



UNIVERSIDADE DO SUL DE SANTA CATARINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AMBIENTAIS

JOÃO MARTINS NETO

**Rhodolith density increases the bioavailability of sedimentary organic matter and
meiofauna biodiversity**



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**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AMBIENTAIS –
MESTRADO**

**ATA Nº09/2020 DE DEFESA PÚBLICA DE DISSERTAÇÃO POR
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Defesa PPGCA Nº23

Aos dez dias do mês de dezembro do ano de dois mil e vinte, às onze horas, na sala online da plataforma digital Zoom: <https://zoom.us/j/96352826346?pwd=eGRQR3FRbHk1WHZKdmlVY2pxZEt0dz09>, realizou-se a sessão pública de apresentação e defesa de Dissertação de Mestrado de João Martins Neto, como requisito para obtenção do título de Mestre em Ciências Ambientais, de acordo com o Regimento Interno do Programa de Pós-Graduação em Ciências Ambientais – PPGCA/UNISUL. Reuniu-se por videoconferência a comissão avaliadora composta pelos seguintes membros: Dr. Sérgio Antonio Netto, orientador e presidente da banca; Dr. Angelo Fraga Bernardino, avaliador externo da Universidade Federal do Espírito Santo (UFES); Dr. Tiago José Pereira, avaliador externo da University of Georgia para, sob a presidência do primeiro, arguirem o mestrando **João Martins Neto**, sobre sua Dissertação intitulada: **“High rhodoliths density increase shelf bioavailability of sedimentary organic matter and meiofauna biodiversity”**, área de concentração “Tecnologia, Ambiente e Sociedade” e linha de pesquisa “Tecnologia & Ambiente”. Após a apresentação, o mestrando foi arguido pelos membros da banca, tendo sido feitos os questionamentos e ouvidas às explicações a comissão avaliadora emitiu o conceito final:

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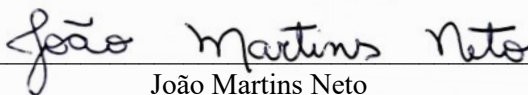
Nada mais havendo a tratar, foram encerrados os trabalhