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REPORT

Effects of increased pCO_2 on zinc uptake and calcification in the tropical coral *Stylophora pistillata*

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Abstract Zinc (Zn) is an essential element for corals. We investigated the effects of ocean acidification on zinc incorporation, photosynthesis, and gross calcification in the scleractinian coral *Stylophora pistillata*. Colonies were maintained at normal pH_T (8.1) and at two low-pH conditions (7.8 and 7.5) for 5 weeks. Corals were exposed to ^{65}Zn dissolved in seawater to assess uptake rates. After 5 weeks, corals raised at pH_T (8.1) exhibited higher ^{65}Zn activity in the coral tissue and skeleton, compared with corals raised at a lower pH. Photosynthesis, photosynthetic efficiency, and gross calcification, measured by ^{45}Ca incorporation, were however unchanged even at the lowest pH.

Keywords Scleractinian corals · Ocean acidification · Radiotracers · Zinc · Calcification

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Introduction

Zinc (Zn) is essential for the health and growth of corals because it is a cofactor of more than 300 enzymes, such as carboxypeptidase, alkaline phosphatase, and carbonic anhydrase, which are involved in all aspects of coral metabolism (Morel et al. 1994). While concentrations of dissolved zinc are often very low in surface waters (ca. 0.1 nM, Martin et al. 1989; Bruland 1989; Lohan et al. 2002), they can be up to 50 times higher in some reefs, such as in the Gulf of Aqaba (5.5 nmol l^{-1}) (Ruiz-Pino et al. 1991). Amongst the metalloenzymes associated with zinc, carbonic anhydrase (CA) contains a complete catalytic site composed of three histidine residues that bind the zinc cofactor. A study made on diatom cultures labeled with ^{65}Zn demonstrates that a large fraction of the cellular Zn is associated with CA (Morel et al. 1994). CA is essential to corals for the acquisition of inorganic carbon (CO_2), which is used by the zooxanthellae for their photosynthesis and by the host for its calcification (Furla et al. 1998). Four classes of CAs have already been found in the different tissue layers of corals, both in the ectodermal and endodermal cells and also in the calicoblastic ectoderm (Furla et al. 2000; deBoer et al. 2006; Moya et al. 2008; Bertucci et al. 2009a, b, 2010a, b). Several experiments have shown that CA inhibitors decrease calcification rates and have suggested that CA is involved in the inorganic carbon supply for calcification and/or the regulation of pH at the calcification site (Tambutté et al. 1996; Furla et al. 2000; Al-Horani et al. 2003; Marshall and Clode 2003). One CA form is also present in the symbiont-containing cells and could play a role in the formation of CO_2 to supply photosynthesis. In this photosynthetic process, zinc is also essential to the superoxide dismutase (SOD), involved in the detoxification of active oxygen species

(Raven et al. 1999). It is therefore possible that some variations in the inputs of this transient metal in corals might induce significant changes in their calcification and photosynthetic processes.

At present, uptake of anthropogenic CO_2 by the oceans alters seawater chemistry; it reduces seawater pH and aragonite saturation state (Ω_{arag}) (Caldeira and Wickett 2003; Orr et al. 2005). The consensus estimate, based on the relationship between net calcification and the aragonite saturation state, is that by the end of this century the rate of calcification in scleractinian corals will decrease by 17–37% as a result of reduced seawater [CO_3^{2-}] due to a doubling of preindustrial levels of atmospheric partial pressure of CO_2 (Gattuso et al. 1999; Kleypas et al. 1999; Langdon and Atkinson 2005). Coral response to acidification is, however, not unequivocal and still puzzling. It has been shown that some tropical species are able to calcify under high pCO_2 levels (Atkinson et al. 1995; Krief et al. 2010), while calcification of the few temperate species studied, up to now, seems to be either unaffected (Rodolfo-Metalpa et al. 2010b) or decreased only when pCO_2 was higher than 900 ppm (Ries et al. 2009, 2010). Rates of photosynthesis were either not affected (e.g., Langdon et al. 2003; Reynaud et al. 2003; Schneider and Erez 2006; Marubini et al. 2008), were slightly increased (e.g., Langdon and Atkinson 2005), or were depressed (Anthony et al. 2008) within the level of pCO_2 expected in 2100. Therefore, if we truly hope to understand and predict the effect of a decrease in seawater pH, we should first understand the biological control of the main physiological processes and then couple that with the predicted change in the seawater carbonate chemistry.

