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Interactive comment on "Food availability and pCO_2 impacts on planulation, juvenile survival, and calcification of the azooxanthellate scleractinian coral, *Balanophyllia elegans*" *by* E. D. Crook et al.

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Reviewer #2

General Comments

Comment 1: One of the major problems with this paper is that the experiment lacks proper replication. Both larval planulation and juvenile growth experiments were conducted in 2 closed beakers including several individuals. Though these two beakers are replicates, each individual within the beaker are pseudo-replication. Since the sta-

tistical analysis used are not entirely sound, it is unclear the definition of replicates and how authors deal with the pseudo-replication problem. Also the result of ANOVA is not fully shown (ANOVA table would be informative), which makes difficult to evaluate the accuracy of the results

Reply: We have edited our methods and results sections to better describe our statistical analyses and demonstrate how we overcome the problem of pseudo-replication (we do in fact have proper replication in the form of 2 separate jars per experimental group). Additionally, we have now included ANOVA tables for each of the analyses (a total of 7 stats tables in all) that include all relevant information to this issue. These data tables make our statistics much more prevalent in the paper. If further confusion exists, we have included a complete description of the analyses used (and motivation behind them) here: For planulation and juvenile mortality, we were able to obtain only a single count per treatment (i.e., for each combination of pCO2 and feeding frequency) for both (1) the number of planulae released and (2) the number of juvenile deaths. Correspondingly, our ANOVA and logistic regression models for these analyses cannot, and do not, assess interactions between the two main factors – doing so would overparameterize the system and not allow for error estimation. Rather, we apply a simple additive model to permit error estimates and reasonable p-values to be obtained. Essentially, "replicates" for each factor occur across all levels of the other factor (e.g., estimates of pCO2 effects occur at both levels of feeding frequency). The assumption that there are no significant interaction terms are supported by supplemental tests: (1) for juvenile mortality, Tukey's Test of Additivity did not reject the assumption (p=0.41), and (2) for planulation (p=1). For weight and volume measurements, we address the pseudo-replication issue by including jars as random nested factors. To simplify calculations in R, we used aggregate data (the jar means of the measurement of interest) for the ANOVA analysis, as recommended by Quinn and McKeough (2002) and Ben Bolker (online discussion). This produces p-values identical to the full nested model for the fixed factors of interest (i.e., CO2 and feeding frequency). For these nested ANOVA models, pCO2 and feeding frequency are the main effects (fixed factors), jars

are random factors nested within the main effects, and individual corals within each jar provide error estimates. We have clarified our description of the nested models in the Methods section. For crystal length and width measurements, we were regretfully unable to match corals to jars (only treatments). We did include corals as a random factor nested within the main effects. Given that coral-to-coral variability was small, we posit that jar-to-jar variability had to also be low and that the significance of our results (especially for CO2; $p<0.001$) would hold regardless.

Comment 2: Another issue is that the seawater carbonate chemistry is not fully shown. (please add salinity, TA and nutrient data). Additionally, since the present study focused on the effect of ocean acidification on corals living in upwelling coast where the seawater chemistry (pH, CO2, DO, TA, temperature, salinity and nutrient) highly change with season, and the present experiment was conducted for 8 months including seasons of high upwelling, the seawater chemistry during the experiment is expected to have strongly oscillated. Therefore, detail information about the seawater chemistry used throughout the 8 months experiment is thought to be critical. Results and interpretation of the results also might be able to be improved by adding these information.

