Effects of Warming and Ocean Acidification
on Calcification and Photosynthesis of Arctic Coralline Red Algae
under Summer Light Conditions

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Abstract

Rising anthropogenic CO₂ emissions since the pre-industrial time are warming the atmosphere and lead to an enhanced uptake of heat and CO₂ into the ocean, resulting in increasing sea surface temperature (ocean warming (OW)), decreasing ocean pH (ocean acidification (OA)) and decreasing carbonate saturation state. Arctic surface waters are projected to become partly undersaturated with respect to aragonite already within this century. Calcified crustose coralline algae (CCA) are regarded sensitive to dissolution due to their high amount of magnesium carbonate, being more soluble than aragonite. Warming might add to CO₂ stress, resulting in potential synergistic effects on calcification in CCA. Knowledge on the calcification process in CCA is limited, however, a linkage to energy generating processes (photosynthesis and respiration) is assumed.

To test for single and synergistic effects of OW and OA on calcification and energy generating processes in Arctic CCA, *Lithothamnion glaciale* was incubated for two months under a cross-factorial design of elevated temperatures (3.5°C, 5.5°C and 7.5°C target values) and elevated pCO₂ (390, 560, 840, 1120 µatm target values) (12 treatment combinations, n = 4 + 1 blank). Light conditions simulated Arctic summer (12 µmol photons m⁻² s⁻¹). In a side experiment, photosynthetic O₂ evolution (O₂E) and respiratory O₂ consumption (O₂C) were measured optode based under control and treatment conditions.

Mean calcification rates decreased over the experimental duration and with increasing pCO₂, except for the lowest temperature, where calcification was constantly reduced with increasing pCO₂. Dissolution occurred first at the end of the experiment and only in the highest temperature treatment. Relative calcification rates at highest temperature increased at low and intermediate pCO₂ levels and decreased first at a pCO₂ >1000 µatm. These results indicate a negative synergistic effect for high pCO₂ and elevated temperature, as well as a positive synergistic effect of intermediate pCO₂ and elevated temperature. Results of relative O₂E and O₂C rates indicate a strong linkage between calcification and energy generating processes.

From this study one can conclude, that within this century the Arctic coralline alga *L. glaciale* might not be negatively affected by elevated temperatures and pCO₂ during the Arctic summer season, but rather might profit from those conditions by increasing its calcification rates. Negative effects of elevated temperatures and pCO₂ on *L. glaciale* during the Arctic summer season are first expected for a combination of temperature and pCO₂, that is not predicted to occur within this century for the Arctic Ocean.
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1 Introduction

Increasing carbon dioxide (CO$_2$) emissions since the industrial age are changing the atmospheric CO$_2$ concentration and owing to radiative forcing the atmospheric heat content. The Earth’s atmosphere is getting warmer and richer in CO$_2$. The ocean takes up heat and CO$_2$ from the atmosphere, resulting in increasing sea surface temperatures (Levitus et al., 2005) (ocean warming) and decreasing ocean pH (Caldeira & Wickett, 2003) (ocean acidification) as well as decreasing carbonate saturation state (Orr et al., 2005).

The Arctic Ocean is considered particular sensitive to the impact of global climate change (ACIA, 2005), mainly due to the existence of feedback processes, which might enhance ocean warming, e.g. the albedo-feedback (Loeng et al., 2005), and a naturally low carbonate saturation state (Orr et al., 2005). The Arctic Ocean is assumed to become faster undersaturated with respect to carbonate saturation than other oceans (Orr et al., 2005). Particular calcifying species are likely threatened by carbonate undersaturation (Guinotte & Fabry, 2008).

Calcifying crustose coralline red algae (CCA) are an important part of the Arctic benthic ecosystem (Adey & Macintyre, 1973). CCA are abundant primary producers and significant contributors to coastal calcium carbonate (CaCO$_3$) deposition (Nelson, 2009). The three-dimensional structure of CCA rhodololiths provides microhabitats for diverse species (Foster, 2001) and increases the biodiversity of the habitat (BIOMAERL Team, 2003). CCA deposit CaCO$_3$ in the form of high magnesium calcite (Mg-calcite), which has a higher solubility than aragonite or calcite (Andersson et al., 2008). Under ongoing CO$_2$ emissions (SRES A2, IPCC 2000), 10% of Arctic surface waters are projected to become undersaturated with respect to aragonite within the coming decades (Steinacher et al., 2009). Based on higher solubility, organisms building up high Mg-calcite, like CCA, are expected to face corrosive conditions even earlier (Andersson et al., 2008). Details on the calcification process in CCA are limited, however, calcification is assumed to be linked to energy generating processes, e.g. photosynthesis or respiration (Borowitzka, 1982; Okazaki, 1977).

So far, the physiological responses of Arctic CCA to the combined effects of ocean acidification (OA) and ocean warming (OW) have not been investigated. Since the future ocean is considered to change in respect to both OW and OA, synergistic effects are likely and might be different to single effects. Therefore, this study aims to answer the following questions: what are the single and synergistic effects of OA and OW on calcification and photosynthesis of the Arctic CCA Lithothamnion glaciale? Does OW interact with reported negative OA effects on calcification? Do effects on calcification rates correlate with photosynthesis rates, arguing for a linkage between both processes? Which implications could be drawn from our findings for the Arctic CCA species L. glaciale under future ocean conditions?
I tested the Arctic CCA *Lithothamnion glaciale* in 12 different combinations of elevated $pCO_2$ and temperature levels on their response in calcification and photosynthesis rates. The experiment lasted three months and simulated Arctic summer conditions of polar day (24 h light).

**Hypothesis and Assumptions**

The experiment was based on the hypothesis that elevated $pCO_2$ and temperature might show single or synergistic effects on calcification and photosynthesis rates in the Arctic CCA *Lithothamnion glaciale* under summer light conditions. The two main questions are:

a. What are the effects of the applied treatment on calcification and photosynthesis, i.e. how are respective rates affected? Calcification and photosynthesis are assumed to be directly affected by elevated $pCO_2$ and temperature due to changes in carbonate chemistry and temperature dependent metabolic activity, compare table 1. Synergistic effects of elevated $pCO_2$ and temperature might change assumed responses.

b. Do both processes respond in accordance with each other, indicating a linkage between calcification and photosynthesis? Assuming that calcification and photosynthesis might be linked by energy, both processes might be indirectly affected by the treatments due to changes in energy availability: if energy storage during photosynthesis (ATP, CO$_2$-fixation in starch) changes, it could affect consequently energy liberation during respiration, depending on available energy, and calcification, being in need of energy.

**Table 1: Assumed impacts of elevated $pCO_2$ and temperature on calcification and photosynthesis.**

<table>
<thead>
<tr>
<th></th>
<th>Calcification</th>
<th>Photosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elevated $pCO_2$</strong></td>
<td>Decreasing concentrations of CO$_3^{2-}$, could reduce the availability as substrate for calcification (Guinotte &amp; Fabry, 2008).</td>
<td>A lowering pH might impair enzyme activity (Calvin-cycle, cellular respiration) and proton-gradients (light-dependent reaction).</td>
</tr>
<tr>
<td></td>
<td>A decreasing carbonate saturation state could lead to dissolution below $\Omega&lt;1$ to CaCO$_3$ (Fabry et al., 2008).</td>
<td>A higher concentration of dissolved inorganic carbon (DIC) could increase the substrate availability for CO$_2$-fixation (Riebesell et al., 2007).</td>
</tr>
<tr>
<td><strong>Elevated temperature</strong></td>
<td>Changes in carbonate chemistry: decreasing gas solubility according to Henry’s law and alteration of temperature-dependent dissociation constants, shifting the relative proportions of inorganic carbon species (Zeebe &amp; Wolf-Gladrow, 2001).</td>
<td>Increasing temperatures generally increase physiological processes (Sommer, 1998), e.g. cellular respiration rates.</td>
</tr>
</tbody>
</table>
State of the Art

Most studies, which investigated the effects of global change (ocean warming and ocean acidification) on CCA, have focused on the response of tropical and temperate CCA to elevated $pCO_2$ alone, revealing an overall negative $CO_2$ effect (Kuffner et al., 2008, Jokiel et al., 2008, Hofmann et al., 2011, Büdenbender et al., 2011). So far, only the study of Martin & Gattuso (2009) investigated the effect of elevated $pCO_2$ in combination with elevated temperature on Mediterranean CCA, revealing synergistic effects of both factors: Net calcification decreased under the combined treatment of high temperature and high $CO_2$, while $pCO_2$ and temperature alone showed no effect. Polar CCA are poorly studied so far: Büdenbender et al. (2011) is until now the only study dealing with polar CCA. Büdenbender et al. (2011) showed, that calcification rates significantly decreased under Arctic summer and winter light conditions. The $CO_2$ level, at which calcification started to decrease, was different between the seasons: Calcification decreased already at a $pCO_2$ of 750 µatm in winter and experienced net dissolution, whereas in summer only CCA in the highest $pCO_2$ treatment (1570 µatm) experienced net dissolution.

A linkage between photosynthesis and calcification has been suggested by Okazaki (1977), Digby (1977) and Borowitzka (1982), who reported light stimulation of calcification, e.g. a 10-fold faster $CaCO_3$ deposition under light than in the dark (Borowitzka, 1982).

1.1 Marine Impacts of Global Climate Change

Global climate change is widely recognized as a consequence of anthropogenic increased emissions of climate relevant greenhouse gases, leading to enhanced radiative heating (Mitchell, 1989). Atmospheric temperatures have already increased stronger than can be explained by natural variability (IPCC, 2001). $CO_2$ and water vapour are the most important greenhouse gases (Mitchell, 1989). Since the industrial revolution $CO_2$ emissions are continuously rising (Raupach et al., 2007), leading to increasing atmospheric $CO_2$ concentrations with a recent concentration of 394 parts per million (ppm) (ESRL/NOAA, March 2012) compared to pre-industrial concentrations of 280 ppm (Canadell et al., 2007). Compared to reconstructed Earth history the recent rate of emitted $CO_2$ is outstanding among the past 22 000 years (Joos & Spahni, 2008).

The main sources for $CO_2$ emissions are anthropogenic fossil fuel combustion, industrial processes and changes in land use, i.e. deforestation. In the coming years, growth of the world population and economy, as well as industrial growth of developing countries will most likely further increase $CO_2$ emissions (Raupach et al., 2007). According to model projections (NCAR CSM 1.4-carbon model) based on the SRES high-emission A2 storyline (IPCC, 2000) future emissions might reach an output
of 30 Gt yr\(^{-1}\), leading to atmospheric CO\(_2\) concentrations of around 840 ppm by the end of this century (Steinacher et al., 2009). A decreasing trend in the capacity of land and ocean sinks for CO\(_2\) uptake has already been observed (Canadell et al., 2007).

With respect to the marine environment, climate change has two impacts: ocean warming as consequence of atmospheric warming and ocean acidification as consequence of a CO\(_2\) equilibration between the atmosphere and the marine system, the so called "other CO\(_2\) problem" (Henderson, 2006).

### 1.1.1 Ocean Warming

Surface ocean and atmospheric heat exchange based on thermodynamic equilibrium is leading to an uptake of atmospheric heat into the ocean (Mitchell, 1989). Significant changes in surface sea temperature (SST) have been recorded since the 1960s (Levitus et al., 2005). In the following 40 years the oceans took up about 84\% of total Earth system heat (Levitus et al., 2005). The strongest changes in SST have been observed in the Atlantic with an 75\% increase in ocean heat content for the upper 300m in the period 1955 till 2003 (Levitus et al., 2005). Barnett et al. (2005) could show that the warming signal was not caused by natural variability, but likely had an anthropogenic origin. According to model projections (NCAR CSM 1.4-carbon model) based on SRES B2 and A2 (IPCC, 2000) global mean SST will further increase by 0.5 - 1.5\(^\circ\)C until the end of this century (Steinacher et al., 2009).

Responses of the ocean to increased SST are complex. Overall, ocean warming is predicted to strengthen the stratification of the surface ocean (Bopp et al., 2001, Sarmiento et al., 2004). A strengthening in stratification could reduce the mixing depth of the surface water column, which most likely reduces the supply of new nutrients from deeper water masses. This could change pelagic productivity with consequences for marine food webs and the deep ocean (Bopp et al., 2001, Sarmiento et al., 2004). Furthermore, microbial activity and physiological responses of both pelagic and benthic flora and fauna are likely affected by elevated SST.

### 1.1.2 Ocean Acidification

The surface ocean and the atmosphere are in a permanent dynamic equilibrium with respect to element concentrations, e.g. [CO\(_2\)]. In consequence, the oceanic CO\(_2\) concentration rises as atmospheric CO\(_2\) concentration rises. The ocean is considered the biggest sink for atmospheric CO\(_2\) and has already taken up about one third of total anthropogenic CO\(_2\) emissions (Sabine et al., 2004). CO\(_2\) that enters the ocean changes the ocean's carbonate system by forming carbonic acid (H\(_2\)CO\(_3\)), which dissociates into bicarbonate HCO\(_3^−\) and carbonate CO\(_3^{−2}\) ions as well as free protons [H\(^+\)] (eq. 1).

\[
CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{−2} + 2H^+ \tag{1}
\]
A part of H\(^+\) is buffered by CO\(_3^{2−}\) ions (Kleypas & Langdon, 2006), which reduces the ocean’s buffer capacity and lowers the carbonate saturation state (Orr et al., 2005, Kleypas & Langdon, 2006, Fabry et al., 2008). Additionally, the carbonate system is shifted to higher HCO\(_3^{−}\) concentrations (Sabine et al., 2004). According to model projections (Steinacher et al., 2008) based on the SRES A2 storyline (IPCC, 2000) the total ocean fraction of oversaturated water might decrease by 17% over this century.

