Authors response to Anonymous Referee #1 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.

1. P 7015, L 26. A lot of work has emerged since the review of Atkinson and Cuet 2008, and now there are few people doing these kinds of studies with the addition of acid and base. Most people use CO_2.

>According to your suggestion, we have changed the related sentence as follows in the revised manuscript (p. 2, L. 41, uploaded as a supplement pdf): "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. The light levels for these experiments (75 µmol/m^2/s) are extremely low and some discussion of this issue is required. As the corals were collected from the reef flat, the parents probably would receive about ~1800 µmol/m^2/s and therefore the low light level is not ecologically relevant, unless the claim can be made that the recruits grow in dark places.

> Coral planulae, of most corals including *Acropora*, often recruit into low-light, cryptic habitats and as they grow extend into high irradiance environment. 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. Is there any information on the genetic identity of the *Symbiodinium* used in the study? Infection with heterologous algae raises some difficulties in evaluating the generality of the statements in this paper. Hopefully the type of symbionts in *Tridacna* are the same as those found in *Acropora*. Also, what features were seen with the dissecting microscope that indicated the symbiosis was established? It would be nice if there was histology to show the association.

> We used the same symbionts in our previous paper (Tanaka et al., 2013); therefore by citing the Tanaka paper, we add information on the genotype of symbionts used (*Symbiodinium* clade A, Tanaka et al., 2013) and the infection levels. We added the following sentence: "In the final day of the experiment, many symbionts (which were identical to the symbionts in Tanaka et al 2013) were observed in infected polyps" (p. 5, L. 102 in the revised manuscript).

4. Expressing growth of the branches as a % change makes it difficult to evaluate net deposition of $CaCO_3$ and to compare to previous work. It would be far batter to express the change in weight as change in dry weight and then standardize to a measure of the surface area of the corals (actual area or biomass).

> We added data on the increase of skeletal buoyant weight (%) per day as well as net $CaCO_3$ deposition rates (mg) per day in the supplementary data (Fig. S1). The difference between initial and final buoyant weight was also converted to dry weight of net $CaCO_3$ deposition using an aragonite density of 2.93 g cm^{-3} (Davies 1989). Seawater density was estimated from temperature and salinity during the measurements using the equation reported by Millero and Poisson (1981). We prepared size of coral branches as nearly equal as possible, and the initial skeletal weight of the all branches was 0.88 +/- 0.18 g (n=250). Ideally, the increase of skeletal weight is needed to standardize to surface area and/or biomass as suggested by the referee. We, however, think the data added in the supplementary materials would be informative to compare our data with other researches' data.

5. Some discussion of the 58% mortality rate of the corals is critical. Clearly

something was wrong with the incubation conditions and this could easily have affected the outcome of the experiments.

>In our experiment, we used 250 fragments and 29 died. Therefore the mortality rate was not 58%, but a relatively small value of 11.6%. We added the information in Materials and Methods of the revised manuscript.

6. Data analysis. Some discussion of the effects of pseudoreplication on the primary polyp work is required. For the branch analysis, I believe both tank (the nested effect) and colony (selected haphazardly) should be treated in the ANOVA as random effects.

>Thank you for valuable comments. For the primary polyp experiment, we incorporated the effect of symbiosis into the ANOVA model as a main effect according to your advice (See "2.4 Data analysis" of "2 Material and methods" in the revised manuscript; p. 7, L. 140). Also, we revised our manuscript by adding some sentences to the first paragraph of *Results* to mention the possibility of pseudoreplication (see p. 8, L. 167-171): "*Because gametes from two colonies were added to each aquarium, genetic differences could not be incorporated into the model. However, it is unlikely that this reverses our conclusion, because the error variance was small compared with the variance that was due to the main treatments effects in our data (see Table S1).*" For the branch experiment, we suppose that both *tank* and *colony* can be considered as a fixed-effect factor, because there is no reason to assume that these effects are normally distributed (i.e., random, and it is obviously unreliable to estimate these parameters using few data).

