in the tissues indicate lower protein synthesis and thereby low growth. Low growth rates observed in 24 ^{o}C in the present study supports this finding. The higher N contents observed in the low irradiance where the growth rate was significantly low, could be due to the higher synthesize of phycobilins to enhance their light absorbing efficiency in the low irradiance. However, phycobilin contents were not analyzed in the present study.

Marine macroalgae are generally exposed to variable irradiance and light quality in the natural environments. Acclimation to such changes is common in macroalgae (Talarico & Maranzana 2000, Figueroa et al. 2003) as well as phytoplankton and seagrasses (Kirk 1994). Decrease in the pigment content in strong light is a general phenomenon in higher plants (Björkman, 1973) and also in phytoplankton (Harris, 1978). On the contrary, shade-acclimated plants have been

reported to synthesize photosynthetic pigments, such as Chlorophyll *a* and phycobilins, to enhance their efficiency in absorbing energy at sub-saturating irradiances (Théry 2001, Figueroa et al. 2003). In the present study, Chlorophyll *a* content decreased in the algae cultured in the optimal irradiance with respect to the algae cultured in the low irradiance. A similar result was found in *Gracilaria lemaneiformis* but its Chlorophyll *a* content was unaffected by the CO_2 levels in the culture (Zou & Gao 2009). This does not agree with our results which showed a significant decrease of Chlorophyll *a* content in *Chondrus crispus* under the high CO_2 treatments compared to the low CO_2 treatments. This effect was however only present when the algae were cultivated in the low irradiance or in the elevated (24 ^{o}C) temperature. Decrease in Chlorophyll *a* contents was also reported in case of *Gracilaria* sp. (Andría et al. 1999, 2001), *G. tenuistipitata* (García-Sanchez et al. 1994) and *Porphyra leucosticta* (Mercado et al. 1999) when they were cultured at high Ci concentration (up to 5% CO_2 in air) compared to normal Ci concentration. Also in case of cyanobacterium *Spirulina platensis* Chlorophyll a was decreased by 20-25% at high CO_2 (1%) (Gordillo et al.1999). The low net photosynthetic rates in *C. crispus* cultured under high CO_2 concentration in the present study could be attributed to the decreased chlorophyll *a* contents in the algae cultured under the high CO_2 concentration together with minimal irradiance or elevated temperature. However, chlorophyll *a* contents of *C. crispus* cultured in 15 ^{o}C and 24 ^{o}C were not statistically different although significantly higher photosynthetic rates were observed in the algae cultured in 24 ^{o}C. This higher photosynthetic rate observed in 24 ^{o}C could be due to other physiological reasons not directly related to the chlorophyll a contents.

5 CONCLUSION

The results from our study showed increased growth of *Chondrus crispus* in the elevated CO_2 concentration. However, this enhancement of growth rate was not attributed to the photosynthetic carbon assimilation of the algae as photosynthetic rate was not increased in the high CO_2 acclimated algae. The growth enhancement in algae was most probably due to the down-regulation of energy consuming CCMs in the elevated CO_2 concentration which had been observed in some other macroalgal species as well. In order to better understand the underlying physiological mechanisms of *C. crispus* leading to increased growth in elevated CO_2 concentration it is necessary to investigate the functioning of CCMs in details under different CO_2 concentrations. *C. crispus* have external and internal carbonic anhydrase (CA) (Smith and Bidwell 1989, Brechignac and Andre 1985) and experiments should be performed with inhibitors of this enzyme. It is also necessary to quantify the amount and activity of CA present in *C. crispus* through biochemical analysis.

In the present study, algae were grown in CO_2 enriched water but photosynthesis of this CO_2 acclimatized was measured in natural seawater (not CO_2 enriched). It would be rather interesting and more realistic to observe their photosynthetic response in CO_2 enriched seawater.

Effects of increased CO_2 in elevated temperature have great implications for the *C. crispus* inhabiting in their southern distribution boundary. In our study, the growth difference between low and high CO_2 concentration was more pronounced in the elevated temperature. It is rather difficult to predict what would be their response to increased CO_2 level in the natural environment under elevated temperature because natural field environments are rather complex system and too many factors act there simultaneously. However, in our study enhanced photosynthesis was observed in elevated temperature although the overall growth was reduced.

Further experiments should be conducted to investigate the CO_2 effect on this macroalgal species in low temperature (<5 ^{o}C). *C. crispus* experience much lower temperature in their northern distribution boundary and their growth is much low there. Moreover, solubility of CO_2 in cold water is higher. Therefore, it would be interesting to know how this species will react to increased CO_2 in low temperature (<5 ^{o}C).

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