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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 may be 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). Shortly after sample collection onboard these corals were labelled with calcium-45. The same experimental approach was used to assess calcification rates and how those changed due to reduced 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<sup>-1</sup> new skeletal growth and average rates of 0.11±0.02% d<sup>-1</sup> (±S.E.). Lowering pH by 0.15 and 0.3 units relative to the ambient level resulted in calcification being reduced by 30 and 56%. Lower pH reduced calcification more in fast growing, young polyps (59% reduction) than in older polyps (40% reduction). Thus skeletal growth of young and fast calcifying corallites suffered more from ocean acidification. Nevertheless, *L. pertusa* exhibited positive net calcification (as measured by <sup>45</sup>Ca incorporation) even at an aragonite saturation state ( $\Omega_a$ ) below 1.

al., 2006). These corals are often also referred to as deep-water corals as they are usually found below the photic zone at depths between 30 and 1000 m where they can build large three-dimensional structures (bioherms). Unlike tropical coral reefs that are usually constructed by a great number of hermatypic (reef-building) corals, the 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 although no such rate estimates are actually available. That is, it is more difficult to assess growth and calcification rates in cold-water than in warm-water scleractinian corals. First, direct access to the usually deep cold-water corals is logistically challenging and frequent or long-term observations are limited. Second, cold-water corals lack the annual bands of high- and low skeletal density that are conveniently produced by reef-building corals. In the latter, it is easy to estimate annual linear growth, which, along with measured skeletal density, allows one 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). Thus it is 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 specimens that have settled and grown on artificial substrates, such as oil and gas platforms in the North Sea. Those cold-water corals have an average linear skeletal extension rate (LSE) of 26 mm yr<sup>-1</sup> (Bell and Smith, 1999; Gass and Roberts, 2006). Growth estimates of specimens maintained in aquaria revealed an annual LSE of 9.4 mm yr<sup>-1</sup> and 15–17 mm yr<sup>-1</sup> for *L. pertusa* collected in

### 1 Introduction

The distribution of cold-water corals is believed to be mostly controlled by temperature (4 to 12°C) although other parameters, such as salinity, current speed and nutrients are important (Rogers, 1999; Hovland et al., 2002; Roberts et



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Norwegian Fjords (Mortensen, 2001) and the Mediterranean Sea (Orejas et al., 2008). There is currently no data available for rates of calcification ( $\text{CaCO}_3$  precipitation rate) in cold-water corals.

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 factors, elevated  $p\text{CO}_2$  and temperature are prominent and have been 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 also 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. A recent study argues that 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 appear particularly vulnerable. Cold-water corals are restricted to high latitudes and 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 below which sea water becomes undersaturated with respect to aragonite (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 (Guinotte et al., 2006). Therefore, not only might calcification of cold-water corals be hampered but also their aragonitic framework could well begin to dissolve posing an additional threat on cold-water coral associated fauna and a loss in biodiversity.

Here our goals are: (1) to measure short-term rates of calcification of the cold-water coral *Lophelia pertusa* and (2) to investigate its response to elevated  $p\text{CO}_2$  (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 reef, we tested a simple experimental set-up to assess calcification rates of *L. pertusa* by labelling freshly collected corals with  $^{45}\text{Ca}$  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. Thus we collected 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 a mobile climate-controlled laboratory container and kept at 7–9°C in small aquaria with 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. The dredge had a triangular opening of 1 m and a mesh size of 2×2 cm and was dredged over a stretch of ca. 150 m during 4 minutes of deployment. 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 $^{45}\text{Ca}$ 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 of seawater that was collected at sampling depth and filtered using 0.2 µm membranes. Caps were placed on top of tubes to avoid contamination by spilling of radioisotope-labelled seawater, but they were not screwed down to be airtight. The samples were not stirred, but ship movement was considered to be adequate to generate enough mixing to avoid stagnant regions near coral polyps. 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 reef in 2006, 30 µl of  $^{45}\text{Ca}$  (1.53 mCi ml<sup>-1</sup>) was added to each tube. The addition of  $^{45}\text{Ca}$  (1.88 mCi ml<sup>-1</sup>) 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.

Corals were incubated for 24 h, after which they were washed three times for 1 h with unlabelled seawater to allow the efflux of unbound  $^{45}\text{Ca}$  from the coelenteron, NaOH-soluble compartment (tissue), and skeleton (Tambutté et al., 1996). Coral branches were then frozen and stored at  $-20^{\circ}\text{C}$  pending analyses.

