Calcification of the Arctic coralline red algae *Lithothamnion glaciale* in response to elevated CO_2

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ABSTRACT: Rising atmospheric CO_2 concentrations could cause a calcium carbonate subsaturation of Arctic surface waters in the next 20 yr, making these waters corrosive for calcareous organisms. It is presently unknown what effects this will have on Arctic calcifying organisms and the ecosystems of which they are integral components. So far, acidification effects on crustose coralline red algae (CCA) have only been studied in tropical and Mediterranean species. In this work, we investigated calcification rates of the CCA *Lithothamnion glaciale* collected in northwest Svalbard in laboratory experiments under future atmospheric CO_2 concentrations. The algae were exposed to simulated Arctic summer and winter light conditions in 2 separate experiments at optimum growth temperatures. We found a significant negative effect of increased CO_2 levels on the net calcification rates of *L. glaciale* in both experiments. Annual mean net dissolution of *L. glaciale* was estimated to start at an aragonite saturation state between 1.1 and 0.9 which is projected to occur in parts of the Arctic surface ocean between 2030 and 2050 if emissions follow 'business as usual' scenarios (SRES A2; IPCC 2007). The massive skeleton of CCA, which consist of more than 80% calcium carbonate, is considered crucial to withstanding natural stresses such as water movement, overgrowth or grazing. The observed strong negative response of this Arctic CCA to increased CO_2 levels suggests severe threats of the projected ocean acidification for an important habitat provider in the Arctic coastal ocean.

KEY WORDS: Calcification · CO_2 · Arctic · Coralline algae · Ocean acidification · Dissolution · *Lithothamnion*

INTRODUCTION

Crustose coralline red algae (Corallinales, Rhodophyta) are abundant and important components of benthic marine communities within the photic zone and are of global significance with respect to coastal calcium carbonate ($CaCO_3$) deposition (Nelson 2009). Their occurrence has been reported for most hard-substratum environments, from tide pools (Dethier & Steneck 2001) and shallow subtidal zones (Paine 1984) to the greatest depths (268 m) recorded for marine algae (Littler et al. 1985). Crustose coralline red algae (CCA) carbonates are important components of tropical reef structures (Adey 1998) and serve as triggers for the settlement and metamor-

phosis of coral larvae (Heyward & Negri 1999). Moreover, CCA are so called 'habitat modifiers' or 'bio-engineers' providing the habitat for entire benthic communities around the globe (Foster 2001, Barbera et al. 2003). The organic basis of these habitats is the unattached growth form of some CCA species, the so called rhodoliths (or maerl) (Foster 2001). In high latitudes, rhodoliths can build up large calcareous beds covering several square kilometers of the sea floor (Freiwald & Henrich 1994, Foster 2001). Rhodoliths significantly increase biodiversity through their 3-dimensional structure and facilitate settlement and recruitment for many invertebrate species, some of economic importance (Steller et al. 2003).

Calcification plays an important role for CCA in multiple ways. The total $CaCO_3$ content varies between algal species and can account for 80 to 90% of the biomass (Bilan & Usov 2001), making calcification an important process for organism growth and protection from grazing. For example, germination during early settlement is secured by hypobasal calcification, which cements the spores to the substrata (Walker & Moss 1984). Calcification also plays an important role in preventing overgrowth and fouling by bacteria and fleshy algae since the main defense mechanism is thought to be the sloughing off and regrowth of their outermost calcified epithelial cell layer (Littler & Littler 1999). Furthermore, calcification was hypothesized to liberate CO_2 for photosynthesis (Borowitzka 1982) and the thickness of the $CaCO_3$ layer in the cell walls was suggested to be relevant for the protection of the photosystem from ultraviolet radiation (Gao & Zheng 2010). CCA precipitate high magnesium calcite (Mg-calcite), e.g. *Lithothamnion glaciale* produces $CaCO_3$ with a fraction of 13 to 25 mol% magnesium carbonate ($MgCO_3$) (Kamenos et al. 2008). Biogenic Mg-calcite (>4 mol% $MgCO_3$; Reeder 1983), within the 'dominant composition range of 12–16 mol% $MgCO_3$', appears to be about 20% more soluble than aragonite (Morse et al. 2006, p. 5818). Therefore, CCA are among the most sensitive calcifying organisms to ocean acidification in terms of solubility. Recent work on Mg:Ca ratios of CCA showed that the Mg-fraction can decrease with increasing atmospheric CO_2 concentration, making algae less soluble at higher CO_2 concentrations (Ries 2011).

