

Authors response to Anonymous Referee #2 comments:

We appreciate your constructive comments on our manuscript entitled: “Symbiosis increases coral tolerance to ocean acidification”. It was recommended that we undertake some changes to the manuscript; the revised version of manuscript is also attached as a supplement pdf file so that the referees can see the changes that were made. Below we summarize our responses to the comments in a point-by-point form. We hope that our responses are judged to have adequately addressed the points made by the reviewers, and that the paper is now acceptable for publication in Biogeosciences.

“This paper addresses a very interesting topic of coral calcification under ocean acidification conditions. However, the title of the paper “Symbiosis increases coral tolerance to ocean acidification (OA),” misleads the reader. Instead the paper really addresses the effects of ocean acidification on the calcification of coral recruits. The implied “tolerance” isn’t really addressed but instead calcification rates of coral recruits with and without symbionts is the focus of the study. Increased tolerance would imply the effects of OA were diminished for symbiotic recruits relative to aposymbiotic recruits. However, there was no statistical difference between aposymbiotic recruits in OA treatments. The differences in calcification are still noteworthy and there is a large body of work pertaining to light enhanced calcification to aid in this discussion. Overall, this paper is still a valuable addition to the field of coral physiology and climate change. In addition to the above issue, I recommend the following changes:”

> According to your suggestion, we changed the title as “Calcification responses of symbiotic and aposymbiotic corals to near-future levels of ocean acidification”.

1. Page 7015 line 8-10 – A recent paper (Comeau et al 2013b) would be a valuable addition to the discussion of carbon source utilized during coral calcification.

> We cited the paper and added explanation as follows: “ It has also been suggested that both the carbonate and bicarbonate ions affect coral calcification under acidified

seawater condition, but the extent of the effect differs in light and dark conditions (Comeau et al. 2013)."

2. Page 7016 line 22 – Were any in situ light measurements taken from the reef flat where the adult colonies were collected? Dufault et al. 2013 found contrasting effects of pCO₂ on calcification and survival of newly settled coral recruits at various light intensities.

> Coral planulae, of most corals including *Acropora*, often recruit into low-light, cryptic habitats and as they grow extend into high irradiance environment. The light levels used in our experiment were designed to be low, and were lower than those in natural conditions where the adult colonies were collected. Although we have previously used higher light levels for coral rearing experiments, however, considering the fact that all fragments showed positive calcification in all treatments, suggests that the present light levels seem to be adequate in our experimental condition.

3. Page 7015 line 23 – I would argue that the use of bubbling CO₂ is now standard in published ocean acidification studies. The discussion of acid vs gas bubbling doesn't really benefit the introduction.

> According to your suggestion, we have removed the discussion of acid vs. gas bubbling from the revised version of manuscript.

4. Page 7018 line 1 – Similar to the previous comment, were any in-situ pCO₂ measurements taken where the adult colonies were collected? Several studies (Ohde and Van Woesik 1999, Kayanne et al. 2008, Bates et al. 2001) have documented large shifts in pCO₂ reefs, some of which were taken within the Ryukyu Islands. Knowledge of current reef conditions may inform the growth trends presented in Fig. 2.

> We referred to Ohde and Van Woesik 1999 and to Kitada et al. (2006), which reported diurnal variation of pCO₂ in reef water in front of our laboratory Sesoko

Station of the University of the Ryukyus. We added the description on the consideration for pCO₂ variation in the revised manuscript (p. 13, L. 266-).

5. Page 7022 line 1-2 – Dufault et al. 2012 found similar results of coral recruits being unaffected by high levels of pCO₂ in experimental treatments.

> In our experiment, we found that high pCO₂ concentration did not affect adult coral calcification although the calcification of juvenile corals was clearly reduced. Related to the comment on 6 below, we cited the Dufault et al 2012 paper and added some descriptions on that. Please see the reply to comment 6 below.

6. Page 7022 line 10-13 – There is a growing body of literature addressing the effects of ocean acidification on newly settled coral recruits, none of which were discussed (Albright et al. 2008, Albright and Langdon 2011, Albright 2011, Cohen et al. 2009, de Putron et al. 2011, Dufault et al. 2012, Dufault et al. 2013). Comparing your results to some of these would greatly aid the discussion.

> We cited the papers and added some descriptions in the revised manuscript (p. 12, L. 243-247): “The same possibility was suggested by other recent studies (Albright et al., 2008; Cohen et al., 2009; Suwa et al., 2010; Albright and Langdon, 2011; Albright, 2011; de Putron et al., 2011; Dufault et al., 2012; Doropoulos et al 2012; Dufault et al., 2013) although comparative studies between aposymbiotic and symbiotic primary polyps is only in its infancy (Inoue et al. 2012; Tanaka et al., 2013)”

7. Fig. 2. Is it possible to use standardized rates of measuring calcification? The relative values limit the reader comparing your results to other published rates.

