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Ocean acidification increases photosynthate translocation in a coral–dinoflagellates symbiosis

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Abstract

This study has examined the effect of an increased seawater pCO_2 on the rates of photosynthesis and carbon translocation in the scleractinian coral species *Stylophora pistillata* using a new model based on ^{13}C-labelling of the photosynthetic products. Symbiont photosynthesis contributes for a large part of the carbon acquisition in tropical coral species and is therefore an important process that may determine their survival under climate change scenarios. Nubbins of *S. pistillata* were maintained for six months under two pHs (8.1 and 7.2). Rates of photosynthesis and respiration of the symbiotic association and of isolated symbionts were assessed at each pH. The fate of ^{13}C-photosynthates was then followed in the symbionts and the coral host for 48 h. Nubbins maintained at pH 7.2 presented a lower areal symbiont concentration, lower areal rates of gross photosynthesis, and lower carbon incorporation rates compared to nubbins maintained at pH 8.1, therefore suggesting that the total carbon acquisition was lower in this first set of nubbins. However, the total percentage of carbon translocated to the host, as well as the amount of carbon translocated per symbiont cell was significantly higher under pH 7.2 than under pH 8.1 (70 % at pH 7.2 versus 60 % at pH 8.1), so that the total amount of photosynthetic carbon received by the coral host was equivalent under both pHs (5.5 to 6.1 $\mu g\,C\,cm^{-2}\,h^{-1}$). Although the carbon budget of the host was unchanged, symbionts acquired less carbon for their own needs (0.6 against 1.8 $\mu g\,C\,cm^{-2}\,h^{-1}$), explaining the overall decrease in symbiont concentration at low pH. In the long-term, this decrease might have important consequences for the survival of corals under an acidification stress.

1 Introduction

Since the beginning of the industrial revolution, the anthropogenic release of carbon dioxide (CO_2) has increased the amount of CO_2 in the atmosphere, but also in the oceans, which absorb a quarter or more of the released CO_2 (Caldeira and Wickett,

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2003). This continuous input of CO_2 changes the seawater chemistry, inducing a significant decrease in the carbonate ion (CO_3^{2-}) concentration, as well as in the saturation states of biologically important calcium carbonate minerals (reviewed in Hoegh-Guldberg et al., 2007; Doney et al., 2009). Such changes also lower the seawater pH, and are consequently termed ocean acidification. They affect many marine organisms, both photosynthetic and calcifying species such as tropical scleractinian corals (Kroeker et al., 2010). The latter build a calcium carbonate skeleton and are particularly affected when seawater becomes under-saturated with respect to calcium carbonate minerals (reviewed in Anthony et al., 2011; Erez et al., 2011). Studies on the effect of ocean acidification on corals were therefore mainly focused on calcification, and generally, showed a decrease in the calcification rates with decreasing pH (reviewed in Erez et al., 2011). On the contrary, the effect of decreasing pH on the physiology of the coral symbionts has been much less studied. However, symbiont photosynthesis contributes for a large part of the carbon acquisition in tropical coral species (Muscatine et al., 1981, 1984), and is therefore an important process that will determine the survival and fitness of corals under the scenario of climate change.

Coral symbionts, called *Symbiodinium*, belong to the eukaryotic algal taxon of dinoflagellates. This taxon is the only one, known to possess form II RuBisCO (Ribulose-1,5 Bisphosphate Carboxylase/Oxygenase), which has a low CO_2 affinity compared to the other algal taxa (reviewed in Tortell, 2000). Corals have therefore developed carbon-concentrating mechanisms (CCM; Leggat et al., 1999) but it has been suggested that the symbionts in hospite are still limited by the host in dissolved inorganic carbon (DIC), because they are dependent on host bicarbonate (HCO_3^-) uptake (Goiran et al., 1996; Marubini and Thake, 1999; Marubini et al., 2008). In theory, an increase in seawater CO_2 concentration, leading to a parallel increase in bicarbonate concentration, should increase the rates of symbiont photosynthesis. However, the few studies performed on the subject led to controversial results, differing according to the coral species studied or to the acclimation length at the lowered pH. Experiments performed on a relatively short-term scale found no effect of acidification (pH of 7.5 to 7.9) on areal

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rates of coral photosynthesis (Scheinder and Erez, 2006; Marubini et al., 2008; Godinot et al., 2011; Houlbrèque et al., 2012) or in the maximum photosynthetic capacity per cell (Pnmax, Crawley et al., 2010). Longer-term experiments at low pH values (7.4 to 7.8) measured a decrease in these rates or in the photosynthetic efficiency of the symbionts (Reynaud et al., 2003; Anthony et al., 2008; Iguchi et al., 2012; Kaniewska et al., 2012), often coupled with a bleaching (i.e. the loss of symbionts; Anthony et al., 2008). Only the temperate species *Cladocora caespitosa* did not significantly change its photosynthesis after being maintained one year under pH 7.8 (Rodolfo-Metalpa et al., 2010). This different inter-specific response to acidification might be related to the symbiont phylotype, as demonstrated in a recent study (Brading et al., 2011), in which the cell-specific productivity of one phylotype out of four was increased under high pCO_2, while it remained unchanged for the others.

In terms of carbon acquisition for the coral host, not only rates of photosynthesis, but also photosynthate translocation rates are important. It is usually assumed that autotrophic carbon acquisition by the host is directly related to the rates of symbiont photosynthesis, because symbionts are supposed to translocate more than 90 % of the photosynthates to the host for its own nutrition (Muscatine et al., 1981). However, percentage of translocation may vary according to the host species (Davies, 1984, 1991; Edmunds and Davies, 1986), the symbiont genotype (Loram et al., 2007), or the environment (Muscatine et al., 1984) and is therefore independent of the production level.

