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Calcification of the cold-water coral *Lophelia pertusa* under ambient and reduced pH

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Abstract

The cold-water coral *Lophelia pertusa* is one of the few species able to build reef-like structures and a 3-dimensional coral framework in the deep oceans. Furthermore, deep cold-water coral bioherms are likely among the first marine ecosystems to be affected by ocean acidification. Colonies of *L. pertusa* were collected during a cruise in 2006 to cold-water coral bioherms of the Mingulay reef complex (Hebrides, North Atlantic). Calcium-45 labelling was conducted shortly after sample collection onboard. After this method proved to deliver reliable data, the same experimental approach was used to assess calcification rates and the effect of lowered pH during a cruise to the Skagerrak (North Sea) in 2007. The highest calcification rates were found in youngest polyps with up to 1% d^{-1} new skeletal growth and average values of 0.11±0.02% d^{-1}(±S.E.). Lowering the pH by 0.15 and 0.3 units relative to ambient pH resulted in a strong decrease in calcification by 30 and 56%, respectively. The effect of changes in pH on calcification was stronger for fast growing, young polyps (59% reduction) than for older polyps (40% reduction) which implies that skeletal growth of young and fast calcifying corallites will be influenced more negatively by ocean acidification. Nevertheless, *L. pertusa* revealed a positive net calcification (as indicated by ^{45}Ca incorporation) at an aragonite saturation state (Ω_a) below 1, which may indicate some adaptation to an environment that is already relatively low in Ω_a compared to tropical or temperate coral bioherms.

1 Introduction

The distribution of cold-water corals is believed to be controlled by temperature (4 to 12 °C). They are mostly distributed below the photic zone at depths between 30 and over 1000 m where they can build large three-dimensional structures (bioherms) (Rogers, 1999; Hovland et al., 2002; Roberts et al., 2006). Unlike tropical coral reefs that are usually constructed by a great number of hermatypic (reef-building) corals, the

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cold-water coral framework is based on the carbonate accretion of single or very few species. Cold-water corals lack photosynthetic endosymbiotic algae (zooxanthellae), which stimulate calcification in reef-building corals (Gattuso et al., 1999). Hence, calcification rates are presumably slower in cold-water corals but no estimate is currently available. Assessing growth and calcification rates is more difficult in cold-water than in warm-water scleractinian corals. Direct access to the usually deep cold-water corals is logistically challenging and frequent or long-term observations are limited. Also, reef-building corals usually reveal annual banding patterns of high- and low-density skeletal bands, making it possible to estimate annual linear growth, which, together with measurement of skeletal density, enable to calculate calcification rates (Knutson et al., 1972). Cold-water corals grow in environments that mostly lack strong annual seasonality in temperature and skeletal density banding does not necessarily relate to annual periodicity (Adkins et al., 2004), making it more difficult to determine age and growth rates of samples. So far, the most reliable and direct growth estimates for *Lophelia pertusa* are derived from specimen that settled and grew on artificial substrates, such as oil and gas platforms in the North Sea with a linear skeletal extension (LSE) of 26 mm yr^{-1} (Bell and Smith, 1999; Gass and Roberts, 2006). Growth estimates of specimen maintained in aquaria revealed an annual LSE of 9.4 mm yr^{-1} and 15–17 mm yr^{-1} for *L. pertusa* collected in Norwegian Fjords (Mortensen 2001) and the Mediterranean Sea (Orejas et al. 2008). There is currently no information of rates of calcification ($CaCO_3$ precipitation through time).

Human-induced threats such as deep bottom trawling are known to destroy large areas of cold-water coral bioherms (Rogers, 1999; Fosså et al., 2002; Hall-Spencer et al., 2002). More recently anthropogenic-induced global changes have been identified as a major threat to cold-water corals (Orr et al., 2005; Guinotte et al., 2006). Among those elevated pCO_2 and temperature are prominent because they were shown to have detrimental effects on reef-building corals, coralline algae and coral communities (Gattuso et al., 1999; Langdon et al., 2000; Leclercq et al., 2000; Kuffner et al., 2007). Similar negative effects have been shown for other benthic calcifiers (Gazeau et al.,

2007; Hall-Spencer et al., 2008). A recent study has shown an important resilience of two temperate zooxanthellate corals in response to ocean acidification (Fine and Tchernov, 2007). Colonies were able to survive without their calcareous skeleton under extremely low seawater pH and resumed calcification when pH was brought back to normal. But even though single species are able to survive, it does not imply that a coral reef can keep up the necessary growth under lower pH and a recent study argues that the predicted ocean acidification will trigger a sixth mass extinction of reef corals within the next couple of centuries (Veron, 2008).