CCA seem to be highly sensitive to ocean acidification because they are found to be the first calcifying organism to disappear in areas with naturally acidified seawater (Hall-Spencer et al. 2008). Furthermore, recent studies found negative effects of ocean acidification on the settlement and recruitment process (Kuffner et al. 2008) and on growth and calcification rates of tropical CCA (Anthony et al. 2008, Jokiel et al. 2008). However, for a Mediterranean CCA species significant pCO_2 effects on net calcification rates were found only in combination with increased temperature (Martin & Gattuso 2009). A significant effect of pCO_2 alone was observed for net dissolution rates. Also a positive effect of increased CO_2 concentrations on calcification rates was reported, but only under conditions where carbonate saturation states are high and CO_2 is possibly limiting algal photosynthesis (Ries et al. 2009). The CO_2 effect became negative when aragonite saturation states were 1.7 and lower (Ries et al. 2009).

The polar oceans have a naturally low $CaCO_3$ saturation state due to low water temperatures (Fabry et al. 2009) and are therefore projected to turn corrosive for calcium carbonate earlier than other oceans (Orr et al. 2005, Steinacher et al. 2009). Decreasing sea ice cover in the Arctic ocean further accelerates the process of ocean acidification by allowing enhanced air–sea CO_2 gas exchange and increasing the freshwater input which is lowering alkalinity (Fabry et al. 2009, Steinacher et al. 2009). Annual mean aragonite subsaturation was projected as early as 2032 for the Arctic surface ocean if anthropogenic CO_2 emissions follow the IPCC (Intergovernmental Panel on Climate Change) 'business as usual' scenario (SRES A2) (Steinacher et al. 2009). Sea surface temperatures of the Arctic Ocean are expected to increase by 0.4 to 1.5°C until 2100 under this scenario (Steinacher et al. 2009).

In view of the ecological importance of CCA (Nelson 2009) and the severe threats to CCA in high latitudes (Andersson et al. 2008), we investigated the effects of ocean acidification and Arctic 'light seasons' on net calcification rates of the predominant rhodolith forming CCA *Lithothamnion glaciale* (Kjellman 1885). *Lithothamnion glaciale* is the most abundant CCA in the North Atlantic and is described from Cape Cod and the British Isles (Adey & Adey 1973) to the northern coast of Svalbard (Teichert et al. in press).

MATERIALS AND METHODS

Rhodolith specimens of *Lithothamnion glaciale* were collected during dives with the manned research submersible JAGO at 40 to 50 m depth near Cape Rubin (80° 32′ 19″ N, 19° 50′ 40″ E) on the north coast of Spitsbergen during cruise No. 2, Leg 3 of RV 'Maria S. Merian', from 31 July until 17 August 2006. Algae were stored in tanks and transferred to the Leibniz Institute of Marine Science (IFM-GEOMAR), where experiments were conducted. Rhodoliths were cultivated in aquaria with natural North Sea water with a salinity of 33 ppt at light intensities of 3 to 10 µmol photons $m^{-2} s^{-1}$ and a temperature of 7°C for 2 yr before experiments were conducted.

For the experimental set up, rhodolith fragments of 22 ± 8 g buoyant weight were cleaned of epiphytic organisms and randomly distributed to 16 acrylglas reactors (radius = 5 cm, height = 40 cm). Four additional reactors were left empty serving as blanks in order to monitor bacterial background activity. The reactors contained 2 l of 0.2 µm filtered North Sea

water (salinity of 33) and were constantly aerated with ambient or premixed air. A water current inside the reactors was created by directing the air bubbles through an internal vertical acrylglas tube (4.5 × 25 cm), (air lift system). The water was renewed weekly to replenish nutrients and total alkalinity (A_T). Changes in nutrients and total alkalinity due to primary production, respiration, calcification and dissolution are accounted for in the calculations of the carbonate system. Premixed air at target pCO$_2$ levels was provided by enriching ambient air with pure CO$_2$ in analytical gas mixing pumps (DIGAMIX 5KA 36A/9, Wösthoff). For the summer experiment, 2 blue fluorescent lamps (Osram 36W) were installed and intensities were controlled with a quantum scalar laboratory irradiance sensor (QSL-2100). Two consecutive experiments were conducted with 2 separate sets of rhodolith fragments, where Arctic summer ('S'; 9.0 ± 0.25°C, 24 h 6.8 ± 0.2 µmol photons m^{-2} s^{-1}) and Arctic winter ('W'; 6.8 ± 0.15°C, 24 h darkness) conditions, in terms of light intensities (Table 1), were simulated at 4 pCO$_2$ levels of ~390, 815, 975 to 1570 ppm (Table 2). pCO$_2$ levels differed slightly between the summer and winter experiment (Table 2). Both experiments (S and W) were run for 4 wk encompassing 4 consecutive experimental phases (1 wk each): (1) first acclimatisation, (2) baseline, (3) second acclimatisation,

and (4) treatment phase. Present day pCO$_2$ conditions (~390 ppm) were applied during the first acclimatisation and the baseline phase to all 16 replicates and 4 blanks, in order to measure the 'baseline' calcification for each rhodolith as a reference for changes in calcification rates in response to elevated pCO$_2$ levels. Elevated pCO$_2$ levels and a control level (Table 2) were applied during the second acclimatisation and the treatment phase each with 4 replicates and 1 blank.