In this study, we measured the fate of the photosynthetically-acquired carbon, as well as the amount of carbon translocated from the symbionts to the host, in colonies of the scleractinian coral *Stylophora pistillata* (Esper, 1797) maintained under two pHs (8.1 and 7.2) for six months. For this purpose, a new model of carbon translocation (Tremblay et al., 2012a), based on the isotopically labelled ^{13}C-bicarbonate, was used. The objectives of our study were to determine the effect of seawater acidification on the autotrophic carbon acquisition by the dinoflagellates in symbiosis with *S. pistillata*, and to relate symbiont productivity to carbon acquisition by the coral host. In other words,

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considering that ocean acidification decreases symbiont productivity in some scleractinian species, do we observe a parallel decrease in carbon acquisition by the host, or does the host maintain its carbon acquisition by increased percentage of carbon translocation?

2 Materials and methods

2.1 Biological material

Three colonies of the scleractinian coral *S. pistillata* (Pocilloporidae) were sampled at 10 m depth in the Gulf of Eilat (Israel). A total of 54 nubbins (18 nubbins per colony) were prepared by cutting the apical branches of the colonies. They were then equally divided (i.e. nine nubbins from each colony) in two tanks (150 l), which were maintained at two pHs: 8.1 (pCO_2 = 387 µatm, ambient conditions) and 7.2 (pCO_2 = 3898 µatm supplied by bubbling of CO_2; Table 1) for six months, described in Cohen and Fine (2012). Nubbins were fed once a week with *Artemia salina* nauplii and mashed fish mix. The tanks were kept under an irradiance of ca. 140 µmol photons m^{-2} s^{-1} (10 h light : 14 h dark photoperiod), in an open flow system (renewal rate of two times the volume of the basin per day) at ambient temperature (ca. 25 °C). From previous studies (Krief et al., 2010), we know that *S. pistillata* grows well under both pHs for years and the difference between 7.2 and 8.1 allows us to clearly established if there is a pH effect on the autotrophic carbon budget of *S. pistillata*.

2.2 Rates of photosynthesis and respiration

Rates of respiration (R) and net photosynthesis (P_n) were measured in each experimental condition using three nubbins (one nubbin per colony). Measurements were performed using temperature-controlled chambers coupled with optodes (ProODO, YSI, Yellow Springs, OH, USA) and filled with 580 ml of 0.65 µm-filtered seawater, at the right pH. Optodes were calibrated before each measurement using sodium bisulfite

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($NaHSO_3$) and water saturated air as 0 and 100 % oxygen saturation values, respectively. Seawater temperature in the chambers was maintained at 25.0 ± 0.5 °C, and was continuously stirred using magnetic stirrers. For each colony, photosynthesis at 140 µmol photons m^{-2} s^{-1} and post-illumination respiration were measured during one hour. Rates of gross photosynthesis (P_g) were calculated by adding R to P_n. Samples were then frozen for later determinations of symbionts, chlorophyll (chl), and protein concentrations. For this purpose, nubbins were thawed and their tissue was detached from the skeleton using an air-brush and 0.2 µm-filtered seawater. The slurry was homogenised using an electric homogenizer (Heidolph DIAX 100 with a Typ 6G/100, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Subsamples were taken for the determination of the symbiont concentration, according to Rodolfo-Metalpa et al. (2006), and protein concentration, according to Smith et al. (1985) using the BCAssay protein quantification kit (Uptima, Interchim) and a Xenius® spectrofluorometer (Safas, Monaco). The remaining sample was used to assess chl *a* and chl c_2 concentrations according to Tremblay et al. (2012b) using a spectrophotometer UVmc²® (Safas, Monaco). Data were subsequently normalised to the skeletal surface area of each nubbin (µmol O_2 cm^{-2} h^{-1}) measured using the wax-dipping technique (Veal et al., 2010) or per symbiont cell (µmol O_2 $cell^{-1}$ h^{-1}).

In addition to the above measurements, respiration rates of freshly isolated symbionts were also determined from three nubbins per condition (one per colony). Symbionts were therefore extracted in 0.65 µm-filtered seawater at the right pH using an air-brush, homogenised and centrifuged at 3000 g for 5 min. The pellet, containing the symbionts was resuspended in 0.65 µm-filtered seawater at the right pH. Respiration rates and symbiont concentration were measured as described above.

The autotrophic carbon acquired (P_C) and respired (R_C) was calculated for each treatment, by converting oxygen fluxes to carbon equivalents using the molar weights, as $P_C = P_g \times 12/PQ$ and $R_C = R \times 12 \times RQ$ (Anthony and Fabricius, 2000), where PQ and RQ are photosynthetic and respiratory quotients equal to 1.1 mol O_2 : mol C and 0.8 mol C : mol O_2, respectively (Muscatine et al., 1981).

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2.3 $H^{13}CO_3$ labelling experiments

The experiments were performed according to Tremblay et al. (2012a). Briefly, corals were placed in $H^{13}CO_3^-$ ($NaH^{13}CO_3$ 98 atom % ^{13}C, #372382, Sigma-Aldrich, St-Louis, MO, USA) enriched seawater, then transferred in non-enriched seawater for various chase periods. At the end, samples were frozen for later ^{13}C enrichment measurements in the symbionts and coral tissue.