The impact of ocean acidification on cold-water corals has not been investigated yet even though they seem to be much more vulnerable. Their physiological requirements restrict the distribution of cold-water corals to high latitudes or to deeper depths, which exhibit lower saturation state of calcium carbonate (Guinotte et al., 2006). Additionally, models project dramatic shallowing in the aragonite saturation horizon (ASH), the depth at which sea water becomes undersaturated with respect to calcium carbonate, both in Southern and North Atlantic Oceans (Orr et al., 2005). It is anticipated that more than 70% of the cold-water coral bioherms will be exposed to waters undersaturated with respect to aragonite by the end of the century. Therefore, not only calcification of cold-water corals will be hampered but also the aragonitic framework will start to dissolve posing an additional threat on cold-water coral associated fauna and a loss in biodiversity.

This study has two goals: (1) measure short-term rates of calcification of the cold-water coral *Lophelia pertusa* and (2) investigate its response to elevated pCO_2 (low pH). The study was carried out at two study sites, at Mingulay (Hebrides, NE Atlantic) and the Skagerrak, where *L. pertusa* is the main frame-building species.

2 Methods

Live colonies or colony branches of *L. pertusa* were sampled during two cruises in 2006 and 2007 at cold-water coral bioherms of the Mingulay reef complex, Hebrides

(56.81° N, 7.43° W) (Roberts et al., 2005; Maier, 2006) and in the Skagerrak, southern Norway at Fjellknausene (59.07° N, 10.74° E) and Soester (59.08° N, 10.76° E) (Maier et al., 2007), respectively.

During the cruise in 2006 to Mingulay, we tested a simple experimental set-up to assess calcification rates of *L. pertusa* by labelling freshly collected corals with ^{45}Ca directly on board the research vessel Pelagia. Because this experimental approach provided reasonable data on *L. pertusa* calcification, it was used during a second cruise to the Skagerrak in March 2007. During that cruise additional experiments were conducted to test the effect of ocean acidification on *L. pertusa* calcification rates.

2.1 Sampling of live *Lophelia pertusa*

Corals were sampled using a box core sampler (50 cm in diameter), which sealed itself off after closing on the bottom. This allows collecting the benthos substrate together with overlying ambient seawater. After box core was back on deck, seawater was siphoned off and live corals were transferred to climate-controlled containers where they were kept at 7–9°C in seawater of which ca. 50% was renewed every second day. During the first cruise to Mingulay, a triangular dredge was once used to collect larger samples for biodiversity determinations. Some of the *L. pertusa* specimens collected with this triangular dredge were used to test the effect of sampling gear on calcification rates (4× box core versus 4× dredge samples).

Sampling depths averaged 150 m±9 m (±S.E., N=10) and 109±3 m (±S.E., N=7) at Mingulay and Skagerrak, respectively. Temperature and salinity were determined by a CTD cast during respective cruises and were 9.9°C and 34.5‰ at Mingulay and 7.5°C and 34.6‰ at Skagerrak at respective sampling depths.

2.2 ^{45}Ca labelling and measurement of calcification rate

Small branches of freshly collected colonies were placed in 50 ml plastic tubes (Fig. 1a) filled with 30 ml seawater that was collected at sampling depth and filtered using 0.2 µm

membranes. Caps were placed on top of tubes, but not screwed air tight to avoid contamination by spilling of radioisotope-labelled seawater. The incubations are nevertheless regarded as a nearly closed system since air flow and thus exchange with external air is greatly reduced by the caps. The samples were unstirred, but ship movement was assumed to sufficiently generate water mixing to avoid stagnant regions close to the coral polyp. The 20 ml headspace provided sufficient oxygen supply for coral respiration, but changes in the carbonate system took place during incubations which have been estimated as described in Sect. 2.5.

During the first labelling experiments at Mingulay in 2006, 30 µl of ^{45}Ca (1.53 mCi ml^{-1}) was added to each tube. The addition of ^{45}Ca (1.88 mCi ml^{-1}) was reduced to 10 µl per 30 ml of filtered seawater in the 2007 experiments. During the second cruise to the Skagerrak (March 2007), two repeated experiments were conducted, each with 3 treatments to assess calcification rates at ambient seawater pH, and at pH lowered by 0.15 and 0.3 pH units. The pH of ambient seawater was measured using a hand-held pH meter calibrated on the N.B.S. scale. The pH treatments were obtained by addition of 1 N and 0.1 N HCl until the required pH value was reached. In general, each treatment consisted of 8 replicates (Table 2), except for Mingulay corals where 4 of the replicates were retrieved by box core and the other 4 by triangular dredge.

Incubation time was 24 h after which corals were washed three times for 1 h with unlabelled seawater to allow the efflux of unbound ^{45}Ca from the coelenteron, NaOH-soluble compartment (tissue) and skeleton (Tambutté et al., 1996). Coral branches were then frozen and stored at −20°C pending analyses.