Calcification rates and water properties (salinity, temperature, pH, total alkalinity (A_T) and inorganic nutrient concentrations) were quantified every second day during the baseline and treatment phase of each experiment (S and W). Salinity, temperature, and pH were measured with a WTW Multi 350i. The pH electrode was calibrated with certified reference material for seawater measurements (Prof. A.G. Dickson, Marine Physical Laboratory, University of California) to the total scale, precision was ±0.01 pH units. Water samples for dissolved inorganic nutrient measurements (nitrogen, NO$_3$, NO$_2$, NH$_4$; phosphate, PO$_4$; silicate, Si) were stored at 4°C and measured photometrically according to Grashoff et al. (1999). Ammonia (NH$_4$) was measured fluorometrically according to Holmes et al. (1999). Water samples for A_T measurements were poisoned with mercuric chloride and stored at 15°C. A_T was determined from duplicate measurements using an automatic titration device (Metrohm Titrando 808). Ten ml of the original A_T sample were 0.2 µm filtered and weighed to the nearest 0.0001g before the measurement, in order to determine the exact subsample volume. Certified reference material measurements (Prof. A.G. Dickson, Marine Physical Laboratory, University of California) were used to correct sample measurement for accuracy. Precision was within 2 µmol kg^{-1}.

Table 1. *Lithothamnion glaciale.* Culturing conditions (means ± SD; n = 20 per season)

Expt	Temperature (°C)	Light (µmol photon m^{-2} s^{-1})	Salinity
Summer	9.0 ± 0.26	6.8 ± 0.2	33 ± 0.2
Winter	6.8 ± 0.17	0	33 ± 0.2

Table 2. *Lithothamnion glaciale.* Summary of carbonate system parameters and nitrogen (as an example for the dissolved inorganic nutrients) from the summer and winter experiment. pH values are the means ± SD of all replicates. Each start and end value reported for total alkalinity (A_T) and nitrogen (NO$_3$ + NO$_2$) is the mean ± SD of 4 replicate water samples. Remaining parameters were calculated using CO2SYS (Seawater scale; K1, K2 from Mehrbach et al. 1973 refitted by Dickson & Millero 1987). $\Omega_{Aragonite}$ and $\Omega_{Calcite}$ refer to the saturation state of seawater with respect to calcium carbonate species

Expt	pH (total scale)	A_T (µmol kg^{-1}) Start	End	NO$_3$ + NO$_2$ (µmol l^{-1}) Start	End	pCO$_2$ (ppm)	$\Omega_{Aragonite}$	$\Omega_{Calcite}$
Summer	8.06 ± 0.03	2419 ± 2	2159 ± 48	1.94 ± 0.12	0.18 ± 0.07	385 ± 26	1.99 ± 0.16	3.14 ± 0.25
	7.74 ± 0.03		2242 ± 51		0.07 ± 0.07	883 ± 49	1.06 ± 0.07	1.67 ± 0.12
	7.70 ± 0.03		2243 ± 62		0.43 ± 0.58	989 ± 57	0.96 ± 0.07	1.51 ± 0.11
	7.52 ± 0.02		2458 ± 31		0.07 ± 0.07	1573 ± 89	0.67 ± 0.03	1.06 ± 0.04
Winter	8.07 ± 0.05	2418 ± 5	2361 ± 11	1.09 ± 0.19	3.03 ± 0.70	388 ± 45	1.92 ± 0.19	3.04 ± 0.30
	7.82 ± 0.04		2453 ± 30		5.68 ± 1.46	754 ± 80	1.18 ± 0.11	1.87 ± 0.17
	7.72 ± 0.05		2450 ± 26		2.42 ± 0.45	958 ± 117	0.96 ± 0.11	1.51 ± 0.17
	7.53 ± 0.05		2601 ± 46		2.53 ± 0.65	1563 ± 187	0.65 ± 0.07	1.03 ± 0.11

A_T was calculated from the Gran function according to Dickson et al. (2003). Carbonate system parameters were calculated with the software CO2SYS (Lewis & Wallace 1998) from A_T, pH (total scale), temperature, salinity and inorganic nutrient concentrations using the constants from Mehrbach et al. (1973) refitted by Dickson & Millero (1987).

