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Biogeosciences Discussions

Interactive comment on "Response of *Halimeda* to ocean acidification: field and laboratory evidence" *by* L. L. Robbins et al.

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Authors response to Anonymous Referees #1, 2, 3

We appreciate the thorough reviews that reviewers #1, 2 and 3 have performed and the manuscript will be much clarified and improved thanks to their comments. Because reviewer #1 had the most extensive comments that were often reiterated by reviewer 2 and 3, we have addressed reviewer #1 comments below and refer to these later in addressing the other reviewer's comments. We have italicized the reviewer comments below and have addressed specific issues following individual comments.

Reviewer #1:

GENERAL COMMENTS

This study deals with the effects of a decrease in pH on the calcification of crystals (morphology and abundance) among different species of the calcareous green alga Halimeda, using scanning electron microscopy. The study comprises (a) results from a laboratory experiment comparing crystal shape and abundance of two species of Halimeda (H. opuntia and H. tuna) maintained at pH 8.1 and pH 7.5 and (b) observations of crystal shape and abundance changes over 40 years (from 1966-1967 and 2008) in two other species of Halimeda (H. incrassata and H. discoidea) obtained from archived samples. The authors showed a shift in crystal size and amount between pH: an increase in crystal concentration and a decrease in crystal width with decreasing pH. They also showed similar changes over a 40 year time period with an increase in crystal concentration and a decrease in crystal width from 1960s to 2008. The authors explained this previous change by a decrease in pH over the last 40 years.

Our paper merges results from two related projects. The first was a pilot study utilizing SEM examination of samples of *Halimeda* species that were observed in abundance at west Florida shelf locations and comparison of those observations with specimens from archived material available in herbarium collections from similar localities. We were asking if consistent, quantifiable differences in some aspect of calcification might be observable in *Halimeda* species – and discovered that they were. A separate study was examining *Halimeda* calcification in response to experimental manipulation of pH, so we decided to check the apical segments of the experimental specimens to see how they responded to pH differences.

The reviewer says that we *"explained this previous change by a decrease in pH over the last 40 years."* That is not true. We described the differences we quantified in the field-collected specimens and then compared them with what we saw in the laboratory specimens. We explained that the trends are consistent, but we do not specifically say that changes in pH over the last 40 years caused the changes.

While the main objective of this study, which is to combine both field and laboratory observations, is really interesting, major issues remain: (1) different species were used for historical data observations and laboratory experiment, (2) variations in pH tested in laboratory (_ = 0.6 pH units, pH 7.5 and 8.1,) do not reflect those from the past 40 years (_$<$0.1 pH unit), (3) there is no information on the environmental parameters,especially on the seawater carbonate chemistry, of the sampling sites and at the time of algal collection (ie. present and archived samples), and (4) there is no evaluation of the intravariability of crystal morphology and density in the sampling area of the west Florida shelf.

(1) Why did the authors use different species for the field work and the laboratory experiment?
The authors showed that Halimeda species present species specific differences in responses to pH changes: number and shape of crystals drastically increased and decreased, respectively, with decreasing pH, for H. opuntia, while these morphological parameters remained similar between pH for H. tuna. In this study, there was no laboratory investigation on the effects of pH on H. incrassata and H. discoidea (archived samples). Accordingly, from this study, we don't know if pH influence the calcification of these species. It is thus not possible to conclude if the morphological changes observed over the 40 year time period are due to pH or to an other environmental parameter.

As noted above, we were comparing the results of two separate studies, which used different species within the same genus. We were looking for quantifiable trends across the genus, *Halimeda*, not direct comparisons that X drop in pH produces Y change in crystal morphology. Since the reviewer clearly misunderstood, we shall more clearly state our objectives in revising our manuscript.

(2) One of the objectives of this study was to investigate the effects of pH between

1960s and 2000s. The decrease in pH over these last 40 years was very low. According to the shift in pH over the last 200 years of 0.1 pH units, the pH variation between 1966-67 and 2008 in the studied area is likely to be less than 0.1 pH units. Why did the authors choose an extreme pH value of 7.5 rather than a value of 8.2 to test their hypothesis? The variation of pH of 0.6 units (from 8.1 to 7.5) tested in the laboratory study is consequently far from that in the field over the last 40 years ($<$ 0.1 pH units).