Back in the laboratory, whole coral branches were dried at 60°C. Single corallites were broken off according to polyp rank (Fig. 1b) and the dry weight was determined. The tissue was removed in 6 N NaOH at 90°C for several hours and subsequently rinsed with MilliQ water. This procedure was repeated until all tissue was removed. The remaining skeleton was dried at 60°C and the skeletal dry weight of each subsample was determined. Tissue dry weight was determined by subtracting skeletal dry weight after removal of tissue from dry weight before tissue was removed. The

remaining skeleton was dissolved in 1 N HCl and neutralized with 1 N NaOH. Eight ml of InstaGel Plus (PerkinElmer) scintillation liquid was added to sub-samples and counts were measured on a Wallace 1211 Rack Beta Scintillation counter with an external standard and corrected against a quenching curve.

2.3 Normalization of calcification rate

The rate of calcification (G) was normalized to the initial skeletal weight and calculated from newly produced calcium carbonate (C_n) and skeletal dry weight at end of experiment (P_n) with G given in percent of initial skeletal weight using the formula:

$$G[\%\mathrm{d}^{-1}] = (C_n/(P_n - C_n))/n \times 100 \quad (1)$$

with n=duration of experiment (*here*: n=1 day).

For comparison with other published studies that have used different units, our units for G used in this study [% d^{-1}] can easily be translated into mg $CaCO_3$ g^{-1} skeleton d^{-1} or mmol $CaCO_3$ (or Ca^{2+}) mol^{-1} skeleton d^{-1} using the average initial skeletal weight given in Table 2.

2.4 Initial parameters of the carbonate system of experiments

2.4.1 Dissolved inorganic carbon (DIC) and total alkalinity (TA)

To characterise the seawater carbonate chemistry of study sites, data for dissolved inorganic carbon (DIC) and total alkalinity (TA) were used from sites close to sampling areas. For the Mingulay area data were derived from the GLODAP (Global Data Analysis Project) database (Key et al., 2004), cruise A24 of the World Ocean Circulation Experiment (WOCE) June, 1997 at 9.334° W; 57.75° N from 151 m water depth. For the Skagerrak site, data were provided by H. Thomas and A. Borges for 58.50° N; 9.50° E sampled in February 2001 at a sampling depth of 100 m (Bozec et al., 2006; Thomas

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et al., 2008). The DIC and TA of the GLODAP data are believed to be consistent to 4 and 6 µmol kg^{-1}, respectively.

2.4.2 Other parameters of the carbonate system

The Seacarb package (Proye and Gattuso, 2003) was used to characterize parameters of the carbonate system of seawater used for ^{45}Ca labelling experiments (Table 1). Dissolved inorganic carbon and TA were used to calculate other parameters (pH, pCO_2 and Ω_a). The hydrostatic pressure was set to zero, because experiments were conducted on board and not at sampling depths. Salinity and temperature were those of ambient seawater at sampling depths (and incubations). For acidified treatments, 0.15 and 0.3 pH were subtracted from pH values derived by Seacarb for ambient pH_T and then used in combination with TA to recalculate the other variables of the carbonate system (DIC, pCO_2 and Ω_a).

For an assumed consistency of DIC and TA of 4 and 6 µmol kg^{-1} (as given by GLODAP), the range of uncertainty of other parameters of the seawater carbonate system was estimated and values between minima and maxima ranged by 0.05 units for ΔpH_T, 45 ppm for ΔpCO_2 and 0.2 units for $\Delta\Omega_a$. These uncertainties are likely underestimates because the TA and DIC data were, despite their proximity to the sampling site, not taken from exactly the same location and at the same time as coral samples.

2.5 Changes in the carbonate chemistry during time of incubation

Coral respiration, calcification and nutrient release into the seawater cause changes in the carbonate system. To estimate the changes that took place during the 24 h incubations, we used a closed-system approach with step-wise (hourly) equilibration between seawater and headspace pCO_2 taking into account the following changes in DIC and TA in seawater caused by respiration, calcification and excretion. Calcification (G) decreases the TA ($\Delta TA=2\times G$) and DIC ($\Delta DIC=G$), respiration (R) does not significantly change TA, but increases DIC ($\Delta DIC=R$) and ammonium excretion increases

TA ($\Delta TA = 14/16 \times E$) (Gattuso et al., 1999).

Hourly coral calcification was recalculated using average G and weight of branches incubated during this study. Respiration were estimated using the data on *L. pertusa* metabolism by Dodds et al. (2007), while data on ammonium release by *L. pertusa* were derived from van Duyl et al. (2005).

An initial pCO_2 of 400 ppm was assumed in the headspace. A stepwise method was used: every hour the TA and DIC were calculated as follows:

$$TA_{t+1} = TA_t - 2G + 0.875E \quad (2)$$

$$DIC_{t+i} = DIC_t + R - G \quad (3)$$

with G, R and E in µmol kg^{-1} seawater h^{-1}.