In this particular context, the aim of this study was to investigate whether ocean acidification could lead to shifts in the uptake of zinc in the zooxanthellate coral *Stylophora pistillata* and thus could affect its main physiological parameters, such as its rates of calcification and photosynthesis as well as the photosynthetic efficiency of its zooxanthellae.

Materials and methods

Aquaria setup

Ten parent colonies of the scleractinian coral *Stylophora pistillata* (Esper, 1797) were collected in the Gulf of Aqaba (Red Sea). After transportation, they were acclimated for 2 months in aquaria to recover. Eighty-four terminal portions of branches (2 cm long fragments) were cut and hung on nylon wires. After 3 weeks, coral fragments were entirely covered with new tissue and were ready for the experiments.

Initial gross calcification (see below) was measured on six colonies, while 30 others were randomly assigned to each of the six tanks (20 l), continuously supplied with clean carbon-filtered seawater, pumped in front of the International Atomic Energy Agency (IAEA) laboratories. Seawater in the aquaria was renewed at a flow rate of 1 l h^{-1}. Ammonium and phosphorus were measured weekly in each tank with an Alliance II autoanalyser following the method of Tréguer and Le Corre (1975). The concentrations remained very low in all tanks (<0.04 µM ammonium, 0–0.09 µM phosphorus and <0.5 µM for nitrate and nitrite). Temperature (26 ± 0.2°C) was kept constant in each tank using heaters connected to electronic controllers (±0.2°C accuracy), and seawater was mixed using a submersible pump (Aquarium system, mini-jet MN 606, Mentor, OH, USA). Corals received a constant irradiance of 170 ± 10 µmol photons m^{-2} s^{-1} (photoperiod was 12 h:12 h light/dark) using metal halide lamps (Philips, HPIT, 400 W, Eindhoven, the Netherlands) and were fed once a week for 1 h with some nauplii of *Artemia salina* (100 ± 34 nauplii l^{-1}). Two tanks were maintained at ambient pH_T (total scale; 8.09 ± 0.04, 378 µatm pCO_2), two at pH_T level projected for the end of the century (7.78 ± 0.06; 903 µatm pCO_2) (Caldeira and Wickett 2003), while the two others were maintained at very low pH_T (7.46 ± 0.04; 2,039 µatm pCO_2). pH_T was controlled using a pH–stat system (IKS, Karlsbad, accuracy ± 0.05 pH unit) by bubbling independently pure CO_2 in each tank that was continuously aerated with CO_2-free air. Colonies were acclimated under these different pCO_2 conditions for 6 weeks. All the measurements were performed after 5 weeks, except the measurements of calcification rates made both after 3 and 5 weeks of incubation.

Seawater carbonate chemistry

pH values of the pH–stat system were adjusted every day from the measurements of pH_T using a pH meter (Metrohm, 826pH mobile) with a glass electrode (Metrohm, electrode plus) calibrated on the total scale using Tris/HCl and 2-aminopyridine/HCl buffer solutions with a salinity of 38 (Dickson et al. 2007). Means pH_T were calculated from hydrogen ion concentrations of each measurement and then re-converted back to pH (Dickson et al. 2007). Water samples were collected in glass bottles and then passed through 0.45 µm pore size filters (GF/F Whatman) and poisoned with 0.05 ml of 50% $HgCl_2$ (Merck, Analar) to avoid biological alteration and stored in the dark at 4°C. Three replicated 20 ml sub-samples were analyzed at 25°C using a titration system composed of a pH meter with a Methrom pH electrode and a 1 ml automatic burette (METHROM). The pH was measured at 0.02 ml increments of 0.1 N HCl. A_T was calculated from the Gran

function applied to pH variations from 4.2 to 3.0 as mEq l^{-1} from the slope of the curve HCl volume versus pH. Titrations of total alkalinity standards provided by A. G. Dickson (batch 99 and 102) were within 0.7 μmol kg^{-1} of the nominal value. Mean A_T of seawater was 2.508 ± 0.016 mmol kg^{-1} ($n = 39$). pCO_2 was calculated from pH_T, mean A_T, temperature, and salinity using the free access CO_2 Systat package.

