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Research papers

Sinkhole-like structures as bioproductivity hotspots in the Abrolhos Bank

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ABSTRACT

We performed a biological survey in the novel system of sinkhole-like structures ("*buracas*") of the Abrolhos Bank, Brazil. We found dissimilar benthic assemblages and higher nutrient concentration, microbial abundance (and activity) and fish abundance inside the *buracas* than in the surrounding rhodolith beds. Our results support the view that these cup-shaped structures trap and accumulate organic matter, functioning as productivity hotspots in the mid and outer shelf of the central portion of the Abrolhos Bank shelf, where they aggregate biomass of commercially important fishes. This distinctive system is being increasingly pressured by commercial fisheries and needs urgent management measures such as fishing effort control and representation in the network of Marine Protected Areas (MPAS).

1. Introduction

The Abrolhos Bank contains the largest and richest coralline reefs of the South Atlantic. This coastal system is well known for its coral assemblages dominated by Brazilian-endemic species (Leão and Ginsburg, 1997) with distinctive mushroom-shaped reef pinnacles (Hartt, 1870; Verrill, 1868; Laborel, 1969), dwelling under high siliciclastic sedimentation. Current knowledge on local reef morphogenesis and ecology is derived mainly from studies on inner-shelf Holocene structures. Recent assessments have shown a high geodiversity in the mid- and outer shelf (Moura et al., 2013), including sinkhole-like structures described by Bastos et al., 2013) and herein.

Mesophotic coralline ecosystems occur in many locations throughout the tropics, from about 30-m depth to the bottom of the photic zone (Lesser et al., 2009; Kahng et al., 2010). However, until recently only scarse information existed on their biodiversity and functioning because of logistic constraints (Hinderstein et al., 2010). Technological advances are now providing easier access to rebreathers and mixed-gas diving, remotely operated vehicles (ROVs) and high-resolution sidescan imagery, making it possible to investigate these environments in more detail.

While hosting some depth-restricted species, mesophotic reefs contain many shallow-water species (Thresher and Colin, 1986; Macintyre et al., 1991), often representing refugia for commercially-important species that are already threatened in shallower reefs (Glynn, 1996; Armstrong et al., 2006). Within the large mesophotic reef realm of the Abrolhos Bank, the unusual sinkhole-like depressions, locally named "buracas" ("buraca" meaning "hole" in Portuguese) are outstanding features with potentially important ecological roles in the mid- and outer shelf. Although diverse submerged sinkholes have been described worldwide, typically found on shallow carbonate platforms, exemplified by the Bahama Banks (Whitaker and Smart, 1998; Reed et al., 2005), as well as on and around the Yucatán Peninsula (Schmitter-Soto et al., 2002), such as at the Great Blue Hole at Lighthouse Reef Atoll, Belize (Gischler et al., 2008), the majority of studies of the underwater

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sinkholes focus on various aspects of their geology (Betzler et al., 2011; van Hengstum et al., 2012; Martin et al., 2012). Few studies focus on the ecology or biology of either the microbial, benthic or fish communities (Reed et al., 2005; Baumberger et al., 2010; Nold et al., 2010).

The numerous novel sinkhole-like structures located off eastern Brazil were initially discovered by local fishermen, as they were anecdotally reported as major fishing grounds. The underlying reasons for such increased fisheries yields were initially unclear, as fishermen also reported different water color and odor, a characteristic "mangrove-like" smell in these very specific spots. Although the "buracas" aggregate fishes (both reef and pelagic) and lobsters, which are important targets for commercial fisheries, those structures are not comprised in the existing network of Marine Protected Areas.

We assessed the main biological and chemical features of these cup-shaped structures in order to evaluate their potential role as productivity hotspots. The specific aims of our study were: (1) to assess the physicochemical characteristics of the "buracas" system, including inorganic and organic nutrient content; (2) to quantify microbes and to describe their diversity through metagenomic analyses and metabolic profiles; (3) to survey the main benthic taxa and fish communities associated with such structures. The mesophotic reefs in the mid- and outer shelf of Abrolhos add complexity to the models explaining the evolution of this unique tropical continental shelf (Laborel, 1969; Leão et al., 2003), and also challenge the understanding of biological processes and cross-shelf connectivity patterns (e.g. Moura et al., 2011), with important implications for natural resources management.

2. Materials and methods

2.1. Study sites and sampling design

The distribution, location and geomorphological characteristics of the sinkhole-like structures are described elsewhere (Bastos et al., this issue). Briefly, a total of 35 circular depressions (17–55 m in diameter, 8—44 m height) occurring between 77 and 198 km off the coast were recently identified within the Abrolhos region, eastern Brazil. Sampling was carried out in two sites randomly selected from the 35 analogous structures. The first site (Buraca 1—B1) is located 150 km offshore (at 50 m depth), with its deepest part at 93 m depth and a diameter of approximately 55 m; the second site (Buraca 2—B2) is located 115 km offshore (at 26 m depth), with its deepest part at 43 m depth and a diameter of approximately 40 m (Fig. 1). Site B1 comprises a single isolated structure, while site B2 comprises two adjacent structures. Water sampling was carried out between 27 and 85 m depth, inside (IN) B1 (1 m from the bottom) and in the largest structure at site B2 (8 m from the bottom), as well as in two control areas outside (OUT) the structures, 200—500 m away from the edge. Other biological sampling (fish counts and benthic assessments) was carried out inside the three structures and in two outside control sites. Field work was conducted in two consecutive summers (2009–2010).

2.2. Chemical analysis and bacterial abundance

Environmental parameters were analyzed by standard oceanographic methods (Grasshoff et al., 1999). Chlorophyll-*a* quantification was performed after vacuum filtration (<25 cm of Hg; cellulose membrane Millipore HAWP 0.45 μm) of 1-L seawater samples. Membranes were frozen in liquid nitrogen until laboratory analysis, when pigments were extracted overnight in 90% acetone at 4 °C and analyzed with a UV–vis Perkin Elmer Lambda 20 spectrophotometer (Perkin Elmer, USA). Water samples were frozen in the field for inorganic nutrients analyses, which were carried out by the following methods: (1) ammonium by indophenol, (2) nitrite by diazotization, (3) nitrate by reduction in Cd–Cu column followed by diazotization, (4) total nitrogen by digestion with potassium persulfate following nitrate determination, (5) orthophosphate by reaction with ascorbic acid, (6) total phosphorous by acid digestion to phosphate, and (7) silicate by reaction with molibdate.

Total bacterial abundance was determined from 2 mL water subsamples (three replicates) immediately fixed in the field with sterile 2% paraformaldehyde for 15 min and further preserved in liquid nitrogen (Gasol and Del Giorgio, 2000). Microbial cells with high (HNA) and low (LNA) nucleic acids content were distinctly quantified through measurement of fluorescence intensity by flow cytometry with Syto-13 (Life Technologies, Carlsbad, CA), with minor modifications (Thompson et al., 2011).