For each condition, 15 beakers were therefore filled with 200 ml of seawater at the right pH, enriched with a concentration of 0.6 mM $NaH^{13}CO_3$ (or 23 % ^{13}C enrichment of the incubation medium), and maintained at the same irradiance and temperature as described previously. Fifteen nubbins (five per colony) were individually incubated during 5 h in the ^{13}C-enriched seawater and thereafter transferred in other 15 beakers containing non-enriched seawater (chase). Three nubbins (one per colony) were removed after 0, 2, 4, 24 and 48 h and immediately frozen at −20 °C. Six control nubbins per condition (two per colony; incubated from the beginning in 200 ml non-enriched seawater) were ran in parallel and two were sampled after 0, 24 and 48 h and immediately frozen at −20 °C. The pH was controlled after addition of the ^{13}C-bicarbonate and during the whole experiment to ensure that it remained at the desired level, especially for the low pH condition.

All nubbins were treated according to Tremblay et al. (2012a). Briefly, tissue was detached from the skeleton using an air-brush in 0.2 µm-filtered seawater. The slurry was homogenised using an electric homogenizer (Heidolph DIAX 100 with a tip 6G/100 Type), and the animal and symbionts fractions separated by centrifugation. Samples were frozen and freeze-dried until analysis. The % ^{13}C, and the carbon content of the animal tissue and symbionts were determined with a mass spectrometer (Delta Plus, Thermofisher Scientific, Bremen, Germany) coupled with a C/N analyzer (Flash EA, Thermofisher Scientific).

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2.4 Autotrophic carbon budget calculations

The equations used to calculate autotrophic carbon budget are fully described in Tremblay et al. (2012a). However, in the present study, ρ_{POC} was not measured and the equations were adjusted accordingly and listed below. The carbon incorporation rate (ρ) in the symbionts (ρ_S) and animal tissue (ρ_H), expressed in $\mu g\,C\,cm^{-2}\,h^{-1}$ was calculated as follow:

$$\rho = \frac{(C_{meas} - C_{nat}) \times M_{sample} \times M_C}{(C_{inc} - C_{meas}) \times (t_{pulse} + t_{chase}) \times S} \quad (1)$$

where C_{meas} and C_{nat} are the percentages of ^{13}C measured in enriched and control samples respectively, C_{inc} is the percent ^{13}C enrichment of the seawater (which varies during the chase; see Tremblay et al., 2012a), M_{sample} is the mass of the sample (mg), M_C is the carbon content per symbiont or host tissue biomass ($\mu g\,mg^{-1}$), S is the surface area (cm^2), t_{pulse} and t_{chase} are the incubation time (h) in the enriched and non-enriched incubation medium, in the light. See Table 2 for a list of symbols and their definitions.

The percentage of fixed carbon remaining (C_R) in symbionts, and host tissue is calculated by dividing ρ_S or ρ_H by the gross photosynthesis expressed in carbon (P_C), and multiplying by 100.

$$C_R = (\rho_S \text{ or } \rho_H / P_C) \times 100 \quad (2)$$

The carbon acquired through photosynthesis (P_C) may have different fates: it is respired by the coral assemblage (R_C), incorporated into the symbiont (ρ_S) and host (ρ_H) biomass, or lost in the surrounding water as POC (ρ_{POC}) and DOC (ρ_{DOC}). Therefore, the carbon budget equation is:

$$P_C = R_C + \rho_S + \rho_H + \rho_{POC} + \rho_{DOC} \quad (3)$$

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R_C includes two components, i.e. symbiont (R_S) and host (R_H) respiration; $R_S + R_H = R_C$.

The above equation does not consider the carbon incorporated in the skeleton because it has been shown that 25 to 30 % comes directly from the external medium as dissolved inorganic carbon and 70 to 75 %, from internal respiration R_C (Erez, 1978; Furla et al., 2000); the first component is external to the equation, and the second component is part of R_C. It follows from Eq. (3) that the amount of carbon lost as combined R_C, ρ_{DOC}, and ρ_{POC} (C_L) is:

$$C_L = R_C + \rho_{DOC} + \rho_{POC} = P_C - \rho_S - \rho_H \quad (4)$$

The amount of carbon translocated by symbionts to the host (T_S) corresponds to the total amount of carbon gained by photosynthesis (P_C) minus the sum of the carbon retained in symbionts (ρ_S) and respired by them (R_S):

$$T_S = P_C - \rho_S - R_S \quad (5)$$

The percentage of carbon lost (C_L) and translocated (T_S) was obtained by dividing C_L or T_S by P_C, and multiplying by 100.

In the present study, ρ_{DOC} and ρ_{POC} were not measured but estimated from carbon lost equation (Eq. 4):

$$\rho_{DOC} + \rho_{POC} = C_L - R_C = (P_C - \rho_S - \rho_H) - R_C \quad (6)$$

2.5 Statistical analysis

All parameters were expressed as average value ± standard error of the mean (s.e.m.). Data were checked for normality using a Kolmogorov-Smirnov's Test with Lilliefors correction and for variance homoscedasticity using a Levene's test. When the conditions were not fulfilled, data were transformed (natural logarithm for protein content). Significant differences in physiological parameters were tested using a t-test. The effect

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of pH on the incorporation rates (ρ) and the C_R in symbionts and coral host, after 0 and 48 h, was tested using a factorial ANOVA with two factors (pH and fraction; fraction are symbionts and host tissue). The effect of pH on C_L and T_S was tested using a t-test. Differences in the amount of carbon lost (C_L) and total respiration rates of the nubbins (R_C) were also tested using a t-test. Differences between factors were considered significant for a p-value < 0.05. Statistics were performed using Systat 13 (Systat Software, Chicago, IL, USA).

