Calcification is not the Achilles’ heel of cold-water corals in an acidifying ocean

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Abstract

Ocean acidification is thought to be a major threat to coral reefs: laboratory evidence and CO$_2$ seep research has shown adverse effects on many coral species, although a few are resilient. There are concerns that cold-water corals are even more vulnerable as they live in areas where aragonite saturation ($\Omega_{\text{ara}}$) is lower than in the tropics and is falling rapidly due to CO$_2$ emissions. Here, we provide laboratory evidence that net (gross calcification minus dissolution) and gross calcification rates of three common cold-water corals, Caryophyllia smithii, Dendrophyllia cornigera and Desmophyllum dianthus, are not affected by pCO$_2$ levels expected for 2100 (pCO$_2$ 1058 µatm, $\Omega_{\text{ara}}$ 1.29), and nor are the rates of skeletal dissolution in D. dianthus. We transplanted D. dianthus to 350 m depth (pH$_T$ 8.02; pCO$_2$ 448 µatm, $\Omega_{\text{ara}}$ 2.58) and to a 3 m depth CO$_2$ seep in oligotrophic waters (pH$_T$ 7.35; pCO$_2$ 2879 µatm, $\Omega_{\text{ara}}$ 0.76) and found that the transplants calcified at the same rates regardless of the pCO$_2$ confirming their resilience to acidification, but at significantly lower rates than corals that were fed in aquaria. Our combination of field and laboratory evidence suggests that ocean acidification will not disrupt cold-water coral calcification although falling aragonite levels may affect other organismal physiological and/or reef community processes.

Introduction

Cold-water corals (CWC) occur from tropical to polar waters, from the shallows to the deep sea and a few build extensive reefs that attract fish and a high diversity of associated invertebrates (Roberts et al., 2009). Despite their diversity and ecological importance we know much less about CWC biogeography than their tropical counterparts. There is a growing body of work on their metabolism, based on monitoring corals in refrigerated aquaria (Dodds et al., 2007; Tsounis et al., 2010; Naumann et al., 2011), but it is difficult to know how these results relate to corals in the wild since very few studies have investigated their metabolism in situ. Field studies have the advantage of being more ecologically realistic, incorporating natural fluctuations in biotic and
abiotic conditions, but are less constrained and logistically more difficult to perform than laboratory experiments. A lack of in situ data on the physiological response of CWC to environmental variations has hindered our ability to project their fate in the face of rising CO$_2$ levels. Ocean acidification is reducing the amount of carbonate ions [CO$_3^{2-}$] available in seawater (Orr et al., 2005), therefore lowering the CaCO$_3$ saturation state towards undersaturated levels ($\Omega < 1$). It is believed, but a matter of debate (McCulloch et al., 2012a), that a decrease in the [CO$_3^{2-}$] in seawater as oceans acidify will reduce coral calcification rates and increase skeletal dissolution (Langdon et al., 2000; Fabricius et al., 2011). Some predict that tropical coral reefs will stop calcifying this century (Hoegh-Guldberg et al., 2007) and others expect that reef-building scleractinian calcification rates will decrease by 17-37% due to a doubling of preindustrial levels of pCO$_2$ (Erez et al., 2011).

It is predicted that ca. 70% of known CWC reefs will be exposed to waters that are corrosive to aragonite before the end of 2100 (Guinotte et al., 2006; Tittensor et al., 2010; Jackson et al., 2014). Here, we assess this issue since many CWC species already live at depths well below the aragonite saturation horizon (Thresher et al., 2011; Lunden et al., 2013) and form habitats at around $\Omega_{\text{ara}} = 1$ in Chilean fjords ( Försterra & Häusserman, 2003).

We combined laboratory and field experiments to investigate responses of Caryophyllia smithii, Desmophyllum dianthus and Dendrophyllia cornigera to waters acidified with CO$_2$. Caryophyllia smithii and D. dianthus are two of the commonest solitary corals in Atlantic Ocean and the Mediterranean Sea; Dendrophyllia cornigera forms colonies and is widespread in the Mediterranean, it also occurs in the Atlantic from Cape Verde and the Azores north to the Bay of Biscay.

Research into the effects of acidification on CWCs in aquaria has provided conflicting results. Form & Riebesell (2012) found a significant decrease in the net calcification of Lophelia pertusa during a one week exposure to high CO$_2$ levels but found that this reef-forming coral was able to acclimate in the long-term (6 months), increasing its calcification rate in seawater that was undersaturated with respect to aragonite compared to saturated conditions. Work on other CWC revealed that their calcification was not affected by acidification during the first 182 and 240 days of incubation (Movilla et al., 2014a; Carreiro-Silva et al., 2014 respectively). On the other hand, D. dianthus had a 70% reduction in skeletal growth rate after 314 days whereas Dendrophyllia
cornigera showed no differences between treatments (Movilla et al., 2014a). Other studies have shown that calcification rates of L. pertusa and Madrepora oculata (which also forms deep-water reefs) were unaffected by future projected $p$CO$_2$ levels in short- (hours-days; Maier et al., 2012) and long-term (weeks-months; Maier et al., 2013a,b) experiments. McCulloch et al. (2012b) showed that CWCs are able to calcify at or close to the aragonite saturation horizon by elevating their internal pH, thus buffering external changes in seawater pH. These studies imply that shoaling of the aragonite saturation horizon may not cause the dramatic declines in coral calcification rates that were first feared (Guinotte et al., 2006; Jackson et al., 2014). However, to what extent undersaturated seawater might affect reef integrity by increasing the dissolution of exposed coral skeletons has not yet been investigated and all studies showing that they maintain calcification rates at high $p$CO$_2$ levels have been carried out in aquaria where feeding may have artificially boosted their energy reserves, likely altering responses to acidification.