7. Page 7020, L 13 – not clear what "substrate medium" means.

>We revised the first sentence of "4 Discussion" section as follows (p. 9, L.185-): "The differences in the skeletal weights between primary polyps with and without symbionts might reflect the difficulty that aposymbiont corals have in acquiring energy and resources, including organic matrix molecules, for calcification."

8. The interpretation of Fv/Fv needs to be revised to be more conservative. The important work of Susanna Enriquez would be most helpful in this regard. Fv/Fm provides a very fine-resolution analysis of how PSII is functioning and the efficiency with which it harvests light and turns it into ATP and reducing agents of use in the Dark Reactions. Excluding any effect of photosynthesis on calcification because Fv/Fm was constant is a bit premature. Likewise the statements regarding photosynthesis in *Acropora* – effects on PSII do not (necessarily) translate linearly to C fixation.

> We appreciate the comment. We changed the description of Fv/Fm in regard of an additional reference on Fv/Fm interpretation (Enriquez et al. 2002). The second paragraph of "4 Discussion" begins with the revised sentence: "Higher calcification in the pre-industrial pCO_2 treatment was most likely attributed to a change in skeletal precipitation by the coral host, because there was no evidence of any dynamic photoinhibition (Enriquez et al. 2002) indicated as the decline in maximum photosynthetic quantum yield among the symbionts in the high-pCO_2 treatments (Fig. 2, Table S4)".

9. Page 7021, L 11. Arguably there has been evidence that zoox promote calcification in corals for nearly 1 a century. The key part is how they are/might be doing this.

>We added an additional description "although the detailed mechanisms have been under investigation" in the sentences (see p. 10, L. 217-).

10. Page 7022, L 10. This statement significantly oversteps what the present data can show. Given the limitations described above, this statement cannot be supported. At the very least, it cannot be written as fact, rather ".. these results suggest that recruitment might be effect, etc.."

>There are several papers supporting the explanation pointed above. In the revised manuscript, we cited the papers and added some explanation while that sentence was rephrased according to the referee's comment (p. 11, L. 239-247): "These results

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

11. Page 7022, L30. The results here do not suggest OA has been on going for 200 y.

> In the revised manuscript, we rephrased the sentence as follows: "Our results also suggest that ocean acidification has had adverse effects on reef corals since the industrial revolution. Ocean acidification, therefore, may not be only a future problem but a direct and present threat to ocean ecosystems (Talmage and Gobler, 2010)." (see p. 12, L. 264-266 in the revised manuscript).

Add refrence: Doropoulos C, Ward S, Diaz-Pulido G, Hoegh-Guldberg O, Mumby PJ (2012). Ocean acidification reduces coral recruitment by disrupting intimate larval-algal settlement interactions. Ecology Letters. 15, 338-346.

Title:

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

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Introduction

As humans are continuing to burn fossil fuels at an unprecedented rate, the concentration of CO_2 in the atmosphere is presently higher than it has been for the last 420,000 years (Hoegh-Guldberg et al., 2007; IPCC, 2007). The oceans uptake a large proportion of that CO_2, forcing them toward more acidic conditions (i.e., with high pCO_2), threatening the very foundation of calcifying marine organisms and coral reefs (Kleypas et al., 2006; Orr et al., 2005; Raven et al., 2005). Indeed, coral reefs support a wealth of calcifying organisms, of which scleractinians corals have been the most essential reef builder since the Triassic (Stanley and Fautin, 2001).

Since the pre-industrial period, we have witnessed a steady increase in pCO_2 concentrations around 100 µatm, which is predicted to reach 200 – 700 µatm above present values (400 µatm) by 2100 (IPCC, 2007). Such an increase in pCO_2 concentrations reduce both the pH and the concentration of carbonate ions in the water column, and increase the availability of bicarbonate ions (Kleypas et al., 1999). Several studies have found that coral calcification rates are directly related to the concentration of carbonate ions in the water column (Anthony et al., 2008; Gattuso et al., 1998; Kleypas et al., 2006; Marubini et al., 2008), whereas other studies have shown a positive relationship between coral growth rates and the availability of bicarbonate ions (Jury et al., 2010). 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). Therefore, the response of coral growth and the state of the ocean's carbonate chemistry is under intensive investigation (Pandolfi et al., 2011).