3 Results

3.1 Effect of seawater acidification on the main physiological parameters

Carbonate chemistry parameters in the different conditions are presented in Table 1. Seawater acidification induced significant differences in several physiological parameters of *S. pistillata* (Figs. 1 and 2). Areal symbiont concentration was 48 % lower at pH 7.2, compared to pH 8.1 (Fig. 1a, t-test $p = 0.0045$; $t = 4.42$; df = 6), combined with a 42 % decrease in areal chl *a* concentration at pH 7.2, compared to pH 8.1 (Fig. 1b, t-test $p < 0.0001$; $t = 12.85$; df = 6). On the contrary, there was no difference in areal chl c_2 concentration between the two treatments (Fig. 1b, t-test $p = 0.8097$; $t = 0.25$; df = 6). Protein content was significantly lower at pH 7.2, compared to pH 8.1 (Fig. 1c, t-test $p = 0.0010$; $t = 6.83$; df = 5).

As a result of low symbiont and chlorophyll concentrations in corals maintained under pH 7.2, aeral rates of gross photosynthesis were 22 % lower at this pH compared to those measured at pH 8.1 (Fig. 2a, t-test $p = 0.0153$; $t = 4.06$; df = 4), whereas the respiration rates were similar (Fig. 2b, t-test $p = 0.1286$; $t = 1.91$; df = 4), corresponding to ca. 72 $\mu g C cm^{-2} d^{-1}$ in both conditions. Gross photosynthesis therefore supplied 22 % less carbon to nubbins maintained under pH 7.2 (79 ± 1 $\mu g C cm^{-2} d^{-1}$) compared to those maintained under pH 8.1 (101 ± 6 $\mu g C cm^{-2} d^{-1}$). When photosynthetic rates were normalised per symbiont cell (cell-specific productivity), they

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were slightly but significantly higher (t-test $p = 0.0347$; $t = 3.15$; df = 4) under pH 7.2 ($11.8 \pm 0.5 \times 10^{-6}$ µg C cell^{-1} h^{-1}) than under pH 8.1 ($9.4 \pm 0.6 \times 10^{-6}$ µg C cell^{-1} h^{-1}; not shown in a figure).

3.2 Carbon translocation between the symbionts and their host

The natural percentage of ^{13}C (measured in non-enriched control samples) was lower at pH 7.2 (1.1224 ± 0.0002 and 1.1222 ± 0.0001 atom % ^{13}C, for symbiont and coral tissue, respectively) than at pH 8.1 (1.1294 ± 0.0014 and 1.1306 ± 0.0004 atom % ^{13}C, respectively). After incubation in ^{13}C-bicarbonate, nubbins from both conditions were however enriched in ^{13}C compared to control samples (atom % ^{13}C ranged between 1.6296 and 3.0147 % in symbionts, and between 1.3015 and 1.3877 % in the host tissue).

Carbon incorporation rates at the beginning of chase interval (0 h) in the symbionts and host tissue were twice lower under pH 7.2 (2.8 and 1.3 µg C cm^{-2} h^{-1}, respectively) than under pH 8.1 (4.9 and 2.7 µg C cm^{-2} h^{-1}, respectively; Fig. 3a, b and Table 3). They were significantly higher in symbionts than in coral tissue (Fig. 3a, b and Table 3). The amount of carbon retained in symbionts decreased during the chase to reach 0.6 and 1.8 µg C cm^{-2} h^{-1} in nubbins maintained at pH 7.2 and 8.1, respectively (Fig. 3a and Table 3). As a consequence, only 8 to 18 % remained in this fraction after 48 h (Fig. 3c). In contrast, carbon retained in the coral tissue remained more or less constant during the whole chase (Fig. 3b) and corresponded to 19–22 % of the initial fixed carbon (Fig. 3d and Table 3).

The major part of the fixed carbon was lost as respiration and excretion of DOC and/or POC. Total losses accounted for 3.8 and 2.4 µg C cm^{-2} h^{-1} at the beginning of the chase for corals under pH 7.2 and 8.1, respectively (Fig. 4a, t-test $p = 0.2298$; $t = 1.42$; df = 4), and reached 6.1 and 5.8 µg C cm^{-2} h^{-1} after 48 h (Fig. 4a, t-test $p = 0.6382$; $t = 0.49$; df = 4), corresponding to 60 to 73 % of the fixed carbon (Fig. 4b, t-test $p = 0.0835$; $t = 2.29$; df = 4). The amount of carbon lost after 48 h (Fig. 4a), at both pHs, was significantly higher than the respiration rates measured using the respirometry

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technique (Fig. 2b, t-test $p = 0.0025$; $t = 6.76$; df = 4 for pH 7.2 and t-test $p = 0.0020$; $t = 7.22$; df = 4 for pH 8.1). These results suggest that the release of autotrophic carbon as DOC and POC must have accounted for a significant fraction of the carbon lost. Indeed, the difference between the C_L and R_C values after 48 h was equal to 3.1 and 2.8 $\mu g\,C\,cm^{-2}\,h^{-1}$ or 39 and 27 % of the fixed carbon for nubbins under pH 7.2 and 8.1, respectively.