Photosynthetic and respiration measurements

Photosynthesis and respiration rates were measured after 5 weeks on 3 colonies taken from each tank ($n = 6$ per treatment). Measurements were always performed at the same time of the day (i.e., early morning) to avoid any error due to diurnal variations (Edmunds and Spencer-Davies 1988). Colonies were placed in respirometric Plexiglass chambers, each containing a Strathkelvin 928R electrode and immersed in a thermostat water bath (26°C). Chambers were filled with seawater at the corresponding pH, and the incubation medium was continuously stirred with a stirring bar. Colonies were incubated for 15 min under their corresponding culture irradiance (170 μmol m^{-2} s^{-1}) or under complete darkness for respiration measurements. The oxygen sensor was calibrated before each experiment against air-saturated seawater and a nitrogen gas (zero oxygen). Oxygen was recorded every 10 s using an acquisition station (Stratkelvin). Rates of photosynthesis and respiration were estimated by regressing oxygen data against time and normalized by chlorophyll a (chl *a*). Net photosynthetic rates were calculated according to the following equation:

$$P_{net} = (V \times \text{slope})/[\text{Chloro}]$$

where P_{net} is the rate of net rate of photosynthesis (μmol O_2 μg chl a^{-1} h^{-1}), V is the volume of the chamber (l), slope is the dissolved oxygen variation (μmol O_2 l^{-1} h^{-1}), and [Chloro] is the amount of chlorophyll *a* (μg).

Photosynthetic efficiency measurements

Photosynthetic efficiency (F_v/F_m) and the electron transport rate (ETR) of the Photosystem II (PSII) of zooxanthellae *in hospite* were measured using a DIVING PAM (Walz, Germany). The initial fluorescence (F_0) was measured by applying a weak pulsed red light (3 μs, LED 650 nm) on dark-adapted colonies. A saturating pulse (800 ms) of bright actinic light (8,000 μmol photons m^{-2} s^{-1}) was then applied to give the maximum fluorescence value (F_m). Variable fluorescence (F_v) was calculated as $F_m - F_o$. rETR was also assessed in the different conditions using the rapid light curve (RLC) function of the PAM fluorometer and by exposing the corals for 10 s to eight increasing light intensities up to 3,000 μmol photons m^{-2} s^{-1}.

Calcification rates measurement

Calcification was assessed at the beginning of the experiment and after 3 and 6 weeks incubation under the different pCO_2 conditions. Three corals from each tank ($n = 6$) were randomly chosen and placed into 6 other 7-l tanks, dedicated to ^{45}Ca incubations. Their gross calcification rates were measured using ^{45}Ca, according to a method adapted from Tambutté et al. (1995). In each tank, seawater was spiked with microliter quantities of the radiotracer ^{45}Ca ($^{45}CaCl_2$, PerkinElmer Life and Analytical Science Products, USA) to reach an activity of 50 KBq l^{-1}. The medium was continuously mixed using a submersible pump (Aquarium system, mini-jet MN 606, Mentor, OH, USA). For each sampling time, colonies have been incubated with ^{45}Ca for 7 h. Light and temperature conditions were similar to those described above. One-milliliter aliquots of seawater were removed at the beginning of the incubation for the determination of the specific radioactivity. At the end of the labeling period, labeled microcolonies were incubated in a beaker containing 30 ml unlabeled seawater for 1 h to achieve isotopic dilution of ^{45}Ca contained in the coelenteron. Tissue was then dissolved completely in 1 N NaOH at 90°C for 20 min, and the skeleton was rinsed twice in 1 ml NaOH solution and once in seawater. Skeletons were then dried, weighed, and dissolved in 1.5 ml of 12 N HCl. Liquid scintillation medium (10-ml; Ultimagold MV Packard) was added to the radioactive samples (seawater and dissolved skeletons), and β-emissions were measured using a liquid scintillation counter (2100 TR Packard; Tricarb). We considered a seawater calcium concentration of 10 mM (Tambutté et al. 1995). Results are expressed in nmol Ca^{2+} g^{-1} dry skeletal mass (Houlbrèque et al. 2003).