As a consequence, it is difficult to understand how calcification response to less than 0.1 pH unit change (historical records; 48 to 70% crystal mean width decrease and 39-62% abundance increase) can be more drastic than the response to a shift of 0.6 pH unit (laboratory experiment, 18 to 43% crystal mean width decrease and 11-65% abundance increase). This point is likely to justify that crystal concentration and morphological changes in the field may be related to other environmental changes than pH.

Three points: 1) we did not say that the change in field-collected specimens was caused by pH change, but only that it is consistent with responses to pH change. 2) The experiment was not designed to test our hypothesis, but was rather a separate experiment to see if changes in calcification could be detected and that began with an admittedly extreme pH difference.

The change in pH on average worldwide has been about 0.1, but in specific areas, differences have been greater. Though pH changes through time on the west Florida shelf have not been documented, this region is the transition between tropical aragonite hypercalcification that occurs in south Florida and temperate conditions of more northerly areas of the Gulf of Mexico, which is our point about a natural laboratory. Moreover, unpublished data collected by the State of Florida for Tampa Bay shows a decrease of average pH from 8.23 in the 1970s to an average of 8.04 in the 2000s, a decrease of nearly 0.2 pH units in the last 40 years.

In addition, in natural seawater in equilibrium with the atmosphere in Hallock's laboratory, pH is consistently about 0.15 lower than 40 years ago. Recent studies in another laboratory have found greater differences in calcification between 8.3 and 8.1 than between 8.1 and 7.7. There is little evidence that calcification responses to pH are linear, rather, that they are likely not linear.

(3) I profoundly regret that the authors could not provide any information on the environmental parameters for the present and past sampling sites in order to provide a more robust test of their hypotheses. What was the evolution of seawater pH in the studied area over the last 40 years? What about the other environmental parameters, which can influence algal calcification, such as temperature, irradiance, seawater chemistry (total alkalinity, nutrient concentration, etc...), physical parameters (wave exposition, flow rate, etc...)? What are the effects of these factors on crystal morphology and density? Additionally, archived samples of H. discoidea used for comparison between recent and past effect of pH were collected at different season: ie. February for the year 2008 and December and August for the years 66-67. Is there any effect of seasonal pH fluctuations or other seasonal environmental changes on the concentration and morphology of crystals in the young apical segments that can develop in a few weeks?

The reviewer's comments are accurate – we could not access a time machine to take us back to 1966 to do water chemistry measurements on the west Florida shelf. As for different seasons, December through March are winter on the west Florida shelf – the interval in which we experience the coldest temperatures, lowest sunlight and highest wave energy. Therefore, comparing a December sample with a February sample does not represent "different seasons". In contrast, August is the warmest time in Florida, so the fact that the December and August samples were more similar in 1966-67 than the December samples were to the February 08 sample should represent a valid temporal comparison. We are revising the manuscript to more completely present our data, with interspecific, geographic, seasonal, interdecadal, and experimental results, which hopefully will address these objections.

(4) Before testing the effects of time on crystal calcification, the authors should have tested the influence of the other environmental parameters. They should have also tested the homogeneity in algal crystal morphology and concentration in the sampling area of the west Florida shelf.

Hindsight is always better than foresight and it is always easy to say what someone "should have" done after the fact. That "should have" is also purely the reviewer's opinion to which he/she is entitled. However, we knew from previous observations that *H. discoidea* and at least some other species with large, fleshy thalli, tend to be more weakly calcified with depth. The environmental parameters associated with that weaker calcification could be reduced temperature, reduced photosynthesis, higher respiration to photosynthesis rates, etc. With access to herbarium specimens, it was completely legitimate to ask the question "are there quantifiable differences between recently collected and 40-year old herbarium specimens from the same area". Some scientists may have access to 100 or 200 year old herbarium specimens, so our asking this question may prompt others with even older material to ask similar questions. Is that not a legitimate goal in science?

I suggest to the author to find and provide data in terms of carbonate chemistry and other environmental parameters in their study site from the last 40 years and to try to correlate their algal calcification results with each environmental parameters. I agree that their "approach provides a tool for predicting future changes" but it is pure speculation to write that "this approach allows evaluation of how ocean acidification has affected green alga" (Introduction, P. 4898, L. 27-28).

As noted above, we have not been able to find appropriate historical carbonate chemistry data for these west Florida shelf sites, so we simply sampled and documented what was available to us. The statement "this approach allows evaluation of how ocean acidification has affected green alga" was not meant to state that we proved influence of ocean acidification, but rather that we demonstrated a technique with promise. Since the reviewer clearly misinterpreted that statement, our objectives will be more carefully restated in the revised manuscript.

