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 Caribbean benthic foraminifera to low carbonate saturation conditions, we analyzed benthic foraminifera abundance and relative distribution in proximity to low carbonate saturation submarine springs and at adjacent control sites. Our results show that the total abundance of benthic foraminifera is significantly lower at the low pH low calcite saturation submarine springs than at control sites, despite higher concentrations of inorganic carbon at the spring sites. The relative abundance of symbiont-bearing foraminifera and agglutinated foraminifera was higher at the low pH low calcite saturation submarine springs compared to control sites. These differences indicate that non-symbiont bearing heterotrophic calcareous foraminifera are more sensitive to the effects of ocean acidification than non-calcifying and symbiont bearing foraminifera, suggesting that future ocean acidification may impact natural benthic foraminifera populations.

1 Introduction

Anthropogenic activities such as deforestation and fossil fuel burning are increasing the concentration of carbon dioxide (CO_2) in the atmosphere. About one third of all the CO_2 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).

Specifically, foraminifera, single celled organisms abundant in the marine water column and sediments that play key roles in many marine ecosystems including being basal contributors to the marine food web and essential elements of the marine

carbonate pump may be impacted (Legendre and Le Fèvre, 1995; Culver and Lipps, 2003; Hain et al., 2014). Several foraminifera species produce calcium carbonate (calcite) tests of diverse shapes and thickness (referred to as calcareous foraminifera), while others build a test made of detrital particles (agglutinant foraminifera) or lack a test (thecate). Some foraminifera harbour photosynthetic algal symbionts while others rely solely on heterotrophic feeding (Murray, 1991). The

5 diversity of life styles and test characteristics of foraminifera suggest that the sensitivity of this group of organisms to changing ocean carbonate chemistry is likely to vary among taxa and/or life style (Fabry et al., 2008; Fujita et al., 2011).

Laboratory culture experiments where benthic foraminifera were maintained under controlled conditions (i.e. partial pressure of CO_2, alkalinity) generally show a decline in foraminifera calcification under high pCO_2 (Erez, 2003; Haynert et al., 2011; Keul et al., 2013). However, this response is not uniform and varies among species (Fujita et al., 2011; McIntyre-Wressnig et

10 al., 2013; Hikami et al., 2011). Field studies at CO_2 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_2, especially of calcareous species; although benthic foraminifera have been found living near CO_2 vents in the northern Gulf of California (Pettit et al., 2013) and near experimentally injected deep-sea CO_2 hydrates (Bernhard et al., 2009).

To shed light on the potential response of Caribbean benthic foraminifera to future increase in CO_2 concentration and associated

15 pH and carbonate ion concentrations decrease, we studied the total and relative abundance of benthic foraminifera living around a series of submarine springs (locally called ojos) that naturally discharge low carbonate saturation state (Ω) groundwater in the Yucatán Peninsula (Mexico) (Crook et al., 2012). The Yucatán peninsula is a karstic region with extensive submarine groundwater springs along the coast that discharge water characterized by low pH and high total inorganic carbon and total alkalinity. Previous studies have determined that the ojos have been discharging low Ω water for millennia (Back et

20 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 Ω. Field studies from this site reported reduced coral species richness and coral colony size at the ojos 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 will also differ between the ojos and control sites, decreasing in overall abundance and having distinct distributions at different Ω sites.

25 ## 2. Materials and methods

2.1 Field sampling

Benthic foraminifera from the upper centimetre of sediment were collected in October 2011 near five submarine groundwater springs (Gorgos, Laja, Mini, Norte and Pargos) at Puerto Morelos reef Lagoon (National Marine Park), in the Mexican Caribbean coast (Fig. 1). At each spring site, five replicate surface sediment samples were collected along with water samples,

30 from near the centre of each submarine spring and at five control sites about two meters away from each 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 μm 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: ± 3 μmol kg^{-1}). Total alkalinity was measured using an automated open-cell, potentiometric titrator (Orion model 950; analytical measurement error: ± 2 μmol kg^{-1}). Certified 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 CO_2Sys (Pierrot et al., 2006) was used to calculate pH, carbonate ion concentrations and the Ω of seawater using 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 ojos and control sites and resulting data has been published in Hofmann et al. (2011) and Crook et al. (2013).

2.3 Foraminiferal analysis

Five replicate sediment samples per site were dried, weighed, washed with deionized water through a 63 μm sieve to remove clay and silt, dried at 50°C and the >250 μm fraction analyzed under an optical microscope (Bausch and Lomb) to determine foraminiferal abundance (number of individuals per gram of sediment). The most abundant genera were *Amphistegina*, *Archaias*, *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 μm sediment size fraction (2 to 122 individuals) were weighted using a micro-balance (Sartorius CP2P) and average weight per specimen determined. This species was chosen because of its abundance in most of the samples and its relatively constant test size.