Zinc bioaccumulation experiments

After 5 weeks of incubation, four corals from each tank ($n = 8$ per treatment) were randomly chosen and placed into three tanks dedicated to trace metal bioaccumulation experiments and setup with the same temperature, light, and pCO_2 treatments as described before. Seawater in each tank was spiked with microliter quantities of the radiotracers ^{65}Zn to reach an activity of 5 kBq l^{-1}. In terms of stable metal concentration, this addition corresponded to an addition of 320 pg l^{-1}. Radiotracer was purchased from Isotope Product Laboratory, USA: ^{65}Zn as $^{65}ZnCl_2$ in 0.5 M HCl, $T_{1/2} = 243.9$ days. Colonies were exposed 7 days to ^{65}Zn. The experimental medium was changed every day to maintain the radiotracer activities at a constant

level and to avoid the depletion of the medium in the different elements (Ferrier-Pagès et al. 2005). Uptake of ^{65}Zn was measured in the whole colony after 6 h, 1, 2, 3, 4, and 6 days. Each colony was then incubated for 30 min in 50 ml of unlabeled seawater to rinse the coelenteric cavity (Tambutté et al. 1995). This cavity contains water with radiotracers that are not incorporated into the animal (Ferrier-Pagès et al. 2005). Then they were carefully blotted dry on absorbent paper to eliminate any adhering radioactive medium and transferred to counting vials containing 50 ml of seawater. After measurement, they were placed back in their aquarium (since the method is a non-destructive measurement). After the last sampling time (day 6), the corals were sacrificed and their tissue was dissolved for 20 min in 1 ml of 1 N NaOH at 90°C. The remaining skeletons were thoroughly washed, dried, and weighed, and the radiotracer activities were determined in the tissue and skeleton fractions.

The gamma emission of ^{65}Zn (1,115.55 keV) in the whole colony, skeleton, tissues, and seawater was determined using a high-resolution γ-spectrometry system consisting of four coaxial Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The detectors were calibrated with an appropriate standard for each counting geometry used, and measurements were corrected for background and physical decay of the radiotracers. Counting times were adapted to obtain relative propagated errors less than 5%, 10 min for the whole colony and 20 min for the tissues and skeletons. ^{65}Zn uptake of the Zn was expressed as changes in concentration factors (CF). CF is the ratio between radiotracer activity in the whole colony, the tissue, or skeleton compartment—Bq g^{-1}—and the mean ^{65}Zn activity measured in seawater—Bq g^{-1} from the beginning of the experiment to the sampling time (Warnau et al. 1996).

^{65}Zn uptake kinetics was best described by using either a linear equation (L) or a saturation exponential equation (E):

$$\text{Linear: } CF_t = k_u t$$

$$\text{Exponential: } CF_t = CF_{ss}\left(1 - e^{-k_e t}\right)$$

where CF_t and CF_{ss} ($CF_{ss} = k_u/k_e$) are the concentration factors at time t (day) and at steady state, respectively, k_e and k_u are the biological depuration and uptake rate constants (day^{-1}), respectively (Whicker and Schultz 1982).

For previous experiments performed in the IAEA laboratories, Zn speciation has been already calculated using the HARPHRQ geochemical speciation code (see Jeffree et al. 2006; Lacoue-Labarthe et al. 2009) using the same physiochemical data (salinity 38 and dissolved organic carbon <1 mg l^{-1}). The results of the speciation modeling indicated that a decrease of pH from 8.10 to 7.60 had only minor influence on the speciation of Zn. In terms of free metal ion concentration, generally considered to represent the bioavailable form of Zn, Zn^{2+}concentration increased from 46 to 56% as the pH decreased from 8.10 to 7.60.

Statistical analysis

All data were tested for the assumptions of normality and homoscedasticity by the Cochran's test and were transformed when required. After verification of the absence of significant differences between tanks in each pH treatment (ANOVA, $P > 0.05$), data were pooled. One-way ANOVAs were used to test the effect of pH (7.5, 7.8, and 8.1) on coral's photosynthesis (P_{net}), respiration rates, photosynthetic efficiency (F_v/F_m), maximum electron transport rate (ETR_{max}), and ^{65}Zn uptake (whole colony, tissue, and skeleton fractions). Two-way ANOVA with fixed effect was performed on gross calcification between pH (7.5, 7.8, and 8.1) and time (0, 3 and 5 weeks). STATISTICA® software (Statsoft) was used. When the ANOVA determined a significant difference, a Tukey's honest significant difference (HSD) test was used to attribute differences between specific factors. All data are expressed as the mean ± standard deviation (SD).