Remaininig free H\(^+\) increase in total H\(^+\) concentration (Orr et al., 2005), which is measurable in the lowering ocean pH; the ocean becomes more acidic, it "acidifies" (Caldeira & Wickett, 2003). Ocean surface pH has already dropped by 0.1 units since pre-industrial times (Caldeira & Wickett, 2003), which translates to an increase in H\(^+\) concentration by 30% (Fabry et al., 2008). The mean surface ocean pH is expected to further decrease by 0.3 - 0.4 units until the end of this century (Caldeira & Wickett, 2003). Assuming unabated CO\(_2\) emissions, ocean pH might even drop by 0.7 units until the year 2300, which would be a lower pH than the ocean has ever experienced in the past 300 million years (Caldeira & Wickett, 2003).

Especially calcifying organisms are expected to suffer from changes in carbonate chemistry (Riebesell et al., 2000, Orr et al., 2005, Fabry et al., 2008): their shells and skeletons made of calcite, aragonite or magnesium-calcite are likely to dissolve in undersaturated water (eq. 2), while the ability to increase calcification rates (eq. 3) could be negatively affected by reduced substrate availability (Guinotte & Fabry, 2008).

\[
CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2HCO_3^{-} \tag{2}
\]

\[
Ca^{2+} + 2HCO_3^{-} \rightarrow CaCO_3 + CO_2 + H_2O \hspace{1cm} \text{respect. } Ca^{+2} + HCO_3^{-} \rightarrow CaCO_3 + H^+ \tag{3}
\]

Geological records revealed that ocean acidification events have happened before in Earth’s history. The major difference between past and recent acidification is the rate of change: while past events occurred slowly over millions of years, e.g. climate change due to plate tectonics (Caldeira & Wickett, 2003), the actual rate of CO\(_2\) uptake into the ocean is much faster (Joos & Spahni, 2008).

1.1.3 The Changing Arctic

The Arctic is considered particular sensitive to the impact of global climate change (ACIA, 2005). In terms of climate warming, the Arctic is assumed to warm faster than the global average (polar amplification) due to cumulative effects of feedback processes (e.g. albedo-, thermohaline- and greenhouse gas-feedback (Loeng et al., 2005)) and a climate, which is strongly associated to sea ice-
atmosphere processes (McBean et al., 2005). Over the past 100 years the mean air temperature has already increased by 0.09°C per decade (based on data form the Global Historical Climatology Network) (McBean et al., 2005), resulting in a thinning and loss of sea ice and melting of glaciers (ACIA, 2005).

In terms of ocean acidification, Arctic surface waters are projected to become faster undersaturated with respect to carbonate saturation than the global ocean due to a naturally low carbonate saturation state and the low water temperature (Orr et al., 2005). Low water temperatures promote gas solubility according to Henry’s law, leading to an enhanced uptake of CO₂ into the Arctic Ocean and a subsequently decreasing pH and carbonate saturation state.

Warming and acidification of surface waters are tightly linked: melting sea ice increases the ocean surface area, which is in direct contact with the atmosphere and consequently takes up CO₂ (Loeng et al., 2005). Freshwater input due to ice melting lowers alkalinity (Steinacher et al., 2009), contributing to low carbonate concentration and buffer capacity. According to model projections based on SRES A2 (IPCC, 2000) warming might increase the uptake of CO₂ by 40%, leading to a 20% higher decrease in saturation state and pH (Steinacher et al., 2009).

In general, the future Arctic Ocean is assumed to become warmer, fresher, stronger stratified and more acidic. In specific, Arctic surface waters are projected to experience a drop in pH by 0.4 units and aragonite undersaturation of nearly the entire water column by the end of this century, when emissions follow SRES A2 (IPCC, 2000) (Steinacher et al., 2008). Already within the coming decades, 10% of the surface water might become undersaturated for at least one month per year (at 409 µatm) or throughout the year (at 450 µatm), establishing corrosive conditions for calcifying organisms made of aragonite or Mg-calcite (Steinacher et al., 2009).

The Arctic Ocean is a marine habitat of extreme conditions, including low water temperatures, partly sea-ice cover, freshwater input, low underwater irradiance and a strong seasonality with respect to light conditions, notably polar day (24 h light) and polar night (24 h darkness) (Freiwald & Henrich, 1994, Loeng et al., 2005). Underwater irradiance is seasonally affected by melt water input, sediment load and stratification in summer as well as sea-ice and snow cover in winter and spring. Reduced transmittance limits the euphotic depth (Svendsen et al., 2002). Productivity follows the prevailing light regime: productivity exhibits a seasonal peak, but is averaged over the year quite low (Loeng et al., 2005). Resident Arctic species are perfectly adapted to these conditions (Loeng et al., 2005), what makes them and their ecosystem particularly sensitive and vulnerable to changes. The adaptation of Arctic species to their extreme habitat likely limits ability to respond to changes (Usher, 2005).
1.2 The Carbonate System of the Ocean

The carbonate system of the ocean is one of the most important chemical equilibria in the ocean. It is responsible for controlling the ocean’s pH (Fabry et al., 2008). In the following, the main aspects of the carbonate system will be given according to Zeebe & Wolf-Gladrow (2001). For further reading and more details see for example Zeebe & Wolf-Gladrow (2001).

Surface ocean and atmosphere exchange CO\(_2\) based on thermodynamic equilibrium. CO\(_2\) that enters the ocean reacts with water and forms true carbonic acid (H\(_2\)CO\(_3\)), bicarbonate (HCO\(_3^-\)) and carbonate (CO\(_3^{2-}\)) ions as well as protons (H\(^+\)) (1.1.2, eq. 1). A small amount of the gaseous CO\(_2\) that is taken up dissolves to aqueous CO\(_2(aq)\). H\(_2\)CO\(_3\) and CO\(_2(aq)\) are generally treated together, e.g. as CO\(_2\). The equilibrium of the carbonate system and therefore the quantities of the ions is controlled by equilibrium constants (K\(_1\) and K\(_2\))(eq. 4).

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \overset{K_1}{\rightleftharpoons} \text{HCO}_3^- + \text{H}^+ \\
\text{HCO}_3^- & \overset{K_2}{\rightleftharpoons} \text{CO}_3^{2-} + \text{H}^+
\end{align*}
\]

The carbonate system in seawater can be described with stoichiometric equilibrium constants (K\(_1^*\), K\(_2^*\)), which are related to concentrations and dependent on temperature, salinity and pressure.

The total dissolved inorganic carbon (DIC) of the ocean is the sum of all dissolved inorganic carbon species (eq. 5).

\[
\text{DIC} = [\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]
\]

The total alkalinity (TA) (eq. 6) is the "capacit of water to neutralize hydrogen ions" (Smith & Key, 1975). Dickson (1981) gives the following definition: "Total alkalinity of a seawater sample is defined as the number of hydrogen ions equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant K ≤ 10\(^{-4.5}\) at 25°C and zero ionic strength) over proton donators (acids with K > 10\(^{-4.5}\)) in 1 kg of sample".

\[
\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] + [\text{OH}^-] - [\text{H}^+] + \text{minor components}
\]

With K\(_1^*\) and K\(_2^*\), DIC (eq. 5) and a simplification of (eq. 6) the carbonate system can be described quantitatively. These four equations describe 6 unknown variables: [CO\(_2\)], [HCO\(_3^-\)], [CO\(_3^{2-}\)], [H\(^+\)], DIC and TA. In consequence, the carbonate system can be calculated from any known two of these six variables. Yet, it has to be kept in mind, that only [CO\(_2\)], [H\(^+\)], DIC and TA can be measured directly.
The carbonate saturation state ($\Omega$) is important for the formation and dissolution of calcium carbonate ($\text{CaCO}_3$) in the ocean. $\Omega$ is a function of $[\text{CO}_3^{2-}]$ (eq. 7, with $K_{sp}^{*}$ being the stoichiometric solubility product for $\text{CaCO}_3$). Based on eq. 7, $\Omega$ is reduced by the reduction of $\text{CO}_3^{2-}$ ions with $\text{H}^+$, which mainly originate from $\text{CO}_2$ uptake in water (eq. 1).

$$\Omega = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]}{K_{sp}^{*}} \quad (7)$$

At $\Omega > 1$, a solution is supersaturated with respect to $\text{CaCO}_3$, favouring biogenic $\text{CaCO}_3$ precipitation. At $\Omega < 1$, a solution is undersaturated with respect to $\text{CaCO}_3$, favouring biogenic $\text{CaCO}_3$ dissolution.

### 1.3 Crustose Coralline Red Algae

Crustose coralline algae (CCA) are calcified benthic macroalgae of the phylum Rhodophyta. They are the most calcified algae in the ocean (Littler, 1976), being a major component of the benthic calcium carbonate production (Nelson, 2009). Their rigid appearance and characteristic purple pink colour make them easy recognizable among other benthic algae (compare fig. 2). Yet, they are regarded as a taxonomically difficult to identifying algae group which might explain their underrepresentation in scientific literature (Adey & Macintyre, 1973). Detailed knowledge on ecological aspects and metabolic functions, e.g. their calcification process, is still rare. Recently, CCA got more attention as biological research object in the light of ocean acidification studies due to their strong calcified tissue.

Due to the good conservation of the calcified tissue, CCA comprise a hugh fossil record (Steneck, 1986). Fossil CCA are used in paleosciences in reconstructions of paleoclimate (Foster, 2001) or paleooceanic conditions, e.g. past oceanic Mg/Ca ratios (Ries, 2009). CCA are commercially used as agent for water filtration and as soil conditioner (Foster, 2001). However, due to their slow growth, CCA are considered a non-renewable resource (BIOMAERL Team, 2003).

**Taxonomic Classification (Guiry & Guiry, 2012)**

In the phylum of Rhodophyta CCA belong to the order of Corallinales, which consists of two families: the Hapalidiaceae and the Corallinaceae. The Hapalidiaceae separate into three subfamilies of which Melobesioideae is the biggest with 180 species. All species are non-articulate, i.e. completely calcified. The Corallinaceae separate into eight subfamilies, of which five consists of non-articulate and three (Amphirioideae, Corallinoideae, Metagoniolithoideae) of articulate, i.e. not completely calcified, species (compare fig.1).
Distribution and Habitat

CCA are worldwide abundant (Adey & Macintyre, 1973). They occur over a broad range of temperature regimes in the benthic photic zone of all oceans from Arctic to Antarctic (reviewed by Steneck, 1986). Their depths distribution ranges within the photic zone from the shallow intertidal to the lower end of the photic zone (Adey & Macintyre, 1973). CCA have a low light requirement and can live at great depths (Foster, 2001) close to light compensation conditions (Schwarz et al., 2005). CCA primarily occur on hard substrata in areas with sufficient water energy (Adey & Macintyre, 1973). Free-living forms can also be found on sediment bottoms with less water energy where they accumulate to large communities (Foster, 2001). In tropical waters CCA are tightly associated with coral reefs (reviewed by Littler, 1972).

Morphology and Growth Forms

CCA morphology and growth forms are divers and influenced by prevailing habitat conditions, e.g. water turbulence and depth (reviewed by Steneck, 1986). The three main growths forms are: crusts (completely attached to a substrate), "leafy"-forms (partly attached with unattached margins) and free-living forms (completely unattached) (Steneck, 1986). According to their degree of calcification, CCA divide into articulate and non-articulate species. Articulate species have uncalcified joints, which make them more flexible (Johansen, 1981). From presently known 605 coralline species 99 are articulate (Guiry & Guiry, 2012). Non-articulate species are completely calcified (Johansen, 1981). They grow attached as thin or thick crusts or free living as maerl and rhodoliths (Steneck, 1986). Maerl originates from fragments of crust protuberances. Those fragments continue to grow freely by protuberance elongation, which leads to a delicate, branched shape (Johansen, 1981). Rhodoliths originate from spore settlement on an unstable organic or inorganic substrate, e.g. mussels, stones (Adey &
Macintyre, 1973). They grow as crust layers around those nuclei, which are either permanent incorporated into the rhodolith or decomposed with time (Johansen, 1981), leaving a characteristic hollow core. Rhodoliths have a three-dimensional, roughly spherical shape with abundant protuberances (Johansen, 1981).

In spite of their free-living lifestyle rhodoliths can accumulate to large communities, so called "rhodolith beds" (Foster, 2001). According their area cover, Foster (2001) ranks rhodolith beds with kelp beds and forests, seagrass meadows and non-articulated coralline reefs. He suggests rhodolith beds to be one of the four major benthic macroalgae communities in the ocean.

1.3.1 Cellular Anatomy and Function (according to Johansen, 1981)

Tissue of CCA is arranged in filaments. Filaments build a thallus that consists of an inner (hypo-) and an outer (perithallus) part. In crusts, hypothallial tissue is build by the lowermost filaments, which are oriented parallel to the substrate. Perithallial tissue is build by the uppermost filaments of the hypothallus. Filaments of the perithallial tissue arch perpendicular away from the hypothallus towards the crust surface and end in small epithallial cells, building a cell layer (epithallium) comparable to an epidermis. In rhodoliths, filaments are similar orientated with the exception, that hypothallial tissue is organized in a central "core" (Woelkerling, 1988), being surrounded by perithallial tissue. Consequently, perithallial filaments arch to all sides (compare fig. 3).

Tissues can be distinguished by the cell length with epithallus cells ≪ perithallus cells ≪ hypothallus cells. Cells of a filament are joined vertically by primary pit-connections, which originate during cell division, being closed by a pit plug. Cells can also be joined laterally by secondary pit-connections and cell fusions. Secondary pit-connections are narrow channels through the cell wall of adjacent cells, closed by a pit plug. Cell fusions link adjacent cells without any plug. They originate as the cell wall
between two cells dissolves and the nuclei of both cells fuse to form a new cell. Both lateral and vertical connections are assumed to be important for the transport of photosynthates from photosynthetic active to unactive (Steneck, 1986) or shaded (Dethier & Steneck, 2001) tissue cells. Algae grow by cell division of meristematic cells in the upper perithallial tissue. Meristematic cells lay either below epithallial cells (intercalary meristem) or at the top end of perithallial cells when epithallial cells are absent (primary meristem). Primary meristems are mostly found where intense growth occurs, e.g. branch tips. Dividing meristematic cells produce both perithallial and epithallial tissue (Steneck, 1986). Dead epithallial cells are discharged in a "sloughing" process (Johansen, 1981) and replaced by new cells. Sloughing is discussed as a way to keep the epithallial surface clean of epiphytes (Borowitzka & Vesk, 1978). Meristematic cells also produce reproductive cells by cell differentiation. Reproductive cells are contained in roofed chambers (conceptacles) at the surface of crusts or protuberances, opening by a single or multiple pores to the surrounding water. Photosynthesis takes place in epi- and upper perithallial tissue, which is indicated by a high number of chloroplasts. Starch grains are stored deeper in the tissue (Borowitzka & Vesk, 1978). Nutrients are taken up by epithallial cells (Johansen, 1981).