Two-sided Student's *t*-test was used for assessing the statistical significance of the difference in nutrient content and bacterial

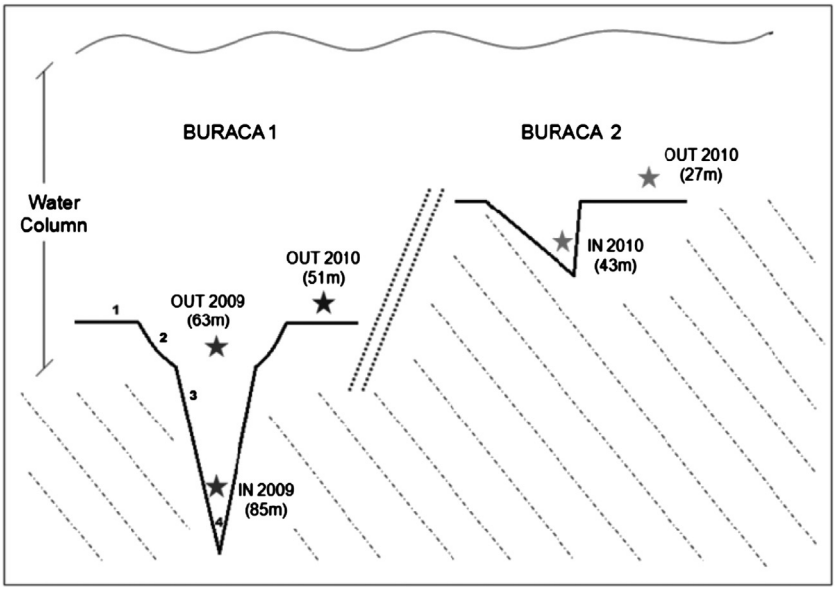

Fig. 1. Schematic design of the sinkhole-like structures (*buracas*) and the sampling design (sampling sites denoted by stars).

Table 1
General features *buracas*.

New Reef Structure	Buraca 1			Buraca 2	
	IN 2009	OUT 2009	OUT 2010	IN 2010	OUT 2010
Geographic location	S17.8561°/W38.119933°	S17.8561°/W38.119933°	S17.91361°/W37.90936°	S17.81399°/W38.24306°	S17.81330°/W38.23744°
Depth (m)	63	85	51	43	27
Ortophosphate (μM)	0.47 ± 0.026 (*N*=3)	0.08 ± 0.006 (*N*=3)	0.17 ± 0.005 (*N*=3)	0.84 ± 0.01 (*N*=3)	0.30 ± 0.01 (*N*=3)
Total phosphorous (μM)	1.62 ± 0.063 (*N*=3)	0.21 ± 0.015 (*N*=3)	0.24 ± 0.007 (*N*=5)	1.03 ± 0.01 (*N*=3)	0.39 ± 0.01 (*N*=5)
Ammonia (μM)	7.73 ± 0.080 (*N*=3)	0.07 ± 0.015 (*N*=3)	0.06 ± 0.023 (*N*=3)	0.87 ± 0.05 (*N*=3)	0.05 ± 0.005 (*N*=3)
Nitrite (μM)	0.45 ± 0.01 (*N*=3)	0.04 ± 0 (*N*=3)	0.05 ± 0 (*N*=3)	0.12 ± 0.01 (*N*=4)	0.03 ± 0.009 (*N*=4)
Nitrate (μM)	1.81 ± 0.05 (*N*=3)	0.75 ± 0.05 (*N*=3)	1.06 ± 0.17 (*N*=3)	1.19 ± 0.08 (*N*=3)	1.30 ± 0.23 (*N*=3)
Total Nitrogen (μM)	14.85 ± 1.12 (*N*=3)	8.6 ± 0.44 (*N*=3)	7.99 ± 0.84 (*N*=3)	27.63 ± 0.63 (*N*=3)	10.28 ± 0.64 (*N*=5)
Silicate (μM)	2.71 ± 0.14 (*N*=3)	1.2 ± 0.017 (*N*=3)	0.82 ± 0.015 (*N*=3)	1.34 ± 0.01 (*N*=3)	0.90 ± 0.64 (*N*=3)
Chlorophyll a (μM)	_	0.18 ± 0.003 (*N*=2)	0.28 ± 0.05 (*N*=2)	4.61 ± 2.05 (*N*=2)	0.15 ± 0.04 (*N*=2)
Phaeophytin (μM)	_	0.02 (*N*=2)	0.19 ± 0.06 (*N*=2)	7.46 ± 3.51 (*N*=2)	0.10 ± 0.00 (*N*=2)
Salinity	37.43 ± 0.10 (*N*=3)	37.22 ± 0.08 (*N*=3)	32.47 ± 3.33 (*N*=9)	37.00 ± 0.07 (*N*=9)	32.70 ± 3.33 (*N*=9)
Bacterial Count (cells/mL)	9.52×10^6 (*N*=3)	5.26×10^5 (*N*=3)	1.74×10^6 (*N*=3)	4.35×10^6 (*N*=3)	1.35×10^6 (*N*=3)
HNA/LNA	2.22	0.29	0.74	3.22	0.92
Metagenome size (Post QC) Mbp	15.76	13.9	13.12	18.07	12.06
Total number of sequences (Post QC)	37.683	32.569	31.975	42.103	28.542
Classified by MG-Rast (GenBank)	26,691 (70.83%)	21,305 (65.41%)	22,355 (69.91%)	30,795 (73.31%)	23,703 (83.46%)
Classified by MG-Rast (Subsystems)	37.607	31.091	33.074	40.964	34.101

count between the inner and outer regions of each structure (B1 2009 IN × OUT; B2 2010 IN × OUT). Only data with p-values < 0.05 were considered statistically different.

2.3. Metagenomic analysis

Five metagenomic profiles were generated, three for Buraca 1 (B1; IN 2009, OUT 2009, OUT 2010) and two for Buraca 2 (B2; IN 2010, OUT 2010) (Table 1). Three 2-L seawater replicates for each point were pre-filtered with a 20 μm net and then filtered using Sterivex filters (0.22 μm). The latter received SET buffer (2 mL) and were stored at −80 °C. DNA extraction was performed as described previously (Thompson et al., 2011). Metagenomic DNA were pyrosequenced by a 454 GS FLX machine (454 Life Sciences, Branford, CT), using a GS FLX Titanium sequencing process. Sequences were submitted to the MG-RAST 3.1 (Metagenomics—Rapid Annotation using Subsytems Technology) server, an automated analysis platform for metagenomes providing quantitative insights into microbial populations based on sequence data (Meyer et al., 2008) and quality filtered. Post-quality control (QC) sequences were annotated using the SEED subsystems for metabolic analyses (Overbeek et al., 2005), and the GenBank database for phylogenetic analyses, both with a maximum 10^{-5} expected value cutoff. The Statistical Analysis of Metagenomic Profiles (STAMP v2.0.0) software was used for statistical analysis (Parks and Beiko, 2010). Statistical significance was calculated using two-sided Fisher's exact test, and the differences between proportions were analyzed using the Newcombe–Wilson method with 95% confidence interval. Data was further subjected to filtering and only data with p-values < 0.05 were considered statistically different.

2.4. Benthic and fish assemblages

Video images were obtained with a Seabotix® LBV 150S remotely operated vehicle (ROV) equipped with a color video camera and two parallel reference laser beams. Footage was recorded ad libitum for at least 40 min during each ROV deployment inside (IN) and outside (OUT) the structures. Footage was transformed to one-frame-per-second still images, with 25 randomly selected frames used to determine benthic coverage at each stratum. Images obtained at the convex margin and in the gentle slope zone of the structures were used for the comparisons with the control rhodolith bed sites. Benthic images were processed with software Coral Point Count with Excel Extension (Kohler and Gill, 2006), using 20 random points per frame. Benthic coverage was estimated by identifying organisms (or categories) immediately below each point. Turf was defined as multispecific consortia of small epilithic algae, microorganisms and detritus. Non-metric multidimensional scaling (MDS) ordination, based on triangular matrices with Bray–Curtis similarity index between each pair of samples, was used to analyze spatial similarities between benthic assemblages. Control sites (OUT) were adjacent rhodolith beds in which the density of rhodoliths (rhodoliths m^{-2}) was determined by counting every individual nodule within each frame. Fish abundance was estimated as sightings-per-minute for each species, also from the ROV footage. Data were $\log_{10}$ transformed to counteract the weight of dominant species without severely diminishing their importance. Reef fish species richness was compiled from both the ROV footage and records made by divers (up to 80 m depth). The ROV sampling was followed by 3–5 mixed-gas (TRIMIX: He–N–O) diving operations in each structure and in the adjacent rhodolith beds, in order to improve the taxonomic resolution of ROV surveys by means of direct observations and collections of selected specimens. Water samples were collected during the dive operations.