2.5 Statistical analysis

Data analysis and visualization were performed using R program version 3.4.3 (Team 2017). Non-parametric Mann-Whitney rank sum test was conducted to determine differences in foraminiferal abundance and weight between each low Ω submarine spring and its corresponding control site. Nonmetric multidimensional scaling (nMDS) ordination was used to visualize the similarity in foraminiferal assemblages among Ω 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

5   Water Ω with respect to calcite was significantly lower in all ojos compared to their corresponding control sites (Table 1). pH and salinity were also lower at the ojos while alkalinity (A_T) and total inorganic carbon (C_T) were higher than at control sites. Temperature (T) was similar at all locations. Comparable results have been previously reported for these sites (Hofmann et al., 2011; Crook et al., 2012; Crook et al., 2013; Crook et al., 2016).

3.2 Foraminifera identification

10   Foraminifera were divided into three functional groups (symbiont-bearing, agglutinated, and calcareous heterotrophic foraminifera) to investigate the effect of physiological characteristics on abundance (Fig. 3). The seven most common taxa in each category were: the symbiont-bearing foraminifera *Archaias angulatus*, *Amphistegina sp.* and *Asterigerina carinata*; the agglutinated genus *Gaudryina*; and the calcareous heterotrophic foraminifera *Discorbis rosea* and several species of *Quinqueloculina* and *Triloculina* genera. These results are consistent with descriptions of benthic foraminifera taxa found in

15   Belize shelf sediments (Wantland, 1967).

3.3 Absolute abundance

Absolute abundance of foraminifera measured as the total number of individuals per gram of sediment was more than 50% higher at the high Ω control sites than at the low Ω springs, for most sites (Table 2 and Fig. 2). Mann-Whitney test revealed that absolute abundances were significantly higher at the control than at the ojo sites for Laja (W =25, p = 0.01), Mini (W =25,

20   p =0.01), Norte (W =25, p = 0.01) and Pargos (W =25, p = 0.01) but, while higher, the difference was not statistically significant at Gorgos (W = 21, p = 0.09524). Average absolute abundance of calcareous heterotrophic foraminifera ranged from 97 to 204 individuals per gram of sediment in high Ω samples and from 6 to 80 individuals per gram of sediment in low Ω samples. Symbiont-bearing foraminifera absolute abundances ranged from 84 to 200 individuals per gram of sediment in high Ω sites and from 48 to 144 individuals per gram of sediment in low Ω sites. Average absolute abundance of agglutinated foraminifera

25   ranged from 5 to 22 individuals per gram of sediment in high Ω samples and from 2 to 10 individuals per gram of sediment in low Ω samples. The average absolute abundances of calcareous heterotrophic foraminifera; symbiont-bearing; and agglutinated foraminifera were higher at high Ω than at low Ω at all sites (Table 2 and Fig. 3). This difference was statistically significant for calcareous heterotrophic foraminifera in four of the five ojos (Laja: W =25, p = 0.01; Mini: W =25, p =0.01; Norte: W =25, p = 0.01; and Pargos: W =25, p = 0.01) but not statistically significant at Gorgos (W = 20, p = 0.1508). The

30   difference was also statistically significant for symbiont-bearing foraminifera in all ojos (Laja: W =25, p = 0.01; Mini: W =25,

p =0.01; Norte: W =24, p = 0.05; and Pargos: W =25, p = 0.01) but Gorgos (W = 19, p = 0.2222). For agglutinated foraminifera, the difference was only significant in Mini (W =25, p =0.01) and Norte (W =24, p =0.01).

3.4 Relative abundance

Relative abundances of foraminifera (also referred to as community structure), measured as a percentage of each functional group within the population, also differed between Ω conditions (Fig. 3). The relative abundance of the calcareous heterotrophic foraminifera was lower at low Ω than at high Ω in all sites (Table 3). This difference was significant in all ojos (Laja: W =25, p = 0.01; Mini: W =25, p =0.01; Norte: W =25, p = 0.01; and Pargos: W =25, p = 0.01) but Gorgos (W = 20, p = 0.1508). In contrast, the relative abundance of symbiont-bearing foraminifera was higher at low Ω in all sites, this difference was significant in ojos Laja, Mini, and Norte (W = 0, p = 0.01) but not in Gorgos (W = 6, p = 0.2222) and Pargos (W = 4, p = 0.09524). Similarly, the relative abundance of agglutinated foraminifera was higher or the same at low Ω compared to high Ω sites and the difference was statistically significant in ojo Laja (W = 2, p = 0.05).

Calcareous heterotrophic foraminifera contributed about 50% of the total number of benthic foraminifera at the high Ω in most of the sites while the relative abundance dropped below 50% at low Ω sites. In contrast, symbiont-bearing foraminifera contributed less than 50% of the population at high Ω sites while the percentage surpassed 50% in the low Ω sites, replacing calcareous heterotrophic foraminifera as the most abundant type at the low Ω ojo sites.