Reply: While we agree that the seawater chemistry along the CA coast may change dramatically with the season, we did not monitor nutrients, DO, and salinity throughout the 8-month experiment for several reasons. First, and most importantly, the seawater used in the experiment was filtered and collected in very large (several hundred liter) batches at 4 different points during the experiment (October, January, March, and May), which means that the water used was not influenced by daily, weekly, or even monthly fluctuations in nutrients, DO, salinity, TA, etc. While we did sample each of our "batches" of water for nutrients and salinity (in addition to our regular TA and DIC sampling), we did not regularly monitor these variables throughout the experiment, as the water was filtered down to 0.2μm and nutrient or salinity values were unlikely to change. Additionally, the water was ventilated during filtering and allowed to equilibrate with air in the room such that oxygen concentrations were at equilibrium with

the tank air (20%). Second, the experiment was not actually conducted during the upwelling season (summer) as the reviewer suggests. Our last batch of water was filtered in early May, and upwelling does not occur until the summer months. Finally, monitoring of nutrients was not a part of this experiment, as the corals are heterotrophs and do not have zooxanthellae that are directly impacted by nutrients in the water column. Therefore, monitoring nutrients would have added no weight to our results or discussion, as the water was filtered and we would not have been able to make the leap from "nutrients" to "nutrition" for our corals. Rather, we monitored nutrient levels in our batches merely for calculation of saturation state, which we have now included in the supplementary data table. We have added the TA, DIC, nutrient, temperature, and salinity data to the supplementary data table, as we too feel this is important. We have also added important information about the filtration of our large batches of water.

Comment 3: The method used for measuring the volume of the juvenile is unclear.

Reply: To make this clearer, we have included a new diagram in the supplementary material that highlights exactly how we took these measurements using calipers and the formula we used to calculate the volume of an elliptical cylinder. Additionally, we included more description in the text, which we hope eradicates previous confusion.

Specific comments

Comment 4: 7762 line 21-23 Description for the conclusion would be more informative than writing what you pretend to discuss.

Reply: We have revised the abstract to make it more concrete and include a data summary.

Comment 5: p.7763 line 22 More basic information about the interaction between nutrition and energetic resources and calcification would be helpful for better understanding the background of the present study.

Reply: In fact, we do discuss this in length in the third paragraph of the introduction

and include several relevant citations.

Comment 6: p. 7763 line 28 Please add more specific information about the seawater chemistry of coastal water around Monterey Bay. In what range seawater pCO2, pH alkalinity, temperature, DO, nutrient, salinity change, duration of upwelling, seasonal change etc.

Reply: While we do cite relevant papers in the introduction, and go into more specifics about nutrient upwelling in the discussion, we have added a short description of Monterey Bay upwelling in the introduction. We do not feel that a lengthy discussion of upwelling waters, including all of the chemical parameters, is relevant to our paper (for the aforementioned reasons), other than how upwelling impacts pH and nutrient levels.

Comment 7: p. 7765 line 1 What you mean by the sentence "coral's energy budget is fixed under normal circumstances"

Reply: We are referring to the fact that the studies mentioned have shown that corals are not able to increase their proton pumping to raise the pH of the calcifying fluid enough to maintain ambient rates of calcification in acidic conditions. We have tried to make this point clearer. "Normal circumstances" is in contrast to the next sentence, which states that when corals are given a surplus of nutrients in addition to low pH conditions, many are shown to maintain calcification rates at nearly 100% compared to ambient.

Comment 8: p. 7765 line 5 None of the papers referenced here sounds to be proper to indicate that "when provided with excess nutrients, some species can maintain 100% of their calcification rates despite under-saturation conditions". The paper such as Edmunds 2011 might be more relevant.

Reply: We do not understand how the papers cited are not relevant. This is one of the main conclusions of each of these citations. Nevertheless, we have added the Edmunds reference as we feel that it is also very relevant.

Comment 9: 7766 line 14-16 Please give any justification for the given amount of food as high food condition (3 day food) and low food condition (3 weeks food). Also please indicate how much amount of artemia was given every time. What was the density of the "concentrated Artemia nauplii"?

Reply: We have updated the manuscript to include an estimation of nauplii concentration used at each feeding ($\sim$10-15,000 nauplii per jar). As far as the separate feeding regimes, this is based on trial and error from our experience with B. elegans in the laboratory. As we mention in the text, they are able to survive for months without food, so we had to make sure that the "Low Food" group was fed minimally, while the "High Food" group received a maximum amount of nutrition (when they are very young, they can only eat a few brine shrimp at a time). As a side note, we have edited the text to "21 days" instead of "3 weeks" to keep our units constant.