3. Results

3.1. Seawater physical-chemistry and microbiological features

Analyses of seawater parameters for the two sites showed higher inorganic nutrient concentration within the structures than outside ($p < 0.001$), except for nitrate in site B2 2010), with maximum values observed in the B2 site (total N 27.63 μM ± 0.63) (Table 1). High chlorophyll-*a* levels were accompanied by high phaeophytin (chlorophyll degradation product) levels in this B2 site. Additionally, total bacterial counts were statistically higher inside than outside both structures ($p < 0.001$) (up to 18-fold increase) (Table 1). Finally, a higher proportion of bacterial cells with high nucleic acid content was recorded inside the structures (HNA/LNA > 2.2) than outside (HNA/LNA < 0.9), possibly indicating more active cellular division inside the structures (Table 1).

3.2. Microbial community structure

A total of 72.91 million non-redundant base pairs (Mbp) was produced in this study, with an average of 34,500 sequences per

metagenome (Table 1). The major contributing domain was *Bacteria* with 88.43% (B1 IN 2009), 88.21% (B1 OUT 2009), 92.81% (B1 OUT 2010), 97.55% (B2 IN 2010), and 97.21% (B2 OUT 2010) of the recorded sequences. Only a very low percentage of the sequences belonged to the *Archaea* domain, ranging from 0.33% (B2 OUT 2010) to 1.09% (B1 OUT 2009), with the *Euryarchaeota* phylum being the most representative (data not shown). Within the *Eukarya* domain different contributions were found between the two sites. A more pronounced number of hits was assigned to eukaryotes in B1 site for all three samples (5.0% in average) when compared to B2 (1.2% in average), with a larger predominance of microalgal groups in the B1 site (data not shown). Viral sequences corresponded to a small fraction of B2 metagenomes (around 0.5%), but phages (mainly cyanophages) were more abundant in metagenomes from site B1 (approximately 4%), suggesting higher ongoing (cyano)bacterial infection in this site (data not shown).

The *Proteobacteria* phylum was the most abundant bacterial group in all samples, corresponding to about 42–53% of metagenomes from site B1, and 63–70% of site B2, with a significantly higher ($p < 0.05$) number of proteobacterial sequences outside than inside both structures (Fig. 2; B1 IN 2009 versus B1 OUT 2009; and B2 IN 2010 versus B2 OUT 2010). Within this phylum, classes *Alphaproteobacteria* and *Epsilonproteobacteria* were more abundant outside and inside both structures, respectively. The latter class was dominant in the B2 IN 2010 metagenome (Fig. 2). The interior of both structures, B1 and B2, consistently exhibited high abundance of *Bacteriodetes*. The B1 site presented a clear predominance of *Cyanobacteria* (Fig. 2).

Taxonomic distinctions between samples obtained inside and outside the cup-shaped structures were more evident in metagenomes of site B2. In this site there was a significant predominance ($p < 0.05$) of *Alphaproteobacteria* orders (*Rickettsiales*, *Rhizobiales*, *Rhodospirillales*, *Sphingomonadales*) outside the structure, except for the *Rhodobacterales* group, which dominated in B2 IN 2010 metagenome (Fig. 3). The heterotrophic taxa *Roseobacter* (*Rhodobacterales*/*Alphaproteobacteria*) was the most common genera inside the structure of site B2 (Supplementary Table S1). Several bacterial groups related to sulfur metabolism were also more abundant inside than outside the structure at site B2 ($p < 0.05$), such as the orders *Desulfuromonadales*, *Desulfovibrionales*, *Desulfobacterales* (*Deltaproteobacteria*) (Fig. 3), as well as the order *Campylobacterales* (*Epsilonproteobacteria*). The genera *Arcobacter*, *Campylobacter* and *Sulfiromonas* were the main campylobacteria representatives in the B2 IN 2010 metagenome (Supplementary Table S1). On the other hand, the B2 OUT 2010 metagenome was richer in sequences belonging to *Gammaproteobacteria* orders, mainly *Alteromonas* (*Alteromonadales*), which was clearly the most abundant group (Supplementary Table S1).

The huge contribution of the cyanobacterial group in site B1 was noticeable down to the genus level, with *Prochlorococcus* and *Synechococcus* being the first and third most abundant genera of each B1 metagenome (B1 IN 2009, B1 OUT 2009, B1 OUT 2010), summing on average 22.5% and 6.4% of bacterial sequences, respectively (Supplementary Table S1). Another very abundant genus in the B1 metagenome was SAR11 (Candidatus *Pelagibacter*). Interestingly, the predominant genera were nearly the same in all B1 metagenomes (even in different years), unlike B2 metagenomes, which presented different profiles inside and outside the Buraca (Supplementary Table S1).

3.3. Metabolic profiles (subsystems classification)

The metagenomes were classified in 26 informative subsystems, revealing a similar pattern for the most predominant metabolic processes in all samples, with the five most abundant responses (carbohydrate, amino acids and derivatives, protein metabolism, cofactors, RNA metabolism) accounting for over 40% of all classified metagenomic sequences (Fig. 4). Inside both structures a significantly higher ($p < 0.05$) number of sequences related to Cell Wall and Capsule metabolism was found, while outside the structures the Stress Response was significantly higher ($p < 0.05$) (Fig. 4). Functional differences between the two sites were more marked than the contrast among the inside and outside of the structures (B1 IN 2009 and B2 IN 2010 versus B1 OUT 2009, B1 OUT 2010 and B2 OUT 2010). Compared as groups, the B1 site samples (B1 IN 2009, B1 OUT 2009, B1 OUT 2010) showed a significant increase ($p < 0.05$) in Photosynthesis, Protein Metabolism and Cofactors subsystems, whereas in B2 site samples (B2 IN 2010, B2 OUT 2010) the categories Membrane Transport, Motility and Chemotaxis were significantly more abundant ($p < 0.05$) (data not shown). A clear difference between inside and outside was found in metagenomes of site B2

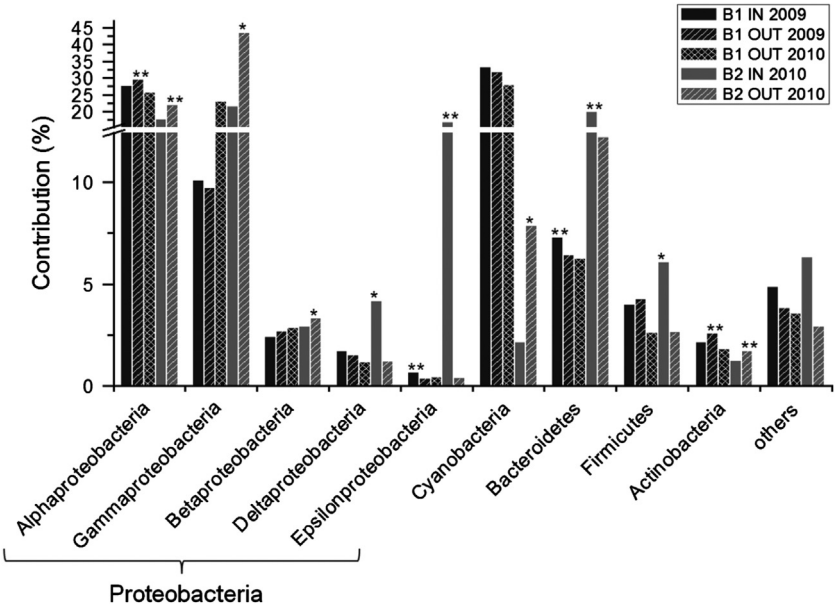

Fig. 2. Relative contribution of different bacterial phyla or classes in the two sampled *buracas*. Taxonomic assignment was performed using MG-Rast. One star represents significant difference ($p < 0.05$) between inside and outside of a single structure, while two stars represent significant difference ($p < 0.05$) between inside and outside of both structures.