The nMDS plots showed a clear clustering of relative abundances between high and low Ω and lack of clear clustering among sites (Fig. 4). The envfit function revealed that areas where calcareous heterotrophic foraminifera are relatively more abundant are characterized by higher pH (R^2 = 0.3531, p = 0.001), salinity (R^2 = 0.4420, p = 0.001), and particularly Ω_c (R^2 = 0.4735, p = 0.001), while areas where calcareous heterotrophic foraminifera are less abundant are characterized by higher alkalinity (represented as arrow A in Fig. 4, R^2 = 0.4420, p = 0.001), and higher total inorganic carbon (represented as arrow C in Fig. 4, R^2 = 0.4261, p = 0.001). Temperature was significant (represented as arrow T in Fig. 4, R^2 = 0.1234, p = 0.036), however it does not explain much of the variance, as the T arrow is not on the main gradient explaining the maximal variance of data, and the temperature difference among sites was at most two degrees Celsius. Calcareous heterotrophic foraminifera relative abundance was associated with high Ω while symbiont-bearing foraminifera and agglutinated foraminifera abundances were related to low Ω sites, consistent with field observations.

3.5 Test weight

The average test weights of *Discorbis rosea* (size fraction 250-355 μm) from low Ω ojos were lower or the same when compared to tests from high Ω control sites, although, the difference was not statistically significant in any of the sites (Table 3 and Fig. 5).

4 Discussion

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 alkalinized, 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 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 Ω submarine springs as the low pH low saturation 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 (due to carbonate dissolution in the aquifer) but lower pH, resulting in lower Ω and carbonate ion concentrations (based on field measurements and CO_2Sys calculations). Toyofuku et al. (2017) proposed that calcification depends on total inorganic carbon (C_T) concentration and not necessarily on Ω. However, in our field study the C_T concentrations were high at the low Ω sites and we still see a decrease in calcareous 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 foraminiferal calcification at low pH, it does not seem to fully counteract the effect of low Ω. The salinity of the discharging water is also different (slightly lower) at the ojos relative to control sites, however, other field studies such as the study at the high CO_2 vents at Papua New Guinea (PNG), where salinity was not different, have found a similar reduction in benthic foraminifera abundance (Fabricius et al., 2011; Uthicke et al., 2013) indicating that Ω was the major parameter affecting benthic foraminifera calcification. Like at our study site and the PNG site, calcareous foraminifera have been found at CO_2 vents with pH ranging from 7.83 to 7.55 in the Gulf of California (Pettit et al., 2013) and near experimental intentional deep-sea carbon dioxide hydrate release sites (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 observe in diverse settings with low pH and low Ω suggest that future reduction in these parameters will negatively affect calcifying benthic foraminifera.

Core-top calibrations used for palaeoceanographic records indicate there is a positive relation between test weight of planktonic foraminifera and carbonate concentration (Barker and Elderfield, 2002) and that the weight of modern planktonic *Globigerina bulloides* tests is 30-35% lower than that of Holocene shells (Moy et al., 2009). Indeed, laboratory experiments at pCO_2 of 1900 ppmv show that shell weight of *Ammonia tepida* decreased with decreasing carbonate ion concentration even though the

5 foraminifera were able to calcify at carbonate undersaturated water (Dissard et al., 2010). The tests of *D. rosea* collected at the low Ω sites in this study had lower or similar average test weight when compared to tests collected at control sites with high Ω. The lack of statistical significance of the difference in weight 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 pCO_2

10 incubations and suggested that this is due to different growth rates. In addition, it has been suggested that body size could influence calcification (Henehan et al., 2017). In our study, the weighted tests were all picked from the 250-355 μm 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 Ω sites, the impact was larger on the

15 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. Foraminifera hosting photosynthetic symbionts may be more resilient to low Ω since they can access additional energy derived from photosynthates translocated from the algae (Hallock, 2000) to increase pH at the calcification site and for alkalinization of seawater vacuoles. In addition, symbiotic algae can promote calcification by removing foraminiferal metabolic N and P which impede crystal formation, as well as by

20 providing organic matter used to synthesize the organic matrix that precedes test growth (Fujita et al., 2011). These mechanisms may explain the significant increase in relative abundance of symbiont-bearing foraminifera (>50%) while calcareous heterotrophic foraminifera relative abundance decreased (<50%) at low Ω sites. Laboratory experiments have shown that the symbiont-bearing benthic foraminifera *Amphistegina gibbosa* was able to survive and grow at 2000 ppmv pCO_2 for 6 weeks although it showed signs of test dissolution (McIntyre-Wressnig, 2013). Similarly, reduced net calcification was detected in

25 the symbiont-bearing foraminifera *Baculogypsina sphaerulata*, *Calcarina gaudichaudii*, and *Amphisorus hemprichii* after a 12-week incubation at pCO_2 of 970 μatm (Fujita et al., 2011), although calcification of *B. sphaerulata* and *C. gaudichaudii* increased at intermediate pCO_2 (580 and 770 μatm). These laboratory studies suggest that benthic symbiont-bearing foraminifera can benefit from slightly high CO_2 to a certain degree but while they might survive at very high pCO_2, their calcification is reduced. Although symbiont-bearing calcareous foraminifera were relatively more abundant (when compared

30 to those without symbionts) at the low Ω sites, we note that their total abundance decreased in comparison with sites at ambient pH, indicating that despite the symbionts, the conditions at the ojos were less favorable than at the high Ω control sites. These results agree with observations by Glas et al. (2012) and Uthicke and Fabricius (2012), who proposed that symbiotic photosynthetic activity does not fully compensate the deleterious effects of ocean acidification on foraminiferal calcification.