Back in the laboratory, whole coral branches were dried at  $60^{\circ}\text{C}$ . 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  $90^{\circ}\text{C}$  for several hours and subsequently rinsed with MilliQ water. This procedure was repeated until all tissue was removed. The remaining skeleton was dried at  $60^{\circ}\text{C}$  and the skeletal dry weight of each sub-sample 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.

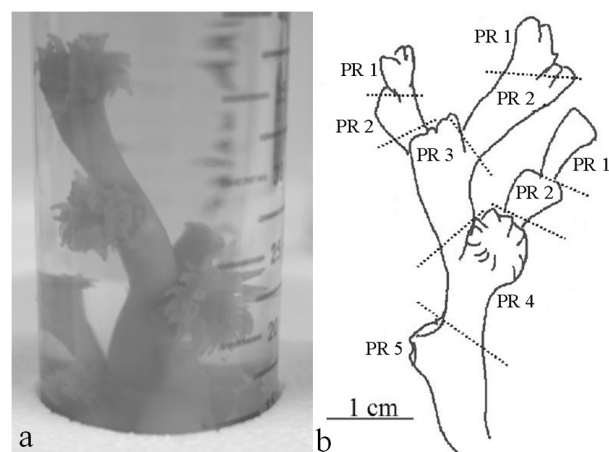
### 2.3 Normalization of calcification rate

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:

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

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  $\text{mg CaCO}_3 \text{ g}^{-1}$  skeleton  $d^{-1}$  or  $\text{mmol CaCO}_3$  (or  $\text{Ca}^{2+}$ )  $\text{mol}^{-1}$  skeleton  $d^{-1}$  using the average initial skeletal weight given in Table 2.



**Fig. 1.** (a) Experimental approach: branch of *Lophelia pertusa* is incubated for 24 h with the radioisotope label  $^{45}\text{Ca}$  in a Greiner tube containing 30 ml of 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.

## 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  $9.334^{\circ}\text{W}$ ;  $57.75^{\circ}\text{N}$  from 151 m water depth. For the Skagerrak site, data were provided by H. Thomas and A. Borges for  $58.50^{\circ}\text{N}$ ;  $9.50^{\circ}\text{E}$  sampled in February 2001 at a sampling depth of 100 m (Bozec et al., 2006 and Thomas et al., 2009). The DIC and TA of the GLODAP data are believed to be consistent to 4 and  $6 \mu\text{mol kg}^{-1}$ , respectively (Key et al., 2004).

### 2.4.2 Other parameters of the carbonate system

The Seacarb package (Gattuso and Lavigne, 2009) was used to characterize parameters of the carbonate system of seawater used for  $^{45}\text{Ca}$  labelling experiments (Table 1). Dissolved inorganic carbon and TA were used to calculate other parameters (pH,  $p\text{CO}_2$  and  $\Omega_a$ ). 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 an assumed consistency of the GLODAP DIC and TA of 4 and  $6 \mu\text{mol kg}^{-1}$  (Key et al., 2004), the range

**Table 1.** Parameters of the carbonate chemistry at the beginning of the incubations.

Site	pH treatment	T (°C)	S	DIC ( $\mu\text{mol kg}^{-1}$ )	TA ( $\mu\text{mol kg}^{-1}$ )	pH <sub>T</sub>	pCO <sub>2</sub> (ppm)	Ω <sub>arag</sub>
Mingulay	ambient	<b>9.9</b>	<b>34.5</b>	<b>2126</b>	<b>2231</b>	<b>8.1</b>	352	2.25
Skagerrak	ambient	<b>7.5</b>	<b>34.6</b>	<b>2143</b>	<b>2231</b>	<b>8.1</b>	386	1.89
Skagerrak	−0.15 pH	<b>7.5</b>	<b>34.6</b>	<b>2143</b>	2255	<b>7.91</b>	544	1.38
Skagerrak	−0.3 pH	<b>7.5</b>	<b>34.6</b>	<b>2143</b>	2203	<b>7.76</b>	791	0.97

\*Values in italics were calculated using the variables in bold and the *R* package seacarb (Gattuso and Lavigne, 2009). Data for DIC and TA were derived from the GLODAP database, WOCE, cruise A24 at 9.334° W; 57.75° N (Key et al., 2004) and from Thomas and Borges for the Skagerrak at 58.50° N; 9.50° E (Bozec et al., 2006 and Thomas et al., 2009).