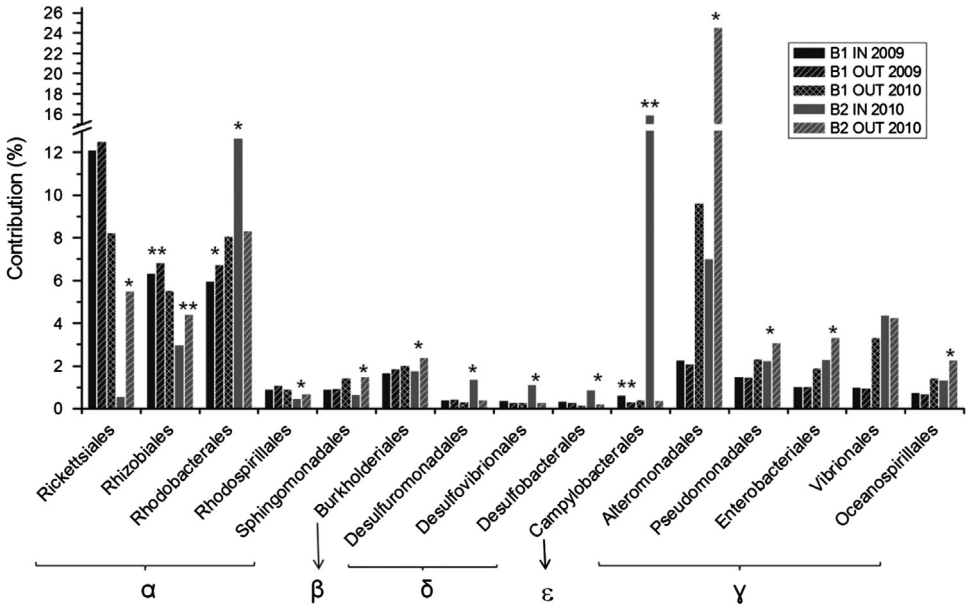

Fig. 3. Relative contribution of the main proteobacterial orders (> 1%) to the *buraca*'s metagenome. Taxonomic assignment was performed using MG-Rast One star represents significant difference ($p < 0.05$) between inside and outside of a single structure, while two stars represent significant difference ($p < 0.05$) between inside and outside of both structures.

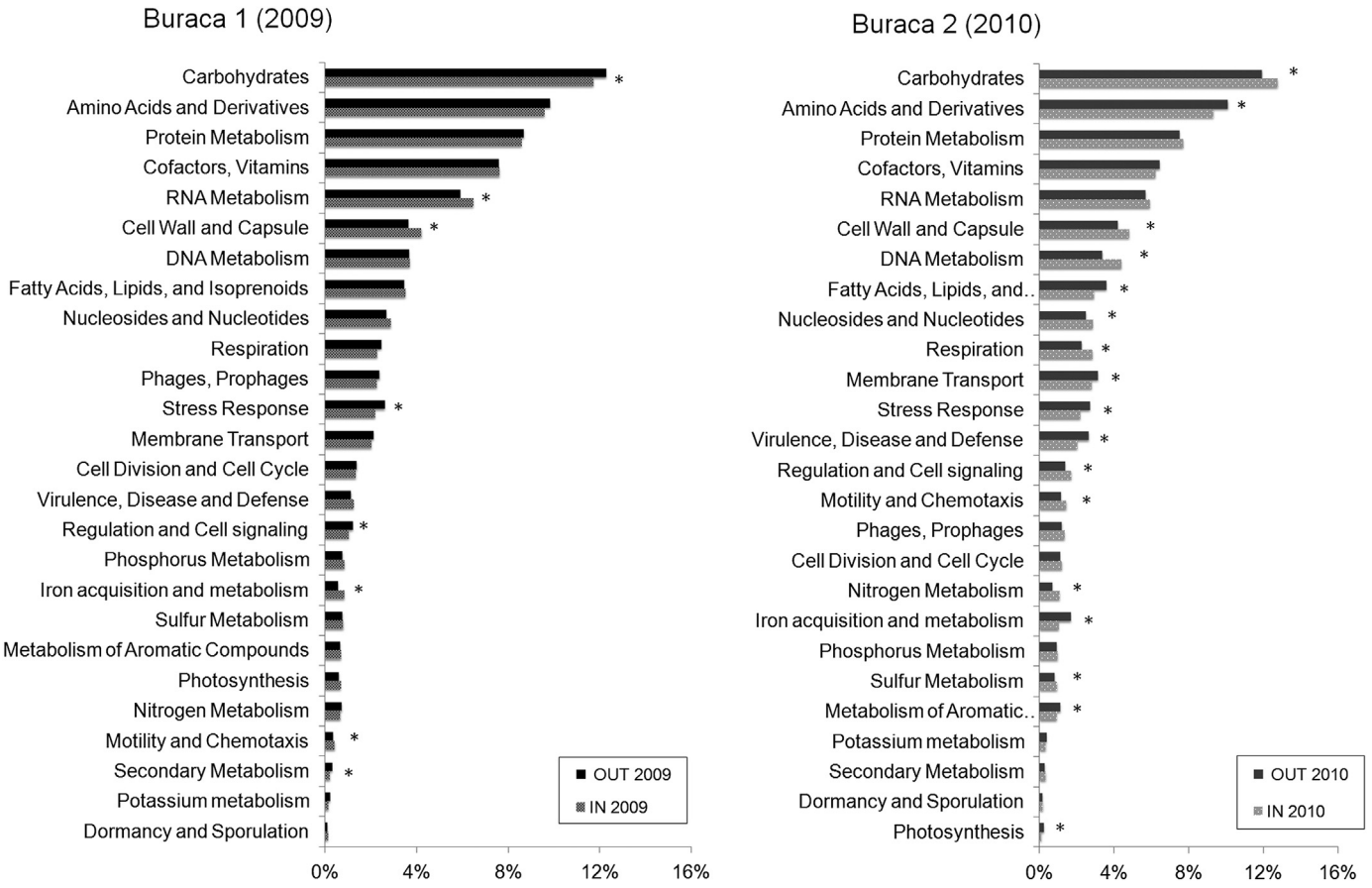

Fig. 4. Relative contribution of subsystems (first level hierarchy) in both sinkhole-like structures investigated. Metabolic assignment was performed using MG-Rast, but sequences assigned as miscellaneous, unknown or clustered based subsystems were not included. Asterisks indicate significant statistical differences ($p < 0.05$).