Carbon translocation rates are represented in Fig. 4c, d. The total amount of carbon translocated per skeletal surface area was similar under the two pHs (Fig. 4c, t-test $p = 0.3648$; $t = 1.02$; df = 4 for 0 h and t-test $p = 0.0624$; $t = 2.56$; df = 4 for 48 h), despite higher areal rates of photosynthesis under pH 8.1 (Fig. 2a), and increased with time. Therefore, the percentage of the total fixed carbon translocated was significantly higher at pH 7.2 than at pH 8.1. Indeed, 70 % of the fixed carbon was translocated to the host after 48 h at pH 7.2 against 60 % at pH 8.1 (Fig. 4d, t-test $p = 0.0079$; $t = 4.93$; df = 4). Moreover, due to lower areal symbiont concentration at pH 7.2 compared to pH 8.1, the amount of carbon translocated per symbiont cell was 40 % higher after 48 h (t-test $p > 0.0001$; $t = 17.54$; df = 4) in symbionts of nubbins maintained at pH 7.2 ($8.8 \pm 0.2 \times 10^{-6}$ $\mu g\,C\,cell^{-1}\,h^{-1}$) than in symbionts of corals under pH 8.1 ($5.0 \pm 0.1 \times 10^{-6}$ $\mu g\,C\,cell^{-1}\,h^{-1}$; not shown in a figure).

4 Discussion

Exposure of *S. pistillata* to an important seawater acidification (pH 7.2) led to a reduction in areal symbiont concentration and subsequently in the areal rates of gross photosynthesis compared to control corals maintained at pH 8.1. However, this decreased capacity in autotrophic carbon acquisition under low pH was counterbalanced by a higher percentage of carbon translocation, leading to an equivalent amount of autotrophic carbon acquired by the host in both pH conditions.

As previously and recently observed for one clade of *Symbiodinium*, clade A2, (Brading et al., 2011), cell-specific productivity in the present study was increased under low

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pH, i.e. under high seawater CO_2 and bicarbonate concentrations. The higher cell-specific productivity observed under pH 7.2 compared to pH 8.1 can be due to the direct effect of increased CO_2/bicarbonate in seawater, or to the indirect "bleaching effect", which decreased areal symbiont concentration in the host tissue and therefore increased the availability of light and DIC for each symbiont cell. This suggests that, in hospite, symbionts of *S. pistillata* are DIC limited. Such limitation was already observed in two previous studies (Goiran et al., 1996; Marubini et al., 2008). Marubini et al. (2008) indeed measured an increase in areal rates of gross photosynthesis of *S. pistillata* when bicarbonate concentration was increased in seawater, independently of the pH conditions. In addition, Goiran et al. (1996) showed that the reduction in the rate of net photosynthesis per chlorophyll in the species *Galaxea fascicularis* was higher in a system in which bicarbonate was decreased rather than in a system with increased bicarbonate and low pH. All together, these results suggest that DIC limitation of the photosynthesis is a general feature for several coral species and their associated symbionts, despite the presence of CCM. It has been shown that CCM efficiency differs between algal species (Tortell, 2000), and coral symbionts might have particularly low efficiencies.

Despite an enhancement of the cell-specific productivity, areal rates of gross photosynthesis were lowered at pH 7.2, because of the lowering of the zooxanthellae concentration in the corals subject to long-term acidification, as previously observed (Krief et al., 2010; Kaniewska et al., 2012). As a consequence, the total autotrophic carbon acquired by the symbiotic association, estimated from the areal rates of gross photosynthesis, was 22 % lower at pH 7.2 than at pH 8.1 ($7.9\,\mu g\,C\,cm^{-2}\,h^{-1}$ instead of $10.1\,\mu g\,C\,cm^{-2}\,h^{-1}$). This decrease in autotrophic carbon acquisition, also observed in previous long-term expositions to a low pH (Reynaud et al., 2003; Anthony et al., 2008; Iguchi et al., 2012; Kaniewska et al., 2012), can have detrimental impacts for the carbon budget and the health of the corals. The model used in this study (summarized in Fig. 5) however shows that the coral host acquired the same amount of autotrophic carbon under both pHs (5.5 and 6.1 $\mu g\,C\,cm^{-2}\,h^{-1}$ for pH 7.2 and 8.1, respectively). This

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was achieved following an increase in the amount of carbon translocated per symbiont cell, and in the percent of translocation in corals maintained at pH 7.2 compared to those at pH 8.1. Since respiration rates of the host and the symbionts remained unchanged under both conditions (ca. 1 and 2 $\mu g C cm^{-2} h^{-1}$ or 12 % and 22 % of the total carbon was respired by the host and the symbionts, respectively, under both pHs), as were the losses of carbon as DOC and POC (ca. 3 $\mu g C cm^{-2} h^{-1}$ under both pHs), the host conserved in both conditions ca. 20 % of the autotrophically acquired carbon in its tissue after 48 h, although with a slightly lower total amount for corals under pH 7.2 than pH 8.1 (1.5 against 2.2 $\mu g C cm^{-2} h^{-1}$). This lower amount matches with lower amount of proteins.