Non-calcifying foraminifera are less influenced by low Ω because they do not rely on calcification to produce their test, and therefore their contribution to the foraminifera population at our study sites does not decrease under low Ω. 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 towards more non-calcareous foraminiferal populations was also reported in the Mediterranean 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., 2010). Similarly, the abundance of non-calcifying thecate and agglutinated foraminifera living in direct contact with the experimentally injected CO_2 hydrate did not decline significantly with decreasing pH (Bernhard et al., 2009). At our study site, we record a small decrease in the absolute abundance of non-calcifying agglutinated foraminifera, but the relative abundance of the agglutinated foraminifera increased, indicating that these foraminifera are less affected by the low pH conditions than the calcareous foraminifera.

5 Conclusion

In summary, our results suggest that low pH low calcite Ω seawater results in lower foraminiferal abundance, even in areas with high concentration of inorganic carbon. The impact is more severe for calcareous heterotrophic foraminifera than for symbiont-bearing foraminifera and agglutinated foraminifera. While symbiont-bearing foraminifera and non-calcifying foraminifera are less affected than calcareous heterotrophic foraminifera by the low pH low Ω, the lower absolute abundance of all benthic foraminifera recorded in this study at the ojo sites suggest that there may be an overall decrease in benthic foraminifera abundance as well as in their shell thickness as a consequence of ocean acidification, with subsequent repercussions on the global carbon cycle and marine food webs. Specifically, planktonic foraminifera efficiently transport biogenic carbon to depth since they sink fast due to their large size and high density (Legendre and Le Fèvre, 1995) and benthic foraminifera are estimated to annually produce 200 million tons of carbonate and, along with planktonic foraminifera, they contribute to around 25% of present-day global ocean carbonate production (Langer, 2008). Finally, 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) hence reduction in their abundance or species distribution may have implications to the ecosystem as a whole.

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 sites (mean ± SD) at the time of sample collection (A_T= total alkalinity; C_T= total inorganic carbon).

Site		A_T ($\mu mol \cdot kg^{-1}$)	C_T ($\mu mol \cdot kg^{-1}$)	*pH	*CO_3^{2-} ($\mu mol \cdot kg^{-1}$)	*$\Omega_{calcite}$	T (°C)	Salinity
Gorgos	control	2325 ± 3	2033 ± 3	7.96	209.42	5.02	27.8	35.9
	ojo	2874 ± 11	2987 ± 8	7.11	94.61	2.38	28.47	31.09
Laja	control	2357 ± 6	2092 ± 1	7.90	193.52	4.63	28.15	36.17
	ojo	2827 ± 9	2756 ± 10	7.51	102.6	2.50	27.9	32.75
Mini	control	2356 ± 3	2049 ± 6	7.99	218.2	5.16	26.4	37.3
	ojo	3108 ± 10	3197 ± 6	7.13	46.3	1.14	27.6	32.41
Norte	control	2354 ± 13	2051 ± 6	7.98	216.2	5.14	27	36.8
	ojo	2611 ± 3	2588 ± 3	7.38	67.0	1.66	27.5	32.21
Pargos	control	2336 ± 4	2012 ± 12	8.01	229.55	5.49	27.65	36.17
	ojo	3000 ± 8	3048 ± 12	7.23	52.70	1.33	27.6	29.95

* Calculated using CO_2Sys

5 **Table 2: Mean absolute abundance of foraminifera (specimens per gram of sediment ± SE, n= 5 samples per site) and abundance of each functional group from size fraction >250 µm.**

Site	Saturation	Foraminifera	Calcareous heterotrophic	Symbiont-bearing	Agglutinated
Gorgos	high	205 ± 23	106 ± 18	92 ± 6	7 ± 3
	low	140 ± 22	63 ± 14	72 ± 9	5 ± 1
Laja	high	190 ± 18	97 ± 12	84 ± 6	8 ± 0
	low	81 ± 8	26 ± 5	48 ± 3	6 ± 1
Mini	high	266 ± 36	157 ± 24	93 ± 8	16 ± 4
	low	18 ± 6	6 ± 2	10 ± 4	2 ± 1
Norte	high	426 ± 21	204 ± 16	201 ± 10	22 ± 3
	low	235 ± 14	80 ± 7	144 ± 9	10 ± 2
Pargos	high	249 ± 15	100 ± 9	144 ± 7	5 ± 0
	low	101 ± 26	33 ± 11	64 ± 16	4 ± 1

Table 3: Mean relative abundance (% ± SE) of foraminifera of different functional groups from size fraction >250 µm and weight of *Discorbis rosea* tests (mean ± SE, n= 5) from size fraction 250-355 µm.