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 (~29 μmol kg^{-1}) than in the Southern Ocean (~18 μmol
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 pCO_2 seawater? And (ii) are juvenile corals, without symbionts, more
54 vulnerable to high pCO_2 seawater than juveniles and adult corals with symbionts?
55
56 Previous experiments that have mimicked the near-future pCO_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 and 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 pCO_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 pCO_2 concentrations in
66 flow-through conditions (Fujita et al., 2011).

Using this system, we examined the effect of pCO_2-adjusted seawater on the calcification rates of *Acropora digitifera,* one of the most common corals in the Pacific Ocean. Calcification was examined in five pCO_2 treatments: (i) pre-industrial pCO_2, < 300 µatm, (ii) present-day pCO_2, 400 µatm, and at three near-future conditions, (iii) 600 µatm, (iv) 800 µatm, and (v) 1000 µatm. Within these treatments, we investigated the response of: (1) primary aposymbiotic coral polyps (i.e., without symbionts), (2) primary symbiotic polyps, and (3) adult symbiotic fragments. It was hypothesized that the calcification process of symbiotic corals was more tolerant to pCO_2 adjustments than aposymbiotic corals.

2 Materials and Methods

2.1 Experimental setup

To produce pCO_2-adjusted seawater, we used a precise pCO_2 control system (Fujita et al., 2011). This system was used to generate five different pCO_2 levels, including one lower than the present level of atmospheric pCO_2: (i) pre-industrial, < 300 µatm, (ii) present-day pCO_2, 400 µatm, and at three near-future conditions, (iii) 600 µatm, (iv) 800 µatm, and (v) 1000 µatm. The pCO_2-adjusted seawater was supplied to duplicate flow-through (150 ml min^{-1}) aquaria systems (12 l). The seawater temperature was maintained at 27^{o}C, with a 12:12 h light:dark photoperiod (of 75 µmol $m^{2}s^{-1}$) under metal-halide lamps (Funnel2 150W, Kamihata, Japan) throughout all treatments. The aragonite saturation state of the seawater was estimated using the CO2SYS program (Lewis and Wallace, 1998) and the variables: temperature, pH, mean salinity, and total alkalinity were measured repeatedly during the experiments. The chemical and physical

89 conditions of each pCO_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 pCO_2 treatments: (i) pre-industrial, < 300 µatm, (ii) present day pCO_2, 400
106 µatm, (iii) 800 µatm, and (iv) 1000 µatm.

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 pCO_2-adjusted seawater
126 was supplied using the flow-through system (two aquaria per pCO_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 pCO_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 pCO_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: pCO_2 (fixed-effect factor), aquarium (nested within pCO_2;
145 fixed-effect factor), colony (fixed-effect factor), initial weight (covariate), and their interactions
146 ($pCO_2 \times$ initial weight, colony $\times$ initial weight, colony $\times pCO_2$, colony $\times pCO_2 \times$ 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 $pCO_2 \times$ 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 pCO_2. The adjusted final weight (W_{AFIN}) for each colony was independently analyzed using
153 an ANOVA model with pCO_2 (fixed-effect factor) and aquarium (nested within pCO_2;
154 fixed-effect factor) as the independent fixed factors. Statistically significant factors ($\alpha = 0.01$)

were subjected to pairwise comparisons (Tukey's HSD tests; $\alpha = 0.01$) to specify significant combinations of treatment levels. The *Fv/Fm* values of adult fragments were analyzed using a one-way ANOVA model with pCO_2 as fixed-effect factors after an arcsine transformation. The subsequent pairwise comparisons among different pCO_2 levels were performed using Tukey's HSD tests ($\alpha = 0.01$).