At each time step, pCO_2 was equilibrated between seawater and the headspace using the following relationship:

$$pCO_{2(eq_{t+1})} = (pCO_{2(air_t)}/a + pCO_{2(t+1)}/b)/(a + b) \times ab \quad (4)$$

where $pCO_{2(eq_{t+1})}$ is the pCO_2 equilibrated between headspace and seawater after each hourly time increment, $pCO_{2(air_t)}$ is the pCO_2 of the headspace before hourly equilibration with seawater pCO_2, and $pCO_{2(t+1)}$ is the pCO_2 of seawater after hourly change in seawater chemistry, but before equilibration with air. For equilibration of pCO_2 between seawater and air, constants (a and b) were used taking into account partial pressure and molecular weight of CO_2 in gas and seawater and the respective volumes of 20 ml and 30 ml of headspace and seawater, respectively. The constants represent the slopes of correlation curves derived by plotting corresponding concentrations of CO_2 and pCO_2 values and multiplication by the respective volume of air or seawater taking into account seawater temperature and density of CO_2 in gas. In this study constants used were a=55 586 and b=34 847.

The DIC after air-sea equilibration and TA were used to calculate the new pCO_2 (corresponds to $pCO_{2(t+1)}$ of Eq. 4), Ω_a and pH using Seacarb and the next hourly time

step was investigated. The average TA, DIC, pH, pCO_2 and Ω_a during incubations were calculated using the data of the 24 hourly time steps (Table 3).

2.6 Statistical analyses

Statistical analyses were conducted using the software package Statistica 7.0. The error is reported as one standard error of mean (S.E.). To test the effects of sampling method, polyp rank, and pH, we used a t-test or a 1-way or 2-way ANOVA depending on the comparisons. Posthoc tests were conducted using the Tukey honest significance test (HSD) for equal or unequal N. One outlier was removed prior to statistical comparisons for Skagerrak experiment 1 at ambient pH and polyp rank 1. This reduced the S.E. from 0.08 to a value of 0.02, with the S.E. after removal of extreme being consistent with S.E. of other treatments (Table 2).

3 Results

3.1 Carbonate system of seawater

3.1.1 Initial conditions of seawater chemistry

The DIC of seawater was 2126×10^{-6} mol kg^{-1} and 2115×10^{-6} mol kg^{-1} for Mingulay and Skagerrak, respectively. Calculated ambient pH_T was, respectively 8.10 and 8.06 at Mingulay and Skagerrak (Table 1). The pCO_2 and Ω_a for ambient pH treatments were 352 ppm and 2.25 for Mingulay and 386 ppm and 1.89 for the Skagerrak treatments. For the Skagerrak experiments, the decrease of pH by 0.15 and 0.3 units caused a decrease of Ω_a to 1.40 and 1.02 and an increase in pCO_2 to 568 and 827 ppm, respectively.

3.1.2 Changes in seawater chemistry during incubation

Changes in the carbonate chemistry as consequence of respiration and calcification rates were estimated for the different treatments and the resulting average values for parameters of the carbonate chemistry during incubation are given in Table 3. As a result of changes in DIC and TA, the pH_T was on average 0.27 units lower than the initial pH, while pCO_2 increased by 495 ppm and Ω a dropped by 0.68 units. According to treatment, this resulted in values for pH_T, pCO_2 and Ω_a between 7.54 and 7.93, 518 and 1419 ppm and 0.68 and 1.47, respectively (Table 3).

3.2 Calcification rates

3.2.1 Bulk calcification rates of *L. pertusa* branches

Lophelia pertusa branches had on average 6.55±0.32 polyps branch^{-1} and the skeletal and tissue dry weights averaged 2855±241 and 189±15 mg branch^{-1}, respectively (Table 2). The average rate of calcification was 0.067±0.019% (N=8) for Mingulay corals but calcification rates spanned 2 orders of magnitude, between 0.0027 and 0.1923% d^{-1}. Calcification at ambient conditions was higher in corals collected at Mingulay than those collected in the Skagerrak. Pooling the Skagerrak data for ambient conditions leads to a mean G of 0.033±0.024% (N=16), which is significantly different from that of Mingulay corals (t-test, $t(1,22)=2.126$, $p=0.045$). A one-way ANOVA reveals a significant effect of pH on calcification rates of *L. pertusa* collected in the Skagerrak ($F2,45=7.03$, $p<0.001$). Subsequent comparison revealed that G was significantly different between ambient seawater and the treatment where pH was lowered by 0.3 units (HSD, $p<0.01$), but relative to treatment with the 0.15 pH unit reduction, there was no significant difference (HSD, $p=0.22$ and $p=0.31$ between ambient and −0.15 pH and between −0.15 pH and −0.30 pH units, respectively).