Moreover, this paper presents major flaws in the methodology, the results and the discussion sections. There is no information on the parameters of seawater carbonate chemistry, except pH. There was no replicate of culture conditions, only one aquarium at pH 7.5 and an other one at pH 8.1 (pseudoreplication). There is no information on the statistical test used to compare calcification results between sites, dates or pH. In addition, there is an absence of a constructive discussion based on the results: the two first paragraphs of the discussion section present a synthesis on Halimeda calcification. Only the third one, limited to 9 lines, is a discussion of the results.

The criticism of the experimental study is valid. Similarly, there is only one west Florida shelf, so samples from there are also pseudoreplicates. Thus, we reported only trends – i.e., means, standard errors, and percent differences. No statistical test results are reported other than visual representation of means and standard errors. As a result of this comment. we have rewritten the discussion section to include more based on the results.

SPECIFIC COMMENTS

Title

The content of the manuscript does not justify the title "Response of Halimeda to ocean acidification: field and laboratory evidence". I agree that the authors showed evident relationships between pH and Halimeda crystal calcification in the laboratory work but the morphological changes observed in the field samples are only dependent on time.The authors do not provide any information on seawater pH (or pCO2) at the sampling sites and the dates of algal collection; accordingly there is no field evidence that Halimeda responds to ocean acidification, this remains a hypothesis but can not be considered as an evidence.

See comments above. No long term information available at sites where the specimens

were collected.

Abstract

P.4896, L.2: delete "In fact". Done

Introduction

Reduce the part on "Halimeda response" which is disproportionate in comparison with the rest of the text and include it in a unique Introduction part. Be more focused on the objectives of this study and present the hypotheses tested. Done

P. 4897, L. 26: Delete fig 1. Picture of Halimeda to illustrate the purpose is useless. Done

P. 4898, L. 24-27: "Our study...with laboratory experiment to simulate predicted carbonate chemistry...in the future". Replace "carbonate chemistry" by "pH": the experiment do not simulate predicted carbonate chemistry but only predicted pH since there is no information on the other parameters of the carbonate chemistry (total alkalinity for example). Done

P. 4898, L. 27-28: I agree that this "approach provides a tool for predicting future changes" but not that "this approach allows evaluation of how ocean acidification has affected green alga" over the last 40 years. Delete this part of the sentence. This sentence has been changed.

P. 4899, L. 2: Which samples were from 1980s? There is no reference to these samples in the Mat & Met and results sections. We have rewritten this section.

Material and methods

P. 4899, L. 10-14: Where the archived samples exactly come from? Specify and give geographic coordinates. Since there is a strong interlocality variability, explain why the locations of the samples were not exactly the same: ie. "Notably, we used samples from the west Florida shelf, off Tampa Bay". Give information on the distance between *sampling sites in this bay. Are the environmental parameters homogenous in this bay?* We have rewritten this section to be clearer where samples came from (outside Tampa Bay). See also comment below.

P. 4899, L. 10: Give the names of the species of archived Halimeda samples. Done

P. 4899, L. 17: Delete Fig. 2 and replace it by the exact geographic coordinates. Done

P. 4899, L. 17: Which species are "purchased from a dealer", H. tuna, H. opuntia or both? Did you have accurate information on the site where the dealer collected the samples? Specify the accurate date of collection both for samples collected in the field and those purchased from a dealer. We have rewritten this to be clearer on where samples were obtained.

P. 4899, L. 17: Why did the authors used different species in the laboratory study (H.tuna and H. opuntia) and in the field study (H. incrassata and H. discoida). Where the two previous species absent at the time of collection? The fact that different species are used in the laboratory study "because of their availability" (P. 4899, L. 18) is not a good argument to justify the choice of the species. Explained below and clarified in text.

P. 4899, L. 18-19: How could you affirm that "the specimens were obtained from sites with pH 8.1"? Did you measure pH? Provide more accurate information on the pH of the sampling site (pH scale, pH variability). We have rewritten this section for clarity.

P. 4899, L. 21-22: Why did the authors present "only the apical data" in this paper? We have explained this in the text.

P.4900, L. 2: Why did not the authors use natural seawater from the study site but "artificial seawater"? Is it an open system or close system? What is the seawater renewal rate in the tanks? All was clarified in the text.

