

## **Response to Referee #1 (Inge van Dijk) Comments**

### **Referee #1 General Comments:**

**Comment 1: The manuscript ‘Impact of carbonate saturation on large Caribbean benthic foraminifera assemblages’ by Martinez and co-authors aims to show the response of the benthic foraminiferal community to by using natural low pH low calcite saturation submarine springs. They show calcareous and agglutinating foraminiferal abundances decrease, but the calcareous non-symbiont bearing species seem to be impacted the most. The manuscript is reasonably well written, and the results are in line with some other similar studies, but I have some concerns about the methodology that could impact the observed trends. Especially lumping porcelaneous and hyaline species together and only using the larger fraction (>250 µm) might bias some of the results. The discussion could use some restructuring and extra depth, by for instance analyzing trends porcelaneous and hyaline species, adding size normalized weights of *Discorbis*, exploring the effect of salinity and different symbiont species.**

Reply: We thanks Dr. van Dijk for recognizing the importance of the study and we appreciate the suggestions to include a more detailed analysis of the foraminifera data. We have included new analyses and interpretation of abundance of porcelaneous and hyaline foraminifera and of symbiotic diatom-bearing and chlorophyte-bearing foraminifera in the new version of the manuscript. We have also included an explanation of why the >250 µm size fraction was used in the analysis and a deeper discussion of the effects of salinity on foraminifera. Although we do not report size normalized weights we did make an effort to select individuals that were similar in size as much as possible and re re-weighted many samples to see if this changes the results which it did not. Regardless we acknowledge this shortcoming of not normalizing the weights. The updated discussion was structured in paragraphs to facilitate readability.

### **Major comments:**

**Comment 2. Page 3 line 14: What kind of substrate was present and was there a difference in substrate near the vents and at the control site?**

Reply: The substrate is coarse sand at all locations, and control and ojo sites were only a few meters apart. We have included this information on the methods section.

**Did you include plants (some benthic species prefer to live on plant debris)?**

Reply: We did not sample plants specifically and any epiphytes that are included were in the upper sediment. Because there are no grass beds right at the springs, we set the control sites to be as similar as possible to the ojo sites avoiding grass beds as this made sense for more direct comparison.

**Did you apply rose bengal staining to only analyze living specimens?**

Reply: We used Rose Bengal but pretty much everything got stained to some degree and it was hard to distinguish dead from live using this stain. Rose Bengal can stain proteins of dead specimens that are not fully decomposed, or proteins of bacteria inside or on the tests, producing

false positives that overestimate abundance of foraminifera (Bernhard et al., 2006; *Paleoceanography*, vol. 21, pa4210, doi:10.1029/2006PA001290, 2006). In addition, it is hard to distinguish the stained specimens in some species with opaque tests such as *Archaias angulatus* (Wantland, 1967). We also tried CellTracker Green but, in this case, only very few forams got stained so that was not useful for the statistical analysis.

**Comment 3. Page 3, line 15: Why did you choose 250 um? Normally 125-150 um is used (Schonfield et al., 2012: Marine Micropaleontology, 94–95), since you might miss the trends in the smaller community now. The trends you observed might be true for larger specimens, but perhaps the smaller specimens tell a different story...**

Reply: We focus on the large size fraction and clearly note this in the title because this size fraction constituted the majority of foraminifera in the samples. Indeed, many foraminifera typically found in tropical lagoons attain large sizes and have mortality rates of above 95% of juveniles until they reach a diameter of 0.5 mm (*Why are larger forams large? Hallock, 1985, Paleobiology*) which may explain the low abundance of the smaller sized forams in our samples. We now described in the methods section that analyses of the <250 um fraction we found only 9-27 specimens per gram sediment while in the >250 um fraction around 300-500 specimens were found. The fraction of >250 represents the adult individuals more prone to be preserved in the sediment (Martin, 1986). We have included an explanation of the size fraction selection in the methods section.

**Comment 4. Page 4, line 13-14 I am not sure about ‘lumping’ low Mg forams together with porcelaneous in one group, since it is known from countless studies they respond different to increased pCO<sub>2</sub>, perhaps due to e.g. solubility of high MgCO<sub>3</sub>. Did you check if both hyaline and porcelaneous species in this group show similar trends? Otherwise you might be skewing your results, especially since you see no significant change in weight of shells of *Discorbis*. I would also be very interested to see (relative) abundances of low (e.g. *Discorbis*), intermediate (*Amphistegina*, *Astergerina*) and high Mg species (*Quinqueloculina*, *Archaias*) between ojos and control. It would bring something new to the existing studies on different sites, especially since you have the opportunity to test it here on species with very contrasting Mg content.**

Reply: We have included the absolute and relative abundance of porcelaneous and hyaline foraminifera as well as of low, intermediate and high magnesium foraminifera in the new version of the manuscript.

**Comment 5. Discussion section: The authors do not (clearly) explain why the abundance of agglutinating foraminifera decreases at the vents. They do not calcify or have symbionts, so the explanations given to explain the calcareous response (proton pumping and symbiont activity) do not apply. Could salinity play a role?**

Reply: The absolute abundance of agglutinating foraminifera did not differ with saturation state in 3 of the 5 sampled submarine springs and we note that in the paper. The relative abundance was higher at low saturation than at high saturation at one site and did not differ in the other 4 sampled sites. We have rewritten these results in a clearer way and we have discussed why agglutinated foraminifera are not sensitive to carbonate saturation as the reviewer indicates. We

also explained why we think salinity is not driving changes in abundance of any of the foraminifera at the springs. In addition, sensors deployed at the springs showed that salinity is >30psu over 90% of the time and it does not drop below 27psu at the sites we sampled. When salinity drops below 30 psu (7% of the time), the low salinity exposure lasts for very short periods of time always less than 1 hour (*Crook et al., Supporting Information, PNAS July 2, 2013 110 (27) 11044-11049; <https://doi.org/10.1073/pnas.1301589110>*). Based on literature the majority of forams we found have very wide salinity tolerance as they are common in settings that have variable salinity such as close to shore and in lagoons.

**Comment 6. Page 7 line 22-29 The authors missed a big overview study by Doo et al., 2014 (Biol. Bull. 226: 169–186.) in which they present a nice overview of response of larger benthic foraminifera to ocean acidification. I think their discussion would benefit from including these observations. For instance, to look at the different kind of symbionts (diatom, dinos) your foraminifera species have and if they follow the general trend of Doo et al., 2014. It would also be informative to add an overview of the response of benthic foraminifera (symbiont/non symbiont) in different studies, like in Keul et al., 2013 to show how your data fits laboratory and field experiments.**

Reply: We have now included the absolute and relative abundance of diatom-bearing (*Ampistegina* and *Asterigerina*) and chlorophyte-bearing foraminifera (*Archaias*). The studies included in the review by *Doo et al., 2014* did not study chlorophyte-bearing foraminifera, therefore we cannot compare our results to the trends seen in other studies that only focused on diatom and dinoflagellate bearing foraminifera. We have included discussion on the potential effects of symbionts on foraminifera calcification.

### Minor comments

**Comment 7. Throughout manuscript pCO<sub>2</sub> (p in italics)**

Reply: We changed p to italics throughout the text.

**Comment 8. Page 2 line 9-10: Keul et al., also contains a nice overview of species-specific responses**

Reply: We have added this relevant reference in the introduction.

**Comment 9. Page 2 line 29: Do other chemical parameters change between ojos and control? Oxygen, sulphates?**

Reply: There are some relatively small differences between ojos, for example the water discharging at ojo Norte has lower oxygen and it is slightly more reducing than the other ojos during very low tide conditions. However, we do not have replicates of ojos that differ from each other (in fact Norte is the only that is slightly different than the other ojos) hence we cannot do a comprehensive analysis on the impact of these differences in chemistry. Specifically, we did not see any unique trends at ojo Norte hence we do not attribute this to the small difference in water chemistry. Regardless we emphasize throughout that there are advantages and disadvantages to conducting field observation with the main issue is that there are confounding variables but on

the other hand the results we obtain cannot be replicated in laboratory settings and are more realistic.

**Comment 10. Page 3, line 08-10: It is more common to use the K1 and K2 values from Lueker et al., 2000. I would suggest recalculating your carbonate parameters with these, since Millero (2010) are known to cause discrepancies in the results amongst programs (for details see Orr et al 2015). Please also specify in more detail what constants were used for carbonate system calculations. For example, what term was used for KHSO<sub>4</sub>? Dickson (1990) is commonly used.**

Reply: Thanks for this important suggestion. We recalculated the carbonate chemistry parameters with K1 and K2 from Lueker et al. 2000 and included a more detailed description of the constants used (KHSO<sub>4</sub> from Dickson 1990 and total boron from Uppström, 1974) in the methods section.

**Comment 11. Page 3, line 14-17: How much gram of sediment was counted?**

Reply: At least 1 gram of sediment and on average 2 grams of sediment (per replicate) was analyzed. We have inserted this information in the methods section.

**Comment 12. Page 3, line 20-23: Even though only specimens from 250-355 um were picked, the test weights have to be normalized for size to be able to compare between sites and studies.**

Reply: We agree with the reviewer that this would be useful. However, we have not done this and in an attempt to resolve this issue we re-analyzed 7 representative samples of the 50 sediment samples we collected for this study (5 replicates at 5 ojos and 5 control sites) normalizing to size, and still did not find any statistically significant difference in the weight. It seemed to be a major waste of time and effort to re-analyze again all 50 samples. We report on that and acknowledge the need to do so in the manuscript.

**Comment 13. Page 4, line 7: There is no seasonality in the output/flux of the vents?**

Reply: Yes, there is an increase in groundwater discharge during the rainy season and during low tide. We refer to a paper that describes the variability at the site. We note however that the foraminifera in the upper sediments represent decades or longer and these organisms grew under all the different conditions at the sites.

**Comment 14. Page 4, line 24-26. The abundance of agglutinating foraminifera is very low already in the control sites. Do you think the numbers are high enough to make big statements of agglutinating foraminifera being more resilient to low calcite saturation state?**

Reply: It is true that the abundance of agglutinating foraminifera is very low already in the control sites and most likely the numbers are not high enough to make big statements of agglutinating foraminifera; however, our data is in agreement with other high pCO<sub>2</sub> field studies

in Papua New Guinea (*Uthicke et al., 2013*) and Italy (*Dias, 2010*). We note that they are rare but still report on the results as we think this is useful.

**Comment 15. Page 5, line 31: Fig 4 not 5**

Reply: Thank you for spotting this mistake, we changed it in the newest version of the manuscript.

**Comment 16. Page 5, Line 17-20-21: Fig 5 not 4**

Reply: We changed this mistake in the newest version of the manuscript.

**Comment 17. Discussion: The discussion needs some restructuring, perhaps adding paragraphs might help?**

Reply: As the reviewer suggested the discussion was divided into different sections to improve the organization of information.

**Comment 18. Page 6 line 25: 3-6 units is in my opinion not a 'slight' but a big difference and should be taken into account or at least discussed**

Reply: We have included further discussion on salinity effects to make it clearer in the manuscript (see reply to comment 5).

**Comment 19. Page 8, line 7-10. There is also evidence from culture experiments showing very species specific response of agglutinating foraminifera with pCO<sub>2</sub> (e.g. van Dijk et al., 2017, JFR).**

Reply: Thank you for the suggestion. This is a relevant paper that has been included in the discussion of impacts of carbonate saturation on agglutinating foraminifera.

**Comment 20. Page 8, line 19-25 This is not really discussed in detail the discussion and has therefore no place in the conclusion. Could you add a paragraph on this in the discussion section.**

Reply: We moved and rewrote these lines in the discussion section.

**Comment 21. Table 1: check number of decimals for consistency. Why is there no error on calculated CO<sub>2</sub>sys values, you could apply a propagating error.**

Reply: We updated the number of decimals for consistency. We included the std on the actual reported values that we measured in the field using these values if anyone is interested the error of the calculated values (pH and carbonate saturation) could be determined. However, we did not include this because the difference between the ojos and control are so large that this will not really change any of the conclusions and discussion.

**Comment 22. Figure 3: Top three panels: Can you put the 0 on the intersection between y and x axis?**

Reply: The new plots have now the same Y-axis scale and are all aligned at 0.

**Is it possible to order the ojos from e.g. South to North or vice versa?**

Reply: The sites are now organized from North (Norte) to South (Gorgos) in plots and tables.

## Response to Anonymous Referee #2

The paper by Martinez et al. describes a very interesting study in which natural variability in carbonate saturation state at submarine springs (ojos) is used to assess the benthic foraminiferal response to ocean acidification. The authors find that proximity to submarine springs impacts the benthic foraminiferal community. In particular, they find a decrease in overall abundances, but also that symbiotic calcareous species are less affected than non-symbiotic bearing species, and agglutinated foraminifera may be least impacted. The paper is overall well written, and the conclusions are interesting. However, in some areas, the complexities and richness of the underlying dataset could be better served. Digging further into some of the complexities here should allow the authors to better support their current conclusions, but I suspect it will also lead to some more specific and novel results. Overall, there are three major (somewhat related) issues, which I would strongly urge the authors to address.