In this study we first measured the effects of acidification on the net and gross calcification rates as well as the respiration rates of three CWC species cultured in aquaria under present and Representative Concentration Pathway (RCP) 8.5 $p$CO$_2$ scenario (IPCC, 2014). In addition, we quantified skeletal dissolution rates of D. dianthus maintained in aquaria during two-month incubations at present and high $p$CO$_2$ levels. Finally, we compared the calcification rate of D. dianthus fed in aquaria in aragonite saturated conditions with those of corals transplanted into undersaturated and saturated conditions off Italy.

Materials and methods

Coral collection and aquaria

Corals were collected using a mini-dredge during three oceanographic cruises. Sixteen samples of Caryophyllia smithii were collected in December 2008 in the Bari Canyon (South Adriatic Sea; 39°27.97’ N; 18°23.29’ E; ca. 600 m depth) during the ARCO cruise (R/V Urania, CNR, Italy). In December 2009, hundreds of Desmophyllum dianthus found growing on an abandoned fishing net, were collected at 830 m depth off Malta (36°44.30’ N; 13°58.48’ E) on the MEDCOR cruise (R/V Urania, CNR, Italy). Finally, three branches of Dendrophyllia cornigera with ca. 20 polyps were retrieved from the Ionian Sea (39°50.49’ N; 16°48.70’ E) at 126 m depth in August 2009, using an ROV operated from R/V Astrea (ISPRA, Italy). These corals were maintained in large, shaded seawater containers held at 13-14°C on board. Seawater was renewed daily, and
continuously mixed with a submersible water pump. At the end of the cruises, the samples were transported to the International Atomic Energy Agency laboratory in Monaco. During the ARCO and MEDCOR cruises, seawater samples next to living corals were collected with a CTD-Rosette system equipped with 12-l Niskin bottles. Depth profiles of temperature, salinity and dissolved oxygen were also acquired. Seawater pH was immediately measured on board (see below for further details) and subsamples for total alkalinity were stored.

The corals were maintained for several months in the dark in flow-through 500 l aquaria held at 13.2°C. The aquaria were supplied with filtered seawater pumped from 30 m depth at a turnover rate of 20% volume h\(^{-1}\). The corals were fed twice per week with frozen krill or freshly hatched _Artemia nauplii_. All corals appeared healthy with polyps expanded. For _D. cornigera_, 4-6 cm long single polyps were separated from the mother colonies using pliers and attached to tagged plastic plates using epoxy glue (HoldFast\textsuperscript{®}, Ohio, USA). Sixteen specimens of both _D. cornigera_ and _C. smithii_, and 80 corallites of _D. dianthus_ were prepared for the experiment in aquaria. As we wanted to examine calcification rates we selected specimens with similar skeletal mass weight (see Carreiro-Silva et al., 2014). Samples were randomly assigned to four 20-l experimental tanks (two tanks for each pH treatment), continuously filled with seawater at 13°C with a turnover rate of 10% h\(^{-1}\). Tanks were also placed in large aquaria containing seawater kept at 13°C that served as water baths. Each tank contained four samples of _D. cornigera_ and _C. smithii_, and 20 samples of _D. dianthus_ which were positioned on the bottom of the tank in a vertical position. To measure skeletal dissolution, 24 dead _D. dianthus_ skeletons were scrubbed clean in seawater and placed in each tank (four specimens for each tank, n = 8 per treatment). These samples had white bare skeletons and appeared normal (not corroded or bored). A submersible pump (micro-jet MC 320, Mentor, OH, USA; flow rate ca 300 l h\(^{-1}\)) provided water circulation inside the tanks. Corals were acclimated for one month to experimental conditions (see Table 1 for seawater parameters). Then, two tanks were set up at ambient pH and pCO\(_2\) (pH\(_T\) = 8.07; pCO\(_2\) = 319 µatm), and the other two at levels projected by the end of the century (pH\(_T\) = 7.70; pCO\(_2\) = 1058 µatm). The pH was controlled using a pH–stat system (IKS, Karlsbad, accuracy ± 0.05 pH unit) by bubbling pure CO\(_2\) into each tank that was continuously aerated with CO\(_2\)-free air. Colonies were maintained under these pCO\(_2\) conditions for three months.
Transplantation