Net calcification rates (G_{net}) were calculated from the change in A_T over time (total alkalinity technique according to Smith & Key 1975). The net calcification rate is given by:

$$G_{net} = -0.5 \, \rho_w V \frac{\Delta A_T}{\Delta t} \qquad (1)$$

where G_{net} is the net calcification rate (µmol $CaCO_3$ d^{-1} $ind.^{-1}$), ρ_w is the seawater density (kg l^{-1}), V is the seawater volume (litres) and $\Delta A_T / \Delta t$ is the rate of change in total alkalinity per unit time (µmol kg^{-1} d^{-1} $ind.^{-1}$). The mean A_T change in the blanks of the 4 pCO_2 levels were below measuring precision and were thus not considered in the calculation of calcification rates. Normalisation of net calcification rates to a feasible standard was necessary for statistical analysis but was complicated by an irregular shape and varying weight to biomass ratio of rhodolith fragments due to varying amounts and type of inclusions, e.g. sand, stones, shells etc. Furthermore, culture organisms were slow growing, of limited availability, and needed to stay alive for further experimental purposes. For statistical analysis we calculated a relative calcification (G_{rel}) for each individual rhodolith fragment according to:

$$G_{rel} = \frac{G_{net,pH,t}}{G_{net,BL,t}} \times 100 \qquad (2)$$

where G_{rel} is the relative calcification (%), $G_{net,pH,t}$ is the net calcification from the treatment phase and $G_{net,BL,t}$ is the net calcification from the baseline phase. G_{rel} is a measure for the impact of elevated pCO_2 levels on algal calcification performance: values >100 % represent higher rates of calcification compared to the baseline phase, values of 100 % represent equal rates of calcification as during the baseline phase, values between 0 and 100 % represent reduced calcification compared to the baseline phase, and negative values represent net dissolution. We tested mean G_{rel} in a 2-way ANOVA for season (combination of light and temperature) and pCO_2 effects as well as possible interactions of these factors. Data were corrected for outliers with a modified Thompson tau test. A Fisher least significant difference (LSD) post-hoc test was applied to identify significant effects between different pCO_2 levels. The general

regression of G_{rel} with respect to elevated pCO_2 levels was derived from a general linear regression analysis.

To estimate rhodolith bed $CaCO_3$ production rates, the maximum length and width of the rhodolith fragments used in experiments were measured with a measuring tape to the nearest 0.5 cm. From this, the area covered by each rhodolith fragment was calculated as a regular rectangle. $CaCO_3$ production of a square meter of rhodolith-covered sea ground was calculated from the mean of the measured areas and the calcification rates from the baseline phase. The standard deviation of the size measurements was used as error approximation for $CaCO_3$ production rates. Annual $CaCO_3$ production was calculated assuming 6 mo of summer and 6 mo of winter net calcification.

RESULTS

Light, temperature, and salinity were constant over time in the individual experiments (summer and winter) (Table 1), whereas dissolved nitrogen (NO_3 + NO_2) concentrations and A_T followed the biological processes of production and remineralisation and net calcification or net dissolution, respectively (Table 2). Dissolved phosphate (PO_4) concentration was 0.08 ± 0.03 µmol l^{-1} (mean ± SD of both experiments) and did not change significantly between the start and end of incubations or between treatments. Estimated annual $CaCO_3$ production per square meter of covered sea ground (mean ± SD) for *Lithothamnion glaciale* was 313.5 ± 78.4 g $CaCO_3$ m^{-2} yr^{-1}.