3.2.2 Effect of polyp rank and sampling method on calcification rates

Calcification rates were evaluated according to polyp rank and sampling method (Fig. 2). Variation between samples was very high ranging from 0.005% and 1.027%. Ranks of higher or equal to 4 were grouped together in order to reach a sample size large enough for statistical comparison. The sampling method had no significant effect on the calcification rates (2-way ANOVA, $F(1,44)=1.16$, $p=0.29$) while polyp rank significantly affected it ($F(3,44)=7.71$, $p<0.01$). The interaction between the sampling method and polyp rank was not statistically significant ($F(3,44)=1.24$, $p=0.31$). The calcification rate was found highest in the youngest polyps (polyp rank 1) with 0.279±0.055% (N=20) and decreased in older polyps with 0.064±0.008% (N=13), 0.034±0.006% and 0.019±0.002% for polyp ranks 2 to 4 and higher, respectively. Posthoc comparison on effect of polyp rank revealed, that calcification rates of polyp rank 1 were significantly different from polyp ranks 2 to 4 (Tukey HSD test for unequal N, $p<0.01$). No significant differences in calcification rates were found between polyp ranks 2 to 4 (HSD for unequal N, $p>0.89$).

3.2.3 Effect of lower pH on calcification

Both experiments carried out on Skagerrak corals showed a decrease of G with decreasing pH for bulk analyses of incubated branches (Table 2). These results were less clear in experiment 2 if calcification rates were analysed according to polyp rank (Fig. 3). Overall, similar results were obtained on colonies collected in the Skagerrak and Mingulay. The rates of calcification decreased with increasing polyp rank. This was found in all pH treatments and for both experiments. The first experiment showed a clear trend of decreasing calcification rates with decreasing pH for all polyp ranks. Changes were more pronounced for young polyps where pH had been lowered by 0.3 units relative to ambient seawater for which calcification decreased to 41 and 46% for polyp ranks 1 and 2, respectively. For polyp ranks 3 and 4 and above, the decrease was less pronounced and calcification at a pH of 0.3 units below ambient were 62 and

60% of the calcification found at ambient seawater.

The results of the second experiment, revealed a less clear picture (Fig. 3b). There was no clear decrease in calcification rates between ambient seawater and treatments with lower pH. Only for polyp ranks 1 and 3 the calcification rates were slightly higher for *L. pertusa* in ambient seawater compared to the treatment with −0.3 pH units. Between treatments of −0.15 and −0.3 pH units, calcification rates were lower at the lower pH level and thus confirm the results of experiment 1.

4 Discussion

Despite several methodological limitations due to our simple experimental approach and the uncertainties with respect to the carbonate chemistry the results appear to show realistic calcification rates of *L. pertusa* with youngest polyps calcifying fastest and of the order of magnitude as the daylight calcification of slow growing tropical corals. Due to decreases in pH as a result of coral respiration and calcification during incubations, our estimated calcification rates may well underestimate the actual calcification rates of cold-water corals under in-situ conditions. Also, the protocol used for ^{45}Ca labelling supposedly underestimates calcification rates by about 12% (Tambutte, 1995).

4.1 Sampling method

For Mingulay corals the effect of sampling *L. pertusa* with different gear (box core or dredge) was tested, because dredging is more damaging to benthos organisms than box coring and may thus affect coral recovery and calcification rates. In this study, the range of calcification rates was lower for dredged corals than those retrieved from box coring (Fig. 2) but mean values were not statistically significant. Thus our specimen of *L. pertusa* branches used were either not more stressed than the box cored corals or they recovered quickly from additional stress induced by dredging. This substanti-

ates aquarium observations showing a high recovery potential to skeletal fragmentation (Maier, 2008).

4.2 Calcification of *Lophelia pertusa* incubated at ambient pH

We show here that calcification rates of *Lophelia pertusa* can be relatively high but also reveal an enormous range dependent on corallite age and size. The youngest polyps had a maximum calcification rate of up to 1% d^{-1}. This rate falls within the range found for tropical, zooxanthellate corals (Erez, 1978; Marubini and Atkinson, 1999; Marubini and Thake, 1999; Reynaud et al., 2003). However, the bulk calcification rate of whole branches was low and in the range of dark calcification of tropical corals (Erez, 1978). Bulk calcification rates average out the extremely fast and slow growth rates of young and old corallites, respectively. Bulk calcification is thus an intermediate value and greatly depends on the proportion of older and younger corallites in a branch. The extremely low growth rates for older corallites are in accordance with aquarium observations where cold-water coral polyps with a diameter larger than 7 mm do not exhibit any linear extension (Mortensen, 2001). Mortensen also observed that skeletal extension occurred as episodic events of rapid growth, which may explain the high range of calcification rates observed in the youngest polyps (polyp rank 1). The finding that the young polyps can calcify extremely fast, suggests that new corallite production (budding) is important for the growth and maintenance of the 3-dimensional structure of cold-water coral bioherms. A high abundance of new coral buds might consequently be a good indicator for the potential of a cold-water coral bioherm to maintain rapid growth and the 3-dimensional structure, which sustains the high biodiversity associated with these ecosystems. A coral branch can produce several new corallites per year (Mortensen, 2001; Orejas et al., 2008), but it is not known which factors trigger budding in *L. pertusa*. It could be intrinsic (genetically controlled) as well as environmentally induced. Food and nutrient availability are suggested to be important factors providing the necessary energy for calcification and reproduction (Spiro et al., 2000), but changes in abiotic environmental parameters such as temperature,

current regimes and carbonate chemistry might further influence new corallite formation. Also, fragmentation and other disturbances may eventually trigger higher budding rates. It has been shown that *L. pertusa* has a high recovery potential and injuries to calyx tissue may result in new polyp formation (Maier, 2008). Sponge bioerosion can induce additional aragonite secretion at younger growth stages of corallites (Beuck et al., 2007). Growth morphologies with densely spaced new corallites and high budding rates were observed in dying *L. pertusa* colonies (Freiwald et al., 1997).