Reply: We thank the reviewer for noting that the paper is interesting and original and suggesting areas for improvement which we have addressed in the revised version.

**Comment 1) First, is the assumption that proximity to “ojos” impacts benthic foraminifera entirely or primarily due to difference in calcite saturation state from the ambient environment. This does not seem like a foregone conclusion to me. These are essentially isolated regions of increased fresh-water influence in a marine context and could be different in a number of ways from the surrounding environment. The authors mention, but then rapidly dismiss, the salinity differences between the ojos and ambient environment (6:24-28). However to entirely dismiss salinity requires either a more detailed quantitative analysis to try to tease apart these covarying parameters, and/or a more in depth discussion of the known sensitivities of different foraminifera and communities to salinity. There could be several additional environmental differences, such as oxygenation, or changes in nutrient or metal concentrations from terrestrial sources that could produce sensitivities in some species (I might look into the literature on benthic foraminifera communities as tracers of metal contamination). Finally, there could be differences in benthic community or environment (substrate? Food source? predation?) between the ojos and control sites. All of this should be discussed.**

Reply: Sensors deployed at these specific ojos determined that salinity is above 30 for 93% of the time, and when it drops below 30, it is for short periods of time of less than 1 hour and does not fall below 27 (Crook et al., *Supporting Information, PNAS July 2, 2013 110 (27) 11044-11049*; <https://doi.org/10.1073/pnas.1301589110>). We emphasize this and refer to the above study. In addition, we note that many of the species in our study have a very broad salinity tolerance range as typical to shallow coastal lagoon and estuary settings. We have included the salinity sensitivities of the major species in our study for which data is available. We also compared our results to results from laboratory experiments where only carbon chemistry is changed to support our idea that the carbonate saturation is the main driver of foraminiferal abundances we see in our field site. Regarding other variables, the ojo and control sites are just a few meters apart with identical substrate (coarse sand), water depth and light, and while we did not monitor at such close proximity we expect that predation and food sources are also similar.

Regarding other chemical differences in the discharging water indeed there are small differences in nutrients and oxygen but not in trace metals. Specifically, ojo Norte has lower oxygen during very low tides and slightly more reducing conditions but there was nothing particularly unique about this ojo in terms of the trends observed. Moreover, the differences among ojos were smaller than the ranges of variability within each ojo (see also reply to reviewer #1). Finally, each ojo is slightly different but we do not have replicates of identical ojos so we cannot deduce statistical differences and attribute them to such conditions. As we write this is the nature of doing field work there are confounding variables, but you get more realistic results.

**Comment 2) A broad view of how major groups of foraminifera respond within the community (symbiotic, agglutinated, etc.) is much needed and well served by the study design. However, it is a shame that it comes at the expense of a discussion of a species, clade, or more finely-defined functional group level response. This study would be more impactful if it also reported the species-level assemblages at each sites. Are there specific species or genera that appear more or less robust to the environmental differences between ojo and “control” environments? Such a discussion is especially warranted given the species-level differences in response to ocean acidification that have repeatedly been shown in culture studies.**

Reply: We have included the contribution and trends in relative abundance of each genus, as well as abundances depending on test type (porcelaneous, hyaline, agglutinated), magnesium content (low, intermediate and high), feeding type (symbiont-bearing, symbiont-barren) and symbiont type (diatom and chlorophyte).

**Comment 3) Finally, there appear to be clear differences between ojo/control pairings which are occasionally mentioned in passing, but never fully addressed. For example, looking at Figure 2, I am immediately greeted with some pretty basic questions such as “Why is there such a large difference in abundance at Mini and its control compared to Gorgos and its control?” and “What is different about Norte that the low-saturation abundance is as high as the high-saturation groups at other sites?” If saturation state is the primary driver of total abundance this should be an unexpected result! Without further information or context about either the assemblages or environments at each site, it is hard to even start thinking about some of these complexities. There is a lot to uncover here that may still require some further analyses.**

Reply: As noted above, each ojo is slightly different than the other in terms of water chemistry and discharge rates but we do not have replicates of identical ojos so we cannot deduce statistical differences and attribute them to such conditions. As we note in our response to reviewer #1 and in the paper, this is the nature of doing field work there are confounding variables which complicate interpretation, but the results you get from such studies are more realistic. We note that despite the differences between ojos there are common trends and we think it is more useful to focus on these observations than to over analyze differences which would be speculative at best to explain. Complexities are the nature of such studies yet we can still glean useful information.

## Minor points:

### **Comment 4. Why the use of the >255 size fraction?**

**Could this have biased the results especially if different size species respond differently? For example the Henehan et al., 2017 paper on weight suggests size may impact species calcification response. Is it possible that smaller species may have differing metabolic requirements?**

Reply: Indeed, size is important and can impact metabolism and calcification response however, tropical benthic foraminifera are characterized by large sizes (*Why are larger forams large? Hallock, 1985, Paleobiology*) and the size fraction >250 um represents the adult individuals likely to be preserved in the sediment (*Martin, 1986*) since juvenile mortality rates are higher than 95% (*Hallock, 1985*). We have inserted this information in the methods section. Specifically, in our samples in the > 250 um size fraction we around 300-500 specimens per gram sediment while only 9-27 specimens were found on the fraction of 125-250um hence the smaller size fraction included at most 10% of the foraminifera. Regardless although the larger size fraction represents ~90% of the forams we specifically refer to Large Foraminifera in the title to be honest to our data. Nonetheless, we have included in the discussion a section on the role of a larger size on increased symbiont concentration and dissolution resistance in sediments (*Hönisch et al, 2004*), which may be responsible for the changes in abundance we see.

### **Comment 5. What was the depth of each site? I almost wonder if this could be contributing to some of the inter-site differences?**

Reply: The depth ranged from ~5 to 7 m at all sites. The depth of each site has been included in the water chemistry table. The setting is quite similar, and all sites have similar light conditions.

### **Comment 6. Can you report all species identified (in addition to the most abundant)? It would be very valuable for assessing assemblages at a finer scale and also for future workers. Ideally, it would be good to see full assemblages reported at each site and represented and compared in a figure.**

Reply: We did exploratory analyses in 10% of the samples to determine what the most abundant genera were, and we have now mentioned other genera present in the samples in the results section (*Borelis, Clavulina, Elphidium, Spiroloculina, Peneroplis, Laevipeneroplis, Planorbulina, Sorites, Vertebralina* and *Heterostegina*). However, we did not analyze the full assemblage of all the species present in all the samples. There is already existing literature on full assemblages in the area which found similar results (*Wantland, 1967; Triffleman et al, 1991; Gischler et al., 2008*).

### **Comment 7. How large were the sediment samples from which forams were measured? Did it differ between sites? And how were they collected? Importantly, how deep into the sediment were samples taken and was consistency in this regard maintained across sites?**

Reply: The samples were collected from the upper centimeter of sediment with a spoon and a centrifuge tube across all sampled sites. We analyzed at least 1 gram of sediment per replicate, with an average of ~2 grams of sediment per replicate. We aimed for at least 300 individuals per



sample; however, due to the low abundances in some of the samples (especially in samples collected at springs), this was not always possible and 24 of the 50 samples had less than 300 individuals per gram. We have included this information in the methods section.

**Comment 8. Page 2, Line 12-3: It is also worth noting that some species also tolerate (even specialize in) high CO<sub>2</sub> environments such as oxygen minimum zones – look into some of the Bernhard papers on this in Santa Barbara Basin– and low salinity (low saturation state) estuaries.**

Reply: We note in the introduction that foraminifera are versatile and that some tolerate high CO<sub>2</sub> settings and low salinity. Note however that most OMZs and also Santa Barbara basin are above the CCD and are super saturated with respect to calcite. Most studies of benthic forams in OMZs and also in SBB focus on the low oxygen rather than high CO<sub>2</sub>.

**Comment 9. Page 3, Line 12: What is your balance error?**

Reply: The analytical micro-balance has an error of  $\pm 5 \mu\text{g}$ . This information has been inserted in the methods section.

**Comment 10. Sections 3.3. and 3.4 raise a lot of questions for the reader about what is producing the reported differences between sites. See major point 3, but I think the conclusions could be made sounder if some of these type of questions are tackled.**

Reply: See reply to major point 3. We agree that it would be nice to address this but we do not think it is possible since the slight differences in other water chemistry parameters are not consistent between ojos so no statistical power to decipher the causes for the difference in magnitude of the responses.

**Comment 11. Section 3.5: Again, it looks as if there may be a difference between sites. Is this statistically significant? Also, this should refer to Figure 4.**

Reply: Thanks for finding the mistake, we have added new plots and changed the number of the figures.

**Comment 12. Page 5L Line 1: “but not Gorgos” - Page 6, Lines 23-34: “Therefore, while abundant CT may help lower the potential impact on foraminiferal calcification at low pH, it does not seem to fully counteract the effect of low .” I don’t think this is quite right. If I understand correctly, the authors are arguing that this important parameter is carbon- ate ion concentration/saturation state, and total inorganic carbon and pH are important drivers of this both intra- and extra-cellularly. If so, this should read along the lines of “Therefore, while abundant CT may increase the availability of carbonate ion, it does not seem to fully counteract the effect of low pH on .”**

Reply: We removed this paragraph from the paper however elsewhere in the paper when we comment about the CT we changed the sentence as suggested.

**Comment 13. Page 7, lines 1-3: This really glosses over the huge history and literature on shell weight and carbonate chemistry in planktonic foraminifera. Have a look at Table 7 in Weinkauf et al., 2016 for a good (though not exhaustive) review of some of this work.**

Reply: Thanks for the reference to this interesting paper, we have rewritten the test weight discussion to be more succinct and straightforward.

**Comment 14. Page 7, lines 8-10: Davis et al., 2017 also shows variable individual responses to saturation state within a population of foraminifera.**

Reply: Thank you for this relevant reference, we have inserted this reference in the discussion of test weights.

**Comment 15- Many of the figures appear low quality and pixelated. This may be the result of embedding, but double check. Also, why not include color as well as gray-scale for this online publication?**

Reply: We have changed the figures to color bar plots and increased image quality to improve readability.

**Comment 16. For figures 2-4, it would be useful to have represented on these plots which groups are significantly different from one another (as in the Results section). Consider including this?**

Reply: We have added asterisk to significant differences ( $p < 0.05$ ) between paired control and spring sites.

### **Response to Referee #3**

**In order to assess the impact of carbonate saturation on the assemblages of large benthic foraminifera in the Caribbean, Martinez et al. compare assemblages at low pH, low calcite saturation submarine spring sites with control sites of higher calcite saturation. This is an important question to tackle given that carbonate saturation will likely decrease in the future due to the increased impacts of ocean acidification. This is a unique experimental setup to take advantage of a natural location where these impacts can be studied. The authors find that at the low pH sites, there is a decrease in total benthic abundance, and increase in symbiont bearing species, and an increase in agglutinated species. Overall, non-symbiont bearing species may be more sensitive to the impacts of ocean acidification. The paper is well written and organized well, and I have only a couple of comments that I believe the authors can easily address.**

We thank the reviewer for this nice summary of our paper she/he brings up similar concerns are the two other reviewers and we have addressed these issues in the new version.

**Key points: (1) One of my main concerns with the study is that the authors are quick to dismiss that there may be other environmental differences between the submarine springs and the control sites, and perhaps too simplistically conclude that the carbonate saturation (and pH) differences are the main control on the foraminifera assemblage differences. For**

**example, there are large salinity differences between the sites that I think warrants more discussion. Are there any differences in food sources, turbidity, depths, etc?**

Reply: We have expanded the discussion on the confounding variables when working in natural settings. Specifically, we note that in selecting sites we tried as much as possible that the ojo and control sites will be as similar as possible in all other aspects (depth, substrate, light, currents, temperatures etc.) but the water carbonate chemistry. Salinity to some degree co-varies with the carbonate parameters but the difference in salinity is relatively small between the ojo water and the controls at the sites we selected. As noted in the response to the other 2 reviewers we expanded the explanation about salinity, mentioned the advantages and limitations of field work and noted that by comparing our results to those obtained in controlled laboratory experiments and at other locations we gain confidence in our conclusions.