In February 2010, 22 specimens (11 per site) of *D. dianthus* were weighed and fixed onto individual plates then transplanted onto a rocky seabaed at CO2 seeps off Ischia (Italy; 40°43.84' N; 13°57.08' E). Specimens were transplanted to the same sites previously studied by Rodolfo-Metalpa *et al.* (2011): 1) station B1 at 3 m depth with a mean pH of 7.43 ± 0.31; 2) and station C at 5 m depth with a mean pH of 8.06 ± 0.07. At both sites corals were positioned calyx upwards inside an open cage made with two PVC plates (60 x 90 cm), mounted using bolts and attached to 30 kg concrete blocks (Fig. 1a). The light irradiance received by the corals was <10 µmol photons m^{-2} s^{-1} as measured by a Li–Cor 4π spherical underwater quantum sensor (LI–193SA). A Hobo Onset logger was positioned inside the cage to monitor seawater temperatures at 15 minute intervals for the entire duration of the transplantation. When checked on February 22nd 2010 the corals appeared healthy with fully expanded polyps that were sometimes seen catching organic detritus. A subsequent storm caused the loss of the cage at station C but corals at the high CO2 site appeared healthy and were collected on April 6th 2010, transported to the laboratory and weighed.

In March 2010, twenty-two samples of *D. dianthus* were suspended at 350 m depth on a mooring in Corsica channel (Aliani & Meloni, 1999) using R/V *Maria Grazia* (CNR, Italy). The mooring had been anchored at 447 m depth (43°01.76' N; 09°41.12' E) since 1985 to monitor seawater temperature, salinity and currents. As at Ischia, corals were weighed and attached to individual plates, which were put into an open cage fixed to the mooring cable (Fig. 1b). Seawater samples were collected at the depth of the transplantation at the beginning (March 14th 2010) and at the end of the experiment (November 27th 2010) using Niskin bottles on a CTD-Rosette system. Details of the pH and total alkalinity measurements are reported below. Temperature, salinity, dissolved oxygen and currents were continuously recorded at 71, 121, 319, 408 m depth by the instruments positioned on the mooring (Aliani & Meloni, 1999). Temperature at corals cage depth was 13.7 °C (± 0.09), salinity was 38.6 (± 0.14) and speed of current was 24 cm s^{-1} (± 15).

Seawater carbonate chemistry

Total alkalinity (*A_T*) and pH expressed on total scale (*pH_T*) were measured from seawater samples collected: 1) on board R/V *Urania* in December 2008 and 2009; 2) at the mooring experiment at
350 m depth during transplantation; 3) at the coastal sites off Ischia, and 4) in the experimental aquaria. The pH$_T$ was measured immediately using a pH-meter and an electrode (Methrom pH mobile) calibrated against the TRIS/HCl and 2-aminopyrididine/HCl buffer solutions. The seawater samples were then passed through Whatman GF/F, treated with 0.05 ml of 50 % HgCl$_2$ (Merck, Analar) and stored in glass bottles the dark at 4°C pending analysis. Three replicate of 20 ml subsamples were analysed at 25°C using a titration system composed of a pH meter with a Methrom pH electrode and a 1 ml automatic burette. Titration values of A$_T$ standards provided by A.G. Dickson were within 0.5 μmol kg$^{-1}$ of the nominal value.

The other parameters of the carbonate system (pCO$_2$, CO$_3^{2-}$, HCO$_3^-$, C$_T$ and Ω$_{ar}$) were calculated from pH$_T$, mean A$_T$, temperature, pressure and mean salinity using the free-access CO$_2$SYS package. Carbonate calculations were carried out using the recommended dissociation constants of carbonic acid in seawater (K$_1$ and K$_2$ from Mehrbach et al., 1973 refitted by Dickson & Millero, 1987). A$_T$ and salinity were constant throughout the laboratory experiments. Averages of pH$_T$ were calculated from hydrogen ion concentrations of each measurement and then re-converted back to pH (Kerrison et al., 2011).

Coral calcification and dissolution

Net calcification rates (gross calcification minus dissolution) of the corals cultured in aquaria were measured during the acclimation period and after three months. At the beginning of the acclimation 46 specimens out of 80 D. dianthus, 14 out of 24 D. cornigera and 14 out of 24 C. smithii were randomly selected and weighed. All samples were weighed both at the end of the acclimation period and at the end of the experiment. In addition net calcification rates of D. dianthus were measured three days before and two days after the transplantation experiments. Corals were weighed using the buoyant weight technique (Davies, 1989) before and after being attached to tagged plastic plates. The difference (plate and glue weight) was recorded, for correction of the total weight. Samples were weighed in seawater of known density using a Mettler AT200 electronic balance with a precision of 0.1 mg. Measurement reproducibility of the buoyant weight system was 0.5 mg (n = 10). The net buoyant weight of the corals was converted into dry weight using the density of the pure aragonite (2.94 g cm$^{-3}$). Using this value we found that our estimations of coral calcification rates were only 5-8 % higher than rates calculated from skeletal density measurements by Movilla et al. (2014a) for D. dianthus and D. cornigera. Daily
growth rates were quantified as the change in dry weight during the incubations, and normalized to the initial dry weight of each specimen (i.e. mg CaCO$_3$ g$^{-1}$ dry skeleton day$^{-1}$). For comparison with other studies, buoyant weights of samples were also calculated in percentage per day (% day$^{-1}$). The same approach as above was used to measure the skeletal dissolution rates of *D. dianthus* maintained in aquaria (7 specimens for each tank, n = 14 per treatment) for two months at normal and high $p$CO$_2$ conditions.