Site	Saturation	% Calcareous heterotrophic	% Symbiont-bearing	% Agglutinated	*D.rosea* (μg test^{-1})
Gorgos	high	51 ± 3	46 ± 3	3 ± 1	40 ± 3
	low	44 ± 3	52 ± 2	4 ± 1	36 ± 1
Laja	high	51± 2	45 ± 2	4 ± 0	35 ± 0
	low	32 ± 3	61 ± 3	7 ± 1	36 ± 2
Mini	high	58 ± 2	36 ± 2	6 ± 1	42 ± 3
	low	36 ± 5	54 ± 5	10 ± 2	36 ± 2
Norte	high	48 ± 2	47 ± 2	5 ± 1	42 ± 1
	low	34 ± 2	61 ± 2	4 ± 1	42 ± 1
Pargos	high	40 ± 1	58 ± 1	2 ± 0	37 ± 1
	low	30 ± 3	66 ± 3	4 ± 2	40 ± 3

11 Figures
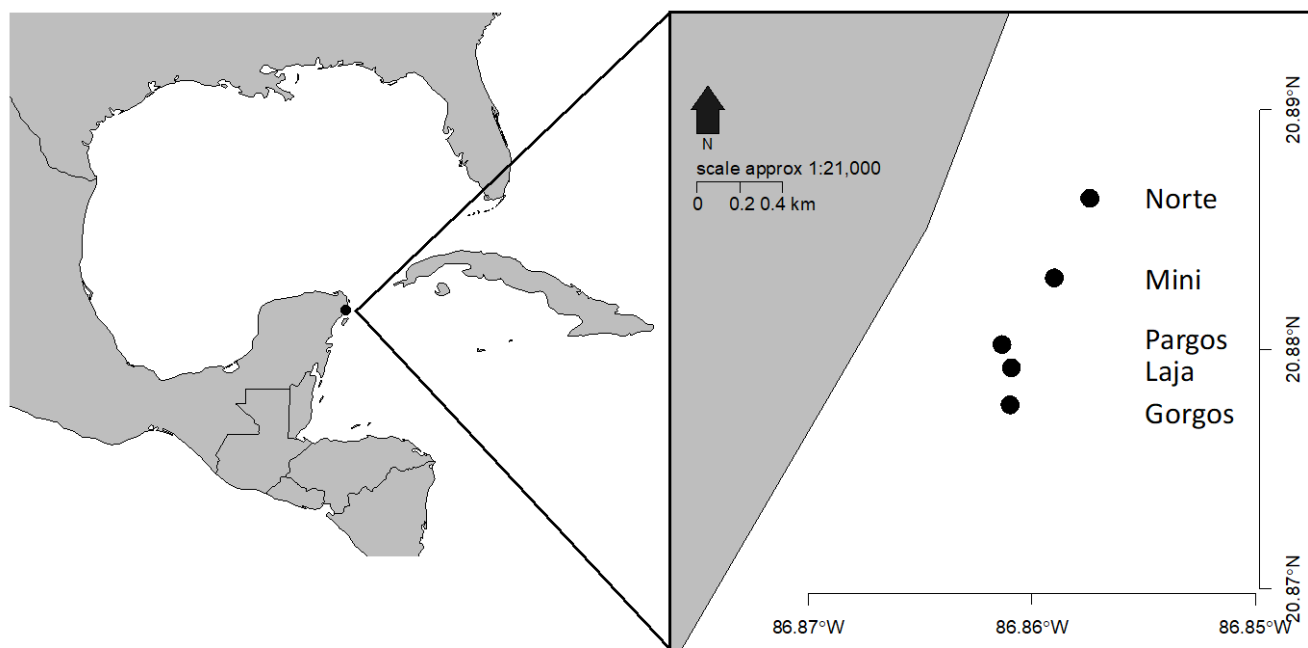
Figure 1: Location of low carbonate saturation state submarine springs
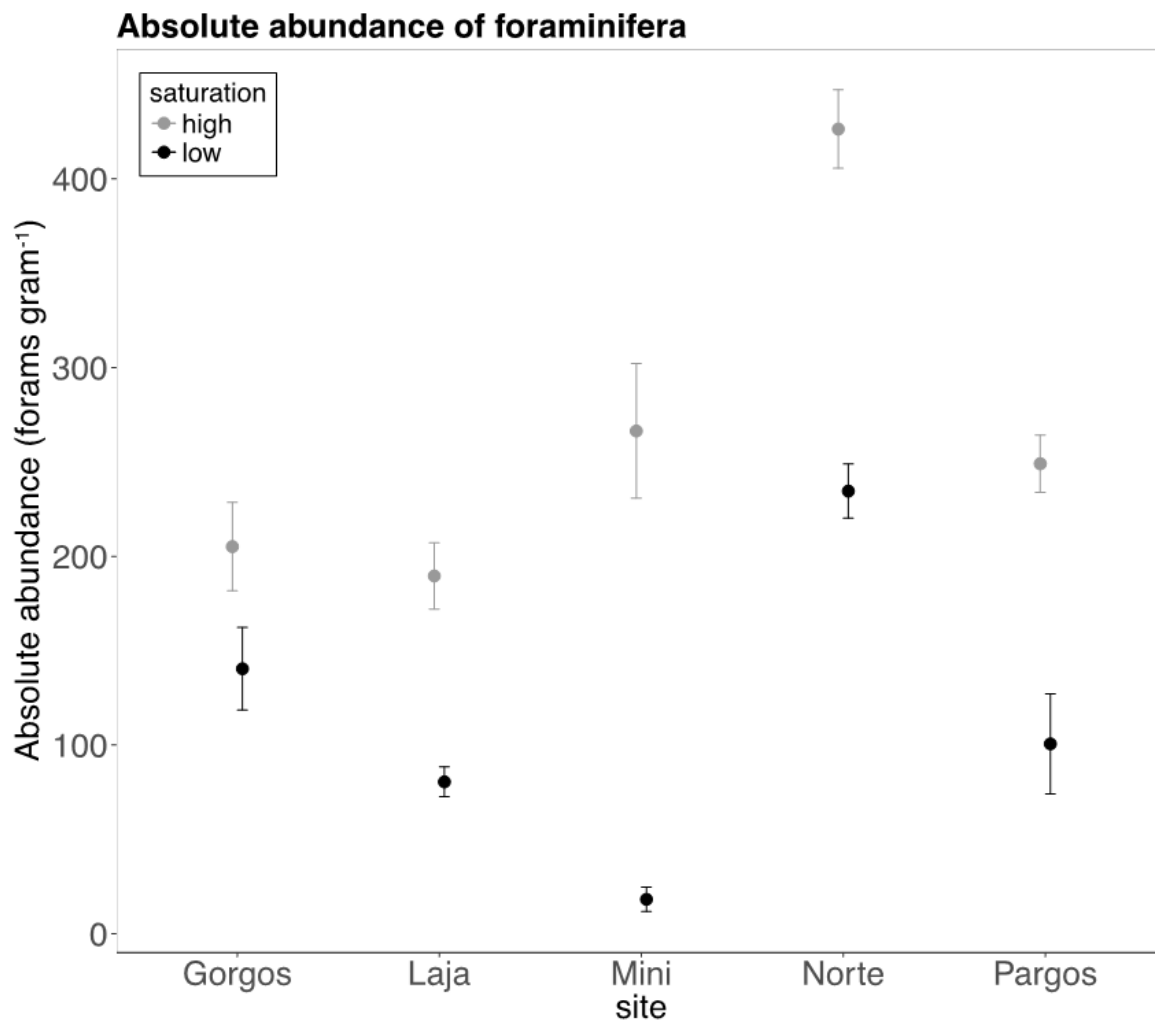
Figure 2: Absolute abundance of foraminifera (number of specimens per gram of sediment) in different submarine springs (low saturation state) and their respective control sites (high saturation state). Data are mean ± SE (n= 5).