**(2) The authors choose to analyze the >250 micrometer fraction of sediment, but do not explain their choice for this. I think that by choosing this fraction, they may be omitting smaller, important foraminifera from their analyses. One of the potential impacts of decreased carbonate saturation is that foraminifera may be smaller. So, it may be that by looking at this larger size fraction, they are missing foraminifera that may be smaller at the submarine spring sites but may still be present. It would be very helpful if the authors can repeat some analyses using a >150 micrometer fraction, for example.**

Reply: We chose the >250 size fraction because it represents the adult foraminifera assemblage likely to be preserved in the sediment (*Martin, 1986*) since tropical benthic foraminifera are characterized by large sizes (*Hallock, 1985*). This fraction comprises 90% of the forams in our samples, probably due to the high mortality rates of juveniles (>95%, *Hallock, 1985*). We have now inserted this information in the text. See detailed response to reviewers 1 and 2.

# Impact of carbonate saturation on large Caribbean benthic foraminifera assemblages

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**Abstract.** Increasing atmospheric carbon dioxide and its dissolution in seawater have reduced ocean pH and carbonate ion concentration with potential implications to calcifying organisms. To assess the response of **large** Caribbean benthic foraminifera to low carbonate saturation conditions, we **analysed benthic foraminifers' abundance and relative distribution in surface sediments** in proximity to low carbonate saturation submarine springs and at adjacent control sites. Our results show that total abundance of **large** benthic foraminifera was significantly lower at the low pH submarine springs than at control sites, **although responses were species-specific**. The relative abundance of **high magnesium, porcelainous** foraminifera was **higher than that of hyaline foraminifera** at the low pH **springs due to the abundant *Archaias angulatus*, a chlorophyte-bearing foraminifer which secretes a large and robust test that is more resilient to dissolution to low calcite saturation. The different assemblages found at the** submarine springs **indicate that calcareous symbiont-bearing** foraminifera are more sensitive to the effects of ocean acidification than **agglutinated** and symbiont-bearing foraminifera, suggesting that future ocean acidification will likely impact natural benthic foraminifera populations.

## 1 Introduction

Anthropogenic activities such as deforestation and fossil fuel burning are increasing the concentration of carbon dioxide (CO<sub>2</sub>) in the atmosphere. About one third of all the CO<sub>2</sub> emitted into the atmosphere by humans over the past 200 years has been absorbed by the oceans (Sabine et al., 2004) causing a change in ocean chemistry, lowering the pH and the concentration of carbonate ions in seawater, collectively referred to as ocean acidification. It is expected that ocean pH will decrease even more, by ~0.4 pH units by year 2100 (Caldeira and Wickett, 2003; Orr et al., 2005) with possible consequences to marine organisms and ecosystems (Raven et al., 2005). Marine calcifying organisms may be particularly sensitive due to the lower availability of carbonate ions which are required for their shell formation (Raven et al., 2005).

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Foraminifera are single celled organisms that are abundant in the marine water column and sediments, playing key roles in many marine ecosystems including being basal contributors to the marine food web and essential elements of the marine carbonate pump (Legendre and Le Fèvre, 1995; Culver and Lipps, 2003; Hain et al., 2014). Calcareous foraminifera produce calcium carbonate tests of diverse shapes and thickness while agglutinated foraminifera build a test made of detrital particles and thecate foraminifera lack a test. The calcification pathway and magnesium content of calcareous foraminifera varies between perforate hyaline and imperforate porcelaneous foraminifera (Brasier, 1980). Some large benthic foraminifera harbour photosynthetic algal symbionts while others rely solely on heterotrophic feeding (Murray, 1991). The diversity of life styles and test characteristics suggest that the sensitivity of this group of organisms to changing ocean carbonate chemistry will be species dependent (Fabry et al., 2008; Fujita et al., 2011).

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Laboratory culture experiments where benthic foraminifera were maintained under controlled conditions (i.e. partial pressure of CO<sub>2</sub>, alkalinity, etc.) generally showed a decline in foraminifera calcification under high pCO<sub>2</sub> (Erez, 2003; Haynert et al., 2011; Keul et al., 2013). However, this response was not uniform and varied among species (Fujita et al., 2011; Hikami et al., 2011; McIntyre-Wressnig et al., 2013). Field studies at CO<sub>2</sub> vents in the Pacific Ocean (Fabricius et al., 2011; Uthicke et al., 2013) and Mediterranean Sea (Dias et al., 2010) reported a decrease in benthic foraminiferal abundance with increasing pCO<sub>2</sub>, especially of calcareous species; nonetheless benthic foraminifera have been found living near CO<sub>2</sub> vents in the northern Gulf of California (Pettit et al., 2013) and near experimentally injected deep-sea CO<sub>2</sub> hydrate (Bernhard et al., 2009), and generally foraminifera can be found in a wide range of environments (Brasier, 1980).

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To shed light on the potential response of large Caribbean benthic foraminifera to future increase of CO<sub>2</sub> concentration and associated decrease in pH and carbonate ion concentrations, we studied the absolute and relative abundance of large benthic foraminifera living around a series of submarine springs that naturally discharge low carbonate saturation state ( $\Omega$ ) saline groundwater in the Yucatan Peninsula (Mexico) (Crook et al., 2012). The Yucatan peninsula is a karstic region with extensive nearshore submarine groundwater springs that discharge water characterized by low pH and high total inorganic carbon and total alkalinity, but only slightly lower salinity and similar temperatures to local marine conditions (Hofmann et al., 2011; Crook et al., 2012; Crook et al., 2013; Paytan et al., 2014; Null et al., 2014; Crook et al., 2016). Previous studies have determined that the springs have been discharging low  $\Omega$  water for millennia (Back et al., 1979); therefore, they serve as a natural laboratory to study the *in-situ* responses of marine organisms and ecosystems to long-term exposure to low  $\Omega$  which may not be captured in short-term experiments (Andersson et al., 2015). Field studies from this site reported reduced coral species richness and coral colony size at the springs compared to control sites (Crook et al., 2012) and 70% less cover of calcifying benthic organisms after 14 months of recruitment experiment (Crook et al., 2016). We hypothesize that benthic foraminifera assemblages will also differ between the springs and control sites, decreasing in overall abundance and having distinct species composition depending on test type, magnesium content, feeding strategy and photosymbiotic associations of foraminifera.

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## 2. Materials and methods

### 2.1 Field sampling

Benthic foraminifera from the upper centimetre of sediment were collected with a spoon and stored in centrifuge tubes in October 2011 near five submarine groundwater springs (Norte, Mini, Pargos, Laja and Gorgos) at Puerto Morelos reef Lagoon (National Marine Park), in the Mexican Caribbean coast off Quintana Roo (Fig. 1). At each spring site, five replicates of surface sediment samples (coarse sand) were collected along with water samples, from near the centre of the submarine spring and at five control sites about two meters away from each spring, outside the impact area of the spring.

### 2.2 Water chemistry

Water temperature and pH were measured in situ with a handheld YSI analyzer (Yellow-spring model 63). Seawater samples were filtered (0.2  $\mu\text{m}$  filter) and split into aliquots for total inorganic carbon ( $C_T$ ), total alkalinity ( $A_T$ ) and salinity measurements following the standard operating procedures described by (Dickson et al., 2007). Total inorganic carbon was analyzed on a CM5011 Carbon Coulometer (UIC, Inc.; analytical measurement error:  $\pm 3 \mu\text{mol kg}^{-1}$ ). Total alkalinity was measured using an automated open-cell, potentiometric titrator (Orion model 950; analytical measurement error:  $\pm 2 \mu\text{mol kg}^{-1}$ ). Certified  $\text{CO}_2$  reference material (from A. Dickson lab at UC San Diego, batch 112) was used to calibrate the instruments. Salinity was analyzed using a portable salinometer (Portasal Model 8410, Guild Line). The program  $\text{CO}_2\text{Sys}$  (Pierrot et al., 2006) was used to calculate pH, carbonate ion concentrations and the  $\Omega$  of seawater ( $\text{CO}_2$  dissociation constants: (Lueker et al., 2000);  $\text{KHSO}_3$ : Dickson; B concentration: (Uppström, 1974)).

### 2.3 Foraminiferal analysis

Five replicate sediment samples per site were freeze dried, weighed, washed with deionized water through a 63  $\mu\text{m}$  sieve to remove clay and silt, dried at 50°C and the >250  $\mu\text{m}$  fraction analyzed under an optical microscope (Bausch and Lomb) to determine foraminiferal abundance measured as individuals per gram of sediment. The >250  $\mu\text{m}$  fraction contains the assemblage of adult individuals which are likely to be conserved in the sediment (Martin, 1986). Small juveniles of species dominating shallow coastal setting have high mortality rates (pre-productive death rate of 99.5% for *A. angulatus*, (Knorr et al., 2015); >99% for *Amphistegina* spp., (Muller, 1974) and mortality rates of large foraminifera drop once their diameter is ~0.5 mm (Hallock and Glenn, 1986). Specifically in our samples the >250  $\mu\text{m}$  fraction typically constituted >80% of the total tests in a sample. Indeed, large-size foraminifera are typical for warm, oligotrophic, well-lit, shallow water assemblages (Hallock, 1985). At least 1 gram of sediment per replicate was analyzed (with 2 grams per replicate for most samples). At least 300 individuals per replicate were picked; however, in 24 of the 50 samples less than 300 individuals per replicate were picked due to low foraminifera abundance. Foraminifera were identified following several taxonomic references (d'Orbigny, 1839; Poag, 1981; Wantland, 1967; Crevison and Hallock, 2001), each individual within a genus was counted, and total foraminiferal

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Deleted: using  $\text{CO}_2$  dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Additionally, several SeapHOx sensors, which continuously measure seawater pH, temperature, salinity and oxygen, were deployed at some of the offshore and control sites and resulting data has been published in Hofmann et al. (2011) and Crook et al. (2013).

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Five replicate sediment samples per site were freeze dried, weighed, washed with deionized water through a 63  $\mu\text{m}$  sieve to remove clay and silt, dried at 50°C and the >250  $\mu\text{m}$  fraction analyzed under an optical microscope (Bausch and Lomb) to determine foraminiferal abundance measured as individuals per gram of sediment. The most abundant genera were *Amphistegina*, *Archaia*, *Asterigerina*, *Quinqueloculina*, *Triloculina*, *Discorbis* and *Gaudryina*. Foraminifera were identified following several taxonomic references (d'Orbigny, 1839; Poag, 1981; Wantland, 1967; Crevison and Hallock, 2001), each individual within a genus was counted, and total foraminiferal and genus abundances were normalized to sediment weight. ¶

2.4 Test weight ¶

Tests of *Discorbis rosea* from the 250-355  $\mu\text{m}$  sediment size fraction (2 to 122 individuals) were weighted using a

and genus abundances were normalized to sediment weight. Only the most common genera (>5% of the assemblage in 10% of the samples) were picked and considered for statistical analyses.

#### 2.4 Test weight

Tests of *Discorbis rosea* from the 250-355  $\mu\text{m}$  sediment size fraction (2 to 122 individuals) were weighted using an analytical micro-balance (Sartorius, model CP2P,  $\pm 5 \mu\text{g}$  error) and average weight per specimen determined. This species was chosen because of its abundance in most of the samples and the relatively constant test size.

#### 2.5 Statistical analysis

Data analysis and visualization were performed using R program version 3.4.3 (Team 2017), and “vegan” package in R (Oksanen et al., 2013). Non-parametric Mann-Whitney rank sum test was conducted to determine differences in foraminiferal abundance and weight between each low  $\Omega$  submarine spring and its corresponding control site. Permutational multivariate analysis of variance (PERMANOVA, 9,999 permutations) was used on the Bray-Curtis dissimilarity matrix after the square root transformed relative abundance of foraminifera to test for differences in community structure between saturation states and sites. Similarity percentages analysis (SIMPER) was used to determine the most important genera that contributed to dissimilarities in community structure. Nonmetric multidimensional scaling (nMDS) ordination was used to visualize the similarity in foraminiferal assemblages among  $\Omega$  levels and sites. nMDS plots were created with metaMDS function on Bray-Curtis dissimilarity matrix of foraminiferal relative abundances and constrained to 2 dimensions. To evaluate the effects of environmental variables on foraminiferal relative abundance, the log-transformed water chemistry data was overlaid using envfit function of vegan library (Dixon, 2003) with 999 permutations.