Gross calcification rates (i.e. the amount of carbonate deposited by an animal over time, and unaffected by the skeletal dissolution) of corals maintained in aquaria under normal and high $p$CO$_2$ conditions were measured at the end of the experiment using the $^{45}$Ca technique (Tambutté *et al.*, 1995). At the end of the incubation, four samples of each species from each tank (n = 8 per treatment) were transferred to four dedicated tanks (6 l volume) set-up at the same experimental temperature (13.4°C) and $p$CO$_2$ conditions (pH$_T$ = 8.07; $p$CO$_2$ = 319 µatm; pH$_T$ = 7.70; $p$CO$_2$ = 1058 µatm). After two days acclimation, the seawater renewal was halted and $^{45}$CaCl$_2$ was added in order to reach a final activity of 50 Bq ml$^{-1}$ in each aquarium. The seawater was renewed and spiked with radiotracer every day to maintain the same [$^{45}$Ca]. This was checked two times a day to determine radioactivity using glass vials containing 1 ml of seawater collected from the aquaria and 10ml of scintillation liquid (Ultima Gold, PerkinElmer). After a week, all corals were collected and processed. At the end of the labelling period, samples were incubated in 6 l tanks containing unlabelled seawater for one hour to achieve isotopic dilution of $^{45}$Ca contained in the coral coelenterons. Samples were blotted dry and the tissue dissolved in 1 to 2 ml 1 N NaOH at 90°C. Each sample was rinsed twice in 1 ml NaOH solution, dried, weighed and transferred onto individual pre-combusted glass vials. Coral skeletons were dissolved in 1.5 ml 12 N HCl, the solutions were evaporated on a hot plate and 10 ml of liquid scintillation medium was added to the radioactive samples. Beta emissions were measured using a liquid scintillation counter (2100 TR Packard; Tricarb). Calculations of calcification were based on a seawater calcium concentration (Tambutté *et al.*, 1995) of 10 mM. Results were normalized to dry skeletal mass (i.e. nmol $^{45}$Ca g$^{-1}$ dry skeleton day$^{-1}$). Non-biological incorporation of $^{45}$Ca (i.e. adsorption) on exposed skeletons (only present for samples of *D. cornigera*) was estimated using an identical protocol. The skeletons of dead *D. dianthus* were incubated and treated as those of the live
samples. The amount of $^{45}$Ca incorporated (less than 7%) was subtracted from the total amount measured on live samples.

Respiration

Respiration rates were measured during the acclimation period and after 10 weeks of incubation. Each of the seven specimens of D. dianthus (n = 14 for each treatment), four D. cornigera (n = 8 for each treatment) and four C. smithii (n = 8 for each treatment) was placed in closed thermostated Perspex chambers (ca. 100 ml volume). Seawater used during the incubations was previously filtered at 0.20 µm and then maintained at the treatment $pCO_2$ and temperature conditions. Seawater in the chamber was continuously stirred with a stirring bar. Six chambers were immersed in a water bath connected to a Ministat 125 (Huber) kept at 13.5 ± 0.2°C. Changes in the concentration of dissolved oxygen were measured using a Strathkelvin oxygen electrode system (Clark-type electrodes connected to a Strathkelvin 928 oxygen meter and a computer). The electrode was calibrated against O$_2$-free (using sodium dithionite) and air-saturated seawater. The O$_2$ concentration at saturation was calculated according to the experimental temperature and the salinity values at the ambient barometric pressure (http://www.unisense.com/Default.aspx?ID=117). Prior to the measurements, the polyps were acclimated for at least 10 min. The respiration rates were measured in the dark during 20-30 min incubations during which the corals were fully expanded. Seawater pH$_T$ was 8.07 and 7.70 at the beginning of the incubations (Table 1) and 8.05 ± 0.02 and 6.65 ± 0.04 at the end. Changes in dissolved O$_2$ were also measured in chambers without polyps (n = 6 for each treatment). All measurements were performed sequentially over a period of one day. Data were normalized to the coral skeleton weights (i.e. dry weights), which were calculated using the buoyant weight technique and expressed in µmol O$_2$ g$^{-1}$ h$^{-1}$.

Statistical analyses

Mixed model GLMs were used to test the effects of $pCO_2$ (fixed factor: normal and high), species (fixed factor where parameter studied in more than one species) and tanks (random factor: two-three levels) on the net, gross calcification, respiration (and dissolution for D. dianthus). Q-Q plots of residuals and Levene’s homogeneity of variances tests were used to confirm conformity to analytical assumptions; net calcification data required a logarithmic transformation and
respiration data a reflected logarithmic transformation to eliminate undesirable structure in
residuals. Stepwise model simplification (criteria for removal of terms \( p > 0.25, \Delta \text{AIC} > 2 \))
subsequently removed the random tank effect in all instances resulting in ordinary GLM
analyses. Effects of \( p\text{CO}_2 \) upon each parameter in each species independently were analysed
using planned linear contrasts where the same parameter was examined in more than one species.
The same approach was used to compare calcification and respiration rates of the three species
measured during acclimation and at the end of the incubation (pooled data), as well as the net
calcification of \textit{D. dianthus} kept in the laboratory (pooled data), at CO\(_2 \) seeps and at 350 m depth.
To control for low experimental power due to small and unbalanced sample sizes in GLM
analyses, observed power was calculated for the \( p\text{CO}_2 \) effect for each species in each parameter
tested and where low power (< 0.2; \( \alpha = 0.05 \)) was indicated the robustness of the analyses was
examined by bootstrapping (\( n = 500 \)) to check for changes in inference based upon \( p \) values; all
test inferences were robust to bootstrapping. Differences in seawater carbonate chemistry
parameters (High CO\(_2 \) vs. Normal CO\(_2 \)) were tested using 1-way ANOVA. All the tests were
performed using SPSS 21 (IBM Corp., New York). Data in the manuscript are reported as
untransformed means \( \pm \) standard deviations.