Net calcification rates (G_{net}) of *Lithothamnion glaciale* under present day pCO_2 conditions (390 ppm) were about 113 % higher during the summer compared to the winter experiment (Fig. 1). With respect to time, G_{net} of algae in the 390 ppm pCO_2 treatment in the summer experiment increased from the baseline to the treatment phase, while it decreased during the winter experiment (Fig. 1). G_{net} values in both experiments (summer and winter) differed clearly between pCO_2 levels during the treatment phase, while they were similar during the baseline phase (Fig. 1). In the summer experiment (Fig. 1a), G_{net} of algae in the 1570 ppm pCO_2 treatment turned negative during the treatment phase, whereas algae in the 815 and 975 ppm pCO_2 treatments maintained G_{net} as high as during the baseline phase. In the winter experiment (Fig. 1b), G_{net} decreased in all pCO_2 treatments. The strongest decrease with a net dissolution during the treatment phase of twice the net cal-
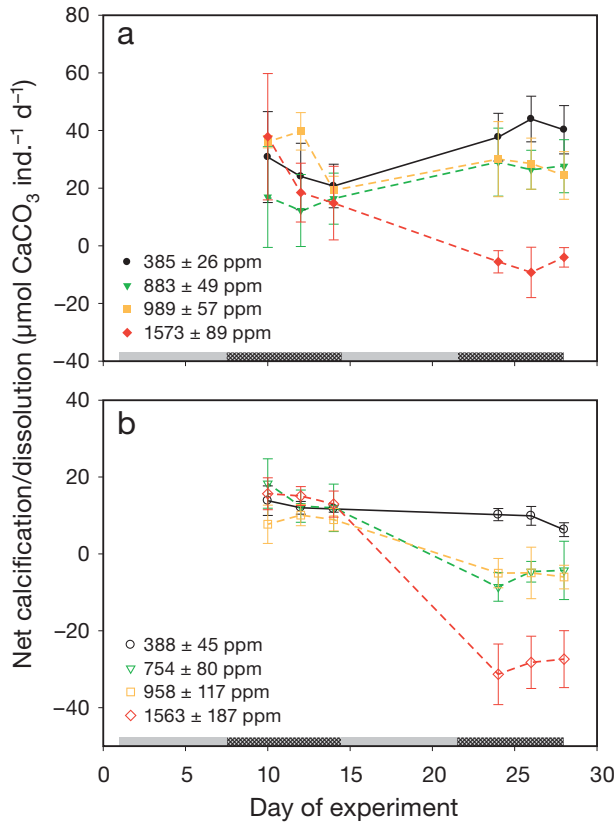
Fig. 1. *Lithothamnion glaciale*. Daily net calcification/dissolution rates from the (a) summer and (b) winter experiments. Data points are means ± SD of 4 replicates. The grey bars on the x-axis indicate acclimatisation (no pattern) and sampling phases (with pattern). From Days 1 to 14 all rhodoliths were exposed to the same pCO$_2$ level of ~390 ppm (baseline) and from Days 14 to 28, 3 sets of 4 rhodoliths were kept under elevated pCO$_2$ levels, as indicated

by increasing CO$_2$ concentrations and the interpolated threshold pCO$_2$ for net dissolution was ~776 ppm lower in the winter compared to the summer experiment (Fig. 2). G$_{rel}$ in the summer experiment still stayed above 100% in the 815 ppm pCO$_2$ treatment due to the fact that G$_{net}$ was higher in the treatment phase than during the baseline phase. Reduced calcification relative to the baseline calcification (G$_{rel}$ < 100%) was observed in the 975 and 1570 ppm pCO$_2$ treatments. Linear interpolation yields a pCO$_2$ level of ~1430 ppm above which net dissolution (G$_{rel}$ < 0%) occurs. G$_{rel}$ in the winter experiment was already decreased (<100%) at 390 ppm and turned to net dissolution (<0%) at a pCO$_2$ value of 654 ppm based on linear interpolation. The linear regression model revealed a high proportion of variation in calcification rates accounted for by pCO$_2$ in both experiments (R^2 = 0.977 and 0.997 for the summer and winter experiment, respectively).

Annual mean relative calcification (Fig. 2) was based on 2 assumptions: first, summer and winter calcification contributed equally to the annual mean, and second, calcification in the 390 ppm pCO$_2$ treatment represents acclimatised calcification rates. Accordingly the annual mean relative calcification estimated for the 390 ppm pCO$_2$ level is set to 100%.

G$_{rel}$ of *Lithothamnion glaciale*, as a function of CaCO$_3$ saturation states for calcite ($\Omega_{Calcite}$) and aragonite ($\Omega_{Aragonite}$), is depicted in Fig. 3. In the present day pCO$_2$ level (390 ppm), water was always supersaturated with respect to $\Omega_{Calcite}$ and $\Omega_{Aragonite}$. In the summer experiment, net calcification occurred at $\Omega_{Aragonite}$ as low as 0.96. Net dissolution was first experienced by the algae when $\Omega_{Calcite}$ was approximately 1 and $\Omega_{Aragonite}$ was at 0.67. In the winter experiment, net dissolution already occurred at $\Omega_{Aragonite}$ of 1.18. Observed responses in G$_{rel}$ were correlated to projected future changes in seawater carbonate chemistry, as reported by Steinacher et al. (2009) (SRES A2 Scenario, IPCC 2007) (Fig. 4). Steinacher et al. (2009)

cification during the baseline phase was observed in the 1570 ppm pCO$_2$ treatment. A lower net dissolution occurred in the 815 and 975 ppm pCO$_2$ treatments, whereas algae kept at 390 ppm pCO$_2$ also had decreased net calcification rates during the treatment phase but experienced no net dissolution.