Comment 10: p. 7767 line 12 Please describe briefly about the environmental condition and how the corals were maintained during the 2 years before used to the experiment.

Reply: We have inserted a brief description of the environmental conditions of the tanks at the marine lab, including how the water is filtered, where the water comes from, etc. The corals were not given any "special" treatment in the years leading up to the study- they were fed approximately once a week and their tanks were cleaned sporadically.

Comment 11: p. 7767 line 18 Please describe in more detail about the used seawater in the present study. From where the ambient seawater was taken? What was the seawater chemistry of the flowing ambient seawater during the collection of larvae? Are these flowing seawater used to prepare the seawater used for the following 8 months experiment?

Reply: We have now included a brief description of the ambient water system at Long Marine Laboratory. We previously reported the pH values of the flowing ambient seawater during the collection of larvae, but we did not specifically sample the wet-lab tank water for other chemical parameters. And yes, as we state in the text, we used

the wet-lab water to obtain our filtered seawater, and we have tried to clarify this point.

Comment 12: p. 7768 line 9 Please add results for the seawater DIC, TA

Reply: DIC and TA results are now reported, but in the results section, not the experimental set-up. They are also now included in Table 1 in the main text.

Comment 13: p. 7768 line 14 Show the seawater carbonate chemistry for the two experiments, Oct 2011- Jan 2012 and Nov 2011-July 2012, separately.

Reply: We are sorry about the confusion. Only the results from a single experiment are reported, because only one experiment was conducted. The experiment came in "2" parts because we tested (1) the impact of pCO2 and feeding on planulation (by placing adults in the experimental jars) and (2) the impact of pCO2 and feeding on survival, growth, etc. of newly settled juveniles. These two parts were run simultaneously in the same jars, and we have made this much clearer.

Comment 14: p. 7768 line 14 Please describe how the seawater salinity was measured and add the data in the result.

Reply: These results have been added to Table 1, but we have now included the laboratory instruments used for these measurements in the text.

Comment 15: p. 7768 I would like more information about the seawater chemistry throughout the 8 months experiment. As authors mentioned in the introduction, since the seawater carbonate chemistry in the coast of California, where the experiment have been conducted, highly change naturally by upwelling event (higher pCO2, low DO, high TA, high nutrient, low temp), information about the seawater carbonate chemistry (TA, pCO2, pH, salinity, nutrient and DO) used through the 8 months experiment would add important information. Additionally, I expect that the seawater pH might change seasonally due to the change of seawater chemistry (salinity, TA, nutrient) if you have controlled the pH by bubbling using a gas with constant CO2 concentration. Please explain that point.

Reply: This was mentioned in our response to the 2nd comment above. First, the experiment was not conducted during the upwelling season. Second, the water was filtered in very large (several hundred liter) batches at 4 different points during the experiment (October, January, March, and May) which means that the water used in the experiment was not influenced by daily, weekly, or even monthly fluctuations in nutrients, DO, salinity, TA, etc. While we did sample each of our "batches" of water for nutrients and salinity (in addition to our regular TA and DIC sampling), we did not regularly monitor these variables throughout the experiment, as the water was filtered down to 0.2μm and nutrient or salinity values were unlikely to change and DO was in equilibrium with the bubbled tank air (20% oxygen). Additionally, monitoring of nutrients was not a part of this experiment, as the corals are heterotrophs and are therefore not directly impacted by nutrients in the water column. Our water chemistry data table (a new Table 1) includes all relevant information to the calculation of saturation state, and gives standard deviations for each measurement, showing the range of data for each of the seawater batches (which incidentally were actually too small to change saturation state). In fact, the greatest change in saturation state occurred due to changes in temperature in the holding tanks, which were not necessarily "ambient" ocean temperatures (we did include temperature in the text previously as well as the supplementary data table, which we have moved to Table 1 in the text).