(Fig. 4). Corroborating the low number of sequences attributed to autotrophs, few hits were assigned to Photosynthesis (Fig. 5). Nitrogen metabolism (ammonia assimilation, nitrate and nitrite ammonification and nitrogen fixation), sulfur metabolism (sulfur oxidation) and respiration were significantly higher ($p < 0.05$) inside the structure (B2 IN 2010), while membrane transport subsystem was relatively higher ($p < 0.05$) outside B2 (B2 OUT 2010) (Fig. 5). Separate analysis of metagenomes from site B1 revealed a more

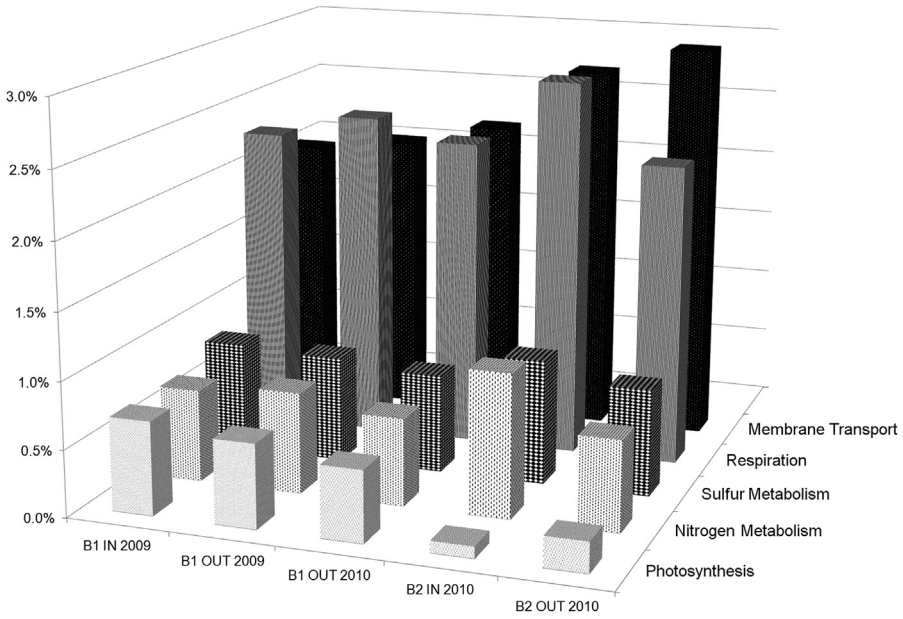

Fig. 5. Relative contribution of five subsystems related to bacterial metabolism in both *buracas*. Higher homogeneity was found in the Buraca 1 metagenome.

homogeneous response for the same categories mentioned above (B1 IN 2009, B1 OUT 2009, B1 OUT 2010) (Fig. 5).

3.4. Benthic and fish assemblages

The three cup-shaped structures sampled for fish and benthic assemblages presented four distinctive zones in terms of biological assemblages (see Bastos et al., this volume, for bathymetric profiles): *Zone* (1) a convex margin with emerging coralline outcrops of up to 2 m and sparse colonies of reef building corals (mainly *Siderastrea* sp. and *Montastraea cavernosa*), flat rhodolith beds intermingling with sandy patches, as well as prominent rhodolith mounds built by tilefish (*Malacanthus plumieri*); *Zone* (2) a gentle slope with exposed limestone encrusted by live coralline algae and sparse rhodoliths, sandy patches, corals and macroalgae; *Zone* (3) a sharper slope of exposed limestone with limited benthic cover and few fish, *Zone* (4) a bottom zone with decomposing organic matter over carbonate sandy mud and dead rhodoliths, and virtually no fish.

In *Zone* 1, rhodolith cover was estimated at $58 \pm 15\%$ (mean $\pm$ SE) and density at 50 ± 20 individuals m^{-2}. In the two control sites rhodolith cover was much higher, estimated at 90–95% of the substrate with a mean density of $100 \pm$ SE 30 individuals m^{-2}. The largest rhodoliths (10.1 ± 2 cm in diameter; discoid in shape) were found in *Zone* 2, while those of *Zone* 1 had 6 ± 3 cm in diameter. Rhodolith vitality decreased drastically from the outside to the inside of the *buracas*, with no living rhodoliths found in *Zone* 4. Rhodoliths were represented by five taxa of crustose coralline algae: *Hydrolithon rupestris*, *Lithothamnion superposistum*, *Lithothamnion* sp., *Neogoniolithon* sp., and *Sporolithon* sp.

The green algae *Cladophora vagabunda* was the main benthic organism attached to rhodoliths in *Zones* 1 and 2. On the other hand, at the two control sites the benthic assemblages were dominated by fleshy macroalgal species, mainly *Sargassum* sp. and *Dictyota* spp. Hard corals (*M. cavernosa*, *Siderastrea* spp. and *Porites branneri*) covered about 1% of the substratum in *Zones* 1 and 2, while encrusting sponges and octocorals (*Plexaurella grandiflora* and *Elisella* sp.) corresponded to less than 1% of the benthic coverage. Commercially important large roving herbivorous fish and large carnivorous fishes were more abundant inside (*Zones* 1 and 2) than in the control sites outside the structures (Fig. 6). A total of 63 reef fish species was recorded associated with the *buracas*. Most fish species belonged to the commercially important families Balistidae, Carangidae, Dasyatidae, Haemulidae, Lutjanidae and Serranidae. The tilefish *M. plumieri* was not particularly abundant, but it was recorded building prominent rhodolith mounds that characterized *Zone* 1. The structure of benthic communities differed markedly between areas inside and outside the structures (Fig. 7), with higher coverage of fleshy macroalgae and sponges inside the structures and higher coverage of crustose coralline algae and hard corals outside.

4. Discussion

4.1. The sinkhole-like structures present higher nutrient content, microbial abundance and metabolism

A three-fold higher nutrient content (total nitrogen and phosphorous) was observed inside both sinkhole-like structures when compared to control sites and other coralline reefs of the Abrolhos Bank (Bruce et al., 2012). High nutrient concentration favors bacterial growth and productivity (synthesis of bacterial biomass), as shown by the higher microbial abundance observed inside the structures. Oligotrophic marine waters usually present bacterioplankton assemblages with relative large proportions of cells with very low metabolic rates, which are dormant, injured or dead (Kirchman, 2008). The total number of prokaryotic cells and the elevated proportion of high nucleic acid prokaryotic cells (i.e. actively growing and replicating) were significantly higher inside than outside the structures, suggesting that the *buracas* function as productivity hotspots.

4.2. Diverse microbial community structuring within the buracas

Sampling in the Buracas region through autonomous diving is a complex and dangerous process, which prevented an optimal sampling design aiming at comprehensive spatial and temporal comparisons. Nevertheless, a clear distinction between sampled structures could be depicted. Site B1 had an impressive homogeneity between samples from control areas OUT 2009 and OUT 2010, and inside the structure (IN 2009). In the B1 site, a higher proportion of autotrophs (both eukaryotic phytoplankton and cyanobacteria) and a more pronounced contribution of the

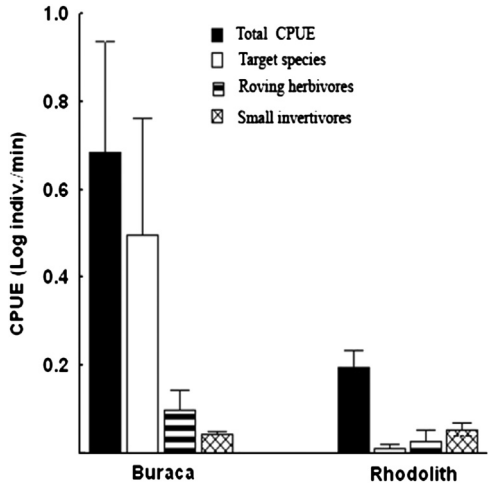

Fig. 6. Relative abundance of reef fishes in the buraca and in the rhodolith beds inferred from CPUE estimates based on ROV sampling (mean and SE).