P.4900, L. 3: Delete Fig. 3. The picture of laboratory experiments already described in the text is useless. We eliminated this picture.

P.4900, L. 3: The aragonite sand substrate contained in the tank can influence carbonate chemistry of seawater in the tanks, especially total alkalinity. The aragonite sand is likely to dissolve faster at pH 7.5 than at pH 8.1 and to cause differential total alkalinity values in the tanks. Accordingly the authors must provide total alkalinity values in their tanks. The calcification of their algae may be profoundly affected by seawater alkalinity. Agreed. We have discussed this in the text and added alkalinity values.

P.4900, L. 7 and 11-12: Specify the variability of pH associated with the mean values of pH 7.5 and 8.1. Were pH values continuously recorded? See comments below.

P.4900, L. 7 and 15-16: Precise which buffers were used for total hydrogen ion concentration pH scale? Clarify and justify the use of two different calibrations, this is unclear in the text. We clarified this in the text.

P.4900, L. 7 and 18-19: The calibration accuracy was confirmed according to Dickson et al. (2007), did you used Dickson standards? Did the authors apply all the recommendation provided by Dickson et al. for spectrophotometric measurements: ie. high quality spectrophotometer, temperature control system for spectrophotometer cell, thermostated bath to within $\pm$0.05_C, etc... Be more specific. We clarified this in the text.

P. 4901, L. 6: Precise which version of Image J software? We noted the version in the revised manuscript.

P. 4901, L. 6: Justify the use of different size of area (10$\times$10 μm and 5$\times$5 μm) to count the crystals. We rewrote this for clarification.

P. 4901, L. 9: Delete the name of the functions used in the software (ie. "measuring tool"). Done.

Results and discussion

Provide two distinct parts. Excellent idea.

Provide information on statistical tests performed to compare calcification data between sites, dates and pH! Are the differences ("differences were observed", "demonstrated a decrease", "showed a trend", etc...) significant or not? See comments below- we have also clarified this in text.

P. 4902, L. 7: Delete "for example". Done.

P. 4902, L. 13: Replace "Fig. 5 a-d" by "Fig. 5". Done.

P. 4902, L. 16-17: Present the results from 1990 and 2000 directly on Fig. 6. Provide standard errors associated to the means in the text. Done.

Discussion

*The two first large paragraphs are only a bibliographic synthesis on Halimeda calcification without any direct relationship with the results. Only the third paragraph (9 lines) discusses the results obtained in this study.*We have rewritten this section.

P. 4903, L. 16: Delete Fig. 10, unuseless .We deleted this figure.

P. 4904, L. 22: Replace "pCO2" by "pH" since there was no measurements pCO2 in this study. Done.

These are all good comments that will be utilized in revising the paper. Specifically:

a) The title will be changed to reflect the observational and methods

b) Recommended word changes will be made, where appropriate. In many cases, whole sentences will be rewritten, often as suggested by all three reviewers.

c) Methods will be more explicit, including names and localities of specimens. Reviewer #1 erroneously thought specimens came from Tampa Bay, so we will rewrite this section to explicitly list localities outside of the bay on the Florida shelf. Our pH stat experiment did not allow us to measure variations in pH, how-

ever, we do have data on the alkalinity of the experiment that we have included in the manuscript.

d) Results (as noted above), will be more comprehensive.

e) We disagree that details concerning *Halimeda* morphology and physiology are unnecessary. However, again, since the reviewer suggests they are, we shall revise the Introduction and Discussion to better integrate those details into the issues we are addressing.

f) We agree that much more experimental testing of possible causes of the trends that we observed are needed. The basic question, which the Editors will have to make, is "are the trends we observed worth reporting at this time so that others can test our hypotheses and perhaps utilize the parameters we quantified?"

Comments of the other two reviewers are consistent with the first reviewer and comments above are pertinent (especially reviewer #2 and 3's comments about historical data on water chemistries). In several cases, additional points made by these reviewers have been incorporated into the revised text, such as explaining that no preservatives are used in herbarium preparations (comment by reviewer 3). That reviewer's (3) comments indicate that even more details about algae, and *Halimeda* in particular, are needed for most readers to fully understand the scope of the work, contrary to Reviewer #1's comments that too much detail about *Halimeda* is included.

Interactive comment on Biogeosciences Discuss., 6, 4895, 2009.