3 Results

The ANOVA indicated that the $pCO_2 \times$ symbiosis interaction was statistically negligible ($p > 0.05$) and the main factors were all significant ($p < 0.0001$). The post-hoc tests demonstrated that the skeletal weights at 300 and 400 µatm were significantly heavier than those at future-level treatments (i.e., 800 and 1000 µatm), regardless of whether polyps contain dinoflagellates or not (Fig. 1). When compared at the same pCO_2 level, the primary polyps with symbionts got heavier than those without dinoflagellates (Fig. 1). Because gametes from two colonies were added to each aquarium, genetic differences could not be incorporated into the model. However, it is unlikely that this reverses our conclusion, because the error variance was small compared with the variance that was due to the main treatments effects in our data (see Table S1). We evaluated the calcification rates of adult fragments of *A. digitifera* under five pCO_2 treatments. The ANOVA on the adult fragment weight adjusted for initial size variation indicated that a higher pCO_2 leads to significantly slower growth rates in four out of the five colonies (Colony b – e; Fig. 2; Table S3). The analysis also suggested that the potential environmental differences between two replicate aquaria were negligible in all five colonies (all $p > 0.05$). 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 pCO_2 conditions
178 in all of the four colonies, showing significant pCO_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 pCO_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 pCO_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 pCO_2 treatments. Moreover, the
192 calcification rates of symbiotic adult *A. digitifera* fragments were higher in the pre-industrial
193 seawater pCO_2 treatment than in the present-day pCO_2 treatment.
194
195 Higher calcification in the pre-industrial pCO_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-pCO_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 pCO_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 pCO_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 pCO_2
206 environments, whereas the primary polyps with symbionts did show reduced calcification rates
207 at high pCO_2. Such results suggest a hierarchical response in tolerance to pCO_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 pCO_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

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

Previous research indicates that acidified seawater increases the concentration of HCO_3^-, possibly followed by the activation of photosynthesis in coral symbionts (Jury et al., 2010; Marubini et al., 2008). In our experiments, however, there was no evidence that acidified seawater activates the photosynthesis of *Acropora digitifera*. The reason why the acidified seawater, with high pCO_2 concentration (1000 µatm), did not affect adult coral calcification and photosynthetic efficiency is unknown. We suspect that there were obvious advantages from symbiosis. For example, the removal of phosphates would facilitate calcification even in acidified seawater. Irrespective of the cellular mechanism involved, our results clearly showed that corals without symbionts were most vulnerable to pCO_2 increases, whereas corals that housed symbionts were more tolerant.

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

near future. 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). This inference on recruitment may be particularly evident in the Indian and Pacific Oceans where most corals are spawners that horizontally transfer symbionts (Harrison and Wallace, 1990), acquiring them after settlement. By contrast, newly settled corals may do better in the Caribbean where most corals are brooders and symbionts are maternally (i.e., vertically) acquired, and the planulae are symbiotic (Harrison and Wallace, 1990).

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

In summary, the increase in pCO_2 of just 100 µatm, between the pre-industrial period and the present, had more effect on the calcification rate of adult *A. digitifera* than the anticipated future increases of several hundreds of micro-atmospheres of pCO_2. Our results also suggest that ocean 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 pCO_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 pCO_2 diurnal variation of 680 – 290 μatm with seasonal variations in
270 reef water in front of Sesoko Station. Thus, the natural pH and pCO_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 pCO_2, < 300 µatm, (ii) present-day pCO_2, 400 µatm, and at two near-future conditions, (iii) 800
481 µatm, and (iv) 1000 µatm. Bars show ± S.E.