Results

\textit{Seawater carbonate chemistry, calcification, dissolution and respiration rates in aquaria}

All of the corals maintained in aquaria survived and had positive calcification rates after four
months of incubation, including a one-month acclimation and a three-month experimental period
(Fig. 2). Corals cultured in elevated CO\(_2 \) conditions were exposed to seawater \( p\text{CO}_2 \) (1058 \( \pm \) 168
\( \mu \text{atm} \)), pH\(_T \) (7.70 \( \pm \) 0.06) and \( \Omega_{\text{ara}} \) (1.29 \( \pm \) 0.17) values that significantly differed (one-way
ANOVA, \( p<0.05 \)) from those at normal \( p\text{CO}_2 \) treatment (Table 1). Both net and gross coral
calcification rates, as measured using buoyant weight and \( ^{45}\text{Ca} \) techniques respectively, did not
significantly differ between normal and high \( p\text{CO}_2 \) treatments (GLM linear contrasts, \( p > 0.05 \);
Table 2). Net calcification rates of \textit{D. dianthus} and \textit{C. smithii} measured at the end of the
experiment were not significantly different from calcification rates measured during the one
month acclimation period (GLM linear contrasts, \textit{D. dianthus} \( F_{1,158} = 0.295, p = 0.588 \); \textit{C. smithii}
\( F_{1,158} = 0.041, p = 0.839 \)), and averaged 0.717 \( \pm \) 0.495 and 1.726 \( \pm \) 0.606 mg CaCO\(_3 \) g\(^{-1} \) dry
skeleton day\(^{-1}\), respectively. In contrast, while during acclimation net calcification rate of *D. cornigera* was 1.125 ± 0.320 mg CaCO\(_3\) g\(^{-1}\) dry skeleton day\(^{-1}\), it significantly decreased by a factor of four both at normal and high *p*CO\(_2\) (GLM linear contrast, F\(_{1,158}\) = 17.246, *p* < 0.001). Gross calcification rates were measured at the end of the experiment, and averaged 774 ± 352 nmol \(^{45}\)Ca g\(^{-1}\) dry skeleton day\(^{-1}\) for *D. dianthus*, 1631 ± 329 nmol \(^{45}\)Ca g\(^{-1}\) dry skeleton day\(^{-1}\) for *C. smithii*, and 1168 ± 311 nmol \(^{45}\)Ca g\(^{-1}\) day\(^{-1}\) for *D. cornigera*.

The skeleton dissolution rates of *D. dianthus* did not significantly differ between normal and high *p*CO\(_2\) treatments (GLM, *p* > 0.05; Table 2) after two months of incubation. Coral dissolution rates were 0.042 ± 0.091 mg g dry CaCO\(_3\) day\(^{-1}\) and 0.094 ± 0.053 mg g dry CaCO\(_3\) day\(^{-1}\) at the normal and high *p*CO\(_2\) respectively.

Respiration rate of *D. dianthus* measured during the acclimation month (1.12 ± 1.23 µmol O\(_2\) g\(^{-1}\) h\(^{-1}\)) was significantly lower (GLM linear contrast, F\(_{1,96}\) = 13.205, *p* < 0.001) than after 10 weeks of incubation both at normal and high *p*CO\(_2\) treatments (1.80 ± 1.19 and 2.14 ± 0.93 µmol O\(_2\) g\(^{-1}\) h\(^{-1}\) respectively; Fig. 3). In contrast, respiration rates of *D. cornigera* and *C. smithii* did not significantly change (GLM linear contrasts, *D. cornigera* F\(_{1,96}\) = 0.769, *p* = 0.384; *C. smithii* F\(_{1,96}\) = 0.002, *p* = 0.964) and averaged 0.32 ± 0.28 and 0.63 ± 0.41 µmol O\(_2\) g\(^{-1}\) h\(^{-1}\) respectively. Overall, *p*CO\(_2\) did not cause changes in the respiration rates of the three species (GLM linear contrasts, *p* > 0.05; Table 2).