Calcification rates of Mingulay *L. pertusa* were significantly higher than those for Skagerrak corals. It is not clear what causes this difference and whether this constitutes a site-specific characteristic or if differences are due to different sampling depth, seasonality or seawater carbonate chemistry. The higher calcification rates of Mingulay corals do reflect the higher initial Ω_a at this site. Also, many of the branches of *L. pertusa* sampled from Skagerrak showed encrustations or overgrowth by the sponge *Hymedesmia coriacea* (van Soest et al., 2005; Maier et al., 2007). Such sponge overgrowth can constitute an additional stress factor. Energy spent for chemical or mechanical defence would consequently be not available for calcification and might have caused reduced calcification of Skagerrak corals.

4.3 Calcification rates in response to reduced pH

The rate of calcification decreased at initial pH levels 0.15 and 0.3 units lower than ambient seawater. The aragonite saturation state (Ω_a) was approximately 1.9 at ambient seawater pH, while it was 1.4 and 1.0 at the lower pH levels (Table 1). This corresponds to a drop of 25 and 46% of Ω_a, which induced a decline of calcification by 29 and 55% at 0.15 and 0.3 units lower than ambient pH, respectively. It is noteworthy that *L. pertusa* still revealed net calcification at an Ω_a close to 1 and even below 1, for average values of Ω_a as a result of biologically-induced changes in seawater chemistry during incubations (Table 3). The average responses of warm-water reef building corals and coral communities exhibit no calcification at Ω_a close to 1 (Gattuso et al., 1998; Langdon and Atkinson, 2005; Schneider and Erez, 2006). That *L. pertusa* still

shows relatively high calcification rates can be an indication of *L. pertusa* being already adapted to lower Ω_a levels as they are found in deeper oceans and at higher latitudes. Nevertheless, a decrease of about more than 50% in calcification rates of *L. pertusa* as response to a decline of 0.3 pH units as anticipated for the end of the century constitutes a drastic decline with respect to coral growth. Nothing is as yet known on the growth rate necessary to build and maintain the 3-dimensional coral framework and this might also be highly dependent on other site-specific factors. There will definitely be regional constraints where higher sedimentation rates require faster calcification to not get buried by the sediment load. Other regions might be more affected by additional rise in temperature where cold-water coral distribution is already at an upper temperature limit, as assumed for cold-water corals in the Mediterranean Sea. Specifically if additional stressors are added to those of climate change, a 50% decrease in calcification rate is likely detrimental to cold-water coral bioherms.

5 Conclusions

This study provides the first measurements of calcification rates in a deep-sea coral. Despite the methodological constraints due to the simple experimental set up used during the onboard experiments, *L. pertusa* showed clear patterns of calcification with youngest polyps calcifying fastest, with rates of the same order of magnitude to those reported in slow growing, reef-building corals. Drastically reduced calcification rates between pH treatments are a clear response to lower pH and increased pCO_2.

This is the first study presenting effects of ocean acidification on calcification rates of a cold-water coral. It is now crucial to optimise experiments for calcification studies and extent work to other species of cold-water corals and a broader geographical and depth scales. It is also pivotal to carry out longer-term experiments that take into account coral adaptation and to test the effects of ocean acidification in combination with elevated temperature to better predict the fate of cold-water coral bioherms.

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Table 1. Parameters of the carbonate chemistry at the beginning of the incubations. Values in italic were calculated using the variables in bold and the software package Seacarb (Proye and Gattuso, 2003). Data for DIC and TA were derived from the GLODAP database (Mingulay) and provided by Thomas and Borges (Skagerrak).

Site	pH treatment	Pressure	T [°C]	S	DIC [mol kg^{-1}]	TA [mol kg^{-1}]	pH_T	pCO_2 [ppm]	Ω_{arag}
Mingulay	ambient	**0**	**9.9**	**34.5**	**2.126×10^{-3}**	**2.231×10^{-3}**	8.10	352	2.25
Skagerrak	ambient	**0**	**7.5**	**34.6**	**2.143×10^{-3}**	**2.313×10^{-3}**	8.06	386	1.89
Skagerrak	−0.15 pH	**0**	**7.5**	**34.6**	2.201×10^{-3}	**2.313×10^{-3}**	**7.91**	568	1.40
Skagerrak	−0.3 pH	**0**	**7.5**	**34.6**	2251×10^{-3}	**2.313×10^{-3}**	**7.76**	827	1.02

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6, 1875–1901, 2009

Table 2. Overview of incubation experiments for different sites and pH treatments. Average number of polyps per branch, tissue dry weight (DW), skeletal weight, and calcification rates normalized to initial skeletal weight of branches.