As G$_{net}$ per individual was not a statistically meaningful normalisation, relative calcification (G$_{rel}$) was used in the statistical analysis. A 2-way ANOVA revealed a highly significant effect of pCO$_2$ and season on G$_{rel}$ (p < 0.001) and no significant interaction between both factors (p = 0.605) (Table 3). Furthermore, post hoc tests (Fisher's LSD) revealed significant and highly significant differences between individual pCO$_2$ treatments (Table 3). G$_{rel}$ was negatively affected

Table 3. *Lithothamnion glaciale*. Results of 2-way ANOVA performed to test the effect of pCO$_2$ and season on algal relative calcification rates. p-values < 0.01 indicate highly significant results. For multiple comparison results (Fisher's LSD test) for the factor pCO$_2$, significant differences are in **bold**, 0 indicates p < 0.001. (1) to (4) indicate pCO$_2$ treatment: (1) 390 ppm; (2) 815 ppm; (3) 975 ppm; (4) 1570 ppm; df: degrees of freedom

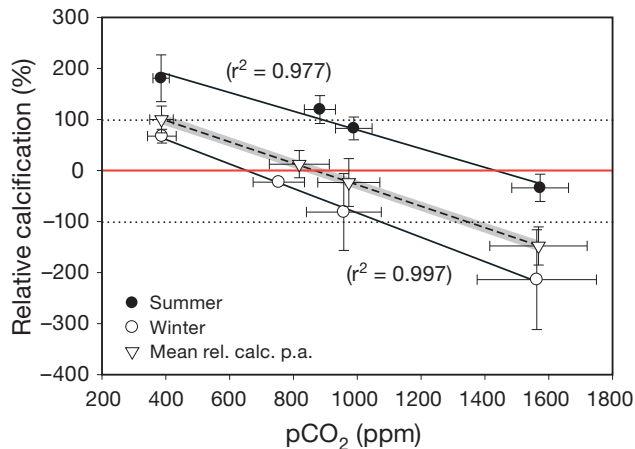| Source of variation	df	F	p		LSD test (pCO$_2$)		
					(1)	(2)	(3)
pCO$_2$	3	32.66	0.000	(2)	**0.012**	–	–
Season	1	63.24	0.000	(3)	**0**	0.098	–
pCO$_2$ × Season	3	0.63	0.605	(4)	**0**	**0**	**0**

Fig. 2. *Lithothamnion glaciale*. Relative calcification as a function of pCO_2 levels from the summer and winter experiment. Data points are means ± SD of the 3 sampling days (see Fig. 1) for each of the 4 treatments. The linear regression analysis (black solid lines) shows trends of calcification with increasing pCO_2 levels for the summer and winter experiment. The upper and lower black dotted horizontal lines represent a relative calcification of 100% and −100% with respect to net calcification rates of the baseline phase (Fig. 1). The red horizontal line indicates zero growth. The black dashed line is the regression for the annual mean relative calcification calculated from the combination of summer and winter data. Annual mean relative calcification of the 390 ppm pCO_2 level was set to 100% by definition. The grey area fills the space between the upper and lower 95% prediction bands

Fig. 4. *Lithothamnion glaciale*. Projected future annual mean relative calcification in the Arctic. Data points are the mean ± SD annual relative calcification rates, which were correlated to projected changes in the aragonite saturation state for the Arctic surface ocean according to Steinacher et al. (2009) and based on a 'business as usual' emission scenario (SRES A2; IPCC 2007). The error in time reflects the variability in the saturation states during the experiment (due to calcification or dissolution processes) translated to years according to the model. The upper *x*-axis gives corresponding aragonite saturation states

projected an annual mean subsaturation for $\Omega_{Aragonite}$ in the Arctic surface ocean by 2032. If we compare their calculations of past and future annual mean $\Omega_{Aragonite}$ levels in the surface ocean surrounding Svalbard (24 grid points) with the levels in our experiments, present annual mean net calcification of *L. glaciale* is already reduced by 40% compared to preindustrial conditions. Annual mean net dissolution could start by 2035 (±5 yr) (Fig. 4).

DISCUSSION

Our results indicate that increasing atmospheric CO_2 partial pressure causes reduced calcification to net dissolution in a key organism of the Arctic coastal ecosystem. The habitat-providing coralline algae *Lithothamnion glaciale* could experience annual mean net dissolution in the Arctic in 20 to 40 yr from today if CO_2 emissions follow a 'business as usual' scenario (SRES A2; IPCC 2007).