> We added data on the increase of skeletal weight per day in supplementary data, together with net CaCO₃ deposition rates (Fig. S1). We think this information would be informative to compare our data with other researches' data.

It was recommended to do some changes in manuscript so the revised version of manuscript is also attached as a supplement pdf file for referees to see the changes.

1 Title:

2 Calcification responses of symbiotic and aposymbiotic corals to near-future levels of
3 ocean acidification

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22 **Introduction**

23 As humans are continuing to burn fossil fuels at an unprecedented rate, the concentration of CO₂
24 in the atmosphere is presently higher than it has been for the last 420,000 years
25 (Hoegh-Guldberg et al., 2007; IPCC, 2007). The oceans uptake a large proportion of that CO₂,
26 forcing them toward more acidic conditions (i.e., with high *p*CO₂), threatening the very
27 foundation of calcifying marine organisms and coral reefs (Kleypas et al., 2006; Orr et al., 2005;
28 Raven et al., 2005). Indeed, coral reefs support a wealth of calcifying organisms, of which
29 scleractinians corals have been the most essential reef builder since the Triassic (Stanley and
30 Fautin, 2001).

31

32 Since the pre-industrial period, we have witnessed a steady increase in *p*CO₂ concentrations
33 around 100 μatm, which is predicted to reach 200 – 700 μatm above present values (400 μatm)
34 by 2100 (IPCC, 2007). Such an increase in *p*CO₂ concentrations reduce both the pH and the
35 concentration of carbonate ions in the water column, and increase the availability of bicarbonate
36 ions (Kleypas et al., 1999). Several studies have found that coral calcification rates are directly
37 related to the concentration of carbonate ions in the water column (Anthony et al., 2008;
38 Gattuso et al., 1998; Kleypas et al., 2006; Marubini et al., 2008), whereas other studies have
39 shown a positive relationship between coral growth rates and the availability of bicarbonate ions
40 (Jury et al., 2010). It has also been suggested that both the carbonate and bicarbonate ions affect
41 coral calcification under acidified seawater condition, but the extent of the effect differs in light
42 and dark conditions (Comeau et al. 2013). Therefore, the response of coral growth and the state
43 of the ocean's carbonate chemistry is under intensive investigation (Pandolfi et al., 2011).

44

45 Moreover, the oceans are not homogeneous, and the temperature gradient from the tropics to the
46 poles sets carbonate ion concentrations naturally higher in the tropics where coral reefs occur.
47 Nevertheless, the decrease in carbonate ion concentrations from the pre-industrial period to the
48 present has been greater in the tropics ($\sim 29 \mu\text{mol kg}^{-1}$) than in the Southern Ocean ($\sim 18 \mu\text{mol}$
49 kg^{-1}) (Orr et al., 2005). Yet, symbiosis is prolific in the tropics, and the self-extending symbiosis
50 theory tells us that organisms harboring symbionts should be more tolerant to environmental
51 change than organisms without symbionts (i.e., aposymbiotic organisms) (Kitano, 2004; Kitano
52 and Oda, 2006). These assertions lead to two pertinent questions: (i) will calcifying coral
53 species survive in high $p\text{CO}_2$ seawater? And (ii) are juvenile corals, without symbionts, more
54 vulnerable to high $p\text{CO}_2$ seawater than juveniles and adult corals with symbionts?

55

56 Previous experiments that have mimicked the near-future $p\text{CO}_2$ conditions on coral reefs have
57 either adjusted the pH of seawater by adding an acid or a base, or by bubbling CO_2 through the
58 seawater in experimental chambers (Atkinson and Cuet, 2008). Adding an acid or a base results
59 in seawater with different alkalinity, bicarbonate, and carbonate ion concentrations than when
60 CO_2 is bubbled through seawater (Atkinson and Cuet, 2008), thus, adding an acid or a base has
61 not been used in recent ocean acidification studies. Although bubbling CO_2 through the
62 seawater more closely reflects near-future conditions than adding acids, it is nevertheless
63 difficult to achieve a stable $p\text{CO}_2$ environment, especially in flow-through systems (e.g.
64 Leclercq et al., 2002; Suwa et al. 2010; Takahashi and Kurihara, 2013). To overcome these
65 problems, our research group developed a system that produced stable $p\text{CO}_2$ concentrations in
66 flow-through conditions (Fujita et al., 2011).