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<sub>T</sub>, 45 ppm for ΔpCO<sub>2</sub> and 0.2 units for ΔΩ<sub>a</sub>. 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.

For treatments that were reduced by 0.15 and 0.3 pH units by the addition of HCl, the ppH function of the seacarb package was used under a closed system approach to estimate the resulting changes in the seawater carbonate chemistry.

## 2.5 Changes in the carbonate chemistry during time of incubation

Coral respiration, calcification and nutrient release into 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 due to gas exchange between seawater and headspace pCO<sub>2</sub> taking into account the following changes in DIC and TA in seawater caused by respiration, calcification and excretion. Calcification (*G*) decreases TA (ΔTA=2×*G*) and DIC (ΔDIC=*G*); respiration (*R*) does not significantly change TA, but increases DIC (ΔDIC=*R*); and ammonium excretion increases TA (ΔTA=14/16×*E*) (Gattuso et al., 1999).

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

An initial pCO<sub>2</sub> of 400 ppm was assumed in the headspace. A stepwise method was used: every hour the TA and DIC were calculated as follows:

$$\text{TA}_{t+1} = \text{TA}_t - 2G + 0.875E \quad (2)$$

$$\text{DIC}_{t+i} = \text{DIC}_t + R - G \quad (3)$$

with *G*, *R* and *E* in  $\mu\text{mol kg}^{-1}$  seawater h<sup>−1</sup>.

At each time step, pCO<sub>2</sub> was equilibrated between seawater and the headspace using the following relationship:

$$p\text{CO}_{2(\text{eq},t+1)} = (p\text{CO}_{2(\text{air},t)}/a + p\text{CO}_{2(t+1)}/b)/(a+b) \times ab \quad (4)$$

where pCO<sub>2(eq,t+1)</sub> is the pCO<sub>2</sub> equilibrated between headspace and seawater after each hourly time increment, pCO<sub>2(air,t)</sub> is the pCO<sub>2</sub> of the headspace before hourly equilibration with seawater pCO<sub>2</sub>, and pCO<sub>2(t+1)</sub> is the pCO<sub>2</sub> of seawater after hourly change in seawater chemistry, but before equilibration with air. For equilibration of pCO<sub>2</sub> between seawater and air, constants (*a* and *b*) were used taking into account partial pressure and molecular weight of CO<sub>2</sub> 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<sub>2</sub> and pCO<sub>2</sub> values and multiplication by the respective volume of air or seawater taking into account seawater temperature and density of CO<sub>2</sub> in gas. In this study, *a*=55586 and *b*=34847.

The TA and DIC after air-sea equilibration were used to calculate the new pCO<sub>2</sub> (i.e. pCO<sub>2(t+1)</sub> of Eq. 4), Ω<sub>a</sub> and pH using Seacarb of consecutive hourly time steps of 24 h. The average TA, DIC, pH, pCO<sub>2</sub> and Ω<sub>a</sub> 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).

**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 <sup>-1</sup>	±S.E.	Tissue DW (mg)	±S.E.	Skeleton (mg)	±S.E.	G (% d <sup>-1</sup> )	±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	0.02	±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<sup>-1</sup> seawater and h<sup>-1</sup> 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	<i>G</i>	<i>R</i>	<i>E</i>	DIC	TA	pH <sub>T</sub>	<i>p</i> CO <sub>2</sub> (ppm)	Ω <sub>arag</sub>
		(μmol kg <sup>-1</sup> h <sup>-1</sup> )			(μmol kg <sup>-1</sup> )				
Mingulay	ambient	11.86	3.82	7.87	2007	2041	7.62	1097	0.82
Skagerrak	ambient	8.40	4.70	7.13	2075	2197	7.93	518	1.47
Skagerrak	-0.15 pH	7.85	7.93	7.13	2117	2152	7.66	1054	0.85
Skagerrak	-0.3 pH	7.72	9.43	9.71	2137	2143	7.53	1389	0.63