Results

Kinetics of zinc uptake

For pH_T 7.8 and 8.1, the time-course of zinc uptake was linear over the 6-day exposure period (Fig. 1; Table 1). The regression lines of experimental data are shown in Fig. 1 and are highly significant (R^2 between 0.982 and 0.994). For both highest pH conditions, colonies of *S. pistillata* kept accumulating ^{65}Zn at a constant uptake rate of $k_u = 86.1 \pm 10.8$ and 106.8 ± 8.2 day^{-1} for pH_T 7.8 and 8.1, respectively (Table 1).

Conversely, the uptake of ^{65}Zn in colonies maintained at the lowest pH_T (7.5) tended to quickly reach a steady state. Zinc accumulation kinetic parameters were determined with the fitting of a simple exponential kinetic model ($R^2 = 0.959$, $P < 0.001$). The calculated zinc concentration factor at steady state (CF_{ss}) was 260.8 ± 70.1 (mean ± asymptotic standard error, $P < 0.001$), with a calculated uptake constant (k_u) of 107.4 ± 10.4 and an elimination rate (k_e) of 0.412 ± 0.244 day^{-1} (Fig. 1; Table 1). At the end of the uptake period, the lowest CF values in the whole colonies were recorded for corals maintained under pH_T 7.5 ($CF = 234.7 \pm 20.4$), corals maintained at pH_T 7.8 and 7.5 showing CFs in the whole

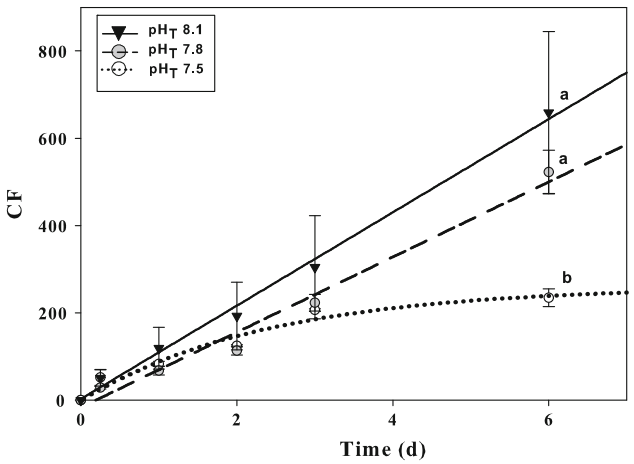

Fig. 1 ^{65}Zn uptake kinetics (CF; mean ± SD; $n = 4$) in total colonies after 5-week exposure to three pH treatments (pH 7.5, pH 7.8, and pH 8.1). Different letters (a, b, c) denote statistically significant differences (Tukey's test, $P < 0.05$) between the different pH treatments

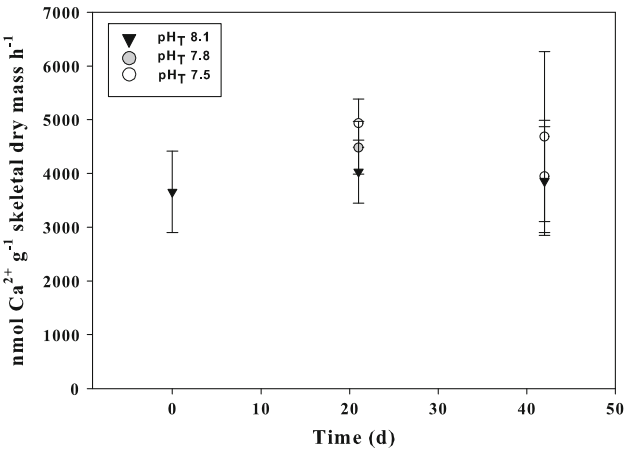

Fig. 2 Rates of calcification per skeletal dry mass (nmol Ca^{2+} g^{-1} skeletal dry mass) for corals maintained under three pH treatments for 21, 28, and 42 days. Data are presented as mean ($n = 4$) and standard deviation. a, b, c: same as Fig. 1

colonies equal to 522.7 ± 24.9 and 658.8 ± 92.8, respectively.