67

68 Using this system, we examined the effect of $p\text{CO}_2$ -adjusted seawater on the calcification rates
69 of *Acropora digitifera*, one of the most common corals in the Pacific Ocean. Calcification was
70 examined in five $p\text{CO}_2$ treatments: (i) pre-industrial $p\text{CO}_2$, $< 300 \mu\text{atm}$, (ii) present-day $p\text{CO}_2$,
71 $400 \mu\text{atm}$, and at three near-future conditions, (iii) $600 \mu\text{atm}$, (iv) $800 \mu\text{atm}$, and (v) $1000 \mu\text{atm}$.
72 Within these treatments, we investigated the response of: (1) primary aposymbiotic coral polyps
73 (i.e., without symbionts), (2) primary symbiotic polyps, and (3) adult symbiotic fragments. It
74 was hypothesized that the calcification process of symbiotic corals was more tolerant to $p\text{CO}_2$
75 adjustments than aposymbiotic corals.

76

77 **2 Materials and Methods**

78 2.1 Experimental setup

79 To produce $p\text{CO}_2$ -adjusted seawater, we used a precise $p\text{CO}_2$ control system (Fujita et al., 2011).
80 This system was used to generate five different $p\text{CO}_2$ levels, including one lower than the
81 present level of atmospheric $p\text{CO}_2$: (i) pre-industrial, $< 300 \mu\text{atm}$, (ii) present-day $p\text{CO}_2$, 400
82 μatm , and at three near-future conditions, (iii) $600 \mu\text{atm}$, (iv) $800 \mu\text{atm}$, and (v) $1000 \mu\text{atm}$. The
83 $p\text{CO}_2$ -adjusted seawater was supplied to duplicate flow-through (150 ml min^{-1}) aquaria systems
84 (12 l). The seawater temperature was maintained at 27°C , with a 12:12 h light:dark photoperiod
85 (of $75 \mu\text{mol m}^{-2}\text{s}^{-1}$) under metal-halide lamps (Funnel2 150W, Kamihata, Japan) throughout all
86 treatments. The aragonite saturation state of the seawater was estimated using the CO2SYS
87 program (Lewis and Wallace, 1998) and the variables: temperature, pH, mean salinity, and total
88 alkalinity were measured repeatedly during the experiments. The chemical and physical

89 conditions of each $p\text{CO}_2$ treatment are summarized in Tables 1 and 2.

90

91 2.2 Primary polyp experiment

92 Several 20 cm *A. digitifera* colonies were collected from a fringing reef of Sesoko Island.

93 Gametes from two colonies, which spawned on 29 May 2010 were combined in a flow-through

94 aquarium, from which we derived several hundred planulae larvae. Primary polyps were

95 prepared following the methods outlined in our previous report (Suwa et al., 2010) using 13

96 day-old planulae. To prepare the symbiotic primary polyps, primary polyps of *A. digitifera* were

97 infected with the dinoflagellate *Symbiodinium* (clade A, Tanaka et al., 2013) that were derived

98 from the giant clam *Tridacna crocea* (a solution of 4×10^5 cells ml^{-1}) because the primary

99 polyps could acquire algae from this bivalve more efficiently than from other hosts, including

100 *Acropora* species (Hirose et al., 2008). Four days after inducing metamorphosis, primary polyps

101 were exposed to the symbiont solution for one day. Three days after exposure to the symbiont

102 solution, we confirmed symbiont infection using a dissecting microscope. In the final day of the

103 experiment, many symbionts (which were identical to the symbionts in Tanaka et al. 2013) were

104 observed in infected polyps. The primary polyps, both with and without symbionts, were

105 subjected to four $p\text{CO}_2$ treatments: (i) pre-industrial, $< 300 \mu\text{atm}$, (ii) present day $p\text{CO}_2$, 400

106 μatm , (iii) $800 \mu\text{atm}$, and (iv) $1000 \mu\text{atm}$.

107

108 Eight 6-well culture plates, containing the settled primary polyps, were placed into each

109 aquarium (i.e., 4 plates for aposymbiotic primary polyps, and 4 plates for symbiotic primary

110 polyps) during 10 days. Twenty polyps per treatment were used to evaluate skeletal growth of

111 polyps. At the end of the experiment, soft tissues were removed from each polyp with a
112 water-pik. The dry weight of each polyp skeleton was measured according to Inoue et al. (2011).
113 The dry weight (μg) of the polyp skeleton, at the end of the experiment, was used to represent
114 the amount of growth of each coral during the experiment.

115

116 2.3 Adult-coral-fragment experiment

117 Five > 30 cm colonies of *A. digitifera* were collected in August 2009 from a shallow (2 m)
118 fringing reef at Sesoko Island, Okinawa, Japan. The colonies which were growing at least 10 m
119 apart were haphazardly selected. The *A. digitifera* colonies were kept in a flow-through
120 aquarium for 3 weeks under natural light conditions at Sesoko Station, Tropical Biosphere
121 Research Center, University of the Ryukyus (Okinawa, Japan). Fifty, 2-3 cm fragments were cut
122 from each parent colony and attached to plastic bolts with superglue. The fragments were kept
123 in a flow-through aquarium for 2 weeks under natural light conditions until the coral tissues
124 started to spread over the surfaces of the plastic bolts. Five of these fragments, from each parent
125 colony, were maintained for 6 weeks in each of ten aquaria to which $p\text{CO}_2$ -adjusted seawater
126 was supplied using the flow-through system (two aquaria per $p\text{CO}_2$ treatment).