Comment 16: p. 7768 line 18 The volume of the corals were measured before or after removing the tissue? If the volume of the skeleton were measured, I am not convinced that this method (measuring by calipers the height and diameter) is accurate enough to measure the skeleton volume. I suggest that authors conduct different measurement principally because the difference between CO2 conditions seems to be very small and authors are concluding that the density of this coral are affected by CO2.

Reply: Skeletal volume was measured after tissue removal. We have made this clearer in the text. The complaint that the use of calipers was not accurate enough for the study seems invalid, as the differences between group averages (see data summary

in supplementary material) were at least 1 mm different between each of the groups. Therefore, a 0.1mm accuracy for measurement was more than sufficient. As stated previously, we have made the way in which we measured the volume of each elliptical cylinder clearer (we measured both the major and the minor axes in addition to height, and we therefore did not assume that each coral was a perfect cylinder). Additionally, we have added a diagram in the supplementary material if further confusion exists, which we hope that it does not.

Comment 17: p. 7768 Please add any reason why the sample for 770 μatm was excluded.

Reply: This sample was excluded due to instrumentation and monetary constraints. In order to observe any possible differences, we used the two most extreme cases (410 and 1230 uatm groups).

Comment 18: p. 7769 The statistics that the authors use to analyze their data are not entirely sound. Please write in detail the "additive model" applied in the present study of planulation.The coral settlement experiments have problem of pseudoreplication. Please write in detail how the authors work with the statistics to eliminate these problems.

Reply: As stated previously (see our reply to comment 1 above), we have tried to clarify our use of statistics in the text, and also included ANOVA tables in the text which should alleviate previous confusions.

Comment 19: p. 7769 line 17 What is the number of data for each results? Define the number of data used to calculate the mean and standard error for each results.

Reply: We have now included N-values in each of our data tables, as we feel that reporting each individual N-value at this point in the paper would be confusing.

Comment 20: p. 7770 line 7 I could not understand how these statics were conducted.

Reply: Please see description above in comment #2. As with other sections of the text,

a new data table has been added.

Comment 21: p. 7770 line 8 How the survival of juvenile corals was evaluated. The method is completely similar to that applied in the long term experiment?

Reply: There were not two experiments being run simultaneously, but rather one experiment with multiple parts (i.e. planulation and juvenile mortality occurred in the same jars, but at different time points). We have edited the text to make this clearer. For reference, juvenile coral survival was determined by counting mortalities as they occurred throughout the duration of the 8-month experiment. We have made this clearer in the text. Additionally, we have switched our wording from juvenile "survival" to "mortality" to fit with the statistics and the new data table.

Comment 22: p. 7770 line 15 Please add ANOVA table for all results

Reply: We agree that this is very important and have now included a new ANOVA table for each analyses.

Comment 23: Please add statistic result in the figures.

Reply: Statistics are now reported in the tables, figures, and text (as a side note, the figures did show error bars previously but we have made them more prevalent.

Comment 24: Image of the juvenile skeleton would be more informative than Fig. 1. Additionally SEM image of the skeletons at Fig. 4 also would be informative.

Reply: In figure 1, we have added an image of the juvenile skeleton, although we still feel that the previous images were important enough to keep. We have also added an SEM image to the supplementary material.

Comment 25: p. 7771 line 8 Why "early stages" of marine organisms in "upwelling regions" are "particularly sensitive" to OA? Or you mean the early stages of marine organisms in upwelling regions are "particularly susceptible" to OA? The all paper referenced here 'Kroeker et al. 2010, 2013, Hetting et al. 2012" seems to be not relevant

since though these papers describe the effect of OA on early stages, these papers are not evaluating the particular sensitivity of marine organisms in upwelling regions.

Reply: We are sorry for the confusion on this point. Actually, we are referring to the fact that upwelling regions experience low pH conditions, and therefore any organism living in an upwelling regime will be more susceptible to OA simply due to their geographic location. Therefore, the papers cited are in fact relevant, because we are referring to the fact that early stages are more sensitive to OA. Thus, early-stage organisms living in upwelling regions are in even greater danger of the impacts associated with OA. We have tried to make this point clearer in the text.