### 3 Results

#### 3.1 Water chemistry

The  $\Omega$ , pH and salinity of water in all springs was lower than their corresponding control sites (Table 1), while alkalinity ( $A_T$ ) and total inorganic carbon ( $C_T$ ) were higher than control sites. Temperature (T) was similar at all locations. These data represent the analyses of discrete water samples collected during sediment sampling; more data including continuous data collected by deployed sensors at some of these sites have been previously published (Crook et al., 2012; Crook et al., 2013; Crook et al., 2016; Null et al., 2014; Paytan et al., 2014; Hofmann et al., 2011) and data reported here are within the range of the published data. The specific spring sites were selected because the salinity at these sites is >30 over 90% of the time and it does not drop below 27; when salinity drops below 30 psu (7% of the time), the low salinity exposure lasts for very short periods of time always less than 1 hour (Crook et al., 2013)

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### 3.2 Absolute abundance of foraminifera

Absolute abundance of foraminifera measured as total number of individuals per gram of sediment was higher at high  $\Omega$  control sites than at low  $\Omega$  springs in Norte ( $W = 25, p < 0.01$ ), Mini ( $W = 25, p < 0.01$ ), Pargos ( $W = 25, p < 0.01$ ) and Laja ( $W = 25, p < 0.01$ ) but not in Gorgos ( $W = 21, p = 0.095$ ) (Fig. 2).

### 3.3 Genus assemblage

The seven most abundant genera were: *Amphistegina*, *Archaias*, *Asterigerina*, *Quinqueloculina*, *Triloculina*, *Discorbis* and *Gaudryina*. Other foraminifera that were present in some of the samples at a smaller abundance (<5% of assemblage) belong to the following genera: *Borelis*, *Clavulina*, *Elphidium*, *Spiroloculina*, *Peneroplis*, *Laevipeneroplis*, *Planorbulina*, *Sorites*, *Vertebralina* and *Heterostegina*. The composition of foraminifera communities (relative abundance of genera) changed significantly between saturation states (PERMANOVA<sub>saturation</sub>,  $F_{1,50} = 12.11, p < 0.0001$ ) and between sites (PERMANOVA<sub>sites</sub>,  $F_{4,50} = 8.15, p < 0.0001$ ). SIMPER analysis revealed that *Archaias* and *Discorbis* genera contributed the most to dissimilarities in community structure between low  $\Omega$  and high  $\Omega$  in most of the sites while *Asterigerina* contributed the most in Pargos (Fig. 3). *Archaias* relative abundance increased at low  $\Omega$  and *Discorbis* and *Asterigerina* relative abundances decreased at low  $\Omega$  in all sites. *Amphistegina* and *Gaudryina* relative abundances increased at low  $\Omega$  in all sites but Norte. *Quinqueloculina* and *Triloculina* combined relative abundance decreased at low  $\Omega$  in Pargos, Laja and Gorgos and increased in Norte and Mini.

### 3.4 Foraminifera test type

Foraminifera were divided into three groups to investigate abundance differences based on test type. The calcareous porcelaneous group included *Archaias angulatus* and several species of *Quinqueloculina* and *Triloculina* genera. The calcareous hyaline group included *Amphistegina*, *Asterigerina* and *Discorbis*. The non-calcareous agglutinated group included individuals of the genus *Gaudryina*. Porcelaneous absolute abundance was lower at low  $\Omega$  at all sites but Gorgos (Fig. 4) (Norte:  $W = 23, p < 0.05$ ; Mini:  $W = 25, p < 0.01$ ; Pargos:  $W = 25, p < 0.01$ ; Laja:  $W = 25, p < 0.01$ ; Gorgos:  $W = 20, p = 0.151$ ). Hyaline absolute abundance was lower at low  $\Omega$  at all sites (Norte:  $W = 25, p < 0.01$ ; Mini:  $W = 25, p < 0.01$ ; Pargos:  $W = 25, p < 0.01$ ; Laja:  $W = 25, p < 0.01$ ; Gorgos:  $W = 25, p < 0.01$ ). The absolute abundance of agglutinated foraminifera was lower at low  $\Omega$  than at high  $\Omega$  in Norte ( $W = 24, p < 0.05$ ) and Mini ( $W = 25, p < 0.01$ ) and did not vary with  $\Omega$  in Pargos ( $W = 16, p = 0.548$ ), Laja ( $W = 21, p = 0.095$ ), and Gorgos ( $W = 11, p = 0.841$ ).

Relative abundance of foraminifera measured as a percentage of each group within the population also differed between  $\Omega$  conditions (Fig. 4). Porcelaneous relative abundance was higher at low  $\Omega$  in Norte and Laja (Norte:  $W = 0, p < 0.01$ ; Mini:  $W = 5, p = 0.151$ ; Pargos:  $W = 5, p = 0.151$ ; Laja:  $W = 0, p < 0.01$ ; Gorgos:  $W = 5, p = 0.142$ ). In contrast, the hyaline relative abundance was lower at low  $\Omega$  in Norte and Laja (Norte:  $W = 25, p < 0.01$ ; Mini:  $W = 20, p = 0.142$ ; Pargos:  $W = 20, p = 0.151$ ; Laja:  $W = 25, p < 0.01$ ; Gorgos:  $W = 20, p = 0.151$ ). The relative abundance of agglutinated foraminifera was higher at

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low  $\Omega$  in Laja (W = 2, p = 0.05) and did not vary with  $\Omega$  in the other four sites (Norte: W = 16, p = 0.548; Mini: W = 6, p = 0.222; Pargos: W = 3, p = 0.056; Gorgos: W = 7, p = 0.310).

### 3.5 Magnesium content in test of calcareous foraminifera

Calcareous foraminifera were divided into three groups based on their magnesium content of their test to evaluate the effect of  $\Omega$  state on abundance. Foraminifera were grouped into low (*Discorbis*), intermediate (*Amphistegina* and *Asterigerina*) and high Mg content (*Archaias*, *Quinqueloculina* and *Triloculina*) tests. The absolute abundance of foraminifera with low Mg test was lower at low  $\Omega$  in all sites (Fig. 5) (Norte: W = 25, p < 0.01; Mini: W = 25, p < 0.01; Pargos: W = 25, p < 0.01; Laja: W = 25, p < 0.01; Gorgos: W = 25, p < 0.01). Similarly, the absolute abundance of intermediate Mg foraminifera was lower at low  $\Omega$  in all sites (Norte: W = 25, p < 0.01; Mini: W = 25, p < 0.01; Pargos: W = 25, p < 0.01; Laja: W = 25, p < 0.01; Gorgos: W = 23, p < 0.05). The absolute abundance of high Mg foraminifera was lower at low  $\Omega$  at all sites but Gorgos (Norte: W = 23, p < 0.05; Mini: W = 25, p < 0.01; Pargos: W = 25, p < 0.01; Laja: W = 25, p < 0.01; Gorgos: W = 20, p = 0.151).

The relative abundance of low Mg foraminifera was lower at low  $\Omega$  in Norte, Mini and Laja (Norte: W = 25, p < 0.01; Mini: W = 25, p < 0.01; Pargos: W = 20, p = 0.151; Laja: W = 25, p < 0.01; Gorgos: W = 20, p = 0.151). The relative abundance of intermediate Mg foraminifera was significantly lower at low  $\Omega$  in Norte and Pargos (Norte: W = 25, p < 0.01; Mini: W = 8, p = 0.421; Pargos: W = 23, p < 0.05; Laja: W = 18, p = 0.309; Gorgos: W = 20, p = 0.151). In contrast, the relative abundance of high Mg foraminifera was higher at low  $\Omega$  in Norte and Laja (Norte: W = 0, p < 0.01; Mini: W = 5, p < 0.142; Pargos: W = 5, p < 0.151; Laja: W = 0, p < 0.01; Gorgos: W = 5, p = 0.151).

### 3.6 Feeding strategy of calcareous foraminifera

Calcareous foraminifera were divided into two groups based on their feeding strategy: heterotrophic, symbiont-barren foraminifera and symbiont-bearing foraminifera. The absolute abundance of calcareous heterotrophic foraminifera was lower at low  $\Omega$  than at high  $\Omega$  at all sites but Gorgos (Fig. 6) (Norte: W = 25, p < 0.01; Mini: W = 25, p < 0.01; Pargos: W = 25, p < 0.05; Laja: W = 25, p < 0.01; Gorgos: W = 20, p = 0.151). The absolute abundance of symbiont-bearing foraminifera was also lower at low  $\Omega$  than at high  $\Omega$  at all sites but Gorgos (Norte: W = 24, p < 0.05; Mini: W = 25, p < 0.01; Pargos: W = 25, p < 0.01; Laja: W < 25, p < 0.01; Gorgos: W = 19, p = 0.222). The relative abundance of heterotrophic foraminifera was lower at low  $\Omega$  than at high  $\Omega$  in all sites but Gorgos (Norte: W = 25, p < 0.01; Mini: W = 25, p < 0.01; Pargos: W = 25, p < 0.01; Laja: W = 25, p < 0.01; Gorgos: W = 20, p = 0.151). In contrast, the relative abundance of symbiont-bearing foraminifera was higher at low  $\Omega$  at all sites but Gorgos (Norte: W = 0, p < 0.01; Mini: W = 0, p < 0.01; Pargos: W = 0, p < 0.01; Laja: W = 0, p < 0.01; Gorgos: W = 5, p = 0.151).

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### 3.7 Symbiont type of calcareous foraminifera

To test the differences among symbiont types on foraminifera abundance at low  $\Omega$ , symbiont-bearing foraminifera were divided into two groups: diatom-bearing foraminifera (*Amphistegina* and *Asterigerina*) and chlorophyte-bearing foraminifera (*Archaias*). The absolute abundance of diatom-bearing foraminifera was lower at low  $\Omega$  at all sites (Fig. 7) (Norte:  $W = 25$ ,  $p < 0.01$ ; Mini:  $W = 25$ ,  $p < 0.01$ ; Pargos:  $W = 25$ ,  $p < 0.01$ ; Laja:  $W = 25$ ,  $p < 0.01$ ; Gorgos:  $W = 23$ ,  $p < 0.05$ ). The absolute abundance of chlorophyte-bearing foraminifera was lower at low  $\Omega$  in Mini, Pargos and Laja and did not vary significantly in Norte and Gorgos (Norte:  $W = 20$ ,  $p = 0.151$ ; Mini:  $W = 25$ ,  $p < 0.01$ ; Pargos:  $W = 24$ ,  $p < 0.05$ ; Laja:  $W = 25$ ,  $p < 0.01$ ; Gorgos:  $W = 12$ ,  $p = 1$ ).

The relative abundance of diatom-bearing foraminifera was lower at all sites but Mini (Norte:  $W = 25$ ,  $p < 0.01$ ; Mini:  $W = 17$ ,  $p = 0.421$ ; Pargos:  $W = 24$ ,  $p < 0.05$ ; Laja:  $W = 25$ ,  $p < 0.01$ ; Gorgos:  $W = 25$ ,  $p < 0.01$ ). Contrastingly, the relative abundance of chlorophyte-bearing foraminifera was higher at all sites but Mini (Norte:  $W = 0$ ,  $p < 0.01$ ; Mini:  $W = 8$ ,  $p < 0.421$ ; Pargos:  $W = 1$ ,  $p < 0.05$ ; Laja:  $W = 0$ ,  $p < 0.01$ ; Gorgos:  $W = 0$ ,  $p < 0.01$ ).

### 3.8 Environmental factors

The nMDS plots showed a clear clustering of relative abundances between high and low  $\Omega$ , while this clustering was not apparent between sites at a specific saturation state (Fig. 8). The envfit function revealed that areas where calcareous heterotrophic foraminifera were relatively more abundant are the control sites, which are characterized by higher pH ( $R^2 = 0.3531$ ,  $p = 0.001$ ), salinity ( $R^2 = 0.4420$ ,  $p = 0.001$ ), and  $\Omega$  (represented as the arrow titled calcite in Fig. 8,  $R^2 = 0.4735$ ,  $p = 0.001$ ), while areas where calcareous heterotrophic foraminifera were less abundant are the spring sites which are characterized by higher alkalinity (represented as arrow A in Fig. 8,  $R^2 = 0.4420$ ,  $p = 0.001$ ), and higher total inorganic carbon (represented as arrow C in Fig. 8,  $R^2 = 0.4261$ ,  $p = 0.001$ ). Calcareous symbiont-bearing foraminifera were relatively more abundant in low  $\Omega$  areas (blue symbols) with higher temperature (represented as arrow T in Fig. 8,  $R^2 = 0.1234$ ,  $p = 0.036$ ), although the temperature is not on the main gradient of variation and the difference among sites was at most two degrees Celsius, which is lower than diurnal or seasonal natural variability within sites. Relative abundance of agglutinated foraminifera did not seem to be affected by the main gradient explaining the maximal variance of data. These trends are consistent with field observations.

### 3.9 Test weight

The average test weights of *Discorbis rosea* (size fraction 250-355  $\mu\text{m}$ ) did not differ among saturation states in any of the sites (Norte:  $W = 13$ ,  $p = 1$ ; Mini:  $W = 13$ ,  $p = 0.2$ ; Pargos:  $W = 7$ ,  $p = 0.309$ ; Laja:  $W = 8$ ,  $p = 0.421$ ; Gorgos:  $W = 20$ ,  $p = 0.151$ ) (Fig. 9).