The higher percent of translocation in corals at pH 7.2 can be the result of a lower carbon demand of the symbionts (in lower areal concentration in the host tissue), and/or to a higher demand from the host through, for example, an increased release of host factors (HRFs) (Muscatine, 1967; Grant et al., 1998; Davy and Cook, 2001). Overall, this observation is beneficial for the host over time, which can maintain a certain protein level (although lower at pH 7.2 than at pH 8.1) but not for the symbionts, which are the loosers under low pH. Indeed, the amount of carbon remaining in the symbionts after 48 h dropped from 18 % (or 1.8 $\mu g C cm^{-2} h^{-1}$) of the photosynthesized carbon in corals at pH 8.1 to 8 % (or 0.6 $\mu g C cm^{-2} h^{-1}$) in corals at pH 7.2 (Fig. 5). In other words, there was a 33 % decrease in the symbiont cell-specific carbon incorporation rate at pH 7.2 compared to pH 8.1 (from 1.5×10^{-6} $\mu g C cell^{-1} h^{-1}$ at pH 8.1 to 0.9×10^{-6} $\mu g C cell^{-1} h^{-1}$ at pH 7.2). Such low carbon incorporation in symbiont cells at pH 7.2 is the best explanation for the bleaching observed. Symbionts have fewer building blocks to construct new biomass, or to grow and replace the dying cells. This observation therefore mitigates the positive effect, on the host carbon budget, of higher percent carbon translocation. Although the coral host can survive for sometime with the same amount of autotrophically acquired carbon, it is at the detriment of its population of symbionts. This population had severely declined after six months at pH 7.2 compared to the population of control corals, and might continue decreasing up to the level

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at which there will be no photosynthate production and translocation anymore. For the host, the increased photosynthate translocation cannot even maintain its biomass on the long-term, since the total protein content has decreased in colonies maintained at pH 7.2. Questions remaining open are whether the symbiotic association can remain on the fragile equilibrium observed in this study on the long-term or if the symbiont physiology will further decline and what will be the cross effect of increased pCO_2 and seawater temperature on the carbon translocation and budget in such symbioses.

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Table 1. Carbonate chemistry parameters of the treatment and the control calculated from pH, total alkalinity (TA), temperature (25°C) and salinity (40) using the program CO2SYS (Lewis and Wallace, 1998). DIC: dissolved inorganic carbon; Ω_{arag}: aragonite saturation state.

pH total scale	TA ($\mu eq\,kg^{-1}$)	DIC ($\mu mol\,kg^{-1}$)	pCO_2 (μatm)	$CO_{2(aq)}$ ($\mu mol\,kg^{-1}$)	HCO_3^- ($\mu mol\,kg^{-1}$)	CO_3^{2-} ($\mu mol\,kg^{-1}$)	Ω_{arag}
8.1	2501	2122	387	10.6	1846	265	4.02
7.2	2501	2544	3898	107.1	2393	44	0.67

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Table 2. List of symbols, definition and units.

Symbol	Definition
C	Carbon
C_{inc}	^{13}C enrichment of the incubation medium (%)
C_L	Amount of C lost ($\mu g\,C\,cm^{-2}\,h^{-1}$ or %)
C_{meas}	^{13}C measured in the sample (%)
C_{nat}	Natural abundance in ^{13}C in control nubbins (%)
C_R	Percentage of fixed C remaining in symbionts, host tissue and POC (%)
M_C	Mass of C per milligram of tissue or symbionts ($\mu g\,mg^{-1}$) or released POC (μg)
M_{sample}	Mass of the freeze-dried sample (mg)
P_C	Gross C fixed photosynthetically by symbionts ($\mu g\,C\,cm^{-2}\,h^{-1}$)
P_g	Oxygen produced by gross photosynthesis ($\mu mol\,O_2\,cm^{-2}\,h^{-1}$)
P_n	Oxygen produced by net photosynthesis ($\mu mol\,O_2\,cm^{-2}\,h^{-1}$)
PQ	Photosynthetic quotient (equal to 1.1 $mol\,O_2$: mol C)
R	Oxygen consummed by respiration of holobiont ($\mu mol\,O_2\,cm^{-2}\,h^{-1}$)
R_C	C respired by holobiont ($\mu g\,C\,cm^{-2}\,h^{-1}$)
R_H	C respired by coral host ($\mu g\,C\,cm^{-2}\,h^{-1}$)
RQ	Respiratory quotient (equal to 0.8 mol C : $mol\,O_2$)
R_S	C respired by symbionts ($\mu g\,C\,cm^{-2}\,h^{-1}$)
S	Nubbin surface area (cm^2)
T_S	Amount of C translocated calculated from the symbiont rates ($\mu g\,C\,cm^{-2}\,h^{-1}$ or %)
t_{chase}	Incubation time of the nubbins in the non-enriched incubation medium in the light (h)
t_{pulse}	Incubation time of the nubbins in the enriched incubation medium (h)
ρ_{DOC}	C incorporation rate in released DOC (not measured)
ρ_H	C incorporation rate in coral tissue ($\mu g\,C\,cm^{-2}\,h^{-1}$)
ρ_{POC}	C incorporation rate in released POC ($\mu g\,C\,cm^{-2}\,h^{-1}$)
ρ_S	C incorporation rate in symbiont ($\mu g\,C\,cm^{-2}\,h^{-1}$)

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Table 3. Results of the factorial analysis of variance (ANOVA) for carbon incorporation rates (ρ) and percentage of fixed carbon (C_R) at the beginning of chase interval (0 h) and at the end (48 h) with two factors (pH and fraction). Significant p-values are in bold.