### 3 Results

#### 3.1 Carbonate system of seawater

##### 3.1.1 Initial conditions of seawater chemistry

The calculations of initial conditions of seawater chemistry (Table 1) were based on DIC and TA of seawater taken at similar depths approximately 100 km away from study sites (Key et al., 2004 and Thomas et al., 2009). Calculated ambient pH<sub>T</sub> was 8.10 at Mingulay and 8.06 at Skagerrak. The calculated *p*CO<sub>2</sub> and Ω<sub>a</sub> 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, a decrease of pH by 0.15 and 0.3 units would consequently cause a decrease of Ω<sub>a</sub> from 2.25 to 1.38 and an increase in *p*CO<sub>2</sub> from 352 to 544; a pH decrease of 0.3 would cause Ω<sub>a</sub> to drop further to 0.97 and *p*CO<sub>2</sub> would rise further to 791 ppm.

##### 3.1.2 Changes in seawater chemistry during incubation

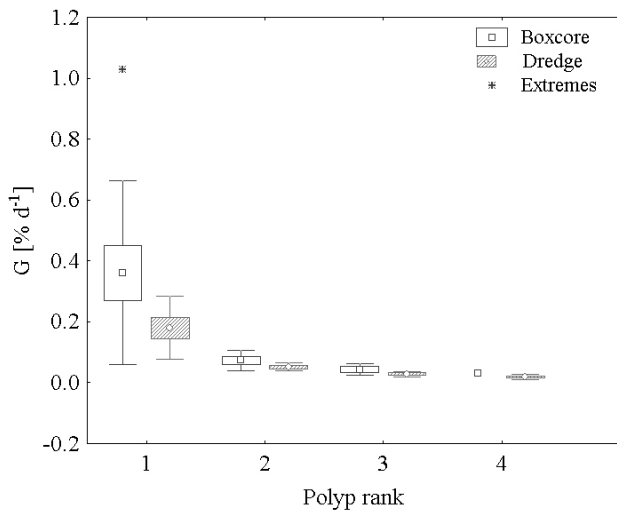
Changes in the carbonate chemistry as consequence of respiration and calcification rates were estimated for the different treatments and the resulting average values for parameters of the carbonate chemistry during incubation and are summarized in Table 3. As a result of changes in DIC by

-55 μmol kg<sup>-1</sup> and TA -117 μmol kg<sup>-1</sup>, the pH<sub>T</sub> was 0.27 units lower than the initial pH, while *p*CO<sub>2</sub> increased by 496 ppm and Ω<sub>a</sub> dropped by 0.68 units on average (Table 4). This resulted in values for pH<sub>T</sub> between 7.53 and 7.93; *p*CO<sub>2</sub> between 518 and 1389 ppm; and Ω<sub>a</sub> between 0.63 and 1.47 (Table 3).

#### 3.2 Calcification rates

##### 3.2.1 Bulk calcification of *L. pertusa* and response to lower pH

*Lophelia pertusa* branches had on average 6.55±0.32 polyps branch<sup>-1</sup> and the skeletal and tissue dry weights averaged 2855±241 and 189±15 mg branch<sup>-1</sup>, respectively (Table 2). The average rate of calcification was 0.067±0.019% d<sup>-1</sup> (N=8) for Mingulay corals but calcification rates spanned 2 orders of magnitude, between 0.0027 and 0.1923% d<sup>-1</sup>. 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*<sub>(1,22)</sub>=2.126, *p*=0.045). A one-way ANOVA reveals a significant effect of pH on bulk calcification rates of *L. pertusa* collected in the Skagerrak (*F*<sub>(2,45)</sub>=7.03, *p*<0.001). Post-hoc comparisons



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

revealed that  $G$  was significantly different between ambient seawater and the treatment where pH was lowered by 0.3 units (HSD,  $p < 0.01$ ). Whereas, for treatments that were different by 0.15 pH units no significant differences with respect to  $G$  could be observed (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).

### 3.3 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  $0.005\% \text{ d}^{-1}$  and  $1.027\% \text{ d}^{-1}$ . Ranks of higher or equal to 4 were grouped together in order to reach sample sizes large enough for statistical comparison. The sampling method had no significant effect on the calcification rates (2-way ANOVA,  $F_{(1,44)} = 1.16$ ,  $p = 0.29$ ) whereas polyp rank did ( $F_{(3,44)} = 7.71$ ,  $p < 0.01$ ). 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 highest in the youngest polyps (polyp rank 1) with  $0.279 \pm 0.055\%$  ( $N = 20$ ) and decreased in older polyps with  $0.064 \pm 0.008\%$  ( $N = 13$ ). Out of the older polyps, those with polyp ranks 2 to 4 had an average calcification rate of  $0.034 \pm 0.006\%$ , those with ranks greater than 4 had an average calcification rate of  $0.019 \pm 0.002\%$ . 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,  $p > 0.89$ ).