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## 4 Discussion

### 4.1 Absolute abundance of calcifying benthic foraminifera decreases at low $\Omega$ springs

The analysis of foraminiferal abundance in surface sediments collected from low  $\Omega$  submarine springs and control sites revealed that the absolute abundance of calcareous foraminifera was lower at springs than at control sites (Fig. 2). Calcification of calcareous foraminifera is a process that depends on the carbonate chemistry of seawater and requires calcite supersaturated conditions at the calcification site (Erez, 2003; Bentov et al., 2009). Foraminifera endocytose seawater to bring calcium and inorganic carbon to the active calcification site (Bentov et al., 2009). In the process, the vacuolized seawater is alkalized to a pH of ~9 to overcome magnesium mediated inhibition of calcite precipitation and to promote the conversion of inorganic carbon speciation from bicarbonate to carbonate ions (de Nooijer et al., 2009). This pH elevation at the site of calcification is achieved by using ATP to pump protons out of the foraminifera protoplasm (Glas et al., 2012b; Toyofuku et al., 2017). If the ambient pH is low, the foraminifera have to devote more energy to rising the intracellular pH to promote calcification, making the conditions at low pH sites less favorable for calcification (de Nooijer et al., 2009). Indeed, this may explain the decrease we see in the total abundance of calcareous porcelaneous and hyaline foraminifera at the low pH, low  $\Omega$  submarine springs. Agglutinated foraminifera absolute abundance was similar between springs and control sites in three of the five sampled sites, and their relative abundance was similar among springs and controls in four of the five sites (Fig. 4), although their abundance was overall low in both springs and control sites. Furthermore, SIMPER analysis revealed that agglutinated *Gaudryina* foraminifera relative abundance increased at low  $\Omega$  in most of the sites (Fig. 3). Since agglutinated foraminifera tests are not made of calcium carbonate, they may be less influenced by the low  $\Omega$  seawater at the springs than calcareous foraminifera. A lesser impact of low pH on agglutinated foraminifera abundance has also been observed in foraminifera present at  $\text{CO}_2$  vents at Papua New Guinea (Uthicke et al., 2013) and Ischia, in the Mediterranean Sea (Dias et al., 2010). Similarly, the abundance of non-calcifying thecate and agglutinated foraminifera living in direct contact with experimentally injected  $\text{CO}_2$  hydrate did not decline significantly with decreasing pH (Bernhard et al., 2009). However, species-specific survival rates of agglutinated foraminifera during a laboratory experiment at 2000 ppm of  $p\text{CO}_2$  suggests that other agglutinated species different than *Gaudryina* may react in a different manner to low  $\Omega$  (van Dijk et al., 2017).

Since many environmental parameters co-vary in natural environments (Andersson et al., 2015), including at our field site, it is possible that the trends in absolute and relative abundances of foraminifera present at the springs are due to species-specific salinity preferences (the only other variable that consistently different at springs and control sites). The salinity of the discharging water at the sampled springs is  $> 30$  for 93% of the time and it is constantly higher than 27 (Crook et al., 2013) as previously mentioned. Although the salinity tolerance ranges are not known for all the species found in the study area, many foraminifera that are abundant in shallow warm coastal waters such as those at our sites, have a very wide salinity tolerance (Brasier, 1980). *Quinqueloculina* spp. has been found at salinity ranges of 12-35 with abundance peaks at 17 and 35 (Horton and Murray, 2007). *Amphistegina lessonii* has been kept between 25 and 45 in a lab experiment (Geerken et al., 2018) and *Archaias* has been reported to be present at salinities of 29-39 (Hallock and Peebles, 1993). Moreover, adaptation to changes

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**Deleted:** Foraminifera calcification is a process that depends on the carbonate ion concentration of seawater and requires supersaturated conditions with respect to calcite at the calcification site (Erez, 2003; Bentov et al., 2009). Bentov et al. (2009) demonstrated that biomineralization of the benthic foraminifera *Amphistegina lobifera* relies on endocytosis of seawater as the mechanism bringing calcium and carbonate ions to the active calcification site. The endocytosed seawater vacuole is alkalized, reaching around 0.5 pH units above ambient extracellular seawater while the cytosol pH is 7.2 to 7.5 (Bentov et al., 2009). De Nooijer et al. (2009) showed that foraminifera increase the pH at the site of calcification by one unit above ambient pH levels to overcome magnesium mediated inhibition of calcite precipitation and to promote the conversion of inorganic carbon speciation from bicarbonate to carbonate ions. This pH elevation at the site of calcification is achieved by pumping protons out of the foraminifera protoplasm by Vacuolar-type  $\text{H}^+$  ATPase (Toyofuku et al., 2017). This proton pumping requires energy released by ATP hydrolysis. If the ambient pH is low, the foraminifera may have to devote more energy to rising the intracellular pH to promote calcification, making the conditions at low pH sites less favorable for calcification (de Nooijer et al., 2009). Indeed, this may explain the decrease we see in the total abundance of calcareous foraminifera at the low  $\Omega$  submarine springs as the conditions at these sites are less favorable. The submarine groundwater discharging at the ojos is characterized by higher than ambient total inorganic carbon and alkalinity but lower pH, resulting in lower  $\Omega$  and carbonate ion concentrations (based on field measurements and  $\text{CO}_2\text{Sys}$  calculations). Toyofuku et al. (2017) proposed that calcification depends on total inorganic carbon ( $C_T$ ) concentration and not necessarily on  $\Omega$ . However, in field study the  $C_T$  concentrations were high at the low  $\Omega$  sites and still see a decrease in foraminiferal absolute abundance. Specifically, even if there is more inorganic carbon available for calcification, carbon must be in the form of carbonate ions to precipitate calcium carbonate tests, which is achieved by increasing the internal pH using energy through proton pumping. Consequently, a decrease in oceanic pH (even at high  $C_T$ ) will require foraminifera to spend more energy to change carbon speciation and promote calcification (de Nooijer et al., 2009; Raven et al., 2005). Therefore, while abundant  $C_T$  may help lower the potential impact on foraminifera calcification at low pH, it does not seem to fully counteract the effect of low  $\Omega$ . The salinity of the discharging water is also different at the ojos relative to control sites (slightly lower), however, other field studies such as the study at the high  $\text{CO}_2$  vent at Papua New Guinea (PNG), where salinity was not different, have found similar reduction in benthic foraminifera densities

in salinity requires increased cellular osmoregulation (McLusky et al., 2004), which is expected to affect both agglutinated and calcareous foraminifera abundance. Since agglutinated foraminifera abundance is similar at the springs and control sites (Fig. 4) and does not seem to be affected by the main gradient of variation in carbonate chemistry and salinity (Fig. 8), we suggest that  $\Omega$  and pH are the main drivers of calcareous foraminifera abundances seen in this study. Consistent with this conclusion, the trends we see in absolute and relative abundance of calcareous and agglutinated foraminifera are in line with observations from other field studies where salinities did not differ between low and ambient pH sampling locations (Fabricius et al., 2011; Uthicke et al., 2013). Hence, the lower abundance of calcareous foraminifera we and others have observed in diverse settings with low  $\Omega$  suggests that future reduction in  $\Omega$  will negatively affect calcareous benthic foraminifera.

#### 4.2 Porcelaneous, high-Magnesium tests foraminifera's relative abundance increases in low $\Omega$ springs

While absolute abundance of both porcelaneous and hyaline foraminifera was lower at low  $\Omega$ , a trend towards higher relative abundance of porcelaneous foraminifera and lower relative abundance of hyaline foraminifera is observed (Fig. 4). The higher relative abundance of porcelaneous (Fig. 4) and high magnesium foraminifera (Fig. 5) is driven by *Archaias angulatus*, which is the most common species found and contributes the most to community dissimilarity in all the sites (Fig. 3). *Archaias angulatus* is well preserved in sediments due to its robust, thick test (Hallock and Peebles, 1993) strengthened by crystal pillars (Martin, 1986), and has been reported to account for more than 20% of the foraminiferal population in the South Florida shelf (Knorr et al., 2015), up to 54% of dead assemblages from North Florida Keys (Martin, 1986) and to be the most common species in Banco Chinchorro, South Yucatan Peninsula (Gischler and Möder, 2009). The lower relative abundance of hyaline, low magnesium foraminifera at low  $\Omega$  (Fig. 4 and 5) is attributed to the decrease of *Discorbis* and *Asterigerina* (Fig. 3). These results are in contrast with the idea that porcelaneous, high magnesium foraminifera would be the "first responders" (Fujita et al., 2011) to ocean acidification, since high Mg calcite is more soluble than low Mg calcite and aragonite at a given  $p\text{CO}_2$  (Morse et al., 2006) and because Mg inhibits calcite crystallization. This can be attributed to the lower solubility of the robust tests.

The calcification pathway of perforate hyaline foraminifera (reviewed by de Nooijer et al., 2014) has been studied in more detail than the calcification process of porcelaneous foraminifera. Hyaline foraminifera capture ions through seawater endocytosis (Bentov et al., 2009; de Nooijer et al., 2009) and transmembrane transport (Nehrke et al., 2013), and store them in separated intracellular reservoirs of inorganic carbon and calcium (Ter Kuile and Erez, 1991; Toyofuku et al., 2008). A perforated test is then secreted extracellularly within a primary organic sheet after intracellular Mg discrimination and pH increase of the vacuolized seawater to a pH of  $> 9$  (Zeebe and Sanyal, 2002; Erez, 2003; de Nooijer et al., 2009). In contrast, porcelaneous foraminifera precipitate calcite needles inside intracellular vesicles (at a pH of  $\sim 9$ ) and are later transported and randomly assembled in an extracellular organic matrix to form a new test chamber (Angell, 1980; Hemleben et al., 1986; Erez, 2003; de Nooijer et al., 2009). These transporting vesicles have been reported to have a pH of 7.5-8.0 (de Nooijer et al., 2009). Since these vesicles have a lower pH, it is possible that less protons are pumped out of the vesicle. In addition, the lack of

**Deleted:** indicating that  $\Omega$  was the major parameter affecting benthic foraminifera calcification. Like at our study site and the PNG site, calcareous foraminifera have been found at  $\text{CO}_2$  vents with pH ranging from 7.83 to 7.55 in the Gulf of California (Pettit et al., 2013) and near intentional deep-sea carbon dioxide hydrate release (Bernhard et al., 2009). (Bernhard et al., 2009) reported that although the calcareous foraminifera were able to survive for short periods of time under pH that was 0.2 units lower than ambient, their mortality was higher. Hence, the lower abundance of calcareous foraminifera we and others have observed in diverse settings with low  $\Omega$  suggest that future reduction in  $\Omega$  will negatively affect calcifying benthic foraminifera. ¶

Palaeoceanographic records indicate there is a positive relation between test weight of planktonic foraminifera and carbonate concentration (Barker and Elderfield, 2002) and that weight of modern planktonic *Globigerina bulloides* tests is 30-35% lower than Holocene shells (Moy et al., 2009). Indeed, laboratory experiments at  $p\text{CO}_2$  of 1900 ppmv show that shell weight of *Ammonia tepida* decreased with decreasing carbonate ion concentration even though the foraminifera were able to calcify at carbonate undersaturated water (Dissard et al., 2010). The tests of *D. rosea* collected at low sites in this study had lower or similar average test weight when compared to tests collected at control sites with high  $\Omega$ . The lack of statistical significance of the difference in weight for *D. rosea* is due to the large variability within populations and individuals but this can also suggest that the impact on weight varies among individuals within a species. Fujita et al. (2011) also reported variable responses of symbiont-bearing foraminiferal weights within clone populations after high  $p\text{CO}_2$  incubations and suggested that this is due to different growth rates. In addition, it has been suggested that body size influence calcification (Henehan et al., 2017). In our study, the weighted tests were all picked from the 250-355  $\mu\text{m}$  sediment fraction but each test was not normalized to shell diameter, hence it is possible that the diversity of sizes and possibly growth rates (which we did not assess) accounted for the wide variability in weights. ¶

While the absolute abundance of all functional groups in our study was lower at the low  $\Omega$  sites, the impact was larger on the calcareous heterotrophic foraminifera compared to the other functional groups as their relative abundance decreased more than that of both the symbiont-bearing and the agglutinated foraminifera. ¶

internal calcium and inorganic carbon pools may require less energy to precipitate calcite tests, which can be a competitive advantage that explains the increase in relative abundance of porcelaneous foraminifera we see at low pH, low  $\Omega$  springs. Another explanation, noted above, could be that lower dissolution rates of the more robust porcelaneous tests (Brasier, 1980; Schmiedl et al., 1997) results in the observed increase in the abundance of these tests. However, further research is needed to test these hypotheses and to better understand the calcification pathway and preservation of porcelaneous foraminifera. These results can guide future controlled experiments in a laboratory setting.