127

128 The weight of each colony was measured as buoyant weight (Davies, 1989), which directly
129 reflects skeletal weight (Anthony et al., 2008). The calcification rate was calculated as the
130 percentage change in final weight relative to the initial weight, during the 6-week experiment
131 (Also see Fig. S1). During the adult fragment experiment, 29 fragments died (11.6% in total 250
132 fragments) and were excluded from the calcification analysis. To evaluate the photosynthetic

133 fitness of zooxanthellae in the adult fragments, the symbionts' maximum photosynthetic
134 quantum yields (F_v/F_m) were measured after 6 weeks using a Diving-PAM Underwater
135 Fluorometer (Walz, Germany) after at least 1 h of darkness.

136

137 2.4 Data analysis

138 Primary polyp experiment: The dry weights of the primary polyp skeleton were analyzed using
139 a two-factor crossed ANOVA, in which $p\text{CO}_2$ (with four levels) and symbiosis (i.e., presence or
140 absence of dinoflagellates) were incorporated into the model as fixed-effect factors. The
141 subsequent pairwise comparisons among different $p\text{CO}_2$ levels were performed using Tukey's
142 HSD tests ($\alpha = 0.01$).

143 Adult-coral-fragment experiment: We used a general linear model to estimate the response of
144 adult-coral calcification to: $p\text{CO}_2$ (fixed-effect factor), aquarium (nested within $p\text{CO}_2$;
145 fixed-effect factor), colony (fixed-effect factor), initial weight (covariate), and their interactions
146 ($p\text{CO}_2 \times$ initial weight, colony \times initial weight, colony $\times p\text{CO}_2$, colony $\times p\text{CO}_2 \times$ initial weight).
147 The result of the F -tests (based on type-III sum of squares) and stepwise backward model
148 selection, suggested that only $p\text{CO}_2 \times$ initial weight, and colony \times initial weight remained as
149 statistically significant interactions (each $\alpha = 0.05$). To remove the variation of covariates, we
150 calculated the adjusted mean final weights relative to the mean initial weight for each colony,
151 assuming that their regression lines were heterogeneous among all the combinations of colony
152 and $p\text{CO}_2$. The adjusted final weight (W_{AFIN}) for each colony was independently analyzed using
153 an ANOVA model with $p\text{CO}_2$ (fixed-effect factor) and aquarium (nested within $p\text{CO}_2$;
154 fixed-effect factor) as the independent fixed factors. Statistically significant factors ($\alpha = 0.01$)

155 were subjected to pairwise comparisons (Tukey's HSD tests; $\alpha = 0.01$) to specify significant
156 combinations of treatment levels. The F_v/F_m values of adult fragments were analyzed using a
157 one-way ANOVA model with $p\text{CO}_2$ as fixed-effect factors after an arcsine transformation. The
158 subsequent pairwise comparisons among different $p\text{CO}_2$ levels were performed using Tukey's
159 HSD tests ($\alpha = 0.01$).

160

161 **3 Results**

162 The ANOVA indicated that the $p\text{CO}_2 \times$ symbiosis interaction was statistically negligible ($p >$
163 0.05) and the main factors were all significant ($p < 0.0001$). The post-hoc tests demonstrated
164 that the skeletal weights at 300 and 400 μatm were significantly heavier than those at
165 future-level treatments (i.e., 800 and 1000 μatm), regardless of whether polyps contain
166 dinoflagellates or not (Fig. 1). When compared at the same $p\text{CO}_2$ level, the primary polyps with
167 symbionts got heavier than those without dinoflagellates (Fig. 1). Because gametes from two
168 colonies were added to each aquarium, genetic differences could not be incorporated into the
169 model. However, it is unlikely that this reverses our conclusion, because the error variance was
170 small compared with the variance that was due to the main treatments effects in our data (see
171 Table S1). We evaluated the calcification rates of adult fragments of *A. digitifera* under five
172 $p\text{CO}_2$ treatments. The ANOVA on the adult fragment weight adjusted for initial size variation
173 indicated that a higher $p\text{CO}_2$ leads to significantly slower growth rates in four out of the five
174 colonies (Colony b – e; Fig. 2; Table S3). The analysis also suggested that the potential
175 environmental differences between two replicate aquaria were negligible in all five colonies (all
176 $p > 0.05$). The subsequent Tukey's HSD tests indicated that the mean final weight of adult

177 fragments, reared at 300 μatm , was significantly greater than those at the other $p\text{CO}_2$ conditions
178 in all of the four colonies, showing significant $p\text{CO}_2$ effects (Fig. 2; Table S3). The maximum
179 photosynthetic efficiencies of the adult fragments were above 0.6, and did not differ
180 significantly among $p\text{CO}_2$ treatments (Fig. 2; Table S4). These observed values indicated that
181 there were negligible or none of light-induced damage caused by lighting system used in the
182 experiment.