Site	Date labelling	PH treatment	N	Polyps branch^{-1}	±S.E.	TissueDW [mg]	± S.E.	Skeleton [mg]	±S.E.	G [%d^{-1}]	±S.E.
Mingulay	20 Jul 06	ambient	8	6.63	±0.65	104.91	±15.53	1597.6	±221.9	0.067	±0.019
Skagerrak	15 Mar 07	ambient	8	5.25	±0.37	112.47	±6.50	1926.0	±170.7	0.046	±0.010
Skagerrak	15 Mar 07	−0.15 pH	8	5.00	±0.78	152.69	±39.07	1896.2	±362.0	0.033	±0.004
Skagerrak	15 Mar 07	−0.30 pH	8	7.75	±0.67	321.75	±26.81	3145.4	±329.8	0.020	±0.003
Skagerrak	17 Mar 07	ambient	8	6.63	±0.96	176.95	±25.05	1992.4	±358.5	0.021	±0.004
Skagerrak	17 Mar 07	−0.15 pH	8	6.63	±0.98	136.23	±21.72	4717.3	±843.2	0.015	±0.003
Skagerrak	17 Mar 07	−0.30 pH	8	8.00	±0.93	319.64	±45.27	4712.3	±663.4	0.010	±0.002

Table 3. Estimated average parameters of the carbonate chemistry changing as result of coral metabolism during experiments. Ammonium excretion (E) as well as rates of calcification (G) and respiration (R) were normalized to kg^{-1} seawater and h^{-1} and used to estimate hourly changes in TA and DIC over the 24 h incubations according to sampling sites and treatments as described in Sect. 2.5.

Site	pH treatment	G	R	E	DIC	TA	pH_T	pCO_2	Ω_{arag}
		[$\mu mol\ kg^{-1}\ h^{-1}$]			[$mol\ kg^{-1}$]			[ppm]	
Mingulay	normal	11.86	3.82	7.87	2.007×10^{-3}	2.041×10^{-3}	7.62	1097	0.82
Skagerrak	normal	8.40	4.70	7.13	2.075×10^{-3}	2.197×10^{-3}	7.93	518	1.47
Skagerrak	−0.15 pH	7.85	7.93	7.13	2.175×10^{-3}	2.210×10^{-3}	7.66	1078	0.87
Skagerrak	−0.3 pH	7.72	9.43	9.71	2.245×10^{-3}	2.244×10^{-3}	7.54	1419	0.68

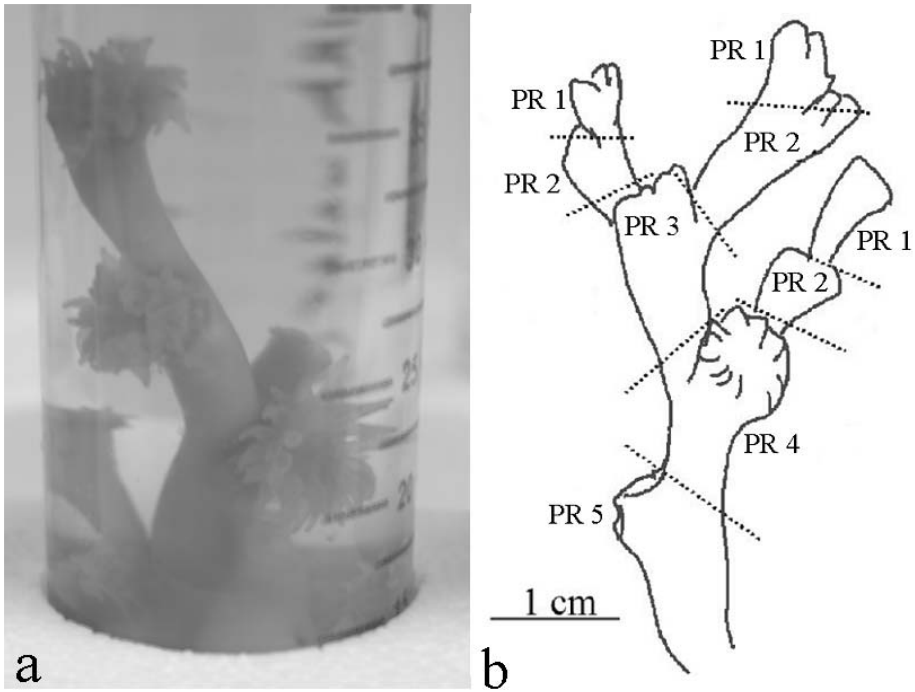

Fig. 1. **(a)** Experimental approach: branch of *Lophelia pertusa* is incubated for 24 h with the radioisotope label ^{45}Ca in a Greiner tube containing 30 ml seawater and a headspace of 20 ml air. **(b)** Branches are sub-sampled according to polyp rank (PR) with youngest polyps corresponding to a PR of 1 and subsequent numbering along the longest branch axis.