*Seawater carbonate chemistry and net calcification rates of Desmophyllum dianthus transplanted in the field*

All the specimens transplanted for 258 days at 350 m depth and for 43 days in aragonite undersaturated water off Ischia survived and grew. Seawater temperatures measured in continuous by temperature loggers at the mooring site and at CO\(_2\) seeps off Ischia were 13.72 ± 0.09°C and 14.41 ± 0.60°C, respectively. Seawater pH and carbonate chemistry were significantly different between the two locations (one-way ANOVAs, *p* < 0.05) with normal levels at the mooring site (pH\(_T\) 8.02; *p*CO\(_2\) 448 µatm, Ω\(_{ara}\) 2.58) and acidified conditions at the
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three coral species calcified in aquaria at the same rates, regardless $p$CO$_2$ treatment. Both gross and net calcification rates were positive, despite the fact that corals grew in seawater with low aragonite saturation ($\Omega_{\text{ara}}$ 1.29 ± 0.17). Two mechanisms have been proposed to explain their ability to cope with low carbonate concentrations. Firstly, coral calcification occurs within the sub-callicoblastic space where the protein Ca$^{2+}$ ATPase actively pumps Ca$^{2+}$ ions from the coelenteron, in exchange for 2H$^+$ ions (e.g. Al-Horani et al., 2003; Cohen & McConnaughey, 2003). This exchange makes conditions favorable for calcification to occur by increasing Ca$^{2+}$ concentration, pH and $\Omega_{\text{ara}}$ in the calcifying space (Allemand et al., 2011 and references therein).

By measuring the boron isotope signature in the skeleton of several CWC species McCulloch et al. (2012b) showed that the corals are able to up-regulate pH at the site of calcification, with respect to the surrounding seawater, therefore facilitating calcification below the aragonite saturation horizon (see also Venn et al., 2013). Second, it has been suggested that at least tropical zooxanthellate corals can use either HCO$_3^-$ directly to support their carbonate needs for calcification or indirectly by converting HCO$_3^-$ to CO$_3^{2-}$ at the calcification site (e.g. Comeau et al., 2013). Coral sensitivity to acidification may also depend on calcification rates; slow-growing corals require less carbonate ions to grow so perhaps carbonate ion concentration is not a limiting factor (Rodolfo-Metalpa et al., 2010).

Net calcification rates of $C$. smithii and $D$. dianthus did not vary between a month at ambient $p$CO$_2$ and three-month incubation at both ambient and high $p$CO$_2$. In contrast, net calcification of $D$. cornigera decreased dramatically after acclimation, both at ambient and at high $p$CO$_2$ conditions. $Dendrophyllia$ cornigera calcified at 0.06 ± 0.04 % day$^{-1}$ during the acclimation period, which is very close to previously reported values for this species (0.04-0.05 % day$^{-1}$) (Orejas et al., 2011; Naumann et al., 2013a,b), and slower s during the three month experiment (0.01-0.02 % day$^{-1}$, pooled data). The decrease in the calcification rates was probably caused by the stress that the samples experienced during the preparation of the replicates from the mother colonies so our data on calcification rates for this species might be a husbandry artifact and should be taken with caution. Although speculative, it is possible that $D$. cornigera samples depleted lipid reserves during the acclimation period, being unable to sustain normal calcification rates during the incubation regardless the $p$CO$_2$ treatment.
This is the first study reporting the growth rate of *C. smithii* (0.070-0.073 % day\(^{-1}\)) whereas the net calcification rates of *D. dianthus* has been already measured in six other studies. Overall, our results for both aquaria (0.023-0.024 % day\(^{-1}\)) and *in situ* experiments (0.019-0.036 % day\(^{-1}\)) agree with previously reported values for *D. dianthus* (0.003-0.25 % day\(^{-1}\)) and for CWCs in general (0.003-0.3 % day\(^{-1}\)). The *D. dianthus* specimens studied in the present study calcified six times slower than those cultured by Naumann *et al.* (2011; 2013b) (0.1-0.3 % day\(^{-1}\)) and by Jentzen *et al.* (2013a) (0.09 % day\(^{-1}\)) but much faster than those cultured by Carreiro-Silva *et al.* (2014) (0.004 % day\(^{-1}\)). The different feeding regimes used during these experiments likely influenced the calcification rates since scleractinian corals allocate a high proportion of the energy derived from food to calcification in tropical (Houlbréque & Ferrier-Pagés, 2009) and cold-water environments (Naumann *et al.*, 2011).

**Calcification rates in situ versus aquaria; the role of food**

To build their skeletons, corals pump protons out of the extracellular calcifying medium to increase internal pH and favour calcification. This is highly energy consuming (Allemand *et al.*, 2011) with an estimated metabolic extra cost of ca. 10 % per 0.1 pH unit decrease in seawater pH (McCulloch *et al.*, 2012b). To meet this energy demand, corals can increase feeding rates and/or draw upon energy reserves. Four previous studies have measured CWC net calcification, respiration rates, and lipid contents at high \(pCO_2\) (Hennige *et al.*, 2013; Maier *et al.*, 2013b; Carreiro-Silva *et al.*, 2014; Movilla *et al.*, 2014a). Hennige *et al.* (2013) found that respiration rates decreased while calcification rates remained the same in specimens of *L. pertusa* exposed to elevated \(pCO_2\); the other studies did not detect any effect of high \(pCO_2\) on coral respiration or calcification (Maier *et al.*, 2013b; Carreiro-Silva *et al.*, 2014; Movilla *et al.*, 2014b) and lipid content (Movilla *et al.*, 2014a). Even if a decrease in the lipid reserves would have been observed, such energy reallocation would only be successful until these lipid reserves run out, likely on a shorter time period than the ones tested during our experiments in aquaria and in the field. Corals could have also maintained elevated calcification rates by reallocating energy dedicated to other metabolic processes such as gamete production, gamete maturation, and spawning, for example. Studies on tropical corals showed that high \(pCO_2\) levels inhibit sperm motility, compromise their ability to move towards unfertilized eggs, therefore affecting fertilization efficiency, decrease larval metabolism and coral post-metamorphic growth (reviewed
by Albright, 2011). Unfortunately, little is known on the reproductive cycle of CWC (references in Movilla et al., 2014) and no study has examined the impact of high $p$CO$_2$ levels on CWC reproduction aspects.