183

184 **4 Discussion**

185 The differences in the skeletal weights between primary polyps with and without symbionts
186 might reflect the difficulty that aposymbiont corals may have in acquiring energy and resources,
187 including organic matrix molecules, for calcification. Yet why would the primary polyps with
188 symbionts be more responsive to pre-industrial treatments than aposymbiotic primary polyps?
189 The increase in calcification in the pre-industrial $p\text{CO}_2$ treatment only occurred in corals that
190 housed symbionts. Indeed, the adult colonies showed the same response as primary polyps with
191 symbionts, clearly increasing calcification rates in low $p\text{CO}_2$ treatments. Moreover, the
192 calcification rates of symbiotic adult *A. digitifera* fragments were higher in the pre-industrial
193 seawater $p\text{CO}_2$ treatment than in the present-day $p\text{CO}_2$ treatment.

194

195 Higher calcification in the pre-industrial $p\text{CO}_2$ treatment was most likely attributed to a change
196 in skeletal precipitation by the coral host, because there was no evidence of any dynamic
197 photoinhibition (Enriquez et al., 2002) indicated as the decline in maximum photosynthetic
198 quantum yield among the symbionts in the high- $p\text{CO}_2$ treatments (Fig. 2, Table S4). Still, there

199 were no differences in calcification rates between present day and near-future concentrations
200 (Fig. 2). We note that this lack of difference in calcification between present day and anticipated
201 future $p\text{CO}_2$ treatments was not apparent for primary polyps (Fig. 1). These differences suggest
202 a number of potential mechanisms that are not mutually exclusive. First, an increase in
203 calcification in low $p\text{CO}_2$ environments was only apparent in the presence of symbionts.
204 Therefore, such phenotypic plasticity in calcification potential was most likely attributed to the
205 presence of the symbionts. Second, the adult colonies did not respond to higher $p\text{CO}_2$
206 environments, whereas the primary polyps with symbionts did show reduced calcification rates
207 at high $p\text{CO}_2$. Such results suggest a hierarchical response in tolerance to $p\text{CO}_2$ environments
208 depending on the density of symbionts, from adult colonies with symbionts as the most tolerant,
209 to symbiotic primary polyps showing some tolerance, to primary polyps without symbionts
210 being the least tolerant to high $p\text{CO}_2$ treatments.

211

212 There is mounting evidence that symbiotic dinoflagellates facilitate calcification within corals
213 through a positive feedback system between the host and the symbionts (Allemand et al., 2004;
214 Muscatine, 1990; Yellowlees et al., 2008) although the detailed mechanisms have been under
215 investigation. The glycerol and oxygen produced by the symbionts facilitate calcification
216 through mitochondrial respiration and ATP production which could be used for ion transport
217 (Allemand et al., 2004; Colombo-Pallotta et al., 2010). CO_2 uptake by photosynthesis is also
218 thought to stimulate calcification by changing the equilibrium of dissolved inorganic carbon
219 (DIC) in coral tissue, although the mechanisms are unresolved (Allemand et al., 2004). Our
220 results also indicate that the primary polyps with symbionts grew faster than aposymbiotic

221 polyps (Fig. 1). Although the primary polyps with symbionts seemed to be more sensitive to
222 acidified seawater than aposymbiotic polyps (Fig. 1), the faster growth induced by symbiosis
223 could compensate for the decrease of calcification by acidified seawater. The reason why
224 coral-algal symbiosis enhances coral calcification is not only attributed to algal photosynthesis
225 but is also potentially related to the removal of substances inhibiting calcification, such as
226 phosphates (Allemand et al., 2004).

227

228 Previous research indicates that acidified seawater increases the concentration of HCO_3^- ,
229 possibly followed by the activation of photosynthesis in coral symbionts (Jury et al., 2010;
230 Marubini et al., 2008). In our experiments, however, there was no evidence that acidified
231 seawater activates the photosynthesis of *Acropora digitifera*. The reason why the acidified
232 seawater, with high $p\text{CO}_2$ concentration (1000 μatm), did not affect adult coral calcification and
233 photosynthetic efficiency is unknown. We suspect that there were obvious advantages from
234 symbiosis. For example, the removal of phosphates would facilitate calcification even in
235 acidified seawater. Irrespective of the cellular mechanism involved, our results clearly showed
236 that corals without symbionts were most vulnerable to $p\text{CO}_2$ increases, whereas corals that
237 housed symbionts were more tolerant.