#### 4.3 Symbiont-bearing foraminifera increase in relative abundance at low $\Omega$ springs

The relative abundance of heterotrophic foraminifera decreased while the relative abundance of symbiont-bearing foraminifera increased in most of the springs (Fig. 6). Foraminifera hosting photosynthetic symbionts may be more resilient to low  $\Omega$  since they can access additional energy derived from photosynthates translocated from the algae (Hallock, 2000) to increase pH at the calcification site and for alkalization of seawater vacuoles. In addition, symbiotic algae can promote calcification by removing foraminiferal metabolic N and P which impede crystal formation, by providing organic matter used to synthesize the organic matrix that precedes test growth (Fujita et al., 2011), and by increasing the pH on the surface of foraminifera (Glas et al., 2012a). These mechanisms may explain the significant increase in relative abundance of symbiont-bearing foraminifera (>50% of the total calcareous population) while calcareous heterotrophic foraminifera relative abundance decreased (<50%) at low  $\Omega$  springs. Although symbiont-bearing calcareous foraminifera were relatively more abundant than symbiont-barren foraminifera at low  $\Omega$  sites, their absolute abundance decreased in comparison with sites at ambient  $\Omega$ , indicating that despite the symbionts, the conditions were less favorable than at ambient conditions. Short laboratory experiments with symbiont-bearing foraminifera cultured at high  $p\text{CO}_2$  have reported reduced net calcification (Fujita et al., 2011; Hikami et al., 2011) and tests dissolution signs (McIntyre-Wressnig et al., 2013). While photosynthetic activity may promote calcification, it does not fully compensate the deleterious effects of elevated  $p\text{CO}_2$  on foraminifera calcification incubated in laboratory (Glas et al., 2012a) and field experiments (Uthicke and Fabricius, 2012). These studies suggest that benthic symbiont-bearing foraminifera can better survive at high  $p\text{CO}_2$ , but their calcification is reduced.

Foraminifera hosting chlorophytes (*Archaias*) were relatively more abundant at springs than those hosting diatoms (*Amphistegina* and *Asterigerina*) (Fig. 7). Hyaline foraminifera hosting diatoms are thought to be more resilient to high  $p\text{CO}_2$  than other symbiont-bearing foraminifera based on a meta-analysis of studies assessing the impacts of acidification on large benthic foraminifera (Doo et al., 2014). However, none of the studies included in the meta-analyses focused on chlorophyte-bearing foraminifera and due to the high variability in methodology, duration and species used in the experiments, it is not possible to make a direct comparison between these studies and an assemblage found at the natural low  $\Omega$  springs in our study. Foraminifera hosting chlorophytes may be more resilient to ocean acidification than those hosting diatoms, or the robustness of *Archaias* tests may be responsible for this difference in relative abundance. It is also plausible that the size of the symbiont-bearing foraminifera influences the survival and preservation under low  $\Omega$  conditions. The relative abundance of *Asterigerina*

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**Deleted:** contribution to the foraminifera population at our study site does not decrease under low  $\Omega$ . This is consistent with observations by Uthicke et al. (2013) who found that in field studies at PNG, the decrease in non-calcareous foraminifera density at low pH was less abrupt than for calcareous foraminifera. A trend toward more non-calcareous foraminiferal populations was also reported at the Mediterranean  $\text{CO}_2$  vents of Ischia, where the foraminiferal assemblages changed from calcareous miliolid dominated taxa at ambient pH to 100% agglutinated taxa at 7.6 pH (Dias et al., 2011). Similarly, the abundance of non-calcifying thecate and agglutinated foraminifera living in direct contact with the

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**Deleted:** At our study site, we record a small decrease in the absolute abundance of non-calcifying agglutinated foraminifera, the relative abundance of the agglutinated foraminifera increased indicating that these foraminifera are less affected by the low pH conditions than the calcareous foraminifera.

**Moved down [2]:** 5 Conclusion  $\nabla$

**Deleted:** In summary, our results suggest that a low calcite saturation state of seawater diminishes foraminiferal abundance, even in areas with high concentration of inorganic carbon. The impact of low  $\Omega$  is more severe for calcareous heterotrophic foraminifera than for symbiont-bearing foraminifera and agglutinated foraminifera. Moreover, while symbiont-bearing

decreased at low  $\Omega$  while *Amphistegina* increased, in spite of both being hyaline foraminifera hosting diatoms (Fig. 3). The larger size of *Amphistegina* in comparison to *Asterigerina* may allow for hosting a larger concentration of photosynthetic algae, as it has been suggested that the number of symbionts increases with test size (Hönisch and Hemming, 2004). In fact, *Archaias* has the largest tests of all the species found at the springs in this study. Furthermore, a larger size has been linked to reduced dissolution due to a smaller surface-volume ratio (Hönisch and Hemming, 2004), which may explain why large foraminifera overall are more abundant than smaller foraminifera at this location.

#### 4.4 *Discorbis rosea* weight did not significantly vary among springs and control sites

The test weight of *D. rosea* did not significantly vary among springs and control sites. This lack of difference may be due to the large variability in test weight within populations and individuals. The variability in tests weights within a species may be due to differential individual growth rates (Fujita et al., 2011), body sizes (Henehan et al., 2017) or genotypes (Davis et al., 2017) with diverse calcification performance under the same  $\Omega$  conditions. In our study, the weighted tests were all picked from the 250-355  $\mu\text{m}$  sediment fraction and we took special care to select individuals of very similar size, however, each test was not normalized to shell diameter, hence the wide variability in test weights may be partially related to the range in test sizes.

#### 4.5 Implications

The reduced absolute abundance of benthic foraminifera at low  $\Omega$  springs suggest that there may be an overall decrease in benthic foraminifera abundance as a consequence of ocean acidification, with subsequent repercussions on the global carbon cycle and marine food web. *Archaias angulatus*, the most common species found in this study, is known to represent a large proportion of the foraminiferal population in different parts of the western tropical Atlantic Ocean (Martin, 1986; Gischler and Möder, 2009; Knorr et al., 2015), being the dominant large benthic foraminifera in the Florida-Bahamas carbonate province (Hallock et al., 1986). A laboratory study with *A. angulatus* reported a 50% decrease in growth rate after 28 days at pH 7.6, and an estimated reduction of 85% of carbonate production by this species in the South Florida reef tract and Florida bay, from 0.27 Mt/yr to 0.04 Mt/yr (Knorr et al., 2015). Besides changes in carbonate production, a decrease in foraminiferal abundance may have cascade effects through the ecosystem since foraminifera are an important link in the marine food web as they prey on bacteria and algae, and are predated on by many animals such as gastropods, bivalves, echinoderms and crustaceans (Culver and Lipps, 2003).

#### 5 Conclusion

The absolute abundance of all large calcareous foraminifera decreased at springs discharging low  $\Omega$ , low pH water. Porcelaneous, high magnesium foraminifera were relatively less impacted compared to hyaline foraminifera at the springs, possibly due to their different calcification mechanism and more robust tests and the lack of internal carbon and calcium pools.

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The relative abundance of symbiont-bearing foraminifera increased while heterotrophic symbiont-barren foraminifera decreased under low  $\Omega$  conditions, which may be explained by the higher energy availability provided by the symbiont to elevate the pH at the site of calcification. Chlorophyte-bearing foraminifera were relatively more abundant than diatom-bearing foraminifera. These trends are driven by the abundant large *Archaias angulatus*, a porcelaneous foraminifera hosting chlorophytes, which may be more resilient to low  $\Omega$  due to its test robustness and large size that can lead to a higher concentration of symbiotic algae and reduced test dissolution. Further laboratory experiments are needed to confirm these results in a controlled setting without covarying environmental variables and to better understand the calcification pathway of porcelaneous foraminifera.

#### 6 Author contribution

Conceived and designed the experiments: AM and AP. Conducted field work: AM AP, MRV, LH. Analyzed the data: AM and AP. Contributed reagents/materials/analysis tools: AM, AP, LH, MRV. AM and AP primarily wrote the paper and LH and MRV provided critical edits.

#### 7 Competing interests

The authors declare that they have no conflict of interest

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10 Tables

Table 1: Carbonate chemistry parameters of discrete water samples collected at low saturation state submarine springs and adjacent high saturation state control sites (mean ± SD) at the time of sample collection (A<sub>T</sub>= total alkalinity; C<sub>T</sub>= total inorganic carbon).

Site	Depth (m)		A <sub>T</sub> (μmol·kg <sup>-1</sup> ) 1)	C <sub>T</sub> (μmol·kg <sup>-1</sup> )	*pH	*CO <sub>3</sub> <sup>2-</sup> (μmol·kg <sup>-1</sup> ) 1)	*Ω calci te	T (°C)	Salinity
Norte	5.8	control	2354 ± 13	2051 ± 6	7.98	216.16	5.14	27.0	36.80
		spring	2611 ± 3	2588 ± 3	7.38	67.03	1.66	27.5	32.21
Mini	4.9	control	2356 ± 3	2049 ± 6	7.9	218.13	5.16	26.4	37.3
		spring	3108 ± 10	3197 ± 6	7.13	46.29	1.14	27.6	32.41
Pargo	6.8	control	2336 ± 4	2012 ± 12	8.01	229.56	5.49	27.6	36.17
		spring	3000 ± 8	3048 ± 12	7.2	52.73	1.33	27.6	29.95
Laja	5.8	control	2357 ± 6	2092 ± 1	7.9	193.55	4.63	28.1	36.17
		spring	2827 ± 9	2756 ± 10	7.5	102.65	2.50	27.9	32.75
Gorgos	7.2	control	2325 ± 3	2033 ± 3	7.96	209.44	5.02	27.8	35.90
		spring	2874 ± 11	2987 ± 8	7.11	94.65	2.38	28.5	31.09