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483 Figure 2. Adjusted mean final weights of coral fragments and mean *Fv/Fm* values from five
484 colonies (Colonies a - e) of *Acropora digitifera* in the five pCO_2 treatments ((i) pre-industrial
485 pCO_2, < 300 µatm, (ii) present-day pCO_2, 400 µatm, and at three near-future conditions, (iii)
486 600 µatm, (iv) 800 µatm, and (v) 1000 µatm. Bars show ± S.E.

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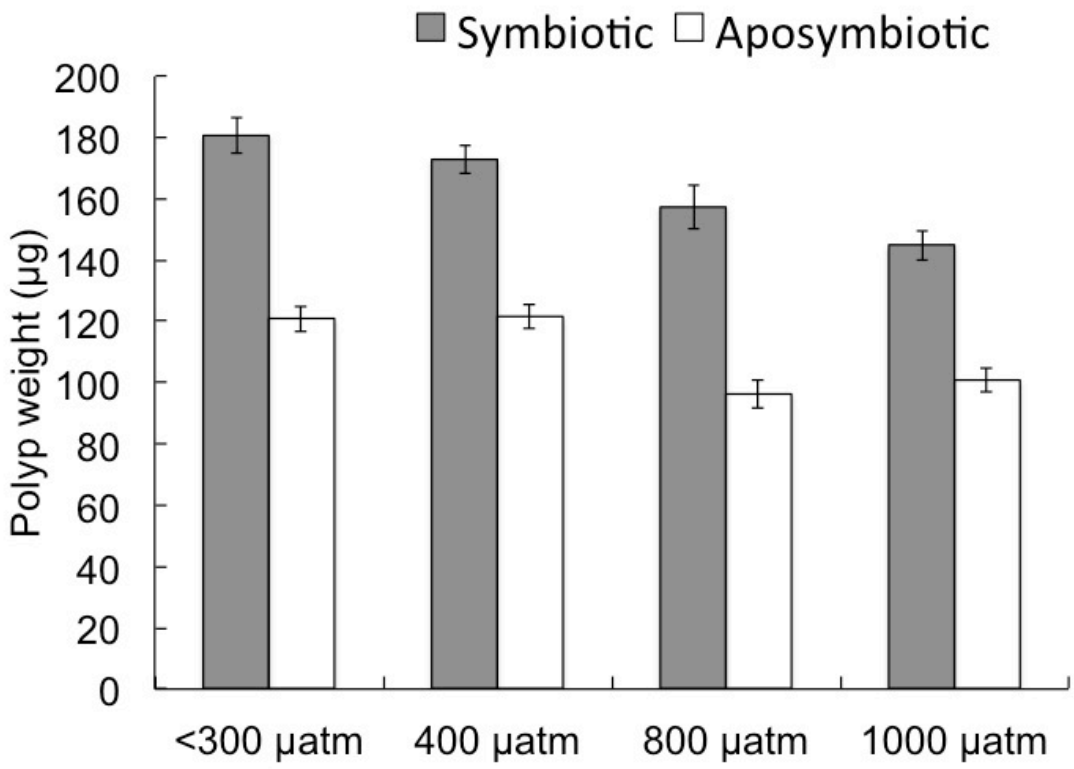
Figure 1