Light and temperature levels during the cultivation were chosen to provide optimum growth conditions (Adey 1970) in order to ensure successful long-term cultivation in the laboratory. Algae used in this study were maintained for 2 yr in culture before experiments were conducted. Adey (1970) reported accli-

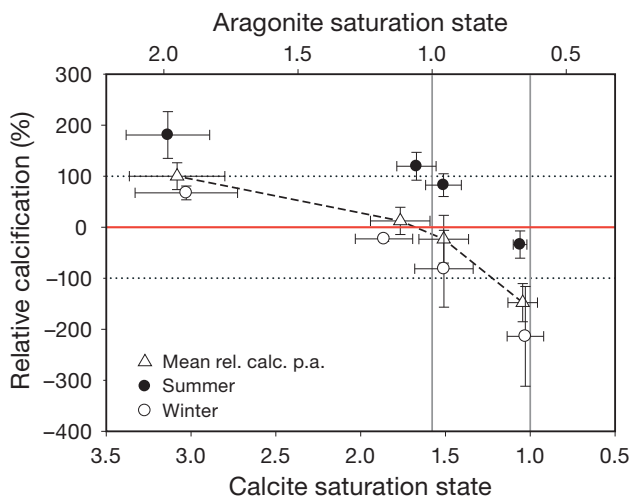Fig. 3. *Lithothamnion glaciale*. Relative calcification ± SD as a function of calcium carbonate saturation states; the 4 data points of each set represent the 4 pCO_2 levels (compare with Fig. 2). Grey vertical lines separate supersaturated from subsaturated conditions for the calcium carbonate mineral phases aragonite and calcite. Black dotted horizontal lines represent relative calcification levels of 100% and −100% with respect to net calcification rates of the baseline phase (see Fig. 1). The red horizontal line indicates zero growth
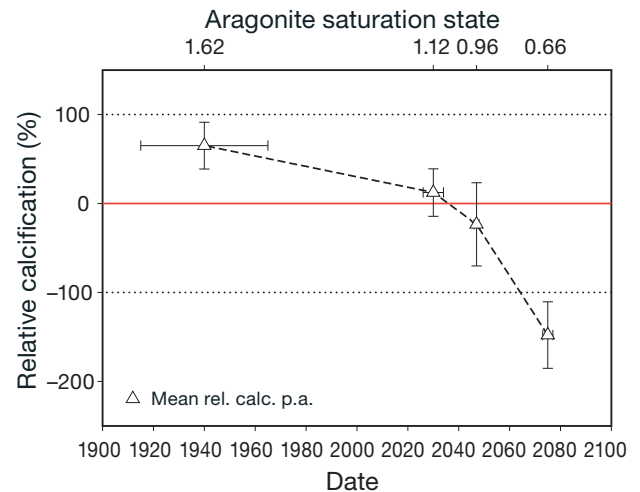
matisation periods of several months for a temperature increase of 5°C. Temperatures of 6.8 and 9.0°C for the winter and summer experiment, respectively, were higher than observed *in situ* water temperatures of −1 to 4°C (J. Büdenbender unpubl. data) but were close to the range of temperatures reported to yield maximum growth rates (8 to 13°C; Adey 1970). Light intensities of 6.8 µmol photons m^{-2} s^{-1} simulated in the summer experiment were generally at the upper limit of measured *in situ* irradiance levels (Teichert et al. in press). To statistically distinguish between pCO$_2$ and season effects, our data were analysed in a 2-way ANOVA with pCO$_2$ and season as factors. As shown in the results, pCO$_2$ and season affected calcification rates but did not interact with each other. This allows us to discuss future pCO$_2$ effects separately from seasonal effects.

Elevated pCO$_2$ levels resulted in decreased net calcification rates. We found a negative linear correlation between pCO$_2$ and algal net calcification rates for the range of pCO$_2$ levels tested (Fig. 2). Additionally, we found an offset in pCO$_2$ of ~776 ppm for interpolated net dissolution between winter and summer experiment (Fig. 2). Possible reasons could be lower carbonate saturation due to higher CO$_2$ dissolution in colder water (which was not detectable in our experiment) and a lowering of the critical threshold pCO$_2$ for net dissolution due to lower temperature and/or absence of photosynthetic activity. Whatever the reasons are, net dissolution can be expected to occur first during the Arctic winter period.

Interestingly, algae in the summer experiment were able to increase (815 ppm) or maintain (975 ppm) their net calcification rates during the experimental period under elevated pCO$_2$ levels (Fig. 2). We attribute the increase or maintenance of net calcification rates in the 390, 815, and 975 ppm treatments in the summer experiment to an acclimatisation to the higher temperature compared to prior long-term cultivation conditions (+2°C). An additional reason could be carbon limitation of photosynthesis (Bowes 1993), possibly leading to CO$_2$ fertilisation at elevated CO$_2$ concentrations in our experiments.