### 3.3.1 Effect of lower pH on calcification according to polyp rank

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 when 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 was lowered by 0.3 units relative to ambient seawater for which calcification decreased to 41% for polyp rank 1 and 46% for polyp rank 2. For polyp rank 3, the decrease was less pronounced and calcification at a pH of 0.3 units below ambient was 62% of that found in ambient seawater; for polyp ranks above 3, the decline was similar, 60%.

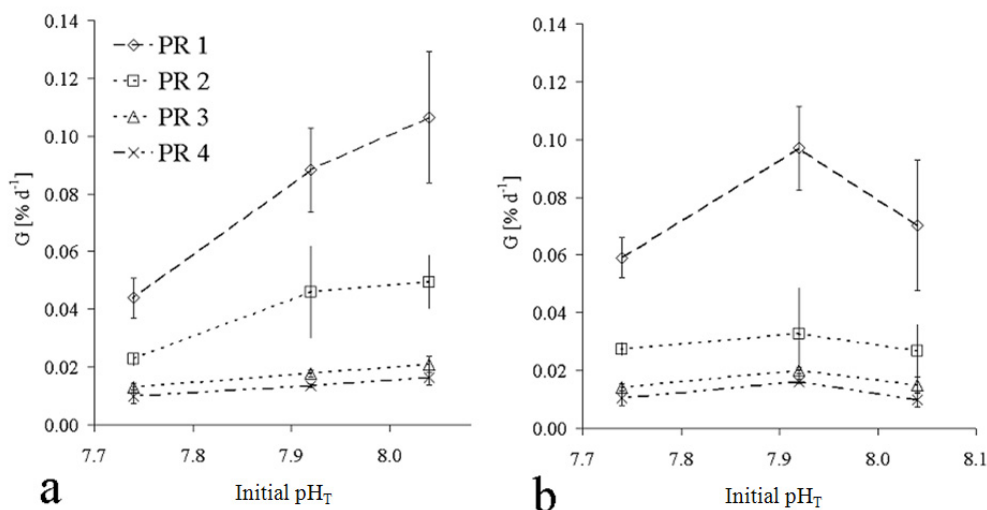
The results of the second experiment are less clear (Fig. 3b). There was no substantial decrease in calcification rates between ambient seawater and treatments with lower pH. Only for polyp ranks 1 and 3 were the calcification rates slightly lower for *L. pertusa* in the treatment with  $-0.3$  pH units relative to ambient seawater. Between treatments of  $-0.15$  and  $-0.3$  pH units, calcification rates were lower at the lower pH level, confirming results from experiment 1.

## 4 Discussion

### 4.1 Methodological constraints

To the best of our knowledge, no physiological experiment has been carried out in situ on deep-sea corals and very few studies have been conducted in the laboratory. Here, colonies were incubated on board ship, using freshly collected specimens and ambient seawater. Although this is as close as the community has come to achieving in situ conditions, experiments at sea do entail several limitations of lab space, time and working with radioactive material. In this section, we examine the methodological constraints experienced and the uncertainties that they generate.

The radioisotope labelling technique is a very sensitive method to determine coral calcification and is thus, well suited for short-term incubations and to measure calcification according to polyp age. For tropical corals it was suggested to grow nubbins completely covered by tissue to avoid non-biologically  $^{45}\text{Ca}$  adsorption by the skeleton (Tambutté et al., 1995). In our experiments, the oldest corallite (of highest polyp rank) was the part that contained the broken off area and had bare skeleton exposed to  $^{45}\text{Ca}$ -labelled seawater. These samples generally exhibited very low calcification



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

rates (<1% of the bulk) demonstrating that non-biologically bound <sup>45</sup>Ca was negligible, despite the bare skeleton.