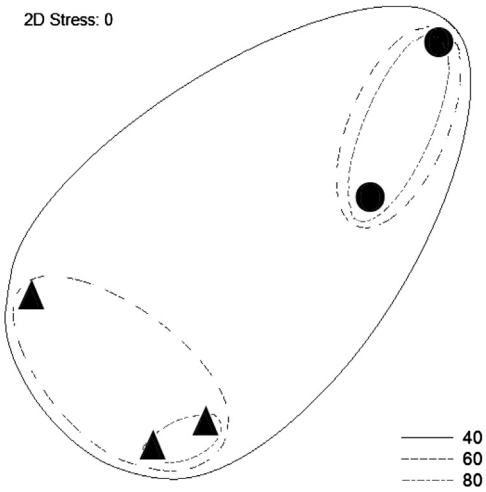

Fig. 7. Multidimensional scaling (MDS) plot based on Bray–Curtis similarities of benthic and fish coverage. Similarities of 40%, 60% and 80% are indicated.

photosynthesis metabolism contrasted with the pattern of site B2, which presented a heterotrophic profile related to nutrient cycling. For instance, the *Roseobacter* group and *Bacteroidetes*, both abundant inside B2, are reported to be dominant in actively growing bacterial communities (Tada et al., 2009). *Roseobacter* clades are known to explore high-nutrient microzones associated with particles and plankton (Moran et al., 2007). They are able to maintain their constant productivity under various environmental conditions because of their nutritional versatility in the use of phytoplankton-derived organic matter as carbon and energy sources (Tada et al., 2009). A recent study addressing potentially bioreactive components of the DOC pool, as well as the taxa that may be responsible for their turnover, has found that half of the expressed DOC transporter sequences in the bacterioplankton community from south-eastern US coastal ocean appeared to originate from just eight taxa: *Roseobacter*, SAR11, *Flavobacteriales* and five orders of δ-*Proteobacteria* (Poretsky et al., 2010), the same prominent taxa from inside structure B2. An expressive abundance of *Epsilonproteobacteria* was also noted inside this structure. Similar depression-like structures, such as Zacatón (northeastern Mexico), the deepest water-filled vertical sinkhole (cenote) in the world, and the submerged sinkholes in the Laurentian Great Lakes also have expressive contribution of this group (Nold et al., 2010; Sahl et al., 2010, 2011). Sequences related to *Arcobacter* and *Sulfuromales* phylotypes, which are important for the cycling of carbon, nitrogen, and sulfurous compounds (Campbell et al., 2006), were also prevalent inside structure B2. These microorganisms have been isolated from sulfide-rich environments (Macalady et al., 2008; Porter and Engel, 2008). It is important to emphasize that the metagenomic approach reveals important data about the more abundant community members and metabolisms, but represents a detailed snapshot of a specific time and place. Experimental testing and time-series studies are needed to better understand microbial dynamics in sinkhole-like structures as described herein.

4.3. *Buracas are productivity hotspots*

Tropical biogenic reefs are generally ranked into morphogenesis-based models ranging from atolls to bank-barrier reefs, the former generally occurring around oceanic islands and the latter most typically associated with continental shelves (Spalding et al., 2001). The architectural complexity provided by coralline structures influences the abundance and diversity of a broad array of organisms (e.g. Luckhurst and Luckhurst, 1978) by providing further habitat for settlement, feeding, and refuge for predators, therefore mediating predation and competition (e.g. Alvarez-Filip et al., 2009). Biogenic reefs in the euphotic zone provide substrate for the growth of benthic algae that, together with zooplankton and detritus, form the basis of reef trophic chains (McClanahan and Branch, 2008). Dinoflagellates, nitrogen-fixing cyanobacteria and alphaproteobacteria found within corals and other keystone organisms are also relevant, yet poorly know elements in reef food webs (Chimetto et al., 2008). Our results suggest that microbial productivity is of foremost trophic relevance in the *buracas* and surrounding environments. While a few highly specialized guilds of reef-associated organisms may be directly dependent on the presence of reef-building corals and coralline algae (e.g. Cole et al., 2008; Francini-Filho et al., 2008), a substantial fraction of the diversity of reef assemblages depend solely on the presence of a hard and complex framework, with size, shape and cross-shelf location of the hard bottom structures being the major drivers of spatial variation among reef-associated communities (e.g. Gladfelter and Gladfelter, 1978; Jones and Syms, 1998; Kendall et al., 2004). In the *buracas* system, increased productivity seems to be conditioned by the cup-like shape of the structures, which favor organic matter trapping and accumulation.

The *buracas* are located within the world's largest continuous rhodolith bed (Amado-Filho et al., 2012; Bastos et al., 2013; Moura et al., 2013), providing complex hard bottom structures that aggregate marine life. However, more than merely providing structural refuges, these distinctive structures also function as primary productivity hotspots in an overall oligotrophic tropical shelf.

The high degree of instability over the rhodolith beds, caused mostly by polar cold fronts that reach the study region (Marins et al., 2012), represents a form of disturbance that injures epibenthic invertebrates and seasonally removes their expressive algae cover (Amado-Filho et al., 2010), with subsequent sinking of organic matter inside the sinkhole-like structures. The increased primary (autotrophs) or secondary (heterotrophic cycling) microbial productivity inside the structures supports the view that nutrient cycling through microbes may influence higher trophic levels, allowing the production of matter and energy to fuel food webs (Crossman et al., 2001). Indeed, higher fish densities and dissimilar benthic coverage were found within the structures (Figs. 6 and 7). These sinkhole-like structures seem to function as traps for macroalgae and other detritus sources, which are likely subject to remineralization through bacterial activity. This, in turn, should promote the accumulation of high nutrient concentration inside the buracas followed by trophic cascading effects that

eventually result in increased fish biomass (Fig. 6). However, these hypotheses need further field observations and experimental testing, including time series studies, to be properly validated.

4.4. Concluding remarks

Our study suggests that the *buracas* have a significant and unforeseen role in the oligotrophic shelf of the Abrolhos Bank, functioning as biodiversity and bioproductivity hotspots. While these distinctive sinkhole-like structures are widespread in the north-eastern part of the mid- and outer shelf of the Abrolhos Bank (Bastos et al., this issue), it is remarkable that the entire system is outside the existing marine protected areas. Because the sinkhole-like structures concentrate marine species of great commercial importance in highly restricted spots, the steadily growing reef fisheries targeted at these specific spots require the development and implementation of appropriate management measures. Fishing in sites that aggregate biomass tend to become highly unsustainable (Sadovy and Domeier, 2005; Pinheiro et al., 2010). Therefore, we suggest that the *buracas* system, as biomass sources, need to be fully protected in the existing network of Marine Protected Areas (MPAs) of Abrolhos, to improve its representativeness and functionality (Crowder and Norse, 2008; Almany et al., 2009; Foley et al., 2010).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.csr.2013.05.011.

References

Almany, G.R., Connolly, S.R., Heath, D.D., Hogan, J.D., Jones, G.P., McCook, L.J., Mills, M., Pressey, R.L., Williamson, D.H., 2009. Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. Coral Reefs, 28, pp. 339–351.

Alvarez-Filip, L., Dulvy, N.K., Gill, J.A., Côté, I.M., Watkinson, A.R., 2009. Flattening of Caribbean coral reefs: region-wide declines in architectural complexity. Proceedings of the Royal Society of London Series B 276 3019-302.

Amado-Filho, G.M., Moura, R.L., Bastos, A.C., Salgado, L.T., Sumida, P.Y., Guth, A.Z., Francini-Filho, R.B., Pereira-Filho, G.H., Abrantes, D.P., Brasileiro, P.S., Bahia, R.G., Leal, R.N., Kaufman, L., Kleypas, J.A., Farina, M., Thompson, F.L., 2012. Rhodolith beds are major CaCO3 bio-factories in the Tropical South West Atlantic. PLoS One 7, e35171.