In our aquarium experiments we also found that calcification and respiration rates were unaffected by acidification, but we suspect that the corals were able to maintain these metabolic rates thanks to the amount of food they received. Currently there is limited information on the natural feeding behavior of deep-sea corals; although we know they form reefs in food rich areas (Findlay et al., 2013), information on the effects of nutrition on cold-water coral metabolism are scarce. It has been experimentally shown that the CWC L. pertusa has an opportunistic feeding strategy and can change diet according to the external food source (Mueller et al., 2014). Up to now, all studies testing the resilience of CWC to ocean acidification used arbitrary feeding rates from no artificial feeding (Maier et al., 2012) to five times per week with Artemia nauplii and/or frozen Cyclops, Mysidacea, minced mussels, fish flakes (Tsounis et al., 2010; Orejas et al., 2011; Naumann et al., 2011; 2013a,b; Carreiro-Silva et al., 2014; Movilla et al., 2014a). Sometimes this diet was used for years before the experiment was carried out (e.g. Movilla et al., 2014a). Corals acclimated to such optimal feeding rates might have their metabolism and behavior completely changed, as well as their energy distribution. For instance, in the presence of artificial food in aquaria CWC tend to be permanently expanded, which is not the case in the field. Corals shown to calcify at reduced seawater $\Omega_{\text{ara}}$ (Maier et al., 2013a; Movilla et al., 2014a; Carreiro-Silva et al., 2014) might only have been able to meet the extra energy demands as they were acclimated to abundant food conditions. This seems to be the case for tropical corals (Edmunds, 2011; Houlbrèque et al., 2015) and for mussels. Thomsen et al. (2013) elegantly demonstrated that only well fed mussels were able to calcify properly at high $p$CO$_2$ levels.

To test whether the ocean acidification responses of artificially fed corals differed from naturally fed corals we transplanted them to field conditions with normal and elevated $p$CO$_2$ where they were seen feeding naturally. In both cases the corals calcified at rates that were 44% lower than corals kept at similar temperature and pH in aquaria but artificially fed. Corals that we held in aquaria calcified faster than those in the field, likely due to greater food availability, but they were able to calcify at predicted levels of increased $p$CO$_2$ both in the field and in the laboratory. Although we recognise that our transplantation experiments do not fully mimic natural
environmental conditions, and that several factors (e.g. food quality, biological competition, hydrodynamics) affect corals calcification rates, this study is a first attempt to measure CWC calcification rates in the field. This helps validate the various aquarium-based observations that cold-water coral calcification is expected to be resilient to ocean acidification.

Dissolution
Coral skeletons are separated from the surrounding environment by soft tissue, allowing corals to create a semi-isolated space where calcification occurs (Allemand et al., 2011), and preventing dissolution in undersaturated seawater (Rodolfo-Metalpa et al., 2011). However, many scleractinians have large portions of their skeleton exposed to surrounding seawater, making tropical coral reefs (Fabricius et al., 2011) and cold-water coral reefs (Jackson et al., 2014) prone to chemical dissolution and bioerosion as oceans acidify. During our 2-month incubation in aquaria, dissolution rates measured using dead samples of D. dianthus did not significantly differ between normal and high pCO₂ treatments. To the best of our knowledge, this is the first study measuring CWC dissolution. Rodolfo-Metalpa et al. (2011) showed that the dissolution of two temperate coral species kept in aquaria under controlled pCO₂ conditions for 21 days, started only at pH < 7.4 (Ω₉₉ = 0.99 ± 0.12). In contrast, van Woesik et al. (2013) measured significant dissolution at pH 7.8 of several perforate and imperforate tropical coral skeletons but they used acetic acid to reach the desired pH which does not simulate ocean acidification conditions. In our laboratory experiments, skeletons were incubated for only two months, which was likely too short to measure significant differences in the dissolution rates between samples maintained under normal and high pCO₂ conditions and never fell below Ω₉₉ 1. However, we found no sign of dissolution on exposed skeletons of D. dianthus transplanted for 43 days at CO₂ seeps off Ischia (Ω₉₉ = 0.76 ± 0.62) and this species grows well despite exposure of the skeleton to aragonite undersaturation in Chile (Försterra & Häusserman, 2009). The time is ripe for experiments on the dissolution and bioerosion of cold-water corals to assess whether deep-sea reefs are as vulnerable to acidification as feared.