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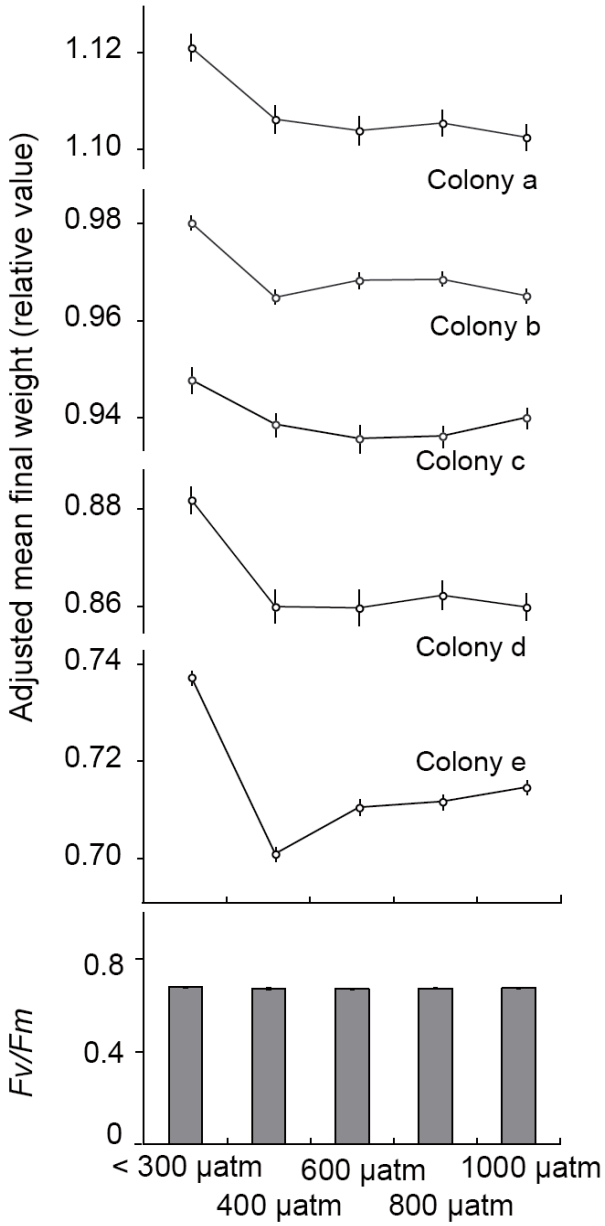

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Table 1. Summary of mean physical and chemical conditions in each pCO_2 treatment of the primary polyp experiment. Standard deviation is shown for pH_T and pCO_2. The mean salinity and total alkalinity measured repeatedly during the experiments were 34.6 and 2257 mol kg^{-1}, respectively. The value of Ωarg was calculated using total alkalinity and pCO_2. (i) pre-industrial pCO_2, < 300 µatm, (ii) present-day pCO_2, 400 µatm, and at two near-future conditions, (iii) 800 µatm, and (iv) 1000 µatm. All values are shown as mean ± standard deviation.

Treatment	Temperature (°C)	pH_T at 25° C	pCO_2 (µatm)	Ωarg
i) Pre-industrial	26.9	8.180 ± 0.009	242 ± 13	4.60
ii) Present	27.2	8.032 ± 0.008	390 ± 21	3.54
iii) 800 µatm	27.2	7.801 ± 0.006	777 ± 9	2.22
iv) 1000 µatm	27.3	7.743 ± 0.003	944 ± 13	1.93

511 Table 2. Summary of mean physical and chemical conditions in each pCO_2 treatment of the
512 adult fragment experiment. Standard deviation is shown for pH_T and pCO_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 Ωarg was calculated using total alkalinity and pCO_2. (i) pre-industrial
515 pCO_2, < 300 µatm, (ii) present-day pCO_2, 400 µatm, and at three near-future conditions, (iii)
516 600 µatm, (iv) 800 µatm, and (v) 1000 µatm. All values are shown as mean ± standard
517 deviation.

518 Treatment	Temperature (°C)	pH_T at 25° C	pCO_2 (µatm)	Ωarg
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 µatm	27.1	7896 ± 0.033	621 ± 24	2.56
522 iv) 800 µatm	27.1	7.793 ± 0.022	842 ± 33	2.05
523 v) 1000 µatm	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 pCO_2 treatments.

Factor	df	SS	*F*	p
pCO_2	3	23912.31	16.3206	<0.0001
Symbiosis	1	116758.83	239.0698	<0.0001
$pCO_2 \times$ 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}).