For all pH_T conditions, most of the ^{65}Zn activity (97.5 ± 3.8% of the total activity measured in each colony) was found in the tissue compartment. Concentration factors in tissues were significantly higher in corals incubated at normal pH (CF = 668.4 ± 144.6) than at the lower pH (CF = 453.1 ± 75.6 and CF = 404.7 ± 63.6 for pH_T 7.8 and pH_T 7.5, respectively) (ANOVA one-way, $F_{1,21} = 37.06$; Tukey, 8.1 > 7.8 > 7.5, $P < 0.001$). The same tendency has been observed for zinc CF in the skeleton compartment with significantly higher activities measured in corals maintained under pH_T 8.1 and pH_T 7.8 (CF = 15.6 ± 2.2 and 17.1 ± 1.5, respectively) compared to pH_T 7.5 (CF = 9.3 ± 2.4) (ANOVA one-way, $F_{1,21} = 9.06$, $P < 0.01$; Tukey, 8.1 = 7.8 > 7.5).

Calcification rates

After 3 and 6 weeks, there was no significant difference in the gross calcification rates between colonies incubated under the three pCO_2 conditions. The two-way ANOVA did not show any significant effect of pH and incubation time ($P > 0.05$, $F_{2,45} = 0.55$ and 1.82, respectively) on gross calcification rates, and there was no significant interaction effect ($P > 0.05$, $F_{2,45} = 0.51$) (Fig. 2). Following 6-week incubation under reduced pH_T conditions, all coral fragments survived and showed positive gross calcification rates, varying from 3,857 ± 1,011 to 4,687 ± 1,045 nmol Ca^{2+} g^{-1} skeletal dry mass h^{-1}, for colonies incubated at pH_T 8.1 and 7.8, respectively (Fig. 2).

Photosynthetic rates and photosynthetic efficiency

Rates of photosynthesis in colonies maintained for 5 weeks under normal pH conditions (0.067 ± 0.021 µmol O_2 µg chl *a* h^{-1}) were equivalent to the rates of colonies maintained in lower pHs for the same period (0.056 ± 0.018 and 0.085 ± 0.018 µmol O_2 µg chl *a* h^{-1} for pH_T 7.8 and pH_T 7.5, respectively) (ANOVA one-way, $F_{1,16} = 1.472$, $P > 0.05$).

High-pCO_2 conditions did not significantly change the $rETR_{max}$ (ANOVA one-way, $F_{1,15} = 2.05$, $P > 0.05$), which ranged from 68.7 ± 40.2 to 100.1 ± 29.7. Photosynthetic efficiency (F_v/F_m) was also not significantly different between pH treatments ($F_v/F_m = 0.64 \pm 0.02$,

Table 1 Parameters of ^{65}Zn uptake kinetics in coral colonies exposed to ^{65}Zn dissolved in seawater (CF; mean ± SD; $n = 4$) after 5-week exposures to three pH levels pH_T 7.5, pH_T 7.8, and pH_T 8.1

pH	Model	k_u ± SD (day^{-1})	CF_{ss} ± SD	k_e ± SD (day^{-1})	R^2
7.5	E	107.4 ± 10.4	260.8 ± 70.1	0.412 ± 0.244	0.959
7.8	L	86.1 ± 10.8	–	–	0.982
8.1	L	106.8 ± 8.2	–	–	0.994

L and E: linear and exponential models, respectively, CF_{ss}: concentration factor at steady state, k_u and k_e: uptake and elimination rate, respectively, SD: standard deviation, R^2: determination coefficient

0.64 ± 0.01, and 0.65 ± 0.01 at pH_T 7.5, 7.8, and 8.1, respectively).

Discussion

This study brings some insights into the ocean acidification consequences on metal uptake in corals and shows that a pCO_2 increase leads to a significant decrease in the uptake of zinc. Although zinc is an essential "substrate" for many physiological mechanisms on corals, the decrease in this essential element at low pH was not reflected in changes in photosynthetic and gross calcification rates.