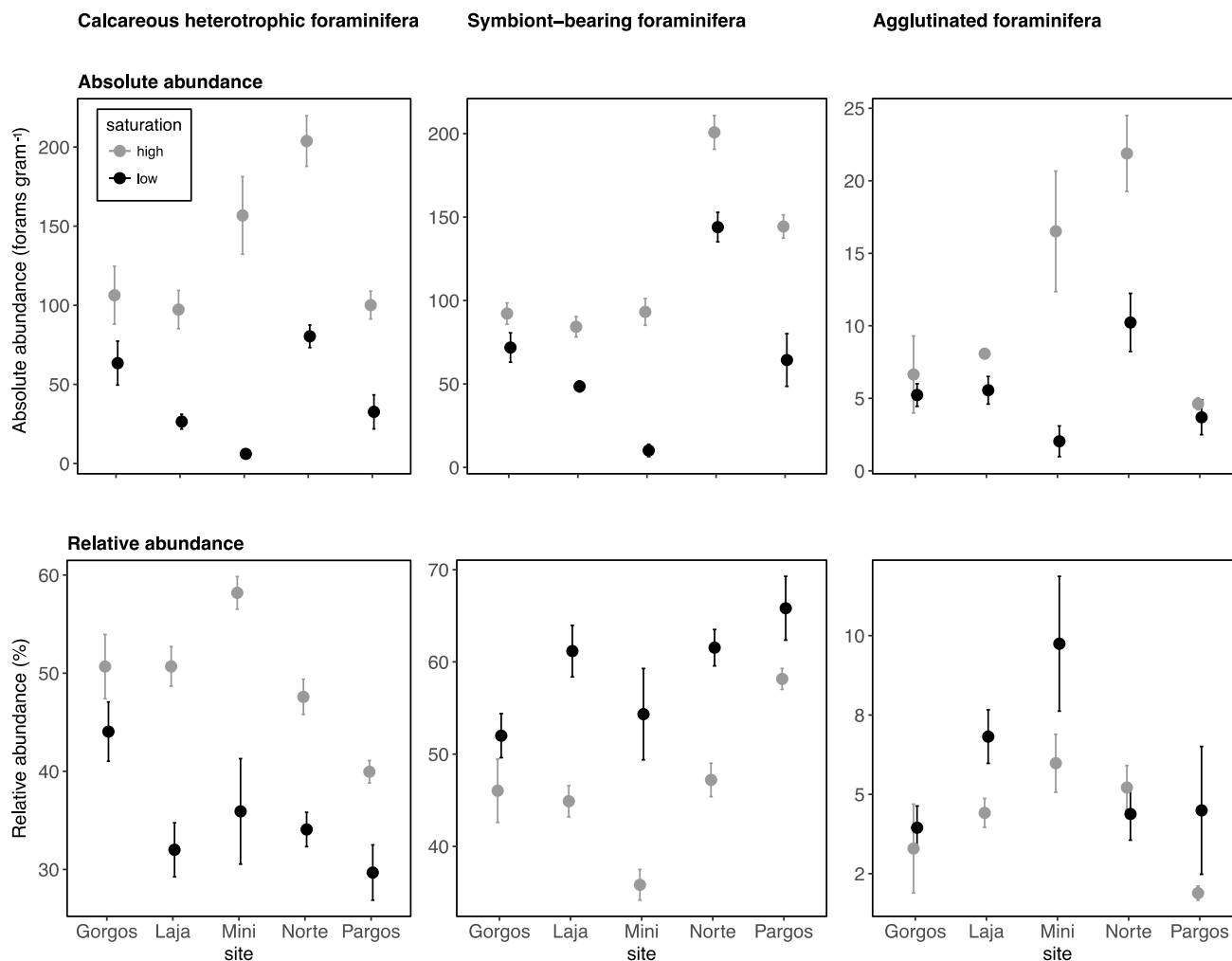
Figure 3: Absolute abundance (specimens per gram of sediment) and relative abundance (percentage) of calcareous heterotrophic, symbiont-bearing, and agglutinated foraminifera. Data are mean ± SE (n= 5). Note the different Y-axis scales.
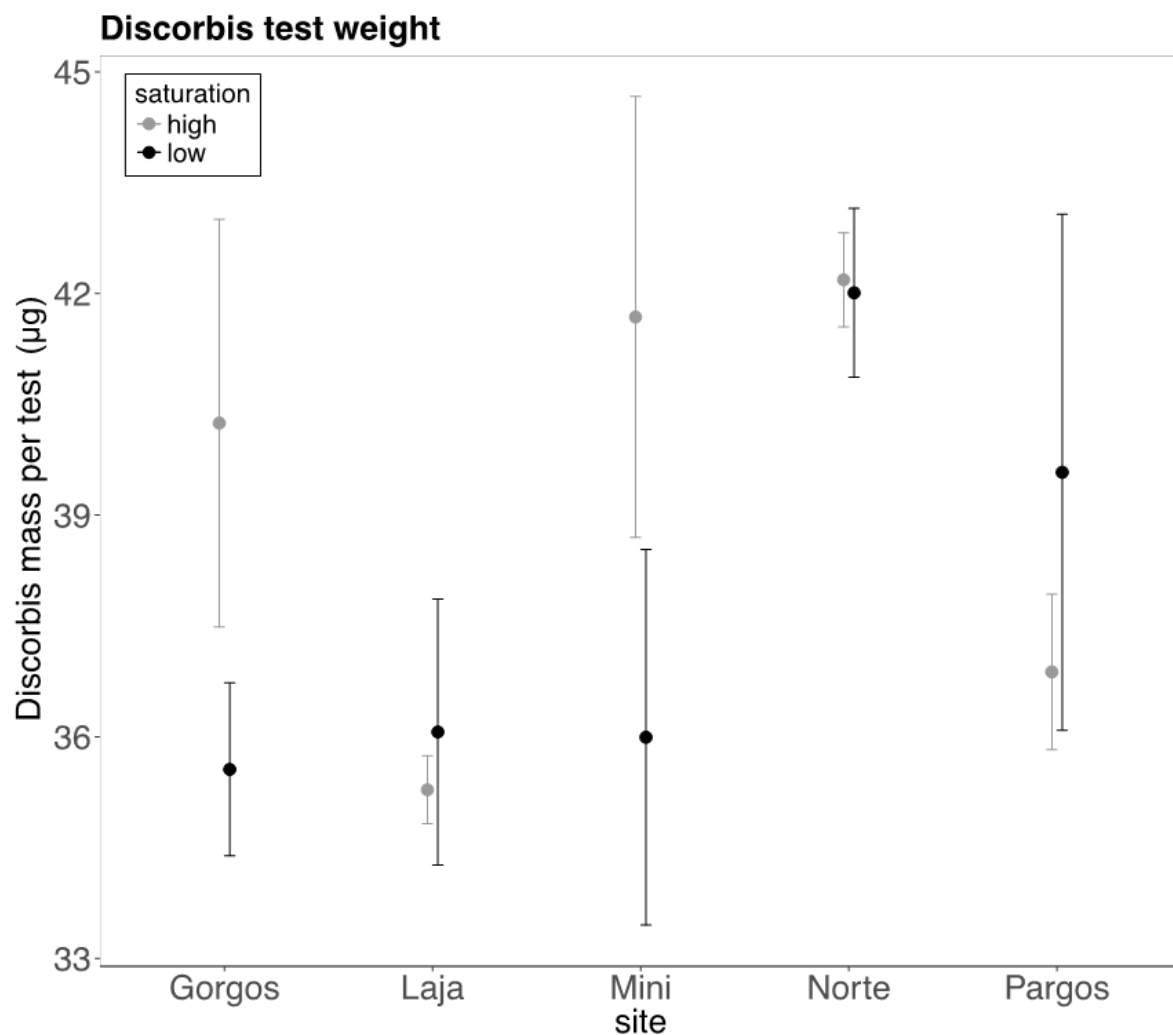