Due to the small volume used (30 ml) we were unable to determine TA and DIC, respiration, and nutrient excretion in the incubations. TA and DIC data were obtained from seawater sampled during other cruises at similar depths in the area of the study sites. A closed system approach was used to model changes in seawater carbonate chemistry assuming that air-exchange between the vials and ambient air was negligible. A small amount of exchange would overestimate the changes in seawater carbonate chemistry. Among the data used to model changes in the seawater chemistry during incubations (Table 3) the respiration and excretion data were taken from the literature (Dodds et al., 2005; van Duyl et al., 2005). A sensitivity analysis was carried out to estimate the uncertainty associated with lower and higher respiration and excretion rates (Table 4).

There are several methods to experimentally change the carbonate chemistry and decrease the pH (Gattuso and Lavigne 2009). For practical reasons, we lowered the pH of ambient seawater adding HCl until the desired reduction (0.15 or 0.3 pH unit) was reached. This is a straight forward method to reach target pH values, but also it generates a decline in total alkalinity that is not expected in the present century. Nor does this approach mimic the projected increase in DIC. In our experiments, the computed deviation from changes expected by the  $p\text{CO}_2$  approach were  $58 \mu\text{mol kg}^{-1}$  for both DIC and TA at a pH reduced by 0.15 units and 108 and  $110 \mu\text{mol kg}^{-1}$  for DIC and TA at a pH reduced by 0.3 units. Consequently, the  $p\text{CO}_2$  was only 24 and 36 ppm below target values, and  $\Omega_a$  was 0.02 and 0.05 higher, which is negligible relative to the  $\Omega_a$  decreases of 0.49 and 0.87 when the pH was lowered by 0.15 and 0.3 units, respectively.

**Table 4.** Estimates of the carbonate chemistry with half and double (bold) the assumed values of  $R$  and  $E$  as given in Table 3 (the 1st row of each treatment in italics contains the values of Tables 2 and 3).

Site	pH treatment	$G$	$R$	$E$	DIC	TA	pH <sub>T</sub>	$p\text{CO}_2$ (ppm)	$\Omega_a$
		( $\mu\text{mol kg}^{-1} \text{h}^{-1}$ )			(mol kg <sup>-1</sup> )				
<i>Mingulay</i>	<i>ambient</i>	11.86	3.82	7.78	$2.01 \times 10^{-3}$	$2.04 \times 10^{-3}$	7.62	1097	0.82
		11.86	<b>7.64</b>	<b>3.94</b>	$2.03 \times 10^{-3}$	$2.00 \times 10^{-3}$	7.4	2047	0.57
		11.86	<b>1.91</b>	<b>3.94</b>	$1.98 \times 10^{-3}$	$2.00 \times 10^{-3}$	7.55	1303	0.73
		11.86	<b>7.64</b>	<b>15.74</b>	$2.06 \times 10^{-3}$	$2.14 \times 10^{-3}$	7.75	798	1.08
		11.86	<b>1.91</b>	<b>17.74</b>	$1.92 \times 10^{-3}$	$2.14 \times 10^{-3}$	7.88	569	1.41
Skagerrak	ambient	8.40	4.70	7.13	$2.08 \times 10^{-3}$	$2.20 \times 10^{-3}$	7.93	518	1.47
		8.40	<b>9.40</b>	<b>3.57</b>	$2.12 \times 10^{-3}$	$2.15 \times 10^{-3}$	7.66	1174	0.94
		8.40	<b>2.35</b>	<b>3.57</b>	$2.05 \times 10^{-3}$	$2.15 \times 10^{-3}$	7.65	593	1.32
		8.40	<b>9.40</b>	<b>14.26</b>	$2.14 \times 10^{-3}$	$2.28 \times 10^{-3}$	7.98	477	1.66
		8.40	<b>2.35</b>	<b>14.26</b>	$2.09 \times 10^{-3}$	$2.28 \times 10^{-3}$	8.1	344	2.12
Skagerrak	-0.15	7.85	7.93	7.13	$2.12 \times 10^{-3}$	$2.152E+00$	7.66	1054	0.85
		7.85	<b>15.86</b>	<b>3.57</b>	$2.17 \times 10^{-3}$	$2.15 \times 10^{-3}$	7.49	1935	0.74
		7.85	<b>3.97</b>	<b>3.57</b>	$2.07 \times 10^{-3}$	$2.15 \times 10^{-3}$	7.83	692	1.21
		7.85	<b>15.86</b>	<b>14.26</b>	$2.20 \times 10^{-3}$	$2.28 \times 10^{-3}$	7.79	817	1.21
		7.85	<b>3.97</b>	<b>14.26</b>	$2.10 \times 10^{-3}$	$2.28 \times 10^{-3}$	8.08	365	2.02
Skagerrak	-0.3	7.72	9.43	9.71	$2.14 \times 10^{-3}$	$2.14 \times 10^{-3}$	7.53	1389	0.63
		7.72	<b>18.86</b>	<b>4.86</b>	$2.20 \times 10^{-3}$	$2.17 \times 10^{-3}$	7.45	2159	0.7
		7.72	<b>4.72</b>	<b>4.86</b>	$2.08 \times 10^{-3}$	$2.17 \times 10^{-3}$	7.84	661	1.25
		7.72	<b>18.86</b>	<b>19.42</b>	$2.24 \times 10^{-3}$	$2.34 \times 10^{-3}$	7.86	685	1.38
		7.72	<b>4.72</b>	<b>19.42</b>	$2.13 \times 10^{-3}$	$2.34 \times 10^{-3}$	8.13	324	2.33