238

239 These results suggest that coral recruitment might be influenced by ocean acidification. Given
240 that globally ~80% of the scleractinian corals are spawners that acquire symbionts from the
241 'wild' after settlement (Baird et al., 2009), vulnerability of primary polyps to ocean acidification
242 upon the first settlement (in particular aposymbiotic polyp) could be at risk of decline in the

243 near future. The same possibility was suggested by other recent studies (Albright et al., 2008;
244 Cohen et al., 2009; Suwa et al., 2010; Albright and Langdon, 2011; Albright, 2011; de Putron et
245 al., 2011; Dufault et al., 2012; Doropoulos et al 2012; Dufault et al., 2013) although
246 comparative studies between aposymbiotic and symbiotic primary polyps is only in its infancy
247 (Inoue et al., 2012; Tanaka et al., 2013). This inference on recruitment may be particularly
248 evident in the Indian and Pacific Oceans where most corals are spawners that horizontally
249 transfer symbionts (Harrison and Wallace, 1990), acquiring them after settlement. By contrast,
250 newly settled corals may do better in the Caribbean where most corals are brooders and
251 symbionts are maternally (i.e., vertically) acquired, and the planulae are symbiotic (Harrison
252 and Wallace, 1990).

253

254 The degree of selective pressure by ocean acidification on newly settled polyps may therefore
255 depend on how rapidly corals are able to support symbionts. Such selective filtering could lead
256 to relative shifts in coral species abundance, changing reefs from those that primarily support
257 spawners, to reefs that primarily support brooders (that maternally acquire symbionts). Similar
258 shifts in species composition have occurred in the Oligocene, when rapidly cooling oceans
259 favored brooding corals over spawning corals in the Caribbean (Edinger and Risk, 1995).

260

261 In summary, the increase in $p\text{CO}_2$ of just 100 μatm , between the pre-industrial period and the
262 present, had more effect on the calcification rate of adult *A. digitifera* than the anticipated future
263 increases of several hundreds of micro-atmospheres of $p\text{CO}_2$. Our results also suggest that ocean
264 acidification has had adverse effects on reef corals since the industrial revolution. Ocean

265 acidification, therefore, may not be only a future problem but a direct and present threat to
266 ocean ecosystems (Talmage and Gobler, 2010). However, we also need to consider that the
267 seawater pH and $p\text{CO}_2$ in coral reefs can be variable over diel time scales (Suzuki et al., 1995;
268 Ohde and van Woesik, 1999; Bates et al., 2001; Santos et al., 2011). Kitada et al. (2006)
269 reported a relatively large $p\text{CO}_2$ diurnal variation of 680 – 290 μatm with seasonal variations in
270 reef water in front of Sesoko Station. Thus, the natural pH and $p\text{CO}_2$ variation in coral reefs
271 should be taken into account to provide more realistic results to predict the effect of ocean
272 acidification. In conclusion, this study showed that the apparent sensitivity of primary polyps to
273 near-future ocean acidification was a consequence of not housing symbionts, and those
274 organisms harboring symbionts, at any life-history stage, are more tolerant to ocean
275 acidification than organisms without symbionts.

276

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283

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477 Figure legends

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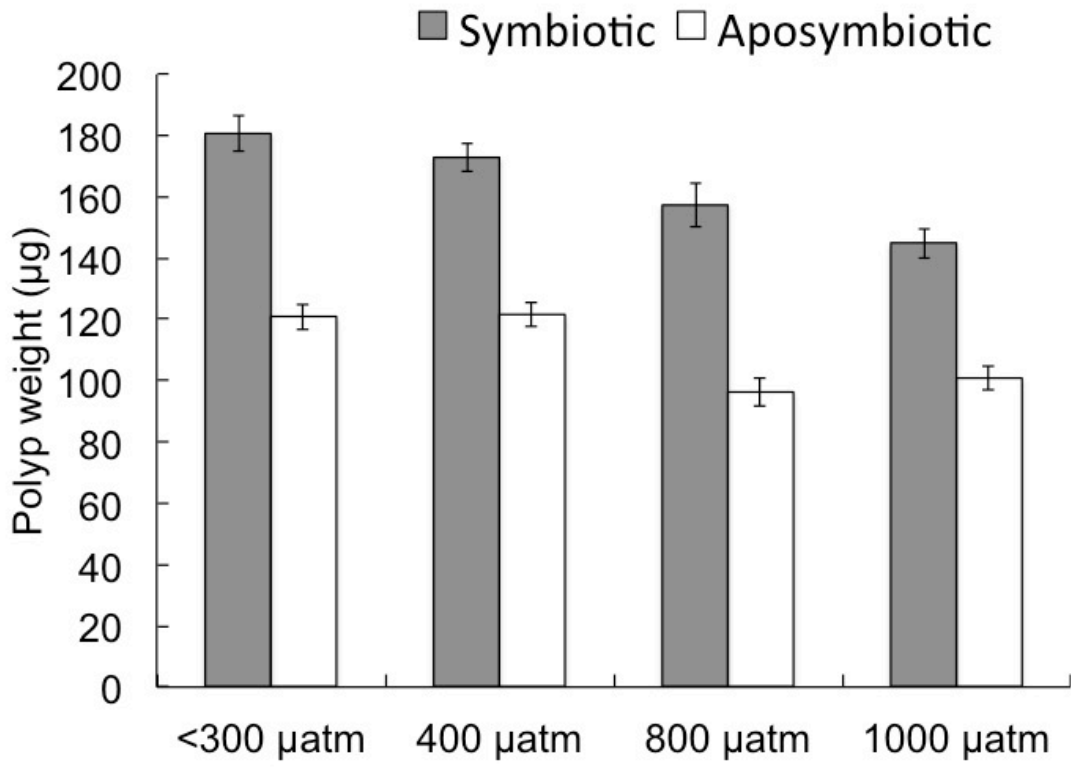
479 Figure 1. Skeletal weights of primary polyps of *Acropora digitifera* in the (i) pre-industrial
480 $p\text{CO}_2$, $< 300 \mu\text{atm}$, (ii) present-day $p\text{CO}_2$, $400 \mu\text{atm}$, and at two near-future conditions, (iii) 800
481 μatm , and (iv) $1000 \mu\text{atm}$. Bars show \pm S.E.