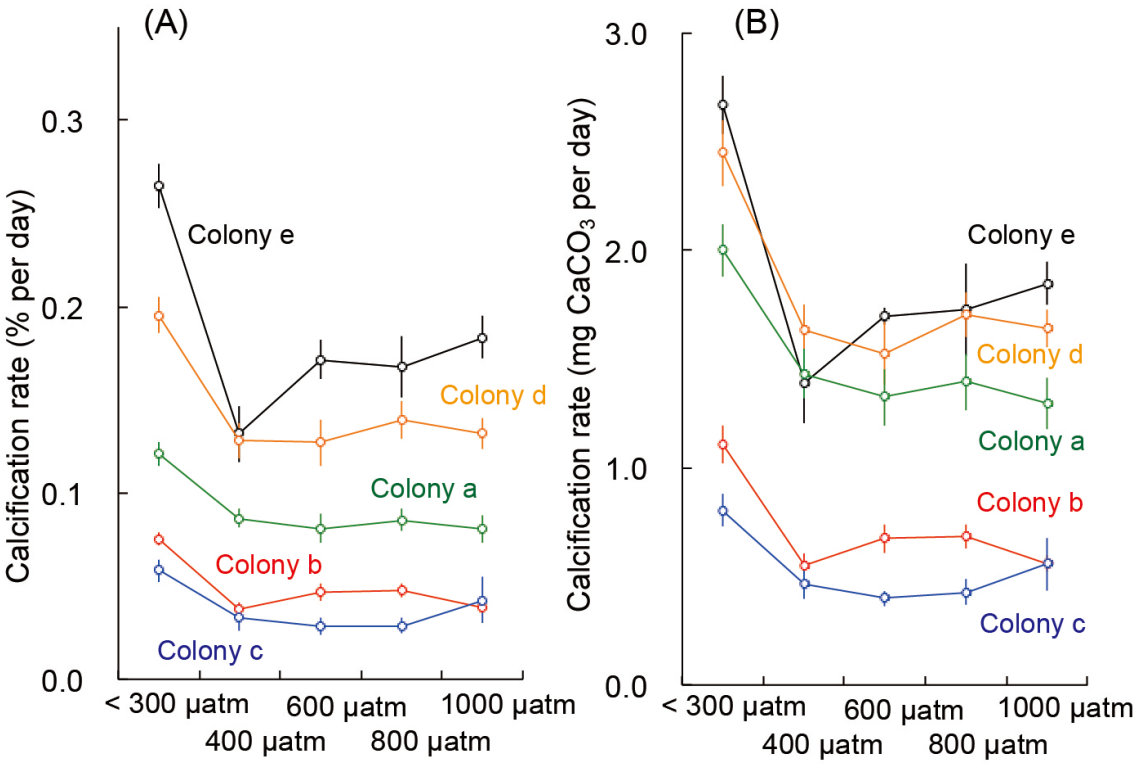
	Factor	df	SS	*F*	p
Colony a	pCO_2	4	0.00055	3.65	0.017
	Aquarium (pCO_2)	5	0.00031	1.62	0.19
	Error	26	0.00099		
Colony b	pCO_2	4	0.0020	7.46	$<10^{-3}$
	Aquarium (pCO_2)	5	0.00072	2.13	0.084
	Error	36	0.0024		
Colony c	pCO_2	4	0.0016	19.10	$<10^{-7}$
	Aquarium (pCO_2)	5	0.000059	0.58	0.71
	Error	38	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 *Fv/Fm* values of coral fragments from five colonies of *Acropora digitifera* under five pCO_2 treatments.

Factor	df	SS	*F*	p
pCO_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 pCO_2 treatments. Calcofocation 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 ± S.E..

Figure S1


(A)
Calcification rate (% per day)
0.3
0.2
0.1
0.0
Colony e
Colony d
Colony a
Colony b
Colony c
< 300 µatm
400 µatm
600 µatm
800 µatm
1000 µatm
(B)
Calcification rate (mg CaCO3 per day)
3.0
2.0
1.0
0.0
Colony e
Colony d
Colony a
Colony b
Colony c
< 300 µatm
400 µatm
600 µatm
800 µatm
1000 µatm