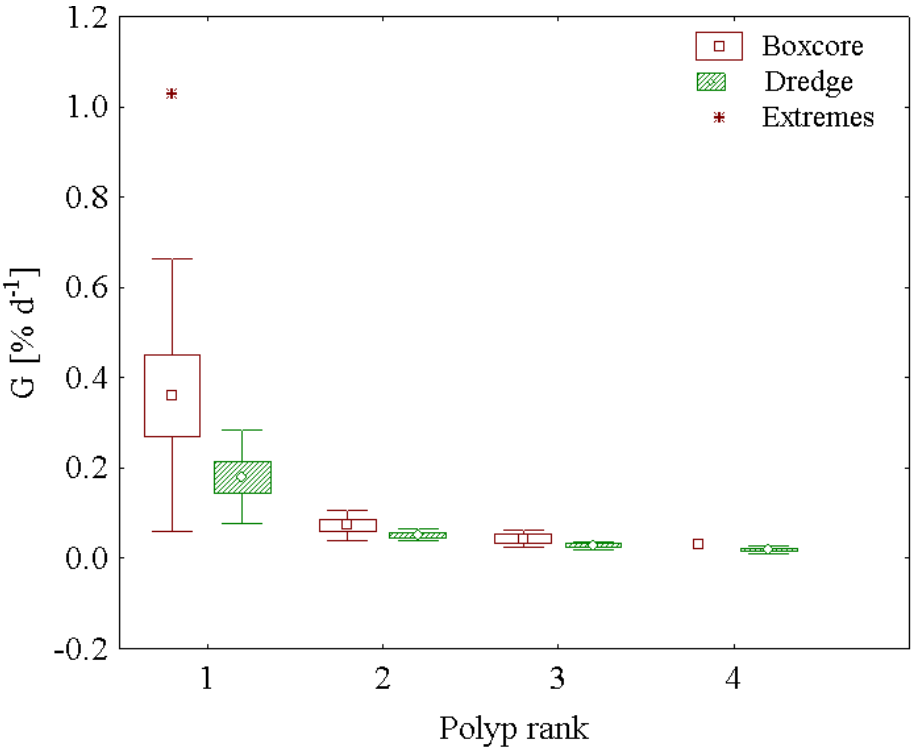

Fig. 2. Calcification rates (*G*) of Mingulay corals collected by box core or dredge as a function of polyp rank. Box and whiskers are ±S.E. and S.D., respectively.

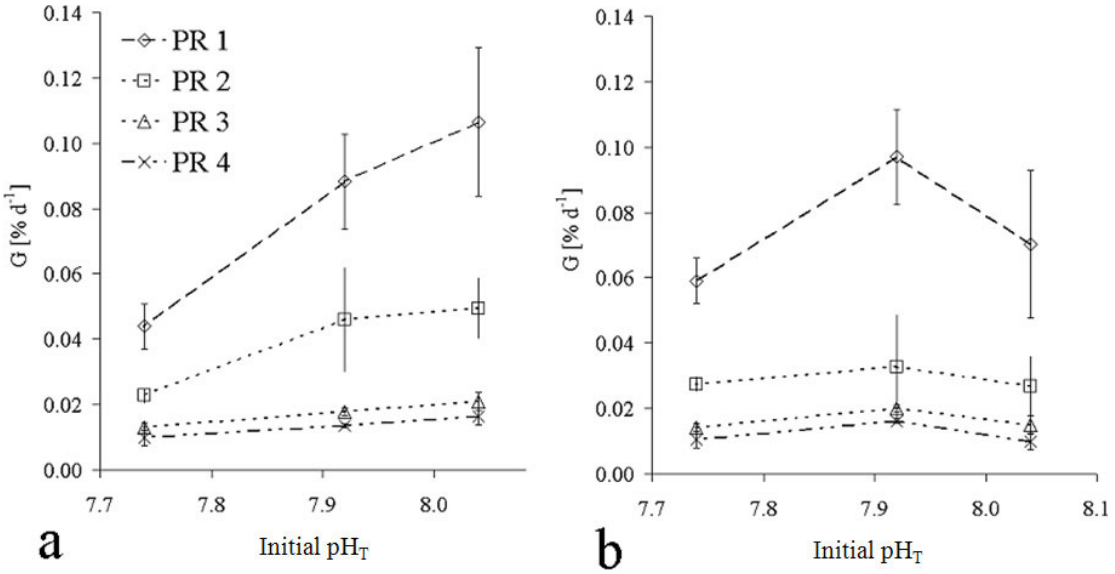

Fig. 3. Calcification rates (*G*) of Skagerrak corals according to polyp rank and as function of pH for experiments 1 **(a)** and 2 **(b)**.