1.3.2 Ecology

Low-Light Adaptation

CCA grow best under low light intensities (Johansen, 1981), which enables them to occupy habitats in great depths up to 200 m or in the shade of other plants (compare Adey & Macintyre, 1973). This low light adaptation might be based on a more efficient absorption of low light and /or a high ability to fix and store CO$_2$ during photosynthesis (Steneck, 1986, Schwarz et al., 2005). Schwarz et al. (2005) suggest that low-light adaptation might be an explanation for the occurrence of CCA in high latitude waters, where irradiance is low and further reduced by ice-cover and strong seasonality regarding polar day and night.

Growth Pattern and Growth Rates

Basic requirements for CCA growth are water motion and low light intensities (Johansen, 1981). Water motion facilitates nutrient supply to the algae and helps mechanical to keep the algal surface clean of epibionts (biofouling)(Steneck, 1986) by rolling rhodoliths against each other. Additionally, biofouling is reduced by grazing of for example snails on CCA surfaces (reviewed by Littler, 1972) and sloughing of epithallial cells (Borowitzka & Vesk, 1978). A clean, unshaded algal surface is important to avoid reductions in photosynthetic productivity (Steneck, 1986). CCA grow via vertical extension, increase in thickness and / or branch elongation (Johansen, 1981). Growth rates are low compared
to uncalcified algae and differ with season, depth and geographical region, i.e. temperature regime (compare an overview given by Foster, 2001). Experimentally estimated growth rates vary between 0.1 - 1.0 mm y$^{-1}$ for temperate maerl (Bosense & Wilson, 2003), 0.4 mm y$^{-1}$ for free-living individuals at depths < 20m (Foster, 2001) and < 1.0 cm y$^{-1}$ for intertidal crust margins (Dethier & Steneck, 2001).

1.3.3 Calcification

CCA deposit calcium carbonate (CaCO$_3$) in the form of high Mg-calcite (Mackenzie et al., 1983) with an average magnesium carbonate (MgCO$_3$) fraction of 11 - 21 Mol %, occasionally up to 36 Mol %, which is the highest MgCO$_3$ content so far described in any calcifying species (Vinogradov, 1953). High Mg-calcite has a higher solubility than aragonite or calcite (Andersson et al., 2008), which are the most dominant CaCO$_3$ forms in calcifying organisms (Littler, 1976). In CCA, calcite deposition takes place within the cell wall (Borowitzka, 1977). Calcification in CCA is assumed to provide mechanical support and protection against grazing and fouling (Littler, 1976). However, the mechanisms of calcification in CCA are still unknown and detailed knowledge is limited, so far. In the following, the main theories about the calcification process in CCA are described.

The "Organic Matrix Theory" (Borowitzka, 1977) suggests that a protein-polysaccharide complex favours the deposition of calcite due to specific substances contained in these complexes (Borowitzka, 1977). This theory is supported by Lind (1970) who proposed initial crystal nucleation to happen within an electron dense cell wall layer close to the membrane.

The "Bicarbonate Usage Theory" (Borowitzka, 1977) suggests a close relationship between photosynthesis and calcification: HCO$_3^-$, taken up for photosynthesis, is assumed to be converted by photosynthetically produced OH$^-$ and e$^-$ to CO$_3^{2-}$, which might subsequently react with Ca$^{2+}$ to CaCO$_3$ (Borowitzka, 1977). The bicarbonate usage theory is not proven so far.

McConnaughey& Whelan (1997) proposed calcification to be a tool for H$^+$ generation, which supports photosynthetic carbon and nutrient uptake, suggesting that CaCO$_3$ production is only a by-product: Calcification produces H$^+$ (eq. 3), which could promote the conversion from HCO$_3^-$ to CO$_2$ for photosynthetic uptake.

Furthermore, McConnaughey& Whelan (1997) proposed that pH-regulation mechanisms could be involved in the calcification process in CCA: elevating the pH at the site of calcification would convert bicarbonate HCO$_3^-$ to carbonate CO$_3^{2-}$ and facilitate calcification. pH-regulation mechanisms are supposed to be energy dependent due active transport of H$^+$ (McConnaughey& Whelan, 1997).
2 Material and Methods

2.1 Material Collection

Crustose coralline algae used in the experiment originated from Kongsfjorden (79°N, 12°E), an Arctic fjord at the west coast of Svalbard, Norway. An extensive review on the physical environment and marine ecosystem of Kongsfjorden is given by Svendsen et al. (2005) and Hop et al. (2002). In the fjord, coralline algae are reported to be abundant on subtidal hard-bottom, being an important part of the benthic primary producers (Hop et al., 2005).

Algal material was collected in June 2010 via scuba diving from 8 - 12 m depth at Kongsfjordneset (78°58’ N 9°30’E) (fig. 4).

![Figure 4: Overview map of Svalbard (right) and detail map of Kongsfjorden (left); the red star marks the sampling location at Kongsfjordneset.](image)

2.1.1 Cultivation and Acclimation in the Lab (GEOMAR)

Algal material was transported from Svalbard to Kiel in 100 l natural fjord water at 3°C. From summer 2010 until spring 2011 algae were kept cultivated in a climate chamber at GEOMAR. Cultivation was done in 2 l natural North Sea water, which was changed every two weeks in order to renew nutrients and total alkalinity. A streaming pump (Hydor, Koralina nano, 1600 l h⁻¹) accounted for a continuous water current in the cultivation water. In order to simulate Arctic winter and summer conditions, algae were kept at no light and 1°C from summer 2010 until spring 2011, and at 6 μmol photons m⁻² s⁻¹ and 4°C from spring 2011 until summer 2011, respectively. Light and temperature between winter and summer season were gradually changed.
2.1.2 Identification

Identification of collected coralline algae (rhodoliths) separated into genus and species identification. For the genus identification rhodolith-forming genera of coralline algae (Harvey & Woelkerling, 2007) were compared with genera lists of the sampling location Spitzbergen (Hansen & Jenneborg, 1996; Vinogradova, 1995 and Athanasiadis, 2006 in Guiry & Guiry, 2012), resulting in four rhodolith-forming coralline genera being described for Spitzbergen: Phymatolithon, Mesophyllum, Lithothamnion and Lithophyllum. Subsequently, diagnostic key features of the selected genera (Harvey & Woelkerling, 2007) were compared to scanning electron microscopy (SEM) pictures of algae specimens. For SEM pictures three randomly chosen algae individuals were bleached in 10% sodium hypochlorite (NaClO) solution and sectioned according to Woelkerling (1988). SEM pictures were taken with CamScan 44/EDX (Institute for Geosciences, Geologie/Paleontology, Christian-Albrechts University Kiel) and Phenom G2 (FEI Company, GEOMAR, Marine Ecology).

For the species identification the mean cell diameter of 17 surface cells of algal specimens was compared to according measurements from Adey et al. (2005). The mean cell diameter was calculated from the longest and shortest distance of surface cells (through the pit-plug until the middle lamella). Since identification of coralline algae is known to be complicated (e.g. Adey & Macintyre, 1973) results of our identification will be genetically validated (Museum National d’Histoire Naturelle, Paris).

2.1.3 Photo Database

Each alga specimen used in the experiment was documented from front- and backside by means of digital photographs. All pictures were collected in a catalogue, allowing an easy identification of algae specimens, e.g. during buoyant weight measurements or water change. The procedure was repeated after the experiment for reasons of visual comparison.

2.2 Experimental Design

The experimental design included two treatment factors (CO$_2$ and temperature) at four, respectively three factor levels (target levels 390, 560, 840, 1120 µatm pCO$_2$ and 3.5, 5.5, 7.5°C) which were cross factorial combined, resulting in 12 distinct treatment combinations.

Each treatment combination was replicated four times with identical incubation units (reactors), containing one alga specimen. Additionally, one reactor per treatment combination contained no alga specimen and served as blank in order to monitor processes in the incubation water, like bacterial activity. The total experimental duration of three months consisted of one month "baseline" and two months treatment and divided into 6 incubation phases of 14 days each, after which sampling took
place and water was renewed. We chose 14 days as duration for the incubation phases as well as a water volume of 2 l for incubation in order to get a significant change in total alkalinity (TA) due to algal calcification.

Baseline

A "baseline" represents in general a response to control condition (Baker & Dunbar, 2000), e.g. the response of an organism to habitat specific in-situ conditions. By comparing an organism’s standardized response with its respond to changed conditions, e.g. due to an applied treatment, the effect of the treatment on the organism can be evaluated as percental deviation from the standardized response (eq. 8) (compare Büdenbender et al., 2011).

\[
X_{rel} (%) = \frac{X_{net (treatment)}}{X_{net (baseline)}} \times 100
\]  

(8)

with \(X_{rel}\) = relative change from the baseline (%), \(X_{net (treatment)}\) = measured net response under treatment conditions and \(X_{net (baseline)}\) = measured net response under baseline conditions. Responses under baseline conditions are represented by 100%. Relative responses \(> 100\%\) represent higher and relative responses \(< 100\%\) represent lower response rates compared to the baseline.

In our experiment, we run a baseline of one month (two incubation phases) prior to the treatment phase of elevated CO\(_2\) and temperature. During the baseline, all replicates were incubated at ambient conditions of 390 \(\mu\)atm and 3.5\(^\circ\)C target levels in order to simulate in-situ Arctic summer conditions. At the end of an incubation phase sampling took place as outlined in 2.4. As coralline algae often contain solid material, like stones (compare 1.3), individual alga specimens used in the experiment could not be standardized to their total weight. Therefore, a baseline was particular important in order to standardize the experimentally used coralline algae.

Treatment

A treatment represents conditions, which are changed compared to ambient, in-situ conditions. In our experiment we implemented the two treatment factors CO\(_2\) and temperature. CO\(_2\) target levels corresponded to present day (ESRL-NOAA data, Mauna Loa Observatory) and two-, three- and four-times pre-industrial CO\(_2\) (Riebesell et al., 2010). CO\(_2\) partial pressure (\(pCO_2\)) was elevated in the incubation water by a constant aeration of the water with premixed air enriched with \(pCO_2\) target levels (Riebesell et al., 2010).

Water temperature target levels corresponded to mean in-situ summer temperatures at habitat depth of collected alga material (F. Cottier, personal communication; mooring site at the outer Kongsfjord
from 2003-05, 2007-11) and a model projected increase in surface sea temperature by 2°C from pre-
industrial time to the end of this century, according to the SRES A2 scenario (Steinacher et al., 2009).
A temperature elevation by 4°C was meant as physiological test for experimentally used algae.

Light Regime

Light-conditions simulated Arctic summer, i.e. polar day with continuous light. Underwater irradiance
was calculated for respective habitat depth of collected alga material (12 m) with eq. 9 (D. Hanelt,
personal communication):

\[
\text{PAR (depth)} = \max \text{PAR} \times \exp (-K_d \times \text{depth})
\]  

with \( \text{PAR} \) = photosynthetic active radiation (400 - 700 nm) and \( K_d \) = vertical attenuation coefficient
of downward irradiance. For a depth of 12 m we used a max PAR of 78 W m\(^{-2}\) and a \( K_d \) of 0.28 m\(^{-1}\). (compare Hanelt et al. (2001) p. 652 fig. 2 (PAR) and p. 653 fig. 5 (K\(_d\))). For the \( K_d \) value we
assumed no melt water input due to the sampling location at the outward fjord. Calculated underwater
irradiance was 12 \( \mu \text{mol} \) photons m\(^{-2}\) s\(^{-1}\).

Experimental Terms

Following terms will be used when referring to the experimental design:

- **Experimental duration**: the total time of the experiment (12 weeks).
- **Incubation phase**: a period of two weeks under experimental conditions, after which sampling
took place and incubation water was renewed; applies to both treatment and baseline phases.
- **Treatment phase**: a period of two weeks under treatment conditions, after which sampling took
place and incubation water was renewed.
- **Baseline phase**: a period of two weeks under baseline conditions, after which sampling took place
and incubation water was renewed.

2.3 Experimental Set-Up

The experimental set-up consisted of three freezer cabinets (in the following: freezers), which were as-
signed to each one target temperature level. Each freezer held 20 identical incubation units (reactors),
which were assigned to four \( p\text{CO}_2 \) levels (fig 5). Reactors were made of acryl glass with a volume of
\( \sim 2.5 \text{ l} \) each. The 20 reactors were placed in a water bath in order to stabilize temperature fluctua-
tions due to cooling and non-cooling intervalls of the freezers. Freezer temperatures were controlled
with independent temperature control units, which measured the temperature inside the freezer and adjusted it to applied target temperatures. The temperature of the water bath was logged (PreSens, F1Box) and verified with an analogue mercury thermometer. Water bath temperatures were regarded representative for temperatures within the reactors. An implemented streaming pump (Hydor, Koralina nano, 1600 l h\(^{-1}\)) maintained a constant water current within each bath.

Reactors were filled with two litres of North Sea water (compare 2.4) and continuously aerated with premixed CO\(_2\) enriched air, which was provided by a gas mixing pump (Vögtlin, Q-flow). Gas with ambient CO\(_2\) originated from compressed air outside of the GEOMAR. Gas was 100% saturated with water vapour by bubbling it through ultrapure water in gas washing bottles (Schott Duran) in order to reduce evaporation in the reactors.