Factor	Degrees of freedom	At the beginning (0 h) p	At the beginning (0 h) F value	At the end (48 h) p	At the end (48 h) F value
Incorporation rates (ρ)					
pH	1	**0.0009**	26.55	**0.0010**	25.26
Fraction	1	**0.0008**	26.95	**0.0101**	11.22
pH*Fraction	1	0.3004	1.23	0.1423	2.65
Error	8	–	–	–	–
Fixed carbon remaining (C_R)					
pH	1	**0.0090**	11.73	**0.0183**	8.73
Fraction	1	**0.0005**	32.12	**0.0085**	12.00
pH*Fraction	1	0.6123	0.28	0.0815	3.97
Error	8	–	–	–	–

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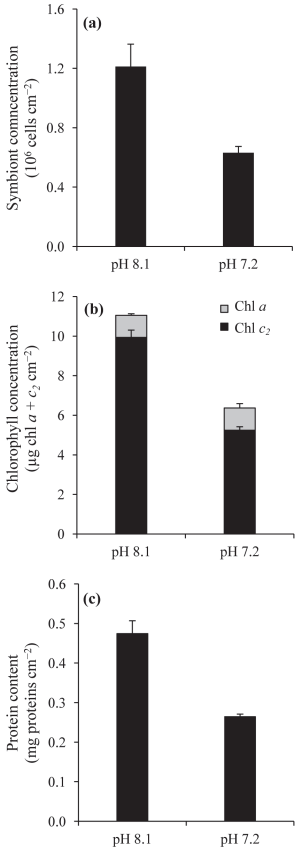

Fig. 1. (a) Symbiont, **(b)** chlorophyll, and **(c)** protein concentrations, for *Stylophora pistillata* at pH 8.1 and 7.2. Data are means ± standard error of means of $n = 4$ measurements.

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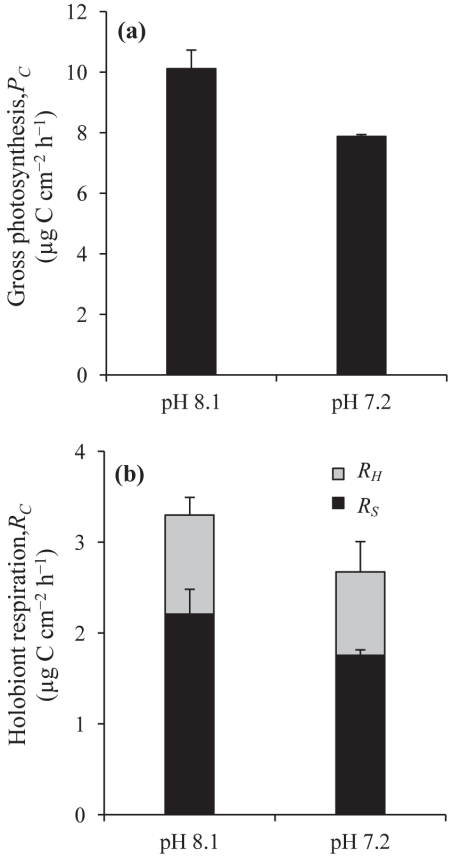

Fig. 2. (a) Gross Photosynthesis (P_C), and **(b)** holobiont respiration ($R_C = R_S + R_H$), for *Stylophora pistillata* at pH 8.1 and 7.2. Data are means ± standard error of means of $n = 3$ measurements.

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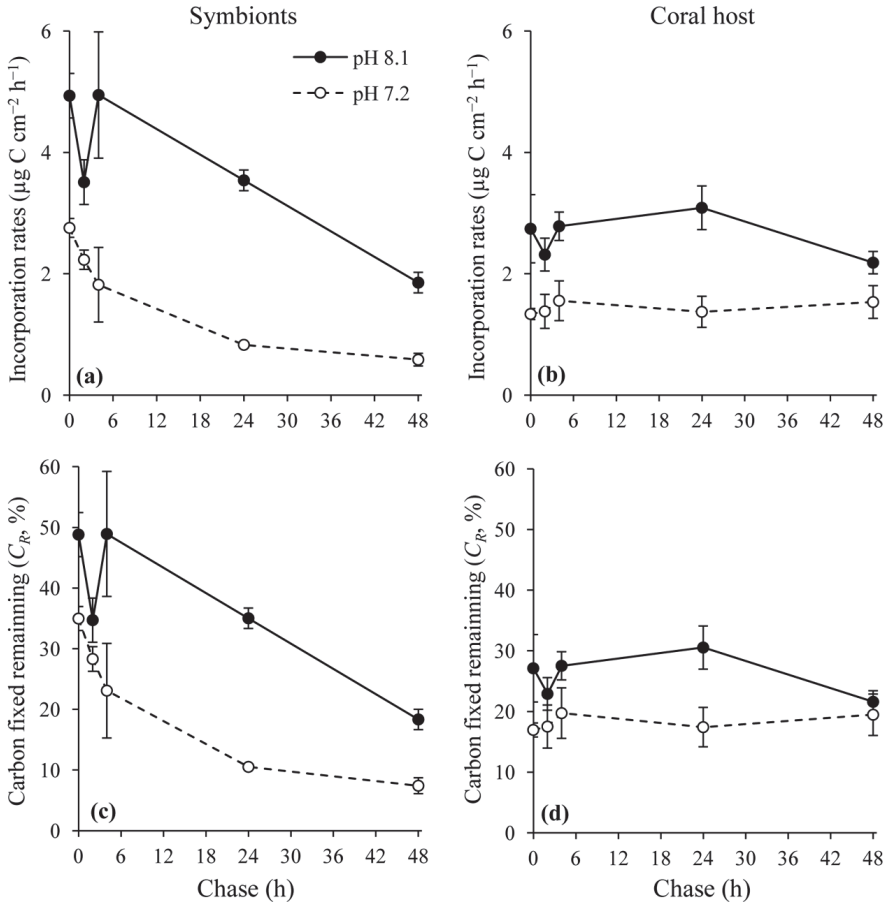

Fig. 3. Carbon incorporation rates (ρ) in **(a)** symbionts; and **(b)** coral host. Percentage of fixed carbon that remains (C_R) in the **(c)** symbionts; and **(d)** host for *Stylophora pistillata* at pH 8.1 and 7.2. Data are means ± standard error of means of n = 3 measurements.