Amado-Filho, G.M., Maneveldt, G.W., Pereira-Filho, G.H., Manso, R.C.C., Bahia, R.G., Barros-Barreto, M.B., Guimarães, S.M.P.B., 2010. Seaweed associated with a Brazilian tropical rhodolith bed. Ciencias Marinas 36, 371–391.

Armstrong, R.A., Singh, H., Torres, J., Nemeth, R.S., Can, A., Roman, C., Eustice, R., Riggs, L., Garcia-Moliner, G., 2006. Characterizing the deep insular shelf coral reef habitat of the Hind Bank Marine Conservation District (US Virgin Islands) using the Seabed autonomous underwater vehicle. Continental Shelf Research 26, 194–205.

Bastos, A.C., Moura, Rodrigo L., Amado-Filho, Gilberto M., D'Agostini, Danielle P., Secchin, Nélio A., Francini-Filho, Ronaldo B., Güth, Arthur Z., Sumida, Paulo Y.G., Mahiques, Michel M., Thompson, Fabiano L., 2013 Buracas: Novel and unusual sinkhole-like features in the Abrolhos Bank. Continental Shelf Research. http://dx.doi.org/10.1016/j.csr.2013.04.035i.

Baumberger, R.E., Brown-Peterson, N.J., Reed, J.K., Gilmore, R.G., 2010. Spawning aggregation of beardfish, Polymixia lowei, in a deep-water sinkhole off the Florida keys. Copeia 2010, 41–46.

Betzler, C., Lindhorst, S., Hübscher, C., Lüdmann, T., Fürstenau, J., Reijmer, J., 2011. Giant pockmarks in a carbonate platform (Maldives, Indian Ocean). Marine Geology 289, 1–16.

Bruce, T., Meirelles, P.M., Garcia, G., Paranhos, R., Rezende, C.E., Moura, L.M., Francine-Filho, R., Coni, E.O.C., Vasconcelos, A.T., Amado-Filho, G., Hatay, M., Schmieder, R., Edwards, R., Dinsdale, E., Thompson, F.L., 2012. Abrolhos Bank reef health evaluated by means of water quality, microbial diversity, benthic cover, and fish biomass data. PLoS One 7, e36687.

Campbell, B.J., Engel, A.S., Porter, M.L., Takai, K., 2006. The versatile epsilon-proteobacteria: key players in sulphidic habitats. Nature Reviews Microbiology 4, 458–468.

Chimetto, L.A., Brocchi, M., Thompson, C.C., Martins, R.C.R., Ramos, H.B., Thompson, F.L., 2008. Vibrios dominate as culturable nitrogen-fixing bacteria of Brazilian coral *Mussismilia hispida*. Systematic and Applied Microbiology 31, 312–319.

Cole, A.J., Pratchett, M.S., Jones, G.P., 2008. Diversity and functional importance of coral-feeding fishes on tropical coral reefs. Fish & Fisheries 9, 286–307.

Crossman, D.J., Choat, J.H., Clements, K.D., Hardy, T., McConochie, J., 2001. Detritus as food for grazing fishes on coral reefs. Limnology and Oceanography 46, 1596–1605.

Crowder, L., Norse, E., 2008. Essential ecological insights for marine ecosystem-based management and marine spatial planning. Marine Policy 32, 772–778.

Francini-Filho, R.B., Moura, R.L., Thompson, F.L., Reis, R.D., Kaufman, L., Kikuchi, R.K. P., Leão, Z.M.A.N., 2008. Diseases leading to accelerated decline of reef corals in the largest South Atlantic reef complex (Abrolhos Bank, eastern Brazil). Marine Pollution Bulletin 56, 1008–1014.

Foley, M.M., Halpern, B.S., Micheli, F., Armsby, M.H., Caldwell, M.R., Crain, C.M., Prahler, E., Rohr, N., Sivas, D., Beck, M.W., Carr, M.H., Crowder, L.B., Duffy, J.E., Hacker, S.D., McLeod, K.L., Palumbi, S.R., Peterson, C.H., Regal, H.M., Ruckelshaus, M.H., Sandifer, P.A., Steneck, R.S., 2010. Guiding ecological principles for marine spatial planning. Marine Policy 34, 955–966.

Gasol, J.M., Del Giorgio, P.A., 2000. Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. Scientia Marina 64, 197–224.

Gischler, E., Shinn, E.A., Oschmann, W., Fiebig, J., Buster, N.A., 2008. A 1500-year Holocene Caribbean Climate Archive from the Blue Hole, Lighthouse Reef, Belize. Journal of Coastal Research 24, 1495–1505.

Gladfelter, W.B., Gladfelter, E.H., 1978. Fish community structure as a function of habitat on West Indian patch reefs. Revista de biologia tropical, 26, pp. 65–4.

Glynn, P.W., 1996. Coral reef bleaching: facts, hypotheses and implications. Global Change Biology 2, 495–509.

Grasshoff, K., Kremling, K., Erhardt, M., 1999. Methods of Seawater Analysis. Wiley-VCH Verlag, Weinheim, Germany.

Hartt, C.F., 1870. Geology and physical geography of Brazil. Boston, Fields, Osgood and Co.

Hinderstein, L.M., Marr, J.C.A., Martinez, F.A., Dowgiallo, M.J., Puglise, K.A., Pyle, R.L., Zawada, D.G., Appeldoorn, R., 2010. Theme section on mesophotic coral ecosystems: characterization, ecology, and management. Coral Reefs 29, 247–251.

Jones, G.P., Syms, C., 1998. Disturbance, habitat structure and the ecology of fishes on coral reefs. Australian Journal of Ecology 23, 287–297.

Kahng, S.E., Garcia-Sais, J.R., Spalding, H.L., Brokovich, E., Wagner, D., Weil, E., Hinderstein, L., Toonen, R.J., 2010. Community ecology of mesophotic coral reef ecosystems. Coral Reefs 29, 255–275.

Kendall, M.S., Christensen, J.D., Caldow, C., Coyne, M., Jeffrey, C., 2004. The influence of bottom type and shelf position on biodiversity of tropical fish inside a recently enlarged marine reserve. Aquatic Conservation: Marine Freshwater Ecosystems 14, 113–132.

Kirchman, D.L., 2008. Microbial ecology of the oceans, second ed.. John Wiley & Sons, Inc., New Jersey.

Kohler, K.E., Gill, S.M., 2006. Coral point count with Excel extensions (CPCe): a visual basic program for the determination of coral and substrate coverage using random point count methodology. Computers & Geosciences 32, 1259–1269.

Laborel, J., 1969. Madréporaires et hydrocoralliaires récifaux des côtes brésiliennes. Systématique, écologie, repartition verticale et géographique. Ann Inst Océanogr Paris 47, 171–229.

Leão, Z.M.A.N., Ginsburg, R.N., 1997. Living reefs surrounded by siliciclastics sediments: the Abrolhos coastal reefs, Bahia, Brazil. Proceedings of the International Coral Reef Symposium 8, 1767–1772.

Leão, Z.M.A.N., Kikuchi, R.K.P., Testa, V., 2003. Corals and coral reefs of Brazil. In: Cortés, J. (Ed.), Latin America Coral Reefs. Elsevier Science, pp. 9–52.

Lesser, M.P., Slattery, M., Stat, M., Ojimi, M., Gates, R.D., Grottoli, A., 2009. Photoacclimatization by the coral *Montastraea cavernosa* in the mesophotic zone: light, food, and genetics. Ecology 375, 1–8.

Luckhurst, B.E., Luckhurst, K., 1978. Analysis of influence of substrate variation on coral reef fish communities. Marine Biology 49, 317–324.