5 \* Calculated using CO<sub>2</sub>Sys

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11 Figures

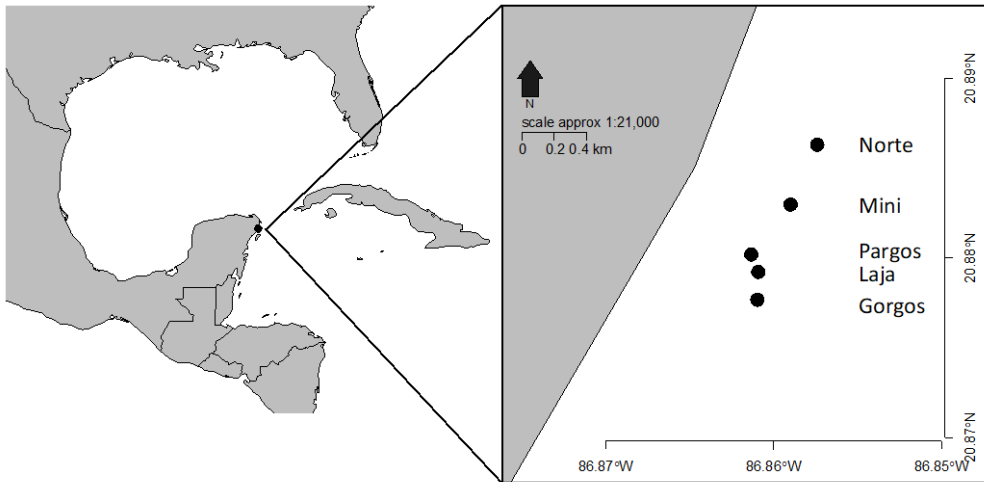


Figure 1: Location of low carbonate saturation state submarine springs

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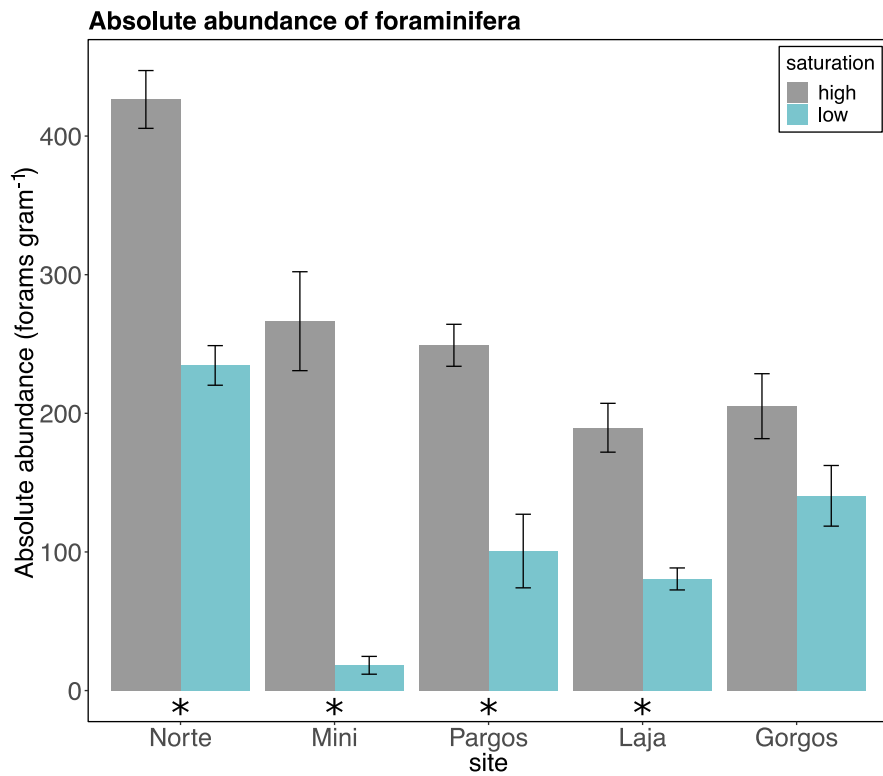
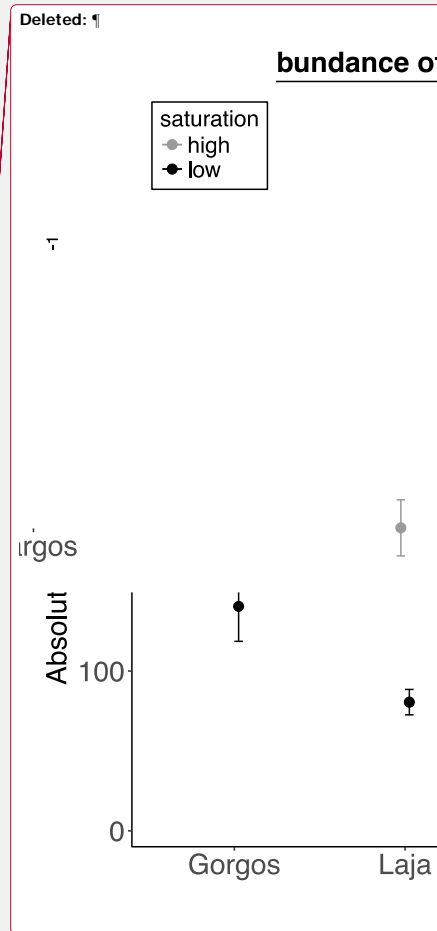
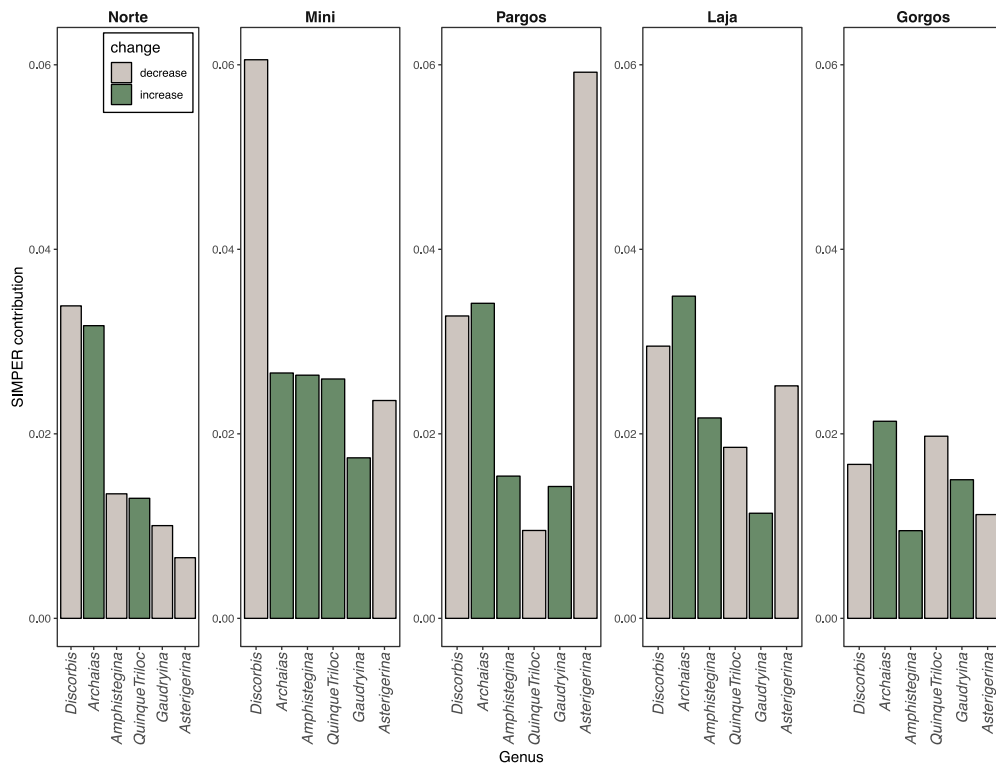
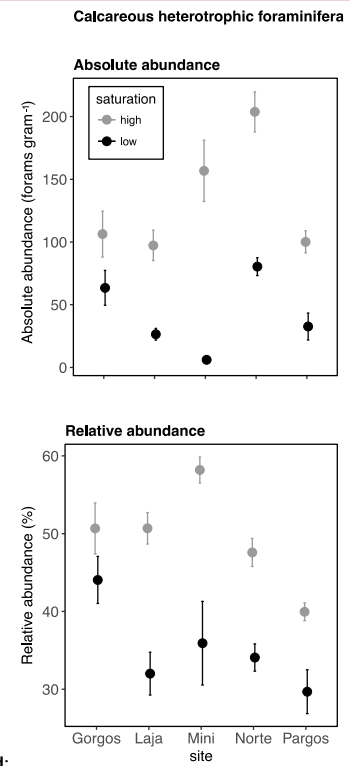


Figure 2: Absolute abundance of foraminifera (number of specimens per gram of sediment) in different submarine springs (low saturation state) and their respective control sites (high saturation state). Data are mean  $\pm$  SE (n= 5). The asterisk demarks a significant difference ( $p < 0.05$ ) in abundance between paired springs and controls at each site according to Mann-Whitney rank sum test.

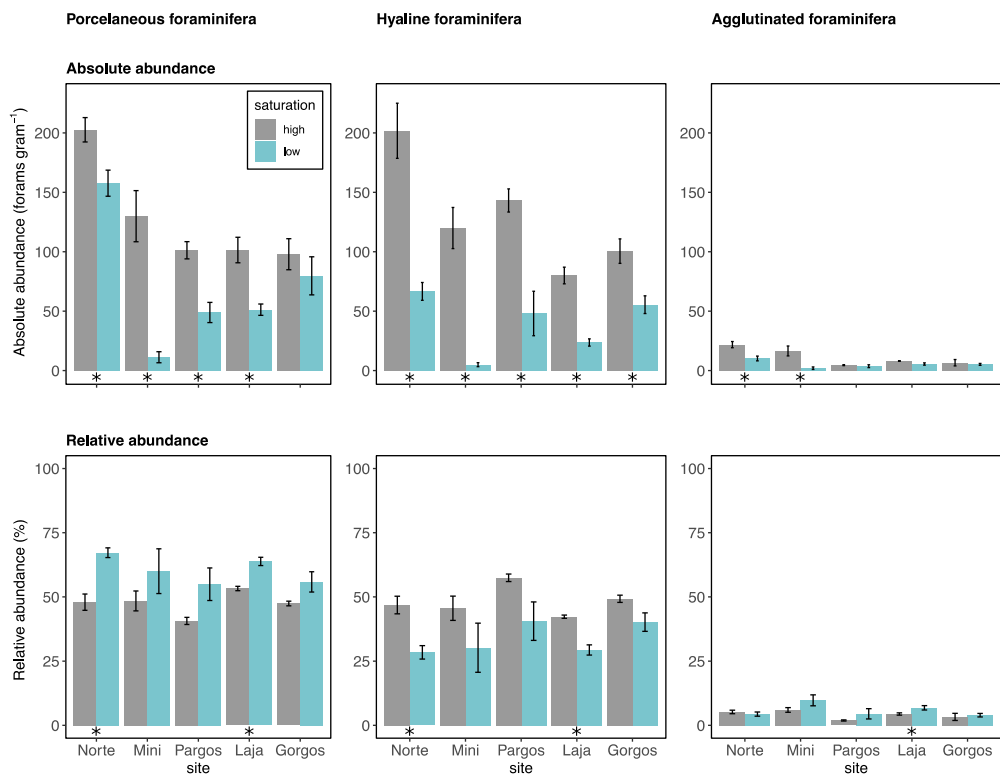




**Figure 3: SIMPER contribution of the most abundant genera. Bar height indicates the mean contribution of each genus to community dissimilarity. Green color represents an increase and grey color represents a decrease in the mean relative abundance of each genus at low saturation springs.**



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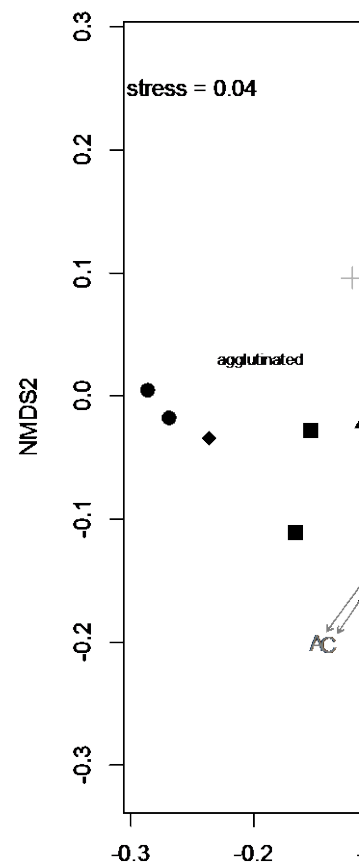
**Figure 4:** Absolute abundance (specimens per gram of sediment) and relative abundance (percentage) of different foraminifera test types (porcelaneous, hyaline, and agglutinated). Data are mean  $\pm$  SE (n = 5). The asterisk demarks a significant difference ( $p < 0.05$ ) in abundance between paired springs and controls at each site according to Mann-Whitney rank sum test.

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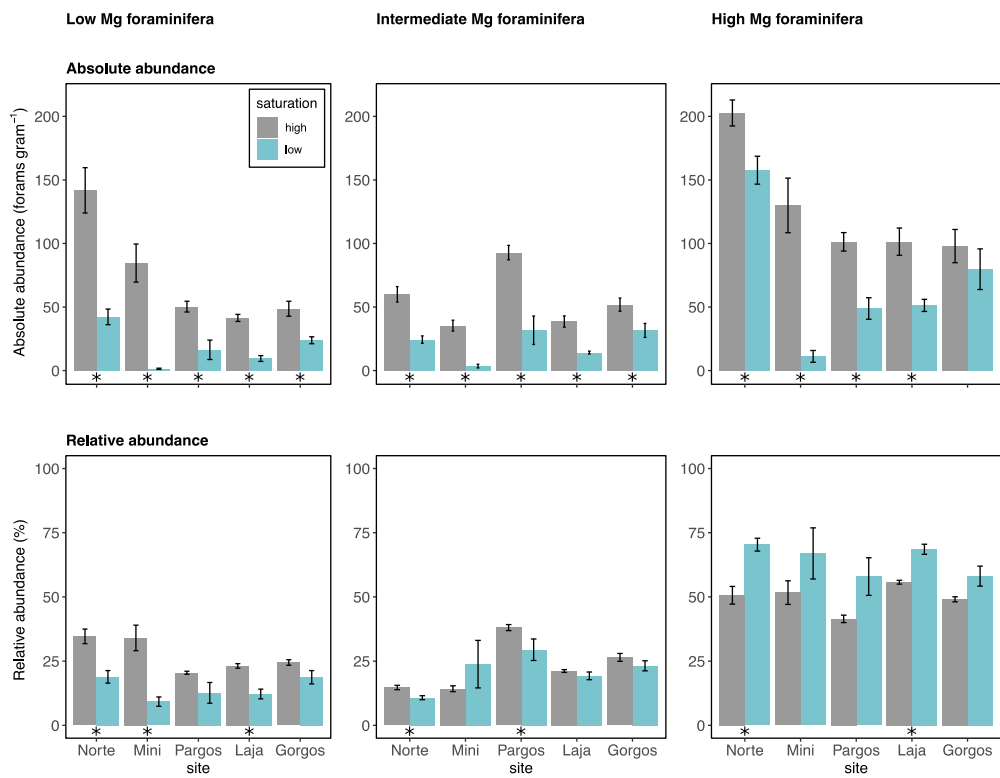
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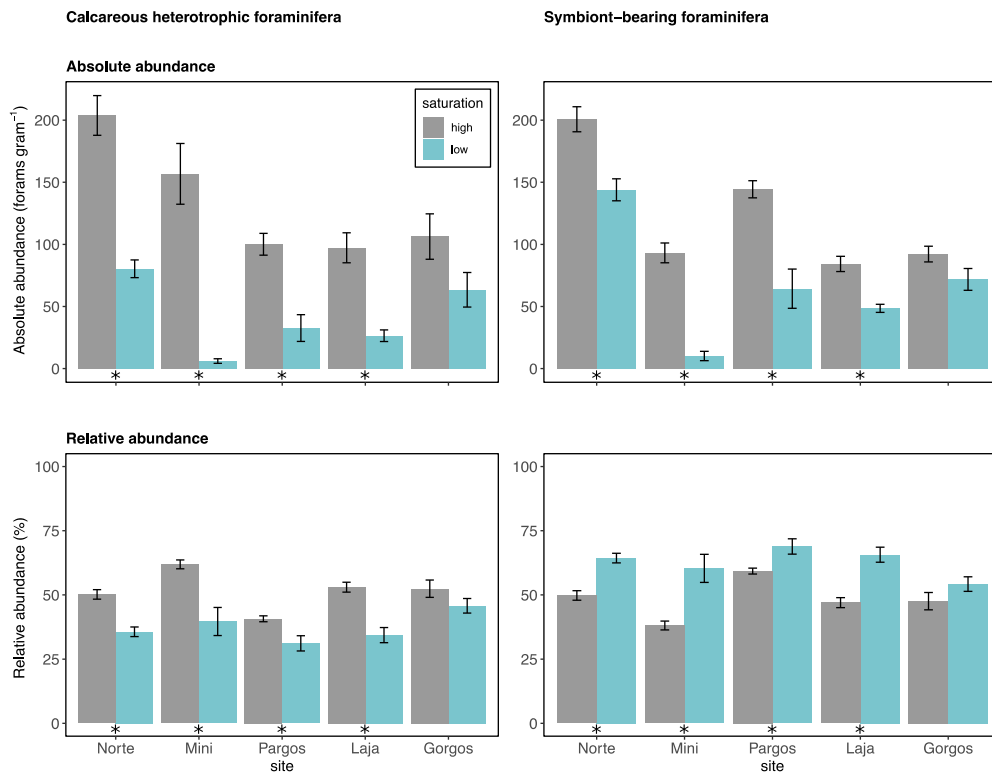