Reactors were of cylindrical shape (radius = 5 cm, height = 40 cm) and made of acryl glass (fig 6). During incubation, reactors were closed by a lid, which contained two holes, closed by rubber plugs: the small hole served for sampling purposes, the bigger hole served for gas supply and outlet by means of two inlets through the rubber plug. Inside the reactor, the gas supply pipe ended in a graphite air stone (aquaria supply), which dispersed the gas. A water current was established by directing the gas bubbles through a vertical acryl glass tube: gas bubbles were released through holes in the upper part of the tube and thereby water was sucked into the tube from its lower end (air lift system) (fig. 6). Target underwater irradiance of 12 \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\) was obtained by installing blue fluorescent lamps (Arcadia, Marine Blue 420 nm Actinic T5) at the inner freezer cover and darkening them with grey foil to the desired irradiance. Underwater irradiance was measured before and after the experiment with a light meter (Licor, Li-250A).

Alga specimens were placed on the reactor bottom. Reactors within a water bath were randomly placed and positions were randomly changed after each sampling. This should ensure homogeneous conditions for all reactors as prevailing temperature and light regime showed small variations within the freezers.
2.4 Experimental Procedure

Experimental procedures included the preparation of incubation water, sampling and change of incubation water to start a new incubation phase. Incubation was done in natural North Sea water with a salinity of 30.4 ± 0.1 before the experiment. Total alkalinity (TA) was increased with sodium carbonate (Na₂CO₃) to around 2366.5 ± 23.6 µmol kg⁻¹ according to Key et al., 2004. Nutrient concentrations of the start water were similar to in-situ concentrations measured in the Kongsfjord in June 2010 (EPOCA mesocosm campaign, unpublished data).

North Sea water was kept in 1000 l tanks from which small volumina were regularly taken for the preparation of start water. Start water refers to the water that was used to start a new incubation phase. In order to ensure a mixed water body within the tank before taking water, tank water was
mixed for one day by using a pump (Eheim Universal). Start water was filtered through 0.2 µm glass fiber filters (GF/B, Whatmann) and distributed to four 20 l polycarbonate bottles (Nalgene). For one day all bottles were cooled to ∼ 7°C in a climate control room and pre-aerated with CO₂ enriched air to the respective target pCO₂ level. Target pH levels of prepared start water were controlled via a portable pH meter (Metrohm, 826pH). Start water for the first treatment phase was not acidified in order to avoid a “CO₂-shock” for the algae. Instead, water was more slowly acidified in the reactors themselves. For the subsequent treatment phases water was pre-acidified.

The routine for sampling procedures and start of a new phase were as follows: at the beginning of the experiment the empty weight of all reactors was determined with a high precision scale (Sartorius, SR3 2001, precision ± 0.1 g). After filling with approximately 2 l of start water the full weight was determined in order to calculate the exact incubation volume of each reactor. Each reactor received one alga specimen and incubation was started by connecting reactors to the gas supply. Samples for nutrient and TA analysis were taken from the start water and represented start conditions for all reactors.

After 14 days, incubation was stopped and reactors were weighted again in order to determine potential loss of water. pH, temperature and salinity were recorded (Metrohm, WTW TetraCon 325 with WTW Multi 350i device). Water samples for nutrient and TA analysis were taken with a syringe. Nutrient samples were stored at -20°C. TA samples were poisoned with 20 µl mercuric chloride (HgCl₂) and stored at room temperature. The incubation water was exchanged with new start water and the next incubation phase was started.

Salinity and differences in reactor weight were recorded to account for possible evaporative losses during incubation, as evaporation influences TA (Zeebe & Wolf-Gladrow, 2001).

### 2.5 Applied Methods and Measurements

According to the study question, we applied methods to determine calcification rates (via buoyant weight and total alkalinity technique) and photosynthetic performance (via O₂ evolution and consumption) in response to the applied treatment. Nutrient measurements were applied to control experimental conditions.

#### 2.5.1 Nutrient Determination according to Seal Analytical

The inorganic nutrients nitrite (NO₂⁻), nitrate (NO₃⁻), phosphate (PO₄³⁻) and silicate (Si) were measured with a nutrient autoanalyzer (Seal Analytical, CFA Quaatro) according to Seal Analytical (NO₂⁻: method no. Q-070-05, NO₃⁻: method no. Q-068-05, PO₄³⁻: method no. Q-064-05, Si: method no. Q-066-05). Detection limits were ± 0.01, 0.03, 0.01 and 0.02 µmol l⁻¹ for NO₂⁻, NO₃⁻, PO₄³⁻.
and Si, respectively. Measurements were single determinations.

In case of measured negative NO$_2^-$, NO$_3^-$, PO$_4^{3-}$ or Si concentrations, the most negative value was set to zero and its difference to zero was added to all other values. For each incubation phase the difference between start and end concentrations was calculated and considered as algal uptake, resulting in uptake rates for each incubation phase. Uptake of dissolved inorganic nitrogen (ΣN) was calculated from pooled NO$_2^-$ and NO$_3^-$ concentrations. Algal uptake was corrected for concentration differences measured in the blank of corresponding treatment combination. Relative consumptions rates were calculated according to (eq. 8) with $X_{rel} = \text{relative uptake of NO}_2^-, \text{NO}_3^-, \text{PO}_4^{3-}$ and Si in each reactor, $X_{net \ (treatment)} = \text{absolute uptake of NO}_2^-, \text{NO}_3^-, \text{PO}_4^{3-}$ and Si in each reactor during the treatment phase and $X_{net \ (baseline)} = \text{absolute uptake of NO}_2^-, \text{NO}_3^-, \text{PO}_4^{3-}$ and Si in each reactor during the mean baseline phase.

### 2.5.2 pH

pH (NBS scale) was measured with a portable pH meter (Metrohm, 826pH, precision ± 0.01 units) in all reactors directly after opening the lid. Reactors were measured in the sequence of their pCO$_2$ level, starting with the lowest level in order to minimize the equilibration time of the pH probe. Each measurement took about 4 minutes until pH readings were constant. The pH probe was regularly calibrated (NBS scale) with buffer solution (Metrohm Ion Analysis) and corrected against certified reference material for ocean carbonate measurements (Prof. A.G. Dickson, Marine Physical Laboratory, University of California).

Measured pH raw data were corrected for actual temperature and certified reference material using eq. 10 according to Dickson et al. (2007). pH (NBS scale) was thereby converted to pH (total scale) ($pH_T$).

$$pH_T(\text{sample}) = pH_T(\text{ref}) + \left( \frac{\text{[measured pH}_{\text{NBS}}]}{1000} \text{ref}_T \right) - \left( \frac{\text{[measured pH}_{\text{NBS}}]}{1000} \text{sample}_T \right) \left[ R \ast (T + 273.15 K) \ast (\frac{L_m}{10}) \right]$$

with $T = \text{temperature at measurement (°C)}$, $R = \text{Revelle factor: 8.315}$, $F = \text{Faraday constant: 96485.340 (C mol}^{-1})$. $pH_T$ (ref) referred to the pH of the reference material (Dickson batch 108, 2011) calculated for the total scale and 20°C from according TA, DIC, salinity, Si and PO$_4^{3-}$ by using the software CO2SYS (Lewis & Wallace, 1998) and following constants: $K_{SO_4}$ from Dickson, and $K_1$ and $K_2$ from Mehrbach et al. (1973), refitted by Dickson & Millero (1987).
2.5.3 Carbonate System

Carbonate system parameters $p\text{CO}_2$ and carbonate saturation state for calcite ($\Omega_{Ca}$) and aragonite ($\Omega_{Ar}$) were calculated for each replicate with the software CO2SYS (Lewis & Wallace, 1998) from pH$_T$ and total alkalinity (TA), salinity, temperature, PO$_4^{3-}$ and Si (Riebesell et al., 2010). We used the constants KSO$_4$ from Dickson, K$_1$ and K$_2$ from Mehrbach et al. (1973), refitted by Dickson & Millero (1987). For pH$_T$ and temperature we used individual measured values, for TA and salinity a mean of start values ($2366.5 \pm 23.6 \mu$mol kg$^{-1}$, 30.4 $\pm$ 0.1), for PO$_4^{3-}$ and Si a mean concentration of 0.140 and 0.030 $\mu$mol l$^{-1}$, respectively.

2.5.4 Buoyant Weight Measurements according to Davies (1989)

The buoyant weighing technique is a non-destructive way to measure the calcium carbonate (CaCO$_3$) amount of living calcified organisms (Davies, 1989). This method was originally established for coral studies (Jokiel et al., 1978), but was also applied to coralline algae (Potin et al., 1990, Jokiel et al., 2008, Martin & Gattuso, 2009, Ries et al., 2009). We used the technique to measure growth and calcification of incubated alga specimens: as the amount of CaCO$_3$ in L. glaciale varies between 80 - 90% of total biomass (Bilan & Ussov, 2001), CaCO$_3$ deposition (calcification) takes a significant part in growth. Therefore, CaCO$_3$ deposition and growth can be regarded as equivalent in L. glaciale.

The key idea of the buoyant weight method is, that organic material, e.g. tissue, has approximately the same density as sea water, whereas mineralized structures, e.g. calcified tissue, have a higher density than sea water. Consequently, by weighing living specimens under water only the weight of included mineralized structures, like CaCO$_3$, contributes to the buoyant weight (Jokiel et al., 1978). An increase in specimen’s weight over time gives the increase in CaCO$_3$ over time, indicating calcification.

From a measured buoyant weight the weight in air is calculated with eq. 11. The density of the actual object can be calculated by rearranging eq. 11, see eq. 12:

\[
\text{Weight of object in air} = \frac{W_{tW}}{1 - \frac{D_{W}}{D_{O}}} \quad (11)
\]

\[
\text{Density of object} = \frac{D_{W}}{1 - \frac{W_{tW}}{W_{ta}}} \quad (12)
\]

with $W_t =$ weight of object, $W =$ water, $A =$ air, $D =$ density, $O =$ object.

In order to determine the buoyant weight of our alga specimens we used an electronic scale (Sarto-
rius, CPA225D, precision ± 0.01 mg), which was mounted on a water bath filled with start water. A weighing platform was immersed into the water bath and connected with the scale by a wire (fig. 7). The wire was made of wolfram in order to reduce water adhesion, possibly interfering with the weighing process. Air bubbles adhering to the platform or the wire were removed.

Salinity and temperature were constantly recorded during weight measurements with a combined salinity probe (Metrohm, WTW TetraCon 325 with WTW Multi 350i device) in order to correct for water density changes. Weight measurements were carried out at constant temperatures of 15°C and during measurement the water bath was covered with a piece of styrofoam in order to ensure constant measurement conditions. A reference object of known density (glass: 2.5 g cm$^{-3}$) was weighted at the beginning and the end of each measuring day and whenever temperature or salinity changed by 0.1 unit.

Precise weight determination was done with the 100 point measurement mode of the scale: the reference object or alga specimen was placed on the under water platform (fig. 8) and automatically weighted 100 times. The scale weight output was the mean of the 100 measurements, being a representative weight of the measured object or alga specimen. Weight measurements were repeated at least once per object, precision was within ± 0.001 g. All alga specimens were measured before and after the experiment.

For density determination of algal CaCO$_3$, 10 specimens were bleached in a 10% solution of sodium hypochloride (NaClO), dried and subsequently weighted in air (Sartorius, BP310P, precision 0.001 g) and water (buoyant weight). In order to obtain constant buoyant weights of dried specimens, these had to be placed in water some time ahead of weighing, because they absorbed water and it took some time until they were water saturated.

Water density was calculated with recorded salinity and temperature data and respective software (Tomczak, 2000 based on Fofonoff & Millard, 1983). Calcite density was calculated with eq. 12. By using water density, mean algal calcite density and the measured buoyant weights, the weight of each algal specimen in air was calculated with eq. 11. Production of CaCO$_3$ over the experimental duration was calculated from the difference between start and end buoyant weight of individual alga specimens.
2.5.5 Total Alkalinity Determination according to Dickson et al. (2003)

Total alkalinity (TA) was determined via open-cell acidimetric titration with an automatic titration device (Metrohm, Titrando 808) and 0.005 N hypochloric acid (HCl). A subsample of 10 ml was extracted with a syringe, weighted (Sartorius, RC210D, precision 0.0001 g) in order to determine its exact volume, 0.2 µm filtered and titrated. Samples were measured at least twice, precision was ± 0.005 µmol kg⁻¹. Samples were corrected with certified reference material for ocean carbonate measurements (Prof. A.G. Dickson, Marine Physical Laboratory, University of California). The electrode was calibrated with a three point calibration and buffer solution (Roth) every third day. TA was calculated with a non linear curve fitting algorithm based on the Gran function, implemented in a Matlab script (according to Dickson et al., 2003).

TA measurements of the sampled water were corrected for the exact water volume and changes in salinity. Changes in TA during incubation time (difference between start and end value of each incubation phase) were used to calculate net calcification rates. According to Smith & Key (1975), calcification and TA concentrations are in a stoichiometric relationship: calcification lowers TA by two equivalents for one mole precipitated CaCO₃ (eq. 3). Therefore, changes in TA concentrations allow the precise calculation of calcification rates, if calcification is the only possible process affecting TA concentrations. Possible other processes affecting the TA concentrations have to be excluded from the system or have to be corrected for (e.g. nutrient concentration changes). Relative calcification rates were calculated according to (eq. 8) with \( X_{rel} = \text{relative calcification (％)} \), \( X_{net \ (treatment)} = \text{absolute} \).
calcification during the treatment phase, and $X_{\text{net}}(\text{baseline})$ = absolute calcification during the mean baseline phase.