482

483 Figure 2. Adjusted mean final weights of coral fragments and mean F_v/F_m values from five
484 colonies (Colonies a - e) of *Acropora digitifera* in the five $p\text{CO}_2$ treatments ((i) pre-industrial
485 $p\text{CO}_2$, $< 300 \mu\text{atm}$, (ii) present-day $p\text{CO}_2$, $400 \mu\text{atm}$, and at three near-future conditions, (iii)
486 $600 \mu\text{atm}$, (iv) $800 \mu\text{atm}$, and (v) $1000 \mu\text{atm}$. Bars show \pm S.E.

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Figure 1

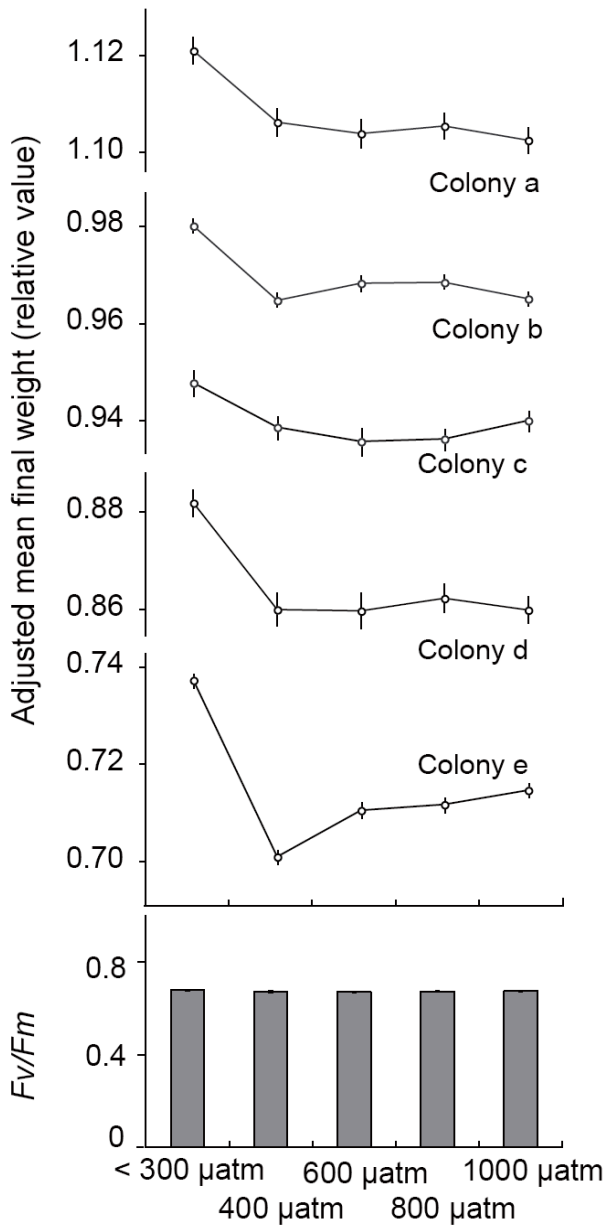


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497 Table 1. Summary of mean physical and chemical conditions in each $p\text{CO}_2$ treatment of the
 498 primary polyp experiment. Standard deviation is shown for pH_T and $p\text{CO}_2$. The mean salinity
 499 and total alkalinity measured repeatedly during the experiments were 34.6 and 2257 mol kg^{-1} ,
 500 respectively. The value of Ω_{arg} was calculated using total alkalinity and $p\text{CO}_2$. (i) pre-industrial
 501 $p\text{CO}_2$, $< 300 \mu\text{atm}$, (ii) present-day $p\text{CO}_2$, $400 \mu\text{atm}$, and at two near-future conditions, (iii) 800
 502 μatm , and (iv) $1000 \mu\text{atm}$. All values are shown as mean \pm standard deviation.