Effects of ocean acidification on zinc uptake

While after 6 days at pH_T 7.8 and 8.1, colonies of *S. pistillata* showed a linear kinetic uptake and kept accumulating ^{65}Zn at a constant uptake rate, colonies at the lowest pH_T (7.5) quickly reached a steady state and even started to slightly lose part of the accumulated zinc. As a consequence, these colonies presented the lowest CF in the whole colony, or in the host tissue and skeleton compared with other conditions. Several reasons can be considered to explain the low incorporation of zinc at low pH:

(1) First, zinc appears to be an essential element required for the synthesis of numerous cell constituents, such as proteins and enzymes (e.g., Vallee and Auld 1990). Higher rates of zinc incorporation at normal pH would mean that the metabolic performances of the coral are at their maximum at pH_T 8.1 with a maximal rate of protein synthesis and zinc requirements. This metal is more particularly a cofactor of carbonic anhydrase (CA), widely involved in the coral calcification process. Through the activity of this enzyme, corals continuously acquire inorganic carbon (CO_2) for photosynthesis and calcification. The fact that in our study, zinc uptake in corals decreased in acidified conditions is quite consistent with the theory of Lane and Morel (2000), showing that the amount of CA activity and the concentration of CA increased markedly in normal conditions. For example, the activity of diatom CA was high in normal conditions (100 and 300 µatm pCO_2) and became undetectable in acidified conditions (1,000 µatm pCO_2) (Morel et al. 1994). In the present experiment, we did not have the opportunity to estimate CA activity in colonies of *S. pistillata,* but our results suggest that less zinc was incorporated in acidified conditions than under more alkaline conditions, since CA would have needed less Zn inputs. (2) Secondly, ocean acidification leads to an increased concentration of H^+. As a result, surface site becomes less available to adsorb metals in the presence of increasing H^+. It was proposed by Santore et al. (2002) that in rainbow trout, in slightly acidic conditions, zinc is displaced by protons from the biotic ligand, which decreases the uptake rate. Therefore, higher zinc uptake can be expected at normal pH conditions due to a reduction in H^+ concentrations. A similar scenario could be considered for the transport of zinc in corals at low pH, for which the transport is active and made through a carrier-mediated component, adapted to the low levels of zinc in seawater (Bruland 1980; Martin et al. 1989; Ferrier-Pagès et al. 2005). (3) Finally, ocean acidification changes the speciation of numerous metal ions in seawater (Byrne 2002; Millero et al. 2009). In terms of free metal ion concentration, which is generally considered to represent the bioavailable form of zinc, Zn^{2+} concentration increases of 10% as the pH decreases from 8.1 to 7.6 (Jeffree et al. 2006; Lacoue-Labarthe et al. 2009), but both forms $ZnOH^-$ and $ZnCO_3$ decrease by a few percent between pH 8.1 and 7.5 (from 5.6 to 1.7% and from 7.2 to 2.3%, respectively) (Millero et al. 2009). Therefore, if corals used predominantly one of these two forms, this might explain why less zinc is incorporated under acidified conditions.

Calcification rates

No change in the gross calcification rates of *S. pistillata* was detected between the different pH conditions. Our results suggest that low zinc inputs (measured at pH_T 7.5) do not seem to influence coral calcification. This observation is contrary to work carried out on the microalga *Emiliana huxleyi*, revealing that $CaCO_3$ production rates per cell remained unaffected by low zinc concentrations (Schulz et al. 2004; Zondervan 2007). In our experiment, this lack of effect may be explained by the fact that (1) the existing initial pool of zinc in coral tissue might be large enough to ensure the functioning of the carbonic anhydrase for some weeks (Harland and Brown 1989; Reichelt-Brushett and McOrist 2003), even if the inputs in this trace metal are reduced, and/or (2) even if the functioning of the carbonic anhydrase is reduced by lower inputs in zinc, calcification is not affected because it has been shown that an inhibition of the carbonic anhydrase activity decreases the coral calcification by only 40% (Tambutté et al. 1996).