2.5.6 Optode-Based Photosynthetic and Respiratory O$_2$ Measurements

Photosynthetic O$_2$ evolution and respiratory O$_2$ consumption under the experimental treatment were measured with optical electrodes (optodes) via changes in O$_2$ concentrations in light and dark in the surrounding medium, indicating photosynthetic performance. The experimental set-up was established by Form (2011) in collaboration with the Institute for Polar Ecology (IPÖ) Kiel. Measurements of O$_2$ concentrations followed every other incubation phase. The first measurement of O$_2$ concentrations was conducted under baseline conditions (target values: 390 $\mu$atm, 3.5°C) following the general experimental design (2.2).

Principle of Optical Sensing of Oxygen according to PreSens (2005)

Applied O$_2$ measurements were based on the dynamic quenching of luminescence by molecular oxygen. The absorption of light elevates luminescent indicator molecules (luminophores) into an excited state, where they emit the gained energy in form of luminescence. When luminophores collide with other molecules while being excited, they transfer their energy to the colliding molecule, themselves being deactivated without emitting light (dynamic quenching). The decrease in luminescence is measurable; its decay time in the presence of O$_2$ is a function of O$_2$ concentration according to the Stern-Volmer-Equation (eq.13):

$$\frac{L_0}{L} = \frac{R_0}{R} = 1 + K_{sv} \cdot O_2$$

with $L_0$ = luminescence intensity in the absence of oxygen, $L$ = luminescence intensity in the presence of oxygen, $R_0$ = luminescence decay time in the absence of oxygen, $R$ = luminescence decay time in the presence of oxygen, $K_{sv}$ = Stern-Volmer constant, $[O_2]$ = oxygen concentration.

We used a 10-channel fiber-optic oxygen meter (PreSens, OXY-10), which regularly excited respective luminophores embedded in sensor spots (optodes) (PreSens) by sinusoidal lightning. Thereby, luminescence decay time was measured by phase modulation: at decay, the luminescence signal is delayed in time and consequently in sinus phase, i.e. decay time is expressed in phase angle between excited state (lightning) and luminescence signal. As the decay time is a function of prevailing O$_2$ concentration so is the measured phase angle.
Measurement Set-Up

The set-up for the measurements of O₂ concentrations consisted of a water baths and eight independent oxygen incubation chambers (fig. 9). To establish experimental treatment temperatures in the water bath, a flow through cooling system was established: water from the water bath was drained into a reservoir bath of similar size, which fed into a cooling aggregate (Aqua Medic, Titan 4000). Cooled water out of the aggregate was pumped back into the water bath. The water circulation was driven by means of a pump (Eheim Universal). To reduce the hysteresis effect of the cooling device on the water temperature, water in the reservoir bath was heated by a titanium immersion heater (Schego, 300W). Heating sped up the warming process of the water and therefore increased the cooling intervals per time, increasing the total time at applied target temperatures. The immersion heater was controlled by a computer (IKS, Aquastar). Cooling aggregate and immersion heater were adjusted in such a way, that target temperature levels within the water bath could be met. Temperatures were logged (PreSens, F1Box). A streaming pump (Hydor, Koralia 1, 1500 l h⁻¹) accounted for water current and mixing in the water bath. The light regime was adjusted with two blue fluorescent lamps (Arcadia, Marine Blue 420 nm Actinic T5), which were placed above the water bath and darkened with grey foil to the desired underwater irradiance of 12 µmol photons m⁻² s⁻¹, according to the main experiment. Underwater irradiance was controlled with a light meter (Licor, Li-250A).

Independent oxygen incubation chambers were of cylindrical shape and made of acryl glass. They enclosed a water volume of 875 ml (without content) and were air tight. Incubation chambers contained a small table, where organisms were placed on, and a magnetic stir bar under the table, establishing a water current. The magnetic stir bars were driven by a low voltage electric motor which was controlled by an external multi channel transformer. A planar O₂ optode (PreSens, SP-PSi3-NAU-D7-YOP) was placed at the inner side of each lid (compare fig. 10, 11). Optodes were activated by light impulses from glass fibre wires from the outer side of the lid. Subsequent O₂ concentrations were measured with a ten channel oxygen meter (PreSens) according to the principle of optical sensing of O₂ (PreSens). The oxygen meter was connected to a standard computer, recording the measurement with respective software (PreSens, OXY 10v3-33FB) (compare Form, 2011).

Measurement

Before the start of O₂ measurements, optodes were calibrated for each temperature level against 100% and 0% O₂ according to PreSens (2005)(two-point calibration). O₂ saturated water was obtained by aeration of water with ambient air. O₂ free water was obtained by mixing water with a supersaturated solution of sodium sulphite (Na₂SO₃). The calibration was recorded by the OXY 10v3-33FB software.
O$_2$ measurements comprised a light (two days) and a dark (one day) phase, in order to measure light and dark O$_2$ changes. The light phase referred to Arctic summer light conditions.

O$_2$ measurements were conducted separately for all temperature levels. For each temperature, two incubation chambers were assigned to one CO$_2$ level: one chamber was filled with all four alga replicates of one treatment combination (2.2), the other chamber was without alga specimens and served as blank, recording oxygen changes in the incubation water, e.g. due to microbial activity. Incubation chambers were filled with filtered, CO$_2$ pre-equilibrated water according to the corresponding treatment (compare 2.4). Lids were closed air tight without enclosing air bubbles. The O$_2$ concentration within the chambers was measured every 15 seconds based on optical sensing, first in light and subsequently in dark without stopping the measurement. After three days the O$_2$ measurement was stopped and algae were exchanged.
The raw data of O\textsubscript{2} concentrations measured within the incubation chambers was divided into light and dark phase. Since the slope of change in O\textsubscript{2} concentrations over the measuring time was constant, i.e. O\textsubscript{2} evolution in light increased linearly and O\textsubscript{2} consumption in the dark decreased linearly, respective rates were considered constant. In order to calculate O\textsubscript{2} evolution and consumption rates for algae groups of each treatment combination, the change of O\textsubscript{2} concentration for the period of 1 day was used.

Concerning the solubility of oxygen in water, respective O\textsubscript{2} concentrations per day and algae group were corrected for calibration values, air pressure at the time of measurement (obtained from regular measurements at the roof of GEOMAR, research unit Marine Meteorology) and recorded temperature during measurement. Absolute O\textsubscript{2} concentrations in \(\mu\text{mol h}^{-1}\) were calculated with respect to actual and standard air pressure, partial pressure (O\textsubscript{2} and water vapour), volume content of O\textsubscript{2} in air, salinity compensated Bunsen absorption coefficient, molecular mass and molar volume of O\textsubscript{2}, water volume of the incubation chambers and incubation time in hours (for more detail see Form, 2011).

Relative O\textsubscript{2} evolution, respectively consumption rates were calculated from obtained absolute rates by using eq. 8 with \(X_{rel} = \text{relative O}_2 \text{ evolution/consumption (\%)}\), \(X_{net (treatment)} = \text{absolute O}_2 \text{ evolution/consumption during the treatment phase}\), and \(X_{net (baseline)} = \text{absolute O}_2 \text{ evolution/consumption during the mean baseline phase}\).

### 2.6 Applied Statistics

Statistical evaluation of results was done with the software Statistica 8. The applied treatment factors "elevated pCO\textsubscript{2}" and "elevated temperature" (target levels as categorical factors) were tested for their statistical significant effects on difference in nutrient uptake, buoyant weight, relative net calcification rates, and relative net O\textsubscript{2} production and respiration rates. The general null hypothesis was that there would be no single or combined effect of the applied factors. Following statistical tests were applied: a two-factorial analysis of variance (MANOVA) for differences in buoyant weight and relative net calcification rates, and a multiple regression analysis for relative nutrient uptake and relative net O\textsubscript{2} production and respiration rates. The regression analysis accounted for a low replicate number of nutrient uptake rates and O\textsubscript{2} rate measurements per treatment combination (only 1 replication, i.e. two data points per combination). All data were tested for assumptions of the respective statistics, i.e. normal distribution (Shapiro-Wilks test) and homogeneous variances (Levene’s test). Following the main statistical tests a post-hoc test (LSD test) was conducted to find out about significant effects between single combinations.
3 Results

3.1 Identification

For genus and species identification of collected algal material three randomly chosen algae individuals were taxonomically identified following recommendations for crustose coralline algae given in Woelkerling (1988). Diagnostic key features of rhodolith-forming genera (Harvey & Woelkerling, 2007) were compared to SEM pictures of algae specimens. Following key features were positively identified on algal SEM pictures: epithallial cells with flattened and flared outermost cell walls, cell-fusions and no secondary pit-connections. Flared epithallial cell walls and cell fusions were identified in vertical (fig. 12), respectively horizontal (fig. 13) sections. Secondary-pit connections could not be identified. Among rhodolith-forming coralline alga genera the combination of no secondary pit-connections and flared epithallial cells is present only in Lithothamnion (Melobesioideae, compare 1.3) (Harvey & Woelkerling, 2007).

Figure 12: Vertical section of Lithothamnion glaciale from Kongsfjorden, Svalbard, Norway. a. Flat and flared epithallial cells in the outermost cell layer (epithallium) b. Meristematic cells below the epithallium (intercalary meristem) c. Vegetative cells of the perithallium. SEM (Phenom G2 pure desktop) GEOMAR, Kiel.

Figure 13: Horizontal section of Lithothamnion glaciale from Kongsfjorden, Svalbard, Norway. a. Primary pit-connections b. Calcified cell walls c. Cell fusions. SEM (CamScan 44/EDX) Institute for Geosciences, Kiel.

Three species of the genus Lithothamnion are described for Spitzbergen according to Hansen & Jenneborg (1996) and Guiry & Guiry (2012): L. flavescens, L. glaciale and L. tophiforme. One diagnostic feature to differentiate between these species is the mean surface cell diameter (fig. 14) (Adey et al., 2005). The mean surface cell diameter of our specimens was 8.51 ± 0.6 µm (7.03 - 10.00 µm) which is in the range of values reported for L. glaciale of 8.0 ± 1.14 µm (Adey et al., 2005). The mean surface cell diameter of L. tophiforme is 11.7 ± 1.15 (Adey et al., 2005). Mean surface cell diameters of L. flavescence are not reported so far.
Following the taxonomic keys and species descriptions for Arctic crustose coralline algae (Hansen & Jenneborg, 1996; Adey et al., 2005; Harvey & Woelkerling, 2007; Guiry & Guiry, 2012) our collected and identified alga specimens are of the species *L. glaciale* Kjellman, 1883. *L. glaciale* is described to be a dominant species of the benthic habitat in the Subarctic, including Spitzbergen (Adey et al., 2005; Hansen & Jenneborg, 1996).

### 3.2 Photo Database

The photo database comprised front- and backside pictures of all experimentally used alga specimens both before and after the experiment. By comparing pre- and post-experimental pictures, changes in surface colour and texture of incubated coralline algae could be visually analysed after the experiment (fig. 15). Post-experimental pictures showed a brown film on the surface of all algae (see fig. 15(b)) as well as abundant white spots (see fig. 15(d)). The brown film was not identified and the abundance of white spots was not qualitative assessed. A subjective impression revealed no tendency regarding the applied treatment.

![Figure 15](image1.png)

Figure 15: Representative pre- and post-experimental pictures of experimentally used *L. glaciale*. Treatment conditions for alga no. 1 and alga no. 48 were 7.5 ± 0.1°C and 1518 ± 136 µatm, respectively 3.4 ± 0.1°C and 569 ± 48 µatm. Fig. 15(a) and fig. 15(c) show *L. glaciale* before, fig. 15(b) and fig. 15(d) after experimental incubation. Red arrows indicate an observed brown film (fig. 15(b)), respectively white spots (fig. 15(d)) on algae surfaces.
3.3 Applied Treatment and Light Regime

The applied CO$_2$ treatment did not meet our expectations in regard to aimed target levels; the applied temperature treatment and light regime did meet our expectations. Applied CO$_2$ treatment levels calculated from pH and total alkalinity (TA) measurements were higher than aimed target levels (tab. 2). In comparison to target levels, the lowest CO$_2$ level had a mean offset of nearly 180 µatm, all elevated CO$_2$ levels had a mean offset of 380 ± 20 µatm. The four CO$_2$ levels were still distinct as standard deviations did not overlap. Resulting mean level $p$CO$_2$ were 569 ± 48, 920 ± 86, 1230 ± 120 and 1518 ± 136 µatm.

Applied temperature treatment levels were with 3.4 ± 0.1, 5.2 ± 0.1 and 7.5 ± 0.1 ºC close to target levels. Mean underwater irradiances at the bottom of each water bath were 11.62 ± 0.81, 12.95 ± 1.36 and 12.1 ± 1.13 µmol photons m$^{-2}$ s$^{-1}$ for temperature levels of 3.4, 5.2 and 7.5ºC, respectively. The variation in irradiance between temperature levels (freezer cabinets) was 8.2 ± 1.8 %. Underwater irradiance was measured at the same depth as algae were located within the reactors. Mean underwater irradiance was close to the target level of 12 µmol photons m$^{-2}$ s$^{-1}$. Irradiance decreased over the experimental duration by ∼ 1.17 µmol photons m$^{-2}$ s$^{-1}$ in all freezer cabinets.

3.4 Nutrient Uptake

The difference between start and end concentrations of measured Si, PO$_4^{3−}$, NO$_2^{−}$ and NO$_3^{−}$ was calculated for each incubation period and regarded as uptake. Uptake of dissolved inorganic nitrogen (ΣN) was calculated from pooled NO$_2^{−}$ and NO$_3^{−}$ concentrations. Relative uptake relates to uptake under ambient control conditions (baseline), according to eq. 8.

Si concentrations remained constant at 0.110 ± 0.054 µmol kg$^{-1}$ over the experimental duration. PO$_4^{3−}$ concentrations were overall low with mean start concentrations of 0.03 ± 0.02 µmol kg$^{-1}$ close to the detection limit of 0.01 µmol kg$^{-1}$. Significant changes during the incubation period could not be identified.