In summary, our observations in aquaria and in field transplants reveal that calcification is not the Achille’s heel of cold-water corals facing ocean acidification. This is not to say that falling aragonite levels are of no concern, since acidification may cause disruptions to coral life cycles or

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widespread degradation of deep-water reef structures. Our study does not consider other likely detrimental factors such as global warming and hypoxia which might interact with acidification causing progressive ecosystem shifts (Pörtner et al., 2005; Lunden et al., 2014). Despite their global distribution, there are still huge gaps in our scientific understanding of the cold-water corals and their resilience to rapidly changing ocean conditions.

Acknowledgements

We thank the crews and the technicians aboard RVs Urania, Maria Grazia and Astrea. Special thanks to Saverio Devoti for assistance during coral collection. François Oberhaënsli and Jean-François Commanducci (IAEA) greatly helped during the experiment in aquaria and coral transplants. The International Atomic Energy Agency is grateful to the Government of the Principality of Monaco for the support provided to its Environment Laboratories. This work contributes to the projects MISTRALS/ENVI-MED BITES and PaleoMex COFIMED. This is ISMAR-CNR Bologna scientific contribution n. 1854. The work contributes to the EU project: 'Mediterranean Sea Acidification under a changing climate' (MedSeA; grant agreement 265103).

Figure legends

Fig. 1. Transplantations of Desmophyllum dianthus on a rocky coast at CO₂ seeps off Ischia (a) and suspended at 350 m depth on a mooring off Corsica (b).

Fig. 2. (a) Net and (b) gross calcification rates of the three Mediterranean cold-water corals held in aquaria during a one month acclimation period at normal pCO₂ levels followed by three months at normal and increased pCO₂ levels. Net calcification was also measured for D. dianthus transplanted to 350 m depth (Mooring) and at CO₂ seeps. Data are mean ± SD. Replicates per treatment are: (a) Acclimation, n = 14, 14 and 46; Normal and High pCO₂, 8, 8, 40 for C. smithii, D. cornigera and D. dianthus respectively; Mooring, 22 and CO₂ seeps 11 D. dianthus. (b) Normal and High pCO₂, n = 8 each species.

Fig. 3. Respiration rates of three cold-water corals maintained in aquaria for one month at ambient seawater pCO₂ (Acclimation) followed by three months at normal and increased pCO₂
(Normal and High $p\text{CO}_2$). Data are mean ± SD. Replicates per treatment are: Acclimation, n = 10, 10 and 16; Normal and High $p\text{CO}_2$, 8, 8, 20 for *C. smithii*, *D. cornigera* and *D. dianthus* respectively.

References


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Table 1. Mean (±SD) seawater temperatures, pH\textsubscript{T} and total alkalinity (\(A\textsubscript{T}\)) measured during the collection of corals (Cruises ARCO and MEDCOR), during experiment in aquaria (acclimation, normal and high \(p\textsubscript{CO}_2\); pooled data between replicated tanks), and during coral transplantation and recover on the mooring and at \(CO\textsubscript{2}\) seeps. Data in square brackets represent the number of measurements. \(A\textsubscript{T}\) replicates were \(n=2\), \(18\), \(36\), \(36\), \(5\), and \(4\) for ARCO, acclimation, normal, high \(p\textsubscript{CO}_2\), \(in\ situ\ 350\ m\), and \(in\ situ\ \text{CO}_2\) seeps respectively. Data for MEDCOR St 30 are from Maier \textit{et al.} (2013a). Mean pH was calculated after conversion of data to hydrogen ion concentrations.

<table>
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<th>Depth (m)</th>
<th>pH\textsubscript{T}</th>
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<th>(p\textsubscript{CO}_2) µatm</th>
<th>(CO_2) µmol kg(^{-1})</th>
<th>(CO_3^2-) µmol kg(^{-1})</th>
<th>HCO\textsubscript{3}- µmol kg(^{-1})</th>
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Table 2.

Summary of GLM (Dissolution in *Desmophyllum dianthus*) or GLM linear contrasts testing the effect of two levels of pCO$_2$ (normal and high) on corals’ physiological parameters in aquaria. NC: net calcification (Ln(x) transformed); GC: gross calcification; Respiration (reflected Ln(x) transformed).

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<th>F-ratio</th>
<th>P-value</th>
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</table>
Acclimation (pH 8.05)
Normal pCO$_2$ (pH 8.07)
High pCO$_2$ (pH 7.70)
Mooring (pH 8.02)
CO$_2$ seeps (pH 7.35)

Net calcification (mg CaCO$_3$ g$^{-1}$ dry skeleton day$^{-1}$)

C. smithii
D. cornigera
D. dyanthus

Gross calcification (nmol $^{45}$Ca g$^{-1}$ dry skeleton day$^{-1}$)

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Respiration (µmol O$_2$ g$^{-1}$ h$^{-1}$)

- High $p$CO$_2$ ($pH_T$ 7.70)
- Normal $p$CO$_2$ ($pH_T$ 8.07)
- Acclimation ($pH_T$ 8.05)

- $C. smithii$
- $D. cornigera$
- $D. dianthus$