Following 5 weeks of incubation under reduced pH_T conditions, all coral fragments survived and showed positive gross calcification rates. Although this result should be taken with caution, since corals were incubated during a short-term period to low pH, it is consistent with some recent studies showing that marine species, including corals, are able to calcify at low-pH seawater (Wood et al. 2008; Jury et al. 2009; Ries et al. 2009; Krief et al. 2010; Rodolfo-Metalpa et al. 2010a, b). In particular, Krief et al. (2010) found 18% decrease in the net calcification (i.e., gross calcification minus dissolution rates) of *S. pistillata* incubated for 14 months at both pH 7.49 and 7.19. It is

likely that this decrease was mainly due to the contribution of the skeleton dissolved in under-saturated water, which was not measured during our incubation, since the method we used allowed a direct measure of the gross calcification. Corals seem to have a strong biological control over their calcification sites allowing them to calcify even under under-saturated seawater. These findings might suggest that some scleractinian coral species will be able to acclimate to a high pCO_2 ocean even if changes in seawater acidity are faster and more dramatic than predicted. While it is estimated that most of the tropical corals will reduce their calcification up to 37% (Kleypas et al. 1999), the species *S. pistillata*, which represents a major builder of coral reefs in the Red Sea, represents one more piece of evidence that some corals can continue, at least for some weeks, to calcify under extremely low [CO_3^{2-}] (Atkinson et al. 1995; Ries et al. 2009; Rodolfo-Metalpa et al. 2010b), likely using HCO_3^- as the primary skeletal carbon source (Jury et al. 2009). Langdon and Atkinson (2005) compiled the results of studies that examined the effects of altered Ω_{arag} on coral net calcification and found that the responses fell into two general groups: a 'low-sensitivity' group where doubled pCO_2 resulted in a 0–18% reduction in calcification rates and a 'high-sensitivity' group where doubled pCO_2 resulted in a 40–83% reduction in calcification rates. Here, our data seem to indicate that *S. pistillata* would fall in the low-sensitivity group. Reynaud et al. (2003) already found that this same species was insensitive to doubled pCO_2 at 25°C but on the other hand experienced a 50% reduction in calcification rate at a higher temperature of 28°C. Based on changes in temperature, some coral species, like *S. pistillata,* shift between insensitivity and high sensitivity to pCO_2 increase (Jury et al. 2009), suggesting a synergetic effect between global warming and ocean acidification (Reynaud et al. 2003; Anthony et al. 2008).

Photosynthetic rates and photosynthetic efficiency

Similarly, rates of photosynthesis (P_{net}) and respiration (not shown) as well as the photosynthetic efficiency of PSII (F_v/F_m) did not seem to be affected by an increase in pCO_2 since they remained equivalent for all pH conditions. Although an increase in zinc concentration in seawater, led to an increase in the $rETR_{max}$ of *S. pistillata* in a previous study (Ferrier-Pagès et al. 2005), we found that the decrease in zinc uptake under high-pCO_2 conditions did not significantly change the $rETR_{max}$. Photosynthetic efficiency (F_v/F_m) was also not significantly different between pH treatments. As in previous studies conducted on other scleractinian corals, photosynthesis had no significant response to changes in the carbonate chemistry of seawater (Burris et al. 1983; Reynaud et al. 2003; Schneider and Erez 2006). These results suggest several possibilities, either the differences in zinc uptake did not impact the carbonic anhydrase activity or this uptake is still sufficient to replenish the large stocks in coral tissue (Harland and Brown 1989; Reichelt-Brushett and McOrist 2003), and to maintain the basic activity of the PSII. Our conclusions are consistent with the assertion that corals do not rely on dissolved CO_2 for their photosynthesis (Gattuso et al. 1999).

In summary, this study showed a strong effect of pH on zinc bioaccumulation in corals. In the context of ocean acidification, it appears that reducing pH leads to a lower incorporation of this element in coral tissue. Zn is considered to be an essential element in the enzymatic system of corals, and a limited input is expected to affect the organism physiology. Even if we did not measure any change in either calcification or photosynthesis processes, a zinc deficiency might affect other physiological processes, including the nitrogen utilization rates or cellular growth, as it has been highlighted in coccolithophorids (Schulz et al. 2004). Our experiment lasted only 6 weeks, so we can only suppose that on a long-term basis, lower uptake of this essential element might have detrimental effects on specific coral physiological functions.

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