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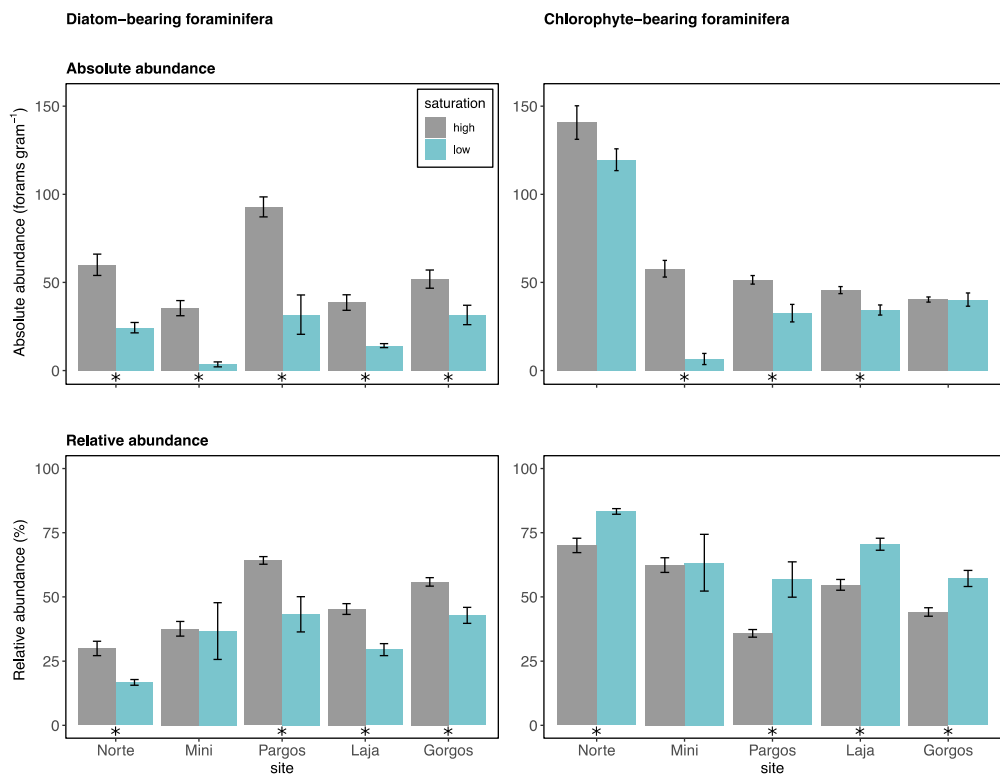
**Figure 5: Absolute abundance (specimens per gram of sediment) and relative abundance (percentage) of foraminifera with different magnesium content tests. Data are mean  $\pm$  SE (n= 5). The asterisk demarks a significant difference ( $p < 0.05$ ) in abundance between paired springs and controls at each site according to Mann-Whitney rank sum test.**



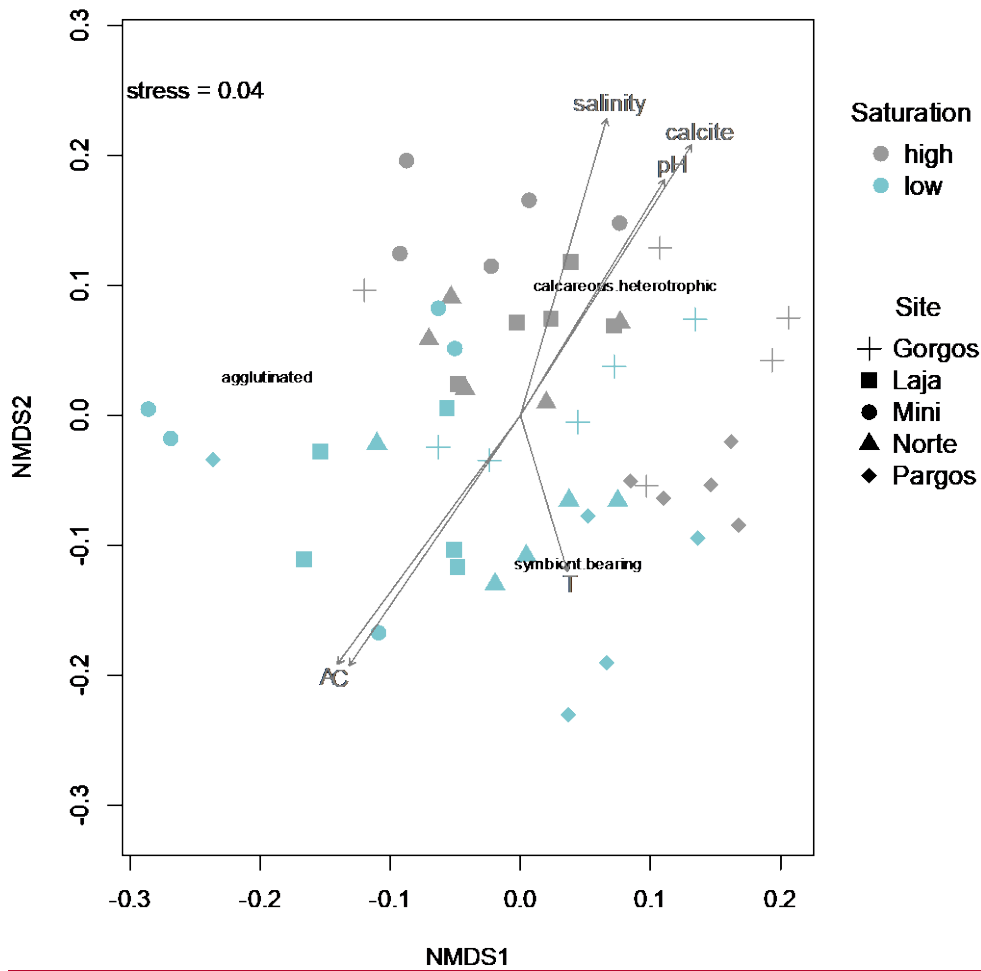


**Figure 6: Absolute abundance (specimens per gram of sediment) and relative abundance (percentage) of different feeding strategies of calcareous foraminifera (symbiont-barren heterotrophic and symbiont-bearing). Data are mean  $\pm$  SE (n= 5). The asterisk demarks a significant difference ( $p < 0.05$ ) in abundance between paired springs and controls at each site according to Mann-Whitney rank sum test.**

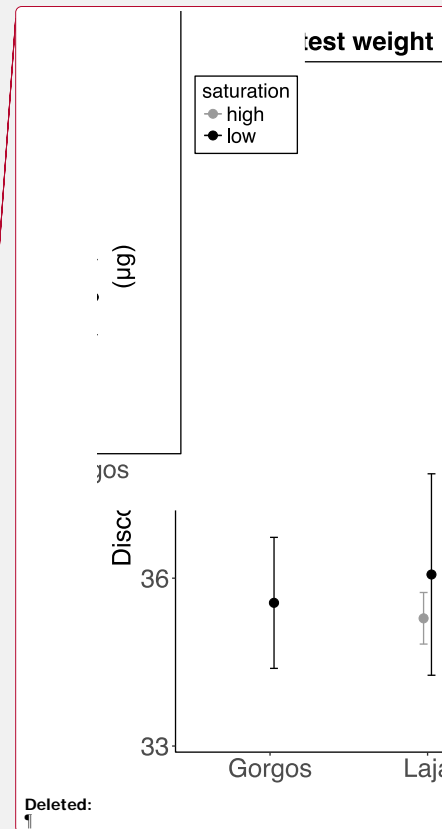
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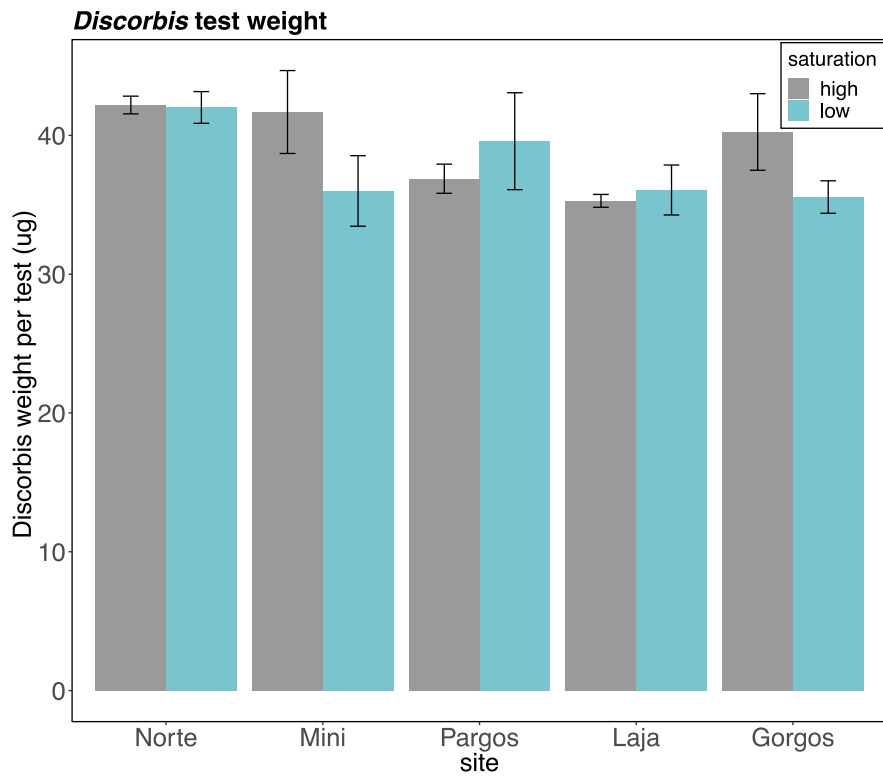


**Figure 7: Absolute abundance (specimens per gram of sediment) and relative abundance (percentage) of large calcareous foraminifera hosting different symbionts (diatoms and chlorophytes). Data are mean  $\pm$  SE (n= 5). The asterisk demarks a significant difference ( $p < 0.05$ ) in abundance between paired springs and controls at each site according to Mann-Whitney rank sum test.**



**Figure 8:** Non-metric Multidimensional Scaling (nMDS) ordination plot for community structure (relative abundance) by carbonate saturation state and site with overlaid environmental parameters (A= total alkalinity; C= total inorganic carbon; T= temperature).





**Figure 9:** Mean weight of *Discorbis rosea* tests (size fraction 250-355  $\mu\text{m}$ ) at low and high saturation at different submarine spring sites. Data are mean  $\pm$  SE.

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