Figure 4: Mean weight of *Discorbis rosea* tests (size fraction 250-355 μm) at low and high saturation at different submarine spring sites. Data are mean ± SE.
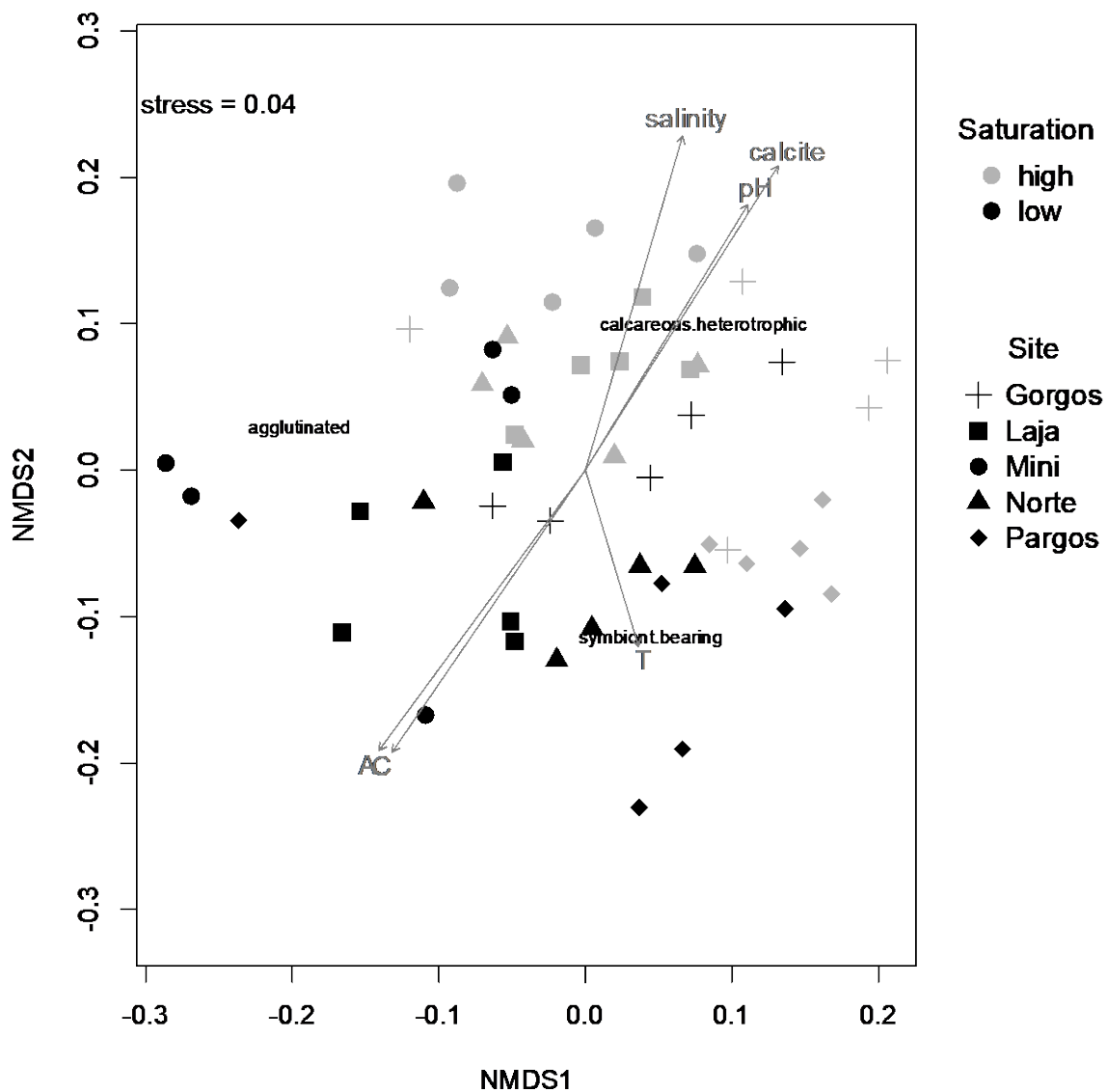
Figure 5: Non-metric Multidimensional Scaling (nMDS) ordination plot for community structure by carbonate saturation state and site with overlaid environmental parameters (A= total alkalinity; C= total inorganic carbon; T= temperature).