As already mentioned, CCA precipitate high Mg-calcite, therefore the solubility is expected to be similar or higher than for aragonite. We calculated an aragonite saturation of 0.96 for the 975 ppm pCO$_2$ level in the summer experiment. Hence, algae were able to maintain net calcification in subsaturated conditions (Fig. 3). In contrast, previous studies found net dissolution in water supersaturated with respect to aragonite (Anthony et al. 2008, Martin & Gattuso 2009). However, this was attributed to bioerosion in

addition to physiochemical erosion by Martin & Gattuso (2009) or to subsaturated conditions with respect to high Mg-calcite by Anthony et al. (2008). In the case of *Lithothamnion glaciale*, net calcification in subsaturated conditions could have been due to enhanced growth rates associated with still ongoing acclimatisation to a temperature higher than during the prior cultivation. Martin & Gattuso (2009) showed that algae living at lower than optimum temperatures are able to benefit from increasing temperatures with respect to growth rates. Since temperatures in our experiments were higher than projected for the Arctic ocean by the end of this century (Steinacher et al. 2009), effects of elevated pCO$_2$ levels on net calcification rates for *L. glaciale* are likely underestimated. Despite the apparent discrepancies, all previous studies reported possible net dissolution of CCA for 'business as usual' CO$_2$ emission until the end of this century (Anthony et al. 2008, Jokiel et al. 2008, Martin & Gattuso 2009, present study).

Acclimatisation to elevated pCO$_2$ was proposed and discussed by Martin & Gattuso (2009), who found a significant pCO$_2$ effect on net growth rates only for the first month of their experiment but not for the following 11 mo. The authors proposed a possible acclimatisation of algal growth rates to increasing CO$_2$ partial pressures but also mentioned that a simultaneous increase in net dissolution likely outbalances this effect. If acclimatisation of calcification rates is possible in Arctic CCA, an outbalancing of this positive effect by a simultaneously increasing dissolution rate is even more likely, because Arctic CCA will face subsaturated conditions much earlier than Mediterranean CCA (Steinacher et al. 2009). A second aspect is the possible modification of the skeletal Mg:Ca ratio and therefore skeletal solubility in response to increasing pCO$_2$ levels (Ries 2011). The observed change in skeletal Mg:Ca ratio would cause subsaturation for CCA carbonates to start at ~1900 ppm instead of ~1700 ppm pCO$_2$ at conditions as applied in Ries (2011). In summary, increasing temperature and/or changing Mg:Ca ratios have the potential to promote calcification or reduce dissolution in arctic CCA and thereby compensate for CO$_2$ stress to some extent.

The annual CaCO$_3$ production rate of *Lithothamnion glaciale* reported here is the first calcification rate reported from a CCA species collected in the high Arctic. Compared to CCA from other geographic regions *L. glaciale* has a production rate lower than a temperate species (Martin et al. 2006) and considerably lower than tropical species (Chisholm 2000) (Table 4). Hence, there could be a gen-

Table 4. CaCO$_3$ production rates of coralline red algae from 3 geographic zones. Annual production rates are estimations from measured calcification rates of isolated algae

Geographical zone	Species	Annual calcification (g CaCO$_3$ m^{-2} yr^{-1})	Source
Tropical	*Hydrolithon onkodes, H. reinboldii, Neogoniolithon brassica-florida, N. conicum*	1500–10 300	Chisholm (2000)
Temperate	*Lithothamnion coralloides*	300–3000	Martin et al. (2006)
Polar	*Lithothamnion glaciale*	235–391	Present study

eral trend of decreasing calcification rates with increasing latitude probably due to decreasing temperature and light availability. Because CaCO$_3$ production rates in this study were obtained for isolated algae and not for *in situ* communities, we only used data for isolated algae in the comparison shown in Table 4.

A characteristic feature of rhodoliths and an important aspect of the unique habitat they are providing is their irregular 3-dimensional structure. This growth form would not exist without a massive skeleton made of calcium carbonate. Based on the data shown in Fig. 4, *Lithothamnion glaciale* could experience annual mean net dissolution in the Arctic in 20 to 40 yr from today under 'business as usual' CO$_2$ emissions. Effects on the recruitment processes of *L. glaciale* have not been investigated yet. However, the early survival of spores, where calcification plays a vital role for settlement and further growth, was shown to be highly susceptible for ocean acidification effects in tropical CCA (Jokiel et al. 2008). Hence, our observations indicate severe consequences for the future survival of *L. glaciale* in the Arctic ecosystem due to increasing atmospheric CO$_2$ concentrations. Moreover, if the observed responses are representative for Arctic CCA in general, our data imply that rhodolith beds, which provide the habitat for diverse benthic communities in the Arctic ecosystem, could shrink or possibly disappear during this century under 'business as usual' CO$_2$ emission, potentially affecting Arctic food webs. Similar projections can be made for tropical and Mediterranean CCA (Anthony et al. 2008, Jokiel et al. 2008, Martin & Gattuso 2009), suggesting a global reduction of CCA in the coming decades in the case of unabated CO$_2$ emissions.

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