Relative uptake of ΣN increased over experimental duration with the highest mean relative uptake of 262 ± 132% after the third treatment phase (fig. 16). Relative uptake of ΣN decreased significantly with increasing temperature (p-value = 0.00). The highest mean relative uptake of ΣN was measured at 3.4ºC. Mean relative uptake of ΣN at 5.5ºC and 7.5ºC was ∼ 30%, respectively ∼ 26% lower than at 3.4ºC (fig. 17). Relative uptake of ΣN at 7.5ºC had large standard deviations, which ranged from ∼ 40% to ∼ 300% (fig. 17). Elevated CO$_2$ had no effect on uptake of ΣN.

Data were normally distributed (Shapiro-Wilks test) after root-transformation. We found a statistical significant effect of elevated temperature on uptake of ΣN (compare tab. 3)
Figure 16: Mean relative uptake of $\Sigma N$ of *L. glaciale* over treatment duration. Datapoints are means of pooled CO$_2$ and temperature treatments (12 combinations with each 2 measurements) from each one treatment phase. Standard deviations ($\pm$ SD) are plotted against treatment duration (time). Relative rates are normalized to rates of each individual alga under ambient conditions ($3.4 \pm 0.1^\circ$C, $568 \pm 41 \mu$atm: baseline). The black dashed reference line at 100% indicates the baseline rate.

Figure 17: Mean relative uptake of $\Sigma N$ of *L. glaciale* with temperature. Data points are means of pooled CO$_2$ treatment (4 CO$_2$ levels with each 2 measurements) from four treatment phases. Standard deviations ($\pm$ SD) are plotted against the applied temperature treatment. Horizontal error bars give the SD of the temperature over the experimental duration. Relative rates are normalized to rates of each individual alga under ambient conditions ($3.4 \pm 0.1^\circ$C, $568 \pm 41 \mu$atm: baseline). The black dashed reference line at 100% indicates the baseline rate.
3.5 Carbonate System

The calculated carbonate system parameters $pCO_2$ (µatm) and saturation state ($\Omega$) for calcite and aragonite are given in Table 2. All actual $pCO_2$ levels were higher than target levels (compare 3.3). $pCO_2$ was highest at the lowest temperature (3.4 °C) and lowest at the highest temperature (7.5 °C). $CO_2$ levels were distinct. The lowest $CO_2$ level (target 390 µatm) was at all temperature levels supersaturated with respect to $\Omega_{Ar}$. All elevated $CO_2$ levels were undersaturated at all temperature levels with respect to $\Omega_{Ar}$. For $\Omega_{Ca}$, the two high $CO_2$ levels (target 840 and 1120 µatm) were undersaturated for 3.4 °C and for the highest $CO_2$ level also at 5.2 °C. All other $CO_2$ levels were supersaturated with respect to $\Omega_{Ca}$ at all temperature levels.

3.6 Calcification

Net calcification was measured via the increase in algal calcium carbonate (CaCO$_3$) content (buoyant weight technique (Davies, 1989)) and via changes in water chemistry (total alkalinity technique (Smith & Key, 1975)). Net calcification rates were in good agreement between both methods (fig. 19, 18). Net calcification rates obtained from both methods were highest at the two lower $pCO_2$ levels and were
decreased with increasing $pCO_2$. Net calcification at 3.4°C was nearly constant at all $pCO_2$ levels and was decreased with increasing $pCO_2$ levels at 5.2°C. Net calcification at 7.5°C was increased at the two lower $pCO_2$ levels and was decreased at the two higher $pCO_2$ levels.

Net calcification rates obtained from the buoyant weight technique were positive at all temperature and $pCO_2$ levels, as well as two to four times higher and with larger standard deviations than net calcification rates calculated with the total alkalinity technique. Net negative calcification in the highest $pCO_2$ at 7.5°C was only visible in calcification rates calculated from the total alkalinity technique.

3.6.1 Buoyant Weight

The CaCO$_3$ content in mg individuum$^{-1}$ of each alga was calculated from measured buoyant weight according to eq. (11). The density of algal calcite was 2.378 g cm$^{-3} \pm 0.288$ g cm$^{-3}$, which was close to the calcite density measured in *L. glaciale* from the Kattegatt of 2.645 g cm$^{-3}$ (A. Form, personal communication). CaCO$_3$ weight increased over the experimental duration, indicating net calcification in all algae. The mean increase in CaCO$_3$ weight (CaCO$_3$ precipitation) was slightly higher at the two lower $pCO_2$ levels compared to the two higher $pCO_2$ levels (fig. 20). Mean CaCO$_3$ precipitation at 569 and 920 µatm was 76 ± 42 mg and 68 ± 34 mg, respectively. Mean CaCO$_3$ precipitation at 1230 and 1518 µatm was 50 ± 14 mg and 51 ± 25 mg, respectively. Looking at the different temperature
levels, mean CaCO$_3$ precipitation at 3.4°C was constant with increasing $p$CO$_2$. At 5.2°C mean CaCO$_3$ precipitation was highest at lowest $p$CO$_2$ and constant lower with increasing $p$CO$_2$. At 7.5°C mean CaCO$_3$ precipitation was high at the two lower $p$CO$_2$ and was decreased with increasing $p$CO$_2$ (fig. 19).

Data were normally distributed (Shapiro-Wilks test) after root-transformation (see tab. 3). We found no statistical significant effect of elevated temperature or CO$_2$ on net calcification (compare tab. 3). Standard deviations of mean CaCO$_3$ precipitation with $p$CO$_2$ level were overlapping.

![Figure 20](image_url)

Figure 20: Mean CaCO$_3$ precipitation of *L. glaciale* with $p$CO$_2$, measured with the buoyant weight technique (Davies, 1989). Data points are means of the pooled CO$_2$ treatment ($n = 12$). Standard deviations (± SD) are plotted against $p$CO$_2$; horizontal error bars give the SD of the $p$CO$_2$ over the experimental duration.

### 3.6.2 Total Alkalinity

Net calcification rates of *L. glaciale* were calculated with the total alkalinity technique (Smith & Key, 1975). Mean net calcification was decreased with increasing $p$CO$_2$ level; the decrease in mean net calcification was different between temperature levels (fig. 19) as described in 3.6.

Relative calcification (%) was calculated according to eq. 8. Mean relative calcification decreased over experimental duration at all temperature levels (fig. 21). Mean relative calcification at 3.4°C and 5.2°C decreased until the third treatment phase to 29 ± 25% and 46 ± 143%, respectively, and increased slightly in the fourth treatment phase; mean relative calcification was reduced compared to
the baseline but positive. Mean relative calcification at 7.5°C increased compared to the baseline until the second treatment phase, and decreased in the subsequent treatment phases. In the last treatment phase (after 8 weeks treatment), mean relative calcification at 7.5°C was zero, i.e. by 100% reduced to baseline calcification.

Mean relative calcification was decreased with increasing $p\text{CO}_2$ levels, being higher at 569 µatm and 920 µatm than at 1230 µatm and 1518 µatm. At 1518 µatm mean relative calcification was negative. Looking at the temperature levels, mean relative calcification at 3.4 °C was reduced at all $p\text{CO}_2$ levels (49 ± 21% at 569 ± 42 µatm, 49 ± 60% at 1654 ± 112 µatm). At 5.2°C mean relative calcification was increased by ~ 50% compared to the baseline at 583 ± 59 µatm, was decreased at intermediate $p\text{CO}_2$ levels and was increased again to the 100% baseline value at the highest $p\text{CO}_2$ level (1479 ± 93 µatm). Mean relative calcification at 7.5°C was increased compared to the baseline at 881 ± 65 µatm and was decreased at the two high $p\text{CO}_2$ levels with a minimum of -14 ± 88% compared to the baseline at 1422 ± 70 µatm (fig. 22).

In summary, highest relative calcification rates occurred in the first two treatment phases and at the lowest and first elevated $p\text{CO}_2$ level (569µatm and 920 µatm) at 5.2°C and 7.5°C. Lowest relative calcification rates occurred in the last treatment phase and at the highest $p\text{CO}_2$ level (1422 ± 70 µatm) at 7.5°C. Relative calcification rates at 3.4°C were constantly reduced over all treatment phases and at all $p\text{CO}_2$ level.

Data were normally distributed (Shapiro-Wilks test). We found no statistical significant effect of elevated temperature or CO$_2$ on net calcification; the effect of elevated CO$_2$ on net calcification was close to significance (p-value = 0.07) (compare tab. 3).
Figure 21: Mean relative calcification of *L. glaciale* over treatment duration (in treatment phases) (total alkalinity technique (Smith & Key, 1975)). Data points are means of pooled CO$_2$ and temperature treatments (12 combinations with each 4 measurements) from each one treatment phase. Standard deviations (± SD) are plotted against treatment duration (phases). Relative rates are normalized to rates of each individual alga under ambient conditions (3.4 ± 0.1°C, 568 ± 41 µatm: baseline). The black dashed reference line at 100% indicates the baseline rate. The red dashed reference line at 0.0 mg CaCO$_3$ d$^{-1}$ indicates zero calcification; negative calcification (dissolution) occurs below 0.0 mg CaCO$_3$ d$^{-1}$.

Figure 22: Mean relative calcification of *L. glaciale* with *p*CO$_2$ (total alkalinity method (Smith & Key, 1975)). Data points are means (n = 24) of pooled CO$_2$ of each temperature treatment over the experimental duration (blue = 3.4°C, grey = 5.2°C, red = 7.5°C). Standard deviations (± SD) are plotted against *p*CO$_2$. Relative rates are normalized to rates of each individual alga under ambient conditions (3.4 ± 0.1°C, 568 ± 41 µatm: baseline). The black dashed reference line at 100% indicates the baseline rate. The red dashed reference line at 0.0 mg CaCO$_3$ d$^{-1}$ indicates zero calcification; negative calcification (dissolution) occurs below 0.0 mg CaCO$_3$ d$^{-1}$.
3.7 Photosynthetic O\textsubscript{2} Evolution and Respiratory O\textsubscript{2} Consumption

Net photosynthetic O\textsubscript{2} evolution (O\textsubscript{2}E) and respiratory O\textsubscript{2} consumption (O\textsubscript{2}C) rates were measured in light, respectively dark. Relative rates (%) were calculated according to eq. 8. Actual mean temperatures of the temperature treatment during the O\textsubscript{2} measurements were 3.9 ± 0.5°C, 4.8 ± 0.7°C and 8.5 ± 0.1°C. Actual temperatures did not meet target temperatures of 3.5°C, 5.5°C and 7.5°C. With regard to their standard deviations, 3.9°C and 4.8°C were still in the temperature range of actual treatment temperatures (compare 3.3), whereas 8.5°C was 1°C warmer than the actual treatment temperature.

Mean relative O\textsubscript{2}C rates were constant over experimental duration and with increasing pCO\textsubscript{2}. Mean relative O\textsubscript{2}C rates were significantly decreased (p = 0.00) with increasing temperature level. Relative O\textsubscript{2}C rates at 3.9°C and 4.8°C were increased compared to the baseline, whereas relative O\textsubscript{2}C rates at 8.5°C were decreased compared to the baseline. At 3.9°C relative O\textsubscript{2}C rates were highest with a rate increase of 113 ± 9%. At 8.5°C relative O\textsubscript{2}C rates were lowest with 92 ± 12%. The rate increase of the intermediate temperature 4.8°C was with 108 ± 8% lower than at 3.4°C and higher than at 8.5°C (fig. 23).

Mean relative O\textsubscript{2}E rates were constant over the experimental duration, but decreased slightly with increasing pCO\textsubscript{2} (fig. 24). Relative O\textsubscript{2}E rates were highest at the lowest and the first elevated pCO\textsubscript{2} level and were slightly decreased in the two highest pCO\textsubscript{2} levels. Looking at the temperature treatment, mean relative O\textsubscript{2}E rates at 3.9°C were reduced (~100%) at all pCO\textsubscript{2} levels. Mean relative O\textsubscript{2}E rates at 4.8°C were highest (190%) at the lowest pCO\textsubscript{2} and decreased with increasing pCO\textsubscript{2} to rates (~100%) in the highest pCO\textsubscript{2}. At 8.5°C, mean relative O\textsubscript{2}E rates were lowest (60%) in the lowest pCO\textsubscript{2} level and increased in the first elevated pCO\textsubscript{2} level to the highest measured rate increase (205%); with increasing pCO\textsubscript{2}, mean relative O\textsubscript{2}E rates decreased and increased again without a constant trend in regard to pCO\textsubscript{2}.

Data of O\textsubscript{2}E and O\textsubscript{2}C were normally distributed (Shapiro-Wilks test) (after root-transformation for O\textsubscript{2}E rates). We found a statistically significant effect of temperature on relative O\textsubscript{2}C rates. We found no statistically significant effect of CO\textsubscript{2} on O\textsubscript{2}C rates, or of CO\textsubscript{2} and temperature on relative O\textsubscript{2}E rates (compare table 3).
Figure 23: Mean relative respiratory O\textsubscript{2} consumption of \textit{L. glaciale} with temperature. Data points are means of pooled CO\textsubscript{2} treatment (4 CO\textsubscript{2} levels with 1 measurements) from two treatment phases. Standard deviations (± SD) are plotted against the temperature treatment. Horizontal error bars give the SD of the temperature over the experimental duration. Relative rates are normalized to rates of a group of four algae under ambient conditions (baseline). The black dashed reference line at 100% indicates the baseline rate.

Figure 24: Mean relative net photosynthetic O\textsubscript{2} evolution of \textit{L. glaciale} with pCO\textsubscript{2}. Data points are means (n = 2) of pooled CO\textsubscript{2} of each temperature treatment over the experimental duration (blue = 3.9°C, grey = 4.8°C, red = 8.5°C). Standard deviations (± SD) are plotted against pCO\textsubscript{2}. Relative rates are normalized to rates of a group of four algae under ambient conditions (baseline). The black dashed reference line at 100% indicates the baseline rate.
Table 3: Results of applied statistics: p-values of normal distribution (normality) and treatment effects (CO$_2$ and temperature) of measurement data. P-values $\geq 0.05$ support null hypothesis ($H_0$), p-values $< 0.05$ do not support $H_0$ and indicate significance.