503	Treatment	Temperature ($^{\circ}\text{C}$)	pH_T at 25°C	$p\text{CO}_2$ (μatm)	Ω_{arg}
504	i) Pre-industrial	26.9	8.180 ± 0.009	242 ± 13	4.60
505	ii) Present	27.2	8.032 ± 0.008	390 ± 21	3.54
506	iii) $800 \mu\text{atm}$	27.2	7.801 ± 0.006	777 ± 9	2.22
507	iv) $1000 \mu\text{atm}$	27.3	7.743 ± 0.003	944 ± 13	1.93

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511 Table 2. Summary of mean physical and chemical conditions in each $p\text{CO}_2$ treatment of the
512 adult fragment experiment. Standard deviation is shown for pH_T and $p\text{CO}_2$. The mean salinity
513 and total alkalinity measured repeatedly during the experiments were 34.7 and 2236 mol kg^{-1} ,
514 respectively. The value of Ω_{arg} was calculated using total alkalinity and $p\text{CO}_2$. (i) pre-industrial
515 $p\text{CO}_2$, $< 300 \text{ } \mu\text{atm}$, (ii) present-day $p\text{CO}_2$, $400 \text{ } \mu\text{atm}$, and at three near-future conditions, (iii)
516 $600 \text{ } \mu\text{atm}$, (iv) $800 \text{ } \mu\text{atm}$, and (v) $1000 \text{ } \mu\text{atm}$. All values are shown as mean \pm standard
517 deviation.

518	Treatment	Temperature ($^{\circ}\text{C}$)	pH_T at 25° C	$p\text{CO}_2$ (μatm)	Ω_{arg}
519	i) Pre-industrial	27.0	8.143 ± 0.014	279 ± 13	4.21
520	ii) Present	27.1	8.040 ± 0.015	391 ± 18	3.47
521	iii) $600 \text{ } \mu\text{atm}$	27.1	7.896 ± 0.033	621 ± 24	2.56
522	iv) $800 \text{ } \mu\text{atm}$	27.1	7.793 ± 0.022	842 ± 33	2.05
523	v) $1000 \text{ } \mu\text{atm}$	27.1	7.719 ± 0.029	1048 ± 44	1.73

Table S1 ANOVA on skeletal weights of symbiotic primary polyp of *Acropora digitifera* under four $p\text{CO}_2$ treatments.

Factor	df	SS	<i>F</i>	p
$p\text{CO}_2$	3	23912.31	16.3206	<0.0001
Symbiosis	1	116758.83	239.0698	<0.0001
$p\text{CO}_2 \times \text{Symbiosis}$	3	1977.95	1.3500	0.2604
Error	152	74234.97		

Table S2 ANOVA on the fragment weight adjusted for initial size variation (W_{AFIN}).

	Factor	df	SS	F	p
Colony a	pCO_2	4	0.00055	3.65	0.017
	Aquarium (pCO_2)	5	0.00031	1.62	0.19
	Error	26	0.00099		
Colony b	pCO_2	4	0.0020	7.46	$<10^{-3}$
	Aquarium (pCO_2)	5	0.00072	2.13	0.084
	Error	36	0.0024		
Colony c	pCO_2	4	0.0016	19.10	$<10^{-7}$
	Aquarium (pCO_2)	5	0.000059	0.58	0.71
	Error	38	0.00078		
Colony d	pCO_2	4	0.0070	20.65	$<10^{-8}$
	Aquarium (pCO_2)	5	0.0010	2.27	0.066
	Error	39	0.0033		
Colony e	pCO_2	4	0.0035	11.90	$<10^{-5}$
	Aquarium (pCO_2)	5	0.00064	1.75	0.15
	Error	32	0.0023		

Table S3 ANOVA on arcsine transformed F_v/F_m values of coral fragments from five colonies of *Acropora digitifera* under five $p\text{CO}_2$ treatments.

Factor	df	SS	F	p
$p\text{CO}_2$	4	0.002791	0.9531	0.4342
Error	216	0.158138		

Fig. S1. Calcification rates of coral nubbins from five colonies (Colonies a to e) of *Acropora digitifera* in the five $p\text{CO}_2$ treatments. Calcification rates were presented in two ways: (A) percentage change in buoyant weight per day, and (B) increase of CaCO_3 weight in air per day. Bars show mean \pm S.E..

Figure S1

