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Interactive comment on "Symbiosis increases coral tolerance to ocean acidification" *by* S. Ohki et al.

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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

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2008, and now there are few people doing these kinds of studies with the addition of acid and base. Most people use CO2.

>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/m2/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 âĹij1800 μmol/m2/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

BGD

10, C4507–C4512, 2013

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 CaCO3 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 CaCO3 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 CaCO3 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.

BGD
10, C4507–C4512, 2013

>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

BGD
10, C4507–C4512, 2013

pre-industrial pCO2 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-pCO2 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.

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> 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.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/10/C4507/2013/bgd-10-C4507-2013-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 10, 7013, 2013.