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<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Total alkalinity</td>
<td>Shapiro-Wilks</td>
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<td>MANOVA</td>
<td>0.07</td>
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<td></td>
</tr>
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<td>Shapiro-Wilks</td>
<td>0.25</td>
<td>Fact. Regress. Analysis</td>
<td>0.98</td>
<td>0.50</td>
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<tr>
<td>Respiratory O$_2$ consumption</td>
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<td>Fact. Regress. Analysis</td>
<td>0.60</td>
<td>0.00</td>
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</tbody>
</table>

3.8 Applied Statistics

Table 3 gives a summary on applied statistical tests and according results.
4 Discussion

4.1 Identification

Algal specimens were identified to be of the genus *Lithothamnion* based on two vegetative features: 1. flared epithallial cells and 2. cell-fusions in the absence of secondary-pit connections. Most diagnostic features of the used identification key from Harvey & Woelkerling (2007) relate to reproductive features, which could not be identified in our algal specimens. However, among rhodolith forming genera the combination of the two identified vegetative features is only present in the genus *Lithothamnion* (Harvey & Woelkerling, 2007). The only other rhodolith forming genus having flared epithalial cells is *Sporolithon*, but in combination with secondary-pit connections (Harvey & Woelkerling, 2007). A mistaken identity between both genera was considered unlikely due to differences in secondary-pit connections and the fact that *Sporolithon* is not described to occur in Spitzbergen (Guiry & Guiry, 2012). Algal specimens were identified by means of the mean surface cell diameter to belong to the species *L. glaciale*.

4.2 Photo Database

Photos of the algal specimens taken at the end of the experiment revealed white spots and a brown film on algal surfaces. "Bleaching" (Webster et al., 2011) of coralline algae is often noticed when algae are environmentally (Foster, 2001) or thermally stressed, e.g. at elevated temperatures (Webster et al., 2011). Bleaching can be associated with the loss of photosynthetic pigments (Wilson et al., 2004) and with dead tissue (Martin & Gattuso, 2009).

By the end of the experiment all incubated algae had white spots on their surfaces, indicating that they were stressed over the experimental duration. White spots did not seem to be particular abundant at elevated temperature compared to control temperature. It was shown that temperature stress increases the abundance of white spots (Webster et al., 2011). Therefore, we would have expected the highest abundance of white spots on alga in the 7.5°C treatment, when associating 7.5°C with thermal stress for the algae. However, it has to be kept in mind, that the abundance of white spots was not quantitatively assessed.

The brown film was considered to result from biofouling, being a common process in the marine habitat (Wahl, 1989) and well known on coralline algae (Lewis et al., 1985, Johnson et al., 1991). Fouling epibionts were assumed to include bacteria (Lewis et al., 1985, Johnson et al., 1991), uni- and multicellular algae (Wahl, 1989). We did not clean algal specimens prior to or during the experiment as we considered it unpossible to remove all epibionts without harming the algae’s surfaces. Therefore, it is assumed, that algal surfaces had already a biofilm of epibionts before the experiment started.
which likely further grew over the experimental duration. In nature, coralline algae rely on grazers and sloughing to keep their surface clean (compare 1.3.2). During the experiment no grazers were present.

### 4.3 Nutrient Uptake

Concentrations of Si and \( \text{PO}_4^{3-} \) did not change over the experimental duration. Si was not taken up during the experiment, indicating that silicifying algae were absent or of minimal abundance in the incubated water and on algal surfaces. Constant Si concentrations were expected as coralline algae do not take up Si. \( \text{PO}_4^{3-} \) concentrations were at the detection limit and therefore no trend could be identified. Arctic waters are known to have low nutrient concentrations (Kirst & Wiencke, 1995), which was also the case during algal collection in Kongsfjorden (EPOCA 2010, unpublished data). Because the calcification process might support nutrient acquisition due to the release of \( \text{H}^+ \), McConnaughey & Whelan (1997) proposed that calcifying plants could live at very low nutrient concentrations.

The uptake of \( \Sigma N \) increased over experimental duration. Most likely, incubated algae where not the source for this increased uptake of \( \Sigma N \), since for example calcification rates, which definitely related to coralline algae, did not increase accordingly (4.6). It is more likely, that the increasing uptake of \( \Sigma N \) was governed by a growing biofilm on the surface of incubated algae as indicated by a brownish film, which was visible on post-experimental pictures (4.2).

The mean relative uptake of \( \Sigma N \) did not change with increasing \( p\text{CO}_2 \) levels, indicating that uptake of \( \Sigma N \) was not affected by a decreasing pH.

The mean relative uptake of \( \Sigma N \) changed with temperature, being higher at 3.4°C than at 7.5°C. This finding was unexpected, as elevated temperatures usually enhance physiological processes (Sommer, 1998). A reason for reduced uptake of \( \Sigma N \) at 7.5°C might be temperature stress of incubated alga *Lithothamnion glaciale*, possibly affecting also its surface associated biofilm.

Uptake of \( \Sigma N \) had a large standard deviation. Assuming that the uptake of \( \Sigma N \) might be mostly governed by an epibiontic biofilm, this variation in uptake might be a consequence of different area covers of the biofilm.

In summary, for two reasons it is most likely that increasing uptake of \( \Sigma N \) resulted from a growing epibiontic biofilm on the surface of incubated coralline algae: 1. growth of a biofilm could be identified by comparing post- with pre-experimental pictures of algae; 2. uptake rates of \( \Sigma N \) did not correlate with coralline algae specific processes, e.g. calcification rates. Low uptake of \( \Sigma N \) at the highest temperature level was in accordance with reduced respiration rates, indicating energy limitation, e.g. for nutrient uptake mechanisms.
4.4 Carbonate System

Actual $p$CO$_2$ levels calculated from pH and total alkalinity (TA) measurements were higher than target levels. The lowest CO$_2$ level was intended to represent in-situ conditions of 390 µatm. Instead, we observed a $p$CO$_2$ of ~ 560 µatm, which was nearly 50% higher than intended. This means, that we had no in-situ CO$_2$ level. Elevated CO$_2$ levels had an offset of ~ 380 µatm each. Standard deviations of mean CO$_2$ levels did not overlap. Therefore, we still obtained four distinct CO$_2$ levels.

For the CO$_2$ treatment, we used CO$_2$ enriched air from gas mixing pumps, whose actual CO$_2$ concentrations were tested with a gas detector (GDZ401, Gas-Detektions-Zentrale) and were found to be in the range of target levels. pH was measured at the end of each incubation period, therefore, CO$_2$ equilibration of incubation water can be considered completed. pH changes due to biological activity were regarded unlikely as water was constantly aerated during incubation. The pH probe was regularly calibrated; measured pH data was corrected with measurements of certified reference material for ocean carbonate measurements (Prof. A.G. Dickson, Marine Physical Laboratory, University of California) (2.5.2). TA measurements of start values had a precision of 2.7 µmol kg$^{-1}$.

Consequently, we consider it most likely, that too high actual $p$CO$_2$ levels were caused by errors in the pH correction measurements of reference material. For example, a too short equilibration time of the pH probe could have resulted in a not representative pH which was subsequently used for the correction of measured sample pH from the experiment. Using a wrong correction factor might explain why all $p$CO$_2$ levels had nearly the same offset.

The incubation water was undersaturated with respect to $\Omega_{Ar}$ at all temperatures in the CO$_2$ enriched treatment levels. Water of the lowest CO$_2$ level was still saturated with respect to $\Omega_{Ar}$ at all temperatures (1.0 - 1.5, see tab. 2). Since L. glaciale contains 13 - 25% MgCO$_3$ (Kamenos et al., 2008), being at least 20% more soluble than aragonite (Morse et al., 2006), it is most likely that incubated algae at all CO$_2$ levels experienced undersaturation with respect to high Mg-calcite.

4.5 Calcification

We measured net calcification with both the buoyant weight (Davies, 1989) and the total alkalinity technique (Smith & Key, 1975). Calcification measured via buoyant weight is a direct measurement of changes in the CaCO$_3$ amount of calcified organisms, whereas calcification measured via total alkalinity is an indirect measurement of changes in carbonate chemistry of incubation water.

We found an overall good agreement of both techniques. The general CO$_2$ trend of calcification was the same between both techniques: calcification rates were highest at the two lowest $p$CO$_2$ levels and decreased with increasing $p$CO$_2$ in the elevated temperature levels.
A difference between both calcification rates was, that dissolution at 7.5°C in the highest pCO₂ level in the last treatment phase, was only visible in the higher temporal resolution of the total alkalinity technique. Since calcification rates calculated from the buoyant weight technique presented start and end changes, only positive rates were measured: Higher net calcification during treatment phase 1 to 3 compared to net dissolution in treatment phase 4 resulted in positive net calcification with respect to the total experimental duration. Furthermore, calcification rates obtained via the buoyant weight technique were two to four times higher than rates obtained with the total alkalinity technique. This finding could not be explained, so far. Which method to use depends on the study question and design.

The high variation in calcification data obtained with the buoyant weight technique is assumed to be caused by different growth rates based on different surface areas of algal specimens. Growth in coralline algae is limited to the meristematic cell-layers close to the surface (Potin et al., 1990) (1.3.1), i.e. the amount of meristematic cells and consequently the potential of growth increases with surface area. Large standard deviations of coralline algal growth obtained with the buoyant weight technique were also found in a study of Jokiel et al. (2008). To compare our algal growth (CaCO₃ deposition) with annual algal growth measured by Jokiel et al. (2008), we calculated only summer growths of tropical algae from Jokiel et al. (2008), since growth rates from our study are not representative for Arctic winter conditions. Tropical coralline algae had calcification rates of 200 ± 100 mg 4 month⁻¹ at ambient pCO₂ compared to calcification rates of 76 ± 42 mg 4 months⁻¹ at the lowest pCO₂ for polar coralline algae in our experiment. Growth in our algae was lower than growth obtained by Jokiel et al. (2008), what was expected due to lower (polar) experimental temperatures.

Treatment effects on L. glaciale were evaluated by looking at relative calcification rates. Relative calcification rates can be quantitatively evaluated since they are normalised to baseline rates for each individual algae (2.2).

Mean relative calcification of coralline algae measured via the total alkalinity technique decreased with experimental duration. This might be due to undersaturation of high Mg-calcite in all treatments. Undersaturated conditions were shown to affect calcification in coralline algae due to enhanced dissolution (Anthony et al., 2008, Büdenbender et al., 2011). Consequently, experimental duration equaled the duration of undersaturation with respect to high Mg-calcite, possibly promoting a decrease in calcification rates. Additionally, a mean decreasing relative calcification could be further promoted by the growing biofilm, e.g. due to shading.

Mean relative calcification decreased with experimental duration differently between temperatures with the strongest decrease at highest temperature, indicating an negative effect of elevated temperatures on calcification rates. A similar response was shown by Anthony et al. (2008) for tropical coralline algae: calcification rates at 3°C elevated temperatures were stronger reduced than at ambient
temperatures.

Mean relative calcification rates at 7.5°C increased until the second treatment phase and from then on decreased over the remaining treatment phases. The same pattern was also visible in the mean relative calcification response with respect to CO$_2$ levels. Relative mean calcification at 7.5°C increased from the lowest (556 ± 70 µatm) to the first elevated pCO$_2$ (881 ± 65 µatm) and decreased in the second and highest pCO$_2$ (1422 ± 70 µatm). This pattern of increasing calcification rates at intermediate pCO$_2$ and net dissolution at highest pCO$_2$ was already described by Ries et al. (2009) as "parabolic response", occurring i.a. in coralline algae (range of intermediate and highest pCO$_2$ given by Ries et al.: 409 ± 6 - 903 ± 12 µatm, respectively 2856 ± 54 µatm). Ω$_{Ar}$ for intermediate and highest treatments applied by Ries et al. (2009) fit to Ω$_{Ar}$ levels calculated for our 5.5°C and 7.5°C treatment (see tab. 2). Therefore, calcification responses are comparable. A parabolic response was also found by Büdenbender et al. (2011) in their study on L. glaciale. Ries et al. (2009) explains parabolic responses with an ability of organisms to regulate pH at the site of calcification (McConnaughey & Whelan, 1997), organic coating of calcified layers and photosynthetic activity. Yet, all algae were of the same species and would consequently all have pH-regulation of calcification and an organic coating (cell membrane) enclosing deposited CaCO$_3$, independent from the temperature of incubation, while only calcification at 7.5°C had a parabolic response. In regard to photosynthetic activity as explanation for a parabolic response, relative net photosynthetic rates at 5.5°C and 7.5°C peaked at the same pCO$_2$ as relative calcification rates did (parabolic response). It could be argued, that at increased net photosynthetic rates more energy (ATP) was produced at respective pCO$_2$, which could be subsequently used for the energy dependent pH regulation mechanism (McConnaughey & Whelan, 1997), enabling algae at 7.5°C to increase their calcification. Yet, inspite of elevated relative net photosynthetic rates at 5.2°C, algae from this treatment did not show a parabolic response. Reasons are unclear.

Mean relative calcification at 3.4°C was not affected by increasing CO$_2$ levels, but rates were reduced compared to the baseline, indicating a constant factor limiting calcification efficiency. That could mean that alga at 3.4°C were possibly negatively affected by elevated pCO$_2$, but were in all four CO$_2$ treatments able to cope with the CO$_2$ stress as relative calcification rates were constant at increasing CO$_2$ levels. Decreased calcification compared to the baseline might also have been affected by overgrowth of the discussed epiphytic biofilm (see 4.2).