#### 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<sup>-1</sup>. 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. This needs to be taken into account when determining calcification rates of bulk samples and not of single corallites (e.g. buoyant weight or total alkalinity anomaly techniques). Our extremely low growth rates for older corallites agree 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, rapid-growth events, which may explain the large range of calcification rates observed in the youngest polyps (polyp rank 1). Our finding that the young polyps can calcify extremely quickly, suggests that new corallite production (budding) is important for the growth and maintenance of the 3-dimensional structure of cold-water coral bioherms. Thus high abundance of new coral buds may consequently be a good indicator for the potential of a cold-water coral bioherm to maintain rapid growth and its 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). Also, sponge bioerosion can induce additional aragonite secretion at younger growth stages of corallites (Beuck et al. 2007), and growth morphologies with densely spaced new corallites and high budding rates have been 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 depths, seasonality, or seawater carbonate chemistry. The higher calcification rates of Mingulay corals may reflect a higher initial  $\Omega_a$  at this site. On the other hand, 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 Sampling gear

For Mingulay corals, the effect of sampling *L. pertusa* with different gear (box core or dredge) was tested, because dredging inflicts more damage on benthic organisms than does box coring. In this study, the range of calcification rates was lower for dredged corals than for those retrieved by box coring (Fig. 2), but mean values were not significantly different. Thus our specimens 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. The latter would be supported by aquarium observations showing a high recovery potential of *L. pertusa* to skeletal fragmentation (Maier, 2008).

### 4.4 Calcification rates in response to reduced pH

The rate of calcification decreased when pH was lowered by 0.15 and 0.3 units relative to ambient seawater. The aragonite saturation state ( $\Omega_a$ ) was approximately 1.9 for ambient seawater, whereas it declined to 1.4 and 1.0 at the lower pH levels (Table 1). These declines of 25 and 46% resulted in corresponding declines in calcification by 29 and 55%. Yet *L. pertusa* exhibited positive net calcification even when  $\Omega_a$  reached values close to 1. Moreover, if biologically-induced changes in seawater chemistry during incubations were taken into account, the positive net calcification would even correspond to an  $\Omega_a$  below 1 (Table 3). In contrast, the average responses of warm-water reef building corals and coral communities exhibit no net calcification at  $\Omega_a$  close to 1 (Gattuso et al., 1998; Langdon and Atkinson, 2005; Schneider and Erez, 2006). That *L. pertusa* still shows positive net calcification rates supports the idea that *L. pertusa* is already adapted to lower  $\Omega_a$  levels, where they live, i.e., in the deeper ocean and higher latitudes. Nevertheless, a decrease of 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 avoid being 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 may well be 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 most rapidly, at rates comparable to those of slow growing, reef-building corals. Dramatically reduced calcification rates at lower pH treatments are a clear response to increased  $p\text{CO}_2$  and lower pH. It is now crucial to optimise experiments for calcification studies and extend work to other species of cold-water corals as well as broaden studies to wider geographical and depth ranges. It is also a priority to carry out longer-term experiments designed to evaluate possible acclimation mechanisms of cold-water corals and to test how cold-water corals will react to the combined effects of ocean acidification and elevated temperature.

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