Comment 26: p. 7771 line 13-15 Why the data showing that the number of planula release increase at high food condition suggest that the female may delay the release of larvae until feeding condition become optimal?

Reply: Larvae are brooded over a 14 month period, and the females were subject to experimental conditions only at the time of planulation, not during the brooding process, so lack of food can only be attributed to larval release, not larval development. This suggests that the females had equal likelihood of producing the same number of larvae in each treatment, but something about the environment was not optimal for larval release. All other conditions being equal, food was the only factor that led to fewer larvae produced. We have tried to make this clearer in the text.

Comment 27: p. 7771 line 25 Justification for the conclusion that, survival of juvenile will decrease at high CO2 (pH 7.6), is enable to be qualified before explanation about the statistical and data analysis.

Reply: We agree that conclusions can't be drawn until stats are explained fully. Hopefully, our new description of the statistics as well as our data tables will eradicate this concern.

Comment 28: p. 7772 line 9 When you say size (volume) is that mean the volume of the

skeleton or skeleton + tissue? According to that the meaning of "density" will change. Since "food amount" is suggested to affect the tissue mass please clear that point. Additionally, if the volume of the skeleton has been measured, the way of measuring the diameter and height with calipers (+0.1mm) assuming that the juveniles are cylinders in shape sounds to be not enough accurate.

Reply: As stated above, volume refers only to the skeleton, and we made no attempt to measure the tissue mass. As mentioned previously (response to comment 16), we believe that our measurements are accurate enough for the aforementioned reasons.

Comment 29: p. 7772 line 15 I could not found results showing the "calcification rate". The value showed at Fig 3 b are calcification rate (g/y) or is just dry weight (g)?

Reply: We do not have a separate figure for calcification, as the plot would look exactly the same as the dry weight plot, just divided by the 8-month duration of the experiment to get g/yr. We feel that this figure would not have added any weight or visual impact to our discussion. We now clarify our reasoning in the text and reinforce that our discussion of calcification refers to the dry weight measurement.

Comment 30: p. 7773 line 22-29 If the aragonite saturation at calcifying fluid does not differ between low and high food concentration, what is the expected mechanism that "excess food counteract the negative impacts of low pH"?

Reply: Actually, we state in the text that High Food does in fact raise saturation state of the CF, just not enough to bring the saturation up to ambient levels. In fact, corals in the High Food group had crystal lengths that were 15% longer than those in the Low Food group at 1220μatm. We go on to mention that "This is consistent with the calcification results and may indicate that excess food enables corals to partially counteract some of the negative impacts of low saturation state." We do not claim that food completely negates the impacts of acidification, particularly at the crystal structure level. We attempt to further clarify our point in the discussion. However, we can remove the discussion of CF from the text entirely, as it is not critical to our discussion, and we

generally speak only of trends in the final paragraphs of the discussion.

Comment 31: p. 7773 line 8-10 This discussion seems to be contradict with the present data and previous discussion that the aragonite saturation was not elevated by food concentration.

Reply: Again, aragonite saturation state in the calcifying fluid was slightly elevated by food concentration, just not enough to bring the CF up to ambient levels. We have attempted to make these trends in the data clearer, although we can remove discussion of CF from the text if further confusion exists.

Comment 32: p. 7774 line 14-16 This discussion seems to be contradict with the sentence wrote at p. 7771 line 3 (early stages of marine organisms in upwelling regions are "particularly sensitive to OA).

Reply: We do in fact agree that these two statements are contradictory. We are trying to argue the point that when given excess food, B. elegans may be able to counteract negative consequences of ocean acidification on calcification by increasing their feeding rate (a finding that has been mirrored in other studies by zooxanthellate corals, including the Edmunds reference provided by the reviewer above). Their "success" (here we have switched our wording to "tolerability") in an upwelling region may explain their presence despite increased susceptibility. This is due to the fact that upwelling regions have greater concentrations of nutrients during certain times of the year, which may increase rates of grazing in corals. We have modified our discussion to make this argument clearer.

Interactive comment on Biogeosciences Discuss., 10, 7761, 2013.