Mean relative calcification at 5.2°C decreased with increasing pCO$_2$. An increased mean relative calcification at the lowest pCO$_2$ compared to baseline rates might indicate a positive temperature effect at low CO$_2$ levels (compare 7.5°C treatment). At the highest CO$_2$ level mean relative calcification was unexpectedly the same as in the baseline, which can not be explained by now.
In summary, calcification rates decreased over experimental duration and responded temperature-dependent differently to increasing $p\text{CO}_2$ levels, indicating a synergistic effect of both treatment factors. Algae at 3.4°C seem to be unaffected of increasing $p\text{CO}_2$ levels but at a reduced but still positive calcification rate. Algae at 7.5°C showed a parabolic response to increasing $p\text{CO}_2$ levels, resulting in net dissolution in the highest $\text{CO}_2$ level. Dissolution was only found in algae at 7.5°C and the highest $p\text{CO}_2$ ($1422 \pm 70 \mu\text{atm}$) at the end of the experimental duration, indicating a synergistic effect of high $\text{CO}_2$ and high temperature levels.

4.6 Photosynthetic $O_2$ Evolution and Respiratory $O_2$ Consumption

Photosynthetic $O_2$ evolution ($O_2E$) and respiratory $O_2$ consumption ($O_2C$) of coralline algae under experimental treatment conditions were measured via changes in $O_2$ concentration in light and dark in the surrounding medium. According to Hofmann et al. (2011) $O_2$ evolution measurements in a closed water volume represent physiological responses as they directly measure a product of photosynthesis ($O_2$).

The highest temperature level during the $O_2$ measurements was 1°C higher than the according temperature level of the experimental treatment, likely due to the different measurement set-up. To compare $O_2$ measurements with calcification and nutrient uptake rates, we will treat actual temperatures of the $O_2$ measurements the same as actual treatment temperatures. Although a temperature difference of 1°C is assumed to likely change net measured rates particular for $O_2C$ rates (statistical significant temperature effect), it might be argued, that $O_2C$ rates would have been similar low at a 1°C lower temperature than at the actual temperature (8.5°C). This might be assumed due to the decreasing trend of $O_2C$ rates (compare 23).

Relative net photosynthetic $O_2$ evolution ($O_2E$) rates were not affected by $\text{CO}_2$. It might have been expected, that relative $O_2E$ rates would increase with increasing $p\text{CO}_2$ due to a higher substrate availability for the photosynthesis with respect to dissolved inorganic carbon (DIC) (photosynthetically used: $\text{CO}_2$ and $\text{HCO}_3^-$) (Riebesell et al., 2007).

We found a similarity between relative $O_2E$ and relative calcification rates: with few exceptions, relative $O_2E$ rates had a similar pattern with increasing $\text{CO}_2$ as relative calcification rates in the respective temperature treatments. Both rates were 20 - 50% reduced at 3.4°C, decreased with increasing $p\text{CO}_2$ at 5.2°C and had a parabolic response at 7.5°C with highest rates at the second $\text{CO}_2$ level. Only relative $O_2E$ at 7.5°C and 1500 $\mu\text{atm}$ and relative calcification at 5.5°C and 1500$\mu\text{atm}$ did not fit to described trends. Similarities between relative $O_2E$ and relative calcification rates might indicate a coupling of both processes, i.e. photosyntheticis and calcification in the coralline alga *L. glaciale* seem to be closely connected. However, the driving factors remain unclear. In order to distinguish whether
calcification, or temperature and CO$_2$ affect photosynthesis, a future experimental design should also include uncalcified algae.

Relative respiratory O$_2$ consumption (O$_2$C) decreased significantly with increasing temperatures. Since physiological processes generally increase with increasing temperature (Sommer, 1998), respiration rates were expected to increase with increasing temperature. In our experiment, we observed the opposite effect: at the highest temperature (7.5°C) the lowest respiration rates were measured. This reverse finding might indicate a temperature dependence of algal respiration rates: *L. glaciale* in our experiment could have been stressed by increasing temperature to an extent where basic physiological activity, like respiration, was negatively affected. Temperature stress in algae at 7.5°C was also already discussed for decreased ΣN uptake rates (4.4) and decreased calcification rates (4.6.2).

Respiration allocates energy for energy consuming processes (Sommer, 1998), like growth or calcification in coralline algae (Okazaki, 1977). Low respiration rates of algae at 7.5°C could consequently mean that less energy was available for their metabolic processes. Limited energy availability at 7.5°C could be an explanation for decreasing calcification rates with increasing CO$_2$. In the same way, sufficient energy availability due to increased respiration at 3.4°C might explain the reduced but constant calcification rates with increasing CO$_2$: Calcification in coralline algae is supposed to be an energy dependent process (Okazaki, 1977). A likely mechanism promoting the calcification process is the active redulation of pH at the site of calcification, being proposed to occur in coralline algae (McConnaughey & Whelan, 1997). An increasing pH might possibly promote the conversion from HCO$_3^-$ to CO$_3^{2-}$, which is an important substrate in the calcification process (Borowitzka, 1982a). pH regulation is assumed to be energy dependent, for example due to active transport mechanisms of H$^+$ (McConnaughey & Whelan, 1997). Consequently, when energy availability is limited, e.g. due to reduced respiration rates, likely only limited energy can be used for the regulation of pH, which could affect CO$_3^{2-}$ availability and therefore the calcification process. At increased CO$_2$ levels pH regulation might be of even higher importance for the calcification process by providing suitable conditions for the conversion of HCO$_3^-$ to CO$_3^{2-}$. Since CO$_3^{2-}$ concentrations are already low, reduced calcification due to energy limitation could further decrease net calcification and possibly result in net dissolution. Increased CO$_2$ levels are likely increased at increased pCO$_2$ levels. This could have been the case for algae at 7.5°C, which had reduced respiration rates and experienced net dissolution at the highest pCO$_2$ (Ω$_{Ar}$ 0.63 ± 0.03). However, it remains unclear, why thermal stress might lead to reduced respiration rates.

In summary, results of photosynthetic O$_2$ evolution indicate a linkage between photosynthesis and calcification. Based on our results, increased temperature seems to impair algae respiration rates.
5 Conclusion

In this study, we investigated single as well as synergistic effects of ocean warming (OW) and ocean acidification (OA) on calcification and photosynthesis of the Arctic coralline red alga *Lithothamnion glaciale* under Arctic summer light conditions. We were interested in whether 1. OW would interact with reported negative OA effects on calcification 2. calcification would correlate with photosynthesis or respiration, indicating a likely linkage between these processes and 3. which implications could be drawn out of our findings for *L. glaciale* under future ocean conditions.

The effects of increasing temperature and increasing \( p\text{CO}_2 \) on *L. glaciale* were tested by incubating algal specimens from the Kongsfjord, Spitzbergen for two months under a treatment of elevated \( p\text{CO}_2 \) (569 - 1518 \( \mu\text{atm} \)) and elevated temperature (3.4 - 7.5°C). Treatment levels were designed to represent future conditions of OW and OA modeled for the Arctic Ocean (Steinacher *et al.*, 2009) based on emission scenario SRES A2 (IPCC, 2000). The extreme conditions of the highest treatment level, being elevated by + 4°C and + 1200 \( \mu\text{atm} \) compared to ambient conditions, were exceptions to model predictions and applied in order to increase the likelihood for the observation of physiological responses. The lowest treatment level was designed to represent in-situ conditions of the Arctic habitat of *L. glaciale* in summer. With respect to \( \text{CO}_2 \) concentrations, we failed to met these conditions because all observed \( \text{CO}_2 \) levels were higher than intended. However, treatment levels were still distinct with respect to \( \text{CO}_2 \). Irradiance simulated in-situ conditions of the Arctic summer season as experienced by investigated algae specimens (12 \( \mu\text{mol photons m}^{-2} \text{s}^{-1} \) for 24 h). The response of *L. glaciale* to the applied treatment was tested in regard to nutrient uptake, calcification and photosynthetic \( \text{O}_2 \) evolution (\( \text{O}_2\text{E} \)) and respiratory \( \text{O}_2 \) consumption (\( \text{O}_2\text{C} \)).

In the highest temperature treatment (7.5°C) relative nutrient uptake (\( \Sigma N \)), relative calcification and relative \( \text{O}_2\text{C} \) rates were decreased compared to the baseline. In contrary, in the lowest temperature treatment (3.4°C) relative uptake of \( \Sigma N \) and relative (\( \text{O}_2\text{C} \) rates were increased. Relative calcification at 3.4°C was reduced compared to the baseline. The temperature treatment effects on algae were particular pronounced in relative calcification and \( \text{O}_2\text{C} \) rates: Calcification rates of algae at 7.5°C turned after an incubation time of 8 weeks only in the highest \( p\text{CO}_2 \) treatment into net dissolution, whereas calcification rates at 3.4°C were at a reduced rate but did not change over experimental duration and also not with increasing \( \text{CO}_2 \) level (min. \( \Omega_{\text{Ar}} 0.45 \pm 0.03 \)). Mean relative \( \text{O}_2\text{C} \) rates at 7.5°C were reduced by 20% compared to rates at 3.4°C, indicating lower energy availability for algae at 7.5°C.

Reduced calcification and respiration rates at 7.5°C could indicate thermal stress, possibly inhibiting...
metabolic efficiency (as discussed). Since *L. glaciale* is a dominant coralline alga species of the Subarctic Ocean (Adey *et al.*, 2005), it can be assumed that *L. glaciale* is likely adapted to low Arctic water temperatures. Therefore, an increase of mean surface sea temperature of circa 4°C was expected to establish temperature stress for *L. glaciale*.

The comparison of relative calcification and O$_2$C rates with respect to temperature levels indicated a possible temperature threshold between 5.2°C and 7.5°C, since *L. glaciale* seemed to be unable to sustain net calcification at high pCO$_2$ concentrations and simultaneously reduced respiration at 7.5°C. However, according to Adey (1970) the optimum growth temperature reported for *L. glaciale* ranges from 9 - 15°C, being higher than our highest temperature level. Büdenbender *et al.* (2011) proposed, that increased temperatures could promote calcification in the Arctic coralline alga *L. glaciale*. This hypothesis was supported by our study, since algae at + 2°C and + 4°C elevated temperature treatments experienced increased calcification rates at low and intermediate elevated CO$_2$ levels. However, at high CO$_2$ levels > 1000 µatm this assumption did not hold true, since calcification at 7.5°C was circa 60% lower than at 3.4°C, resulting in net dissolution of respective algae.

With high CO$_2$ decreasing relative calcification rates and reduced O$_2$C rates at 7.5°C might indicate, that calcification rates could be affected by thermal stress via energy limitation. Energy is allocated during respiration (Sommer, 1998). Reduced O$_2$C rates could indicate reduced energy availability. Since calcification is assumed to be an energy dependent process, *e.g.* due to pH-regulation mechanisms as proposed by McConnaughey & Whelan (1997), calcification is likely affected by reduced energy availability. Energy limitation due to reduced O$_2$C caused by ocean warming could likely enhance effects of additional stressors, *e.g.* high CO$_2$ concentrations. Since energy limitation due to temperature stress had only an effect on calcification rates at very high pCO$_2$ (> 1000 µatm), a synergistic effect between elevated temperatures and very high CO$_2$ is likely.

Within the temperature range of 3.4°C - 5.2°C relative O$_2$C rates were not reduced, what could mean that energy allocation was not reduced as well. Calcification in this temperature range was reduced but constant over experimental duration and reduced calcification rates could have been the result of undersaturated conditions with respect to high Mg-calcite. However, the constance in reduced calcification with CO$_2$ seems to indicate, that alga could withstand increasing pCO$_2$ stress, possibly due to sufficient energy availability.

Relative calcification and photosynthetic O$_2$ evolution (O$_2$E) rates showed a similar pattern, indicating a close linkage between both processes. Since photosynthesis is ultimately the energy providing process in transferring light energy into organic energy, the linkage could be the same as discussed for relative O$_2$C rates.
In summary, calcification in *L. glaciale* seems to be linked to energy availability and therefore to energy generating processes, like photosynthesis and respiration. Under reduced energy availability, calcified algae as *L. glaciale* seem to be more susceptible to net dissolution at high $p$CO$_2$, as shown in this study at 7.5°C and 1518 µatm. This finding indicates a synergistic effect of temperature and $p$CO$_2$ on calcification.

Since highest simulated OW and OA of +4°C and +1200 µatm are not expected to occur in the Arctic surface ocean in the near future (Steinacher *et al.*, 2009), *L. glaciale* might not experience reduced calcification during the summer season within this century. In contrast, calcification of *L. glaciale* could be promoted by a temperature elevation of +2°C at moderately increased CO$_2$ concentrations (up to 560 µatm).

However, it has to be kept in mind, that given results relate to Arctic summer light conditions of 24 h irradiance, being the highest amount of light available in the course of a year. Responses to the applied treatment are likely different under Arctic winter conditions of 24 h darkness. Without light, photosynthesis and consequently energy fixation cannot take place. Respiration rates are assumed to be reduced in order to limit energy consumption as stored photosynthates have to last for the whole winter period. Due to continuous energy limitation, calcification rates of *L. glaciale* are expected to be strong affected with respect to CO$_2$ during the Arctic winter. To evaluate implications for the Arctic benthic ecosystem, the same study should be repeated under Arctic winter conditions.

From this study one can conclude, that within this century the Arctic coralline alga *Lithothamnion glaciale* might not be negatively affected by elevated temperatures and $p$CO$_2$ during the Arctic summer season, but rather might profit from those conditions by increasing its calcification rates.

Negative effects of elevated temperatures and $p$CO$_2$ on *L. glaciale* during the Arctic summer season are first expected for a combination of temperature and $p$CO$_2$, that is not predicted to occur within this century for the Arctic Ocean.
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Declaration on Oath

I hereby declare that I have completed the present master thesis "Effects of Warming and Ocean Acidification on Calcification and Photosynthesis of Arctic Coralline Red Algae under Summer Light Conditions" on my own without using others than the stated sources and auxiliary means.

The electronic version corresponds to this written version.

I assure that this thesis has not been submitted at other places to achieve the master's degree (M.Sc.).

I agree, that a copy of this thesis will be made available in the library of the Christian-Albrechts University, Kiel.

Kiel, 28th April, 2012

Dana Hellemann