Macalady, J.L., Dattagupta, S., Schaperdoth, I., Jones, D.S., Druschel, G.K., 2008. Niche differentiation among sulfur-oxidizing bacterial populations in cave waters. ISME Journal 2, 590–601.

Macintyre, I.G., Rützler, K., Norris, J.N., Smith, K.P., Cairns, S.D., Bucher, K.E., Steneck, R.S., 1991. An early Holocene reef in the western Atlantic: submersible investigations of a deep relict reef off the west coast of Barbados, WI. Coral Reefs 10, 167–174.

Marins, B.V., Amado-Filho, G.M., Barreto, M.B.B., Longo, L.L., 2012. Taxonomy of the southwestern Atlantic endemic kelp: *Laminaria abyssalis* and *Laminaria brasiliensis* (*Phaeophyceae*, *Laminariales*) are not different species. Phycological Research 60, 51–60.

Martin, J.B., Gulley, J., Spellman, P., 2012. Tidal pumping of water between Bahamian blue holes, aquifers, and the ocean. Journal of Hidrology 416, 28–38.

McClanahan, T.R., Branch, G.M., 2008. Food Webs and the Dynamics of Marine Reefs. Oxford University Press.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E.M., 2008. The metagenomics RAST server—a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9, 386.

Moran, M.A., Belas, R., Schell, M.A., Gonzalez, J.M., Sun, F., Sun, S., Binder, B.J., Edmonds, J., Ye, W., Orcutt, B., Howard, E.C., Meile, C., Palefsky, W., Goesmann, A., Ren, Q., Paulsen, I., Ulrich, L.E., Thompson, L.S., Saunders, E., Buchan, A., 2007. Ecological genomics of marine Roseobacters. Applied and Environment Microbiology 73, 4559–4569.

Moura, R.L., Francini-Filho, R.B., Chaves, E.M., Minte-Vera, C.V., Lindeman, K.C., 2011. Use of riverine through reef habitat systems by dog snapper (*Lutjanus jocu*) in eastern Brazil. Estuarine, Coastal and Shelf Science 95, 274–278.

Moura, R.L., Secchin, Nélio Augusto., Amado-Filho, Gilberto Menezes., Francini-Filho, Ronaldo Bastos., Freitas, Matheus Oliveira., Minte-Vera, Carolina Viviana., Teixeira, João Batista., Thompson, Fabiano Lopes., Dutra, Guilherme Fraga., Sumida, Paulo Yukio Gomes., Guth, Arthur Zigliatti., Lopes, Rubens Mendes., Bastos, Alex Cardoso., 2013. Spatial patterns of benthic megahabitats and conservation planning in the Abrolhos Bank. Continental Shelf Research, http://dx.doi.org/10.1016/j.csr.2013.04.036i.

Nold, S.C., Pangborn, J.B., Zajack, H.A., Kendall, S.T., Rediske, R.R., 2010. Benthic bacterial diversity in submerged sinkhole ecosystems. Applied and Environment Microbiology 76, 347–351.

Overbeek, R., Begley, T., Butler, R.M., Choudhuri, J.V., Chuang, H.Y., Cohoon, M., de Crecy-Lagard, V., Diaz, N., Disz, T., Edwards, R., Fonstein, M., Frank, E.D., Gerdes, S., Glass, E.M., Goesmann, A., Hanson, A., Iwata-Reuyl, D., Jensen, R., Jamshidi, N., Krause, L., Kubal, M., Larsen, N., Linke, B., McHardy, A.C., Meyer, F., Neuweger, H., Olsen, G., Olson, R., Osterman, A., Portnoy, V., Pusch, G.D., Rodionov, D.A., Ruckert, C., Steiner, J., Stevens, R., Thiele, I., Vassieva, O., Ye, Y., Zagnitko, O., Vonstein, V., 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Research 33, 5691–5702.

Parks, D.H., Beiko, R.G., 2010. Identifying biologically relevant differences between metagenomic communities. Bioinformatics 26, 715–721.

Pinheiro, H.T., Joyeux, J.C., Martins, A.S., 2010. Reef fisheries and underwater surveys indicate overfishing of a Brazilian coastal island. Natureza & Conservação 8, 151–159.

Poretsky, R.S., Sun, S., Mou, X., Moran, M.A., 2010. Transporter genes expressed by coastal bacterioplankton in response to dissolved organic carbon. Environmental Microbiology 12, 616–627.

Porter, M.L., Engel, A.S., 2008. Diversity of uncultured Epsilonproteobacteria from terrestrial sulfidic caves and springs. Applied and Environment Microbiology 74, 4973–4977.

Reed, J.K., Pomponi, S.A., Weaver, D., Paull, C.K., Wright, A.E., 2005. Deep-water sinkholes and bioherms of South Florida and the Pourtalès Terrace—Habitat and Fauna. Bulletin of Marine Science 77, 267–295.

Sadovy, Y., Domeier, M., 2005. Are aggregation-fisheries sustainable? Reef fish fisheries as a case study. Coral Reefs 24, 254–262.

Sahl, J.W., Fairfield, N., Harris, J.K., Wettergreen, D., Stone, W.C., 2010. Novel microbial diversity retrieved by autonomous robotic exploration of the world's deepest vertical phreatic sinkhole. Astrobiology 10, 201–213.

Sahl, J.W., Gary, M.O., Harris, J.K., Spear, J.R., 2011. A comparative molecular analysis of water-filled limestone sinkholes in north-eastern Mexico. Environment Microbiology 13, 226–240.

Schmitter-Soto, J.J., Comín, F.A., Escobar-Briones, E., Herrera-Silveira, J., Alcocer, J., Suárez-Morales, E., Elías-Gutiérrez, M., Díaz-Arce, V., Marín, L.E., Steinich, B., 2002. Hydrogeochemical and biological characteristics of cenotes in the Yucatan Peninsula (SE Mexico). Hydrobiologia 457, 215–228.

Spalding, M.D., Ravilious, C., Green, E.P., 2001. World Atlas of Coral Reefs. University of California Press, Berkely, California.

Tada, Y., Taniguchi, A., Hamasaki, K., 2009. Phylotype-specific productivity of marine bacterial populations in eutrophic seawater, as revealed by bromodeoxyuridine immunocytochemistry combined with fluorescence in situ hybridization. Microbes Environment 24, 315–321.

Thompson, F.L., Bruce, T., Gonzalez, A., Cardoso, A., Clementino, M., Costagliola, M., Hozbor, C., Otero, E., Piccini, C., Peressutti, S., Schmieder, R., Edwards, R., Smith, M., Takiyama, L.R., Vieira, R., Paranhos, R., Artigas, L.F., 2011. Coastal bacterioplankton community diversity along a latitudinal gradient in Latin America by means of V6 tag pyrosequencing. Archives of Microbiology 193, 105–114.

Thresher, R.E., Colin, P.L., 1986. Trophic structure, diversity and abundance of fishes of the deep reef (30-300 m) at Enewetak, Marshall Islands. Bulletin of Marine Science 38, 253–272.

van Hengstum, P.J., Scott, D.B., Gröcke, D.R., Charette, M.A., 2012. Sea level controls sedimentation and environments in coastal caves and sinkholes. Marine Geology 286, 35–50.

Verrill, A.E., 1868. Notes of the radiates in the Museum of Yale College, with descriptions of new genera and species. 4. Notes of the corals and echinoderms collected by Prof. C.F. Hartt at the Abrolhos reefs, Province of Bahia, Brazil. Connecticut Academy of Arts and Sciences. Transact. 1, 351–371.

Whitaker, F.F., Smart, P.L., 1998. Groundwater circulation and geochemistry of a karstified bank–marginal fracture system, South Andros Island, Bahamas. Journal of Hydrology 197, 293–315.