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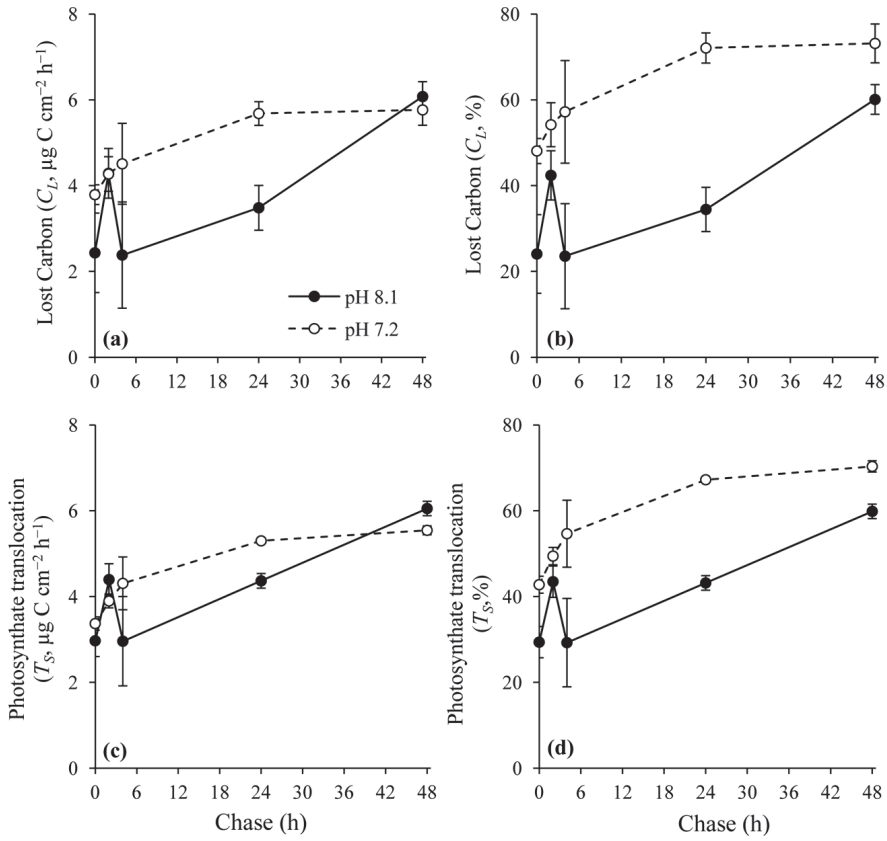

Fig. 4. (a) Amount and **(b)** percentage of carbon lost by symbiosis (C_L); **(c)** amount and **(d)** percentage of photosynthesized carbon translocated to the host by symbionts (T_S) for *Stylophora pistillata* at pH 8.1 and 7.2. Data are means ± standard error of means of n = 3 measurements.

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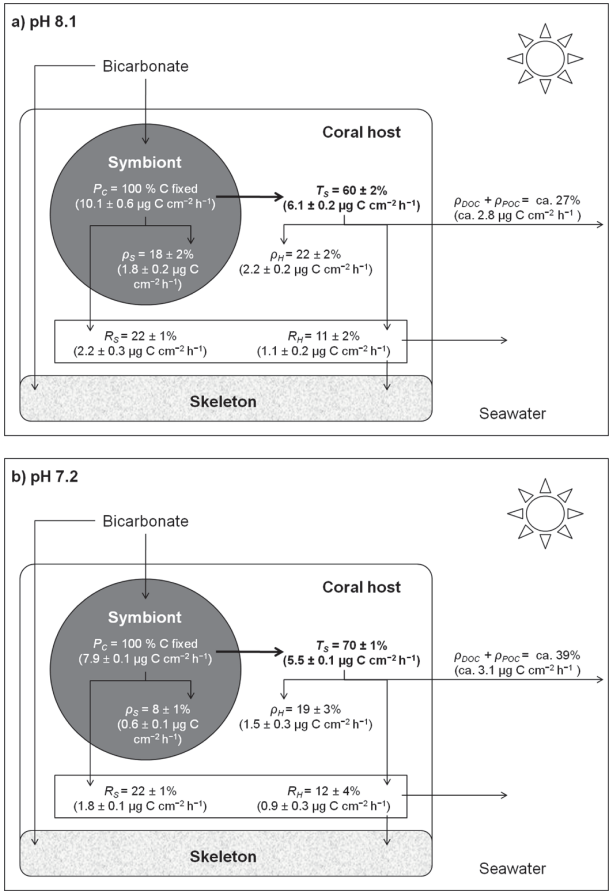

Fig. 5. Mass-balanced results of photosynthate translocation and carbon budget in *Stylophora pistillata* under **(a)** pH 8.1, and **(b)** pH 7.2 based on ^{13}C experiments after 48 h. Symbols are defined in the text and summarised in Table 2. Data are means ± standard error of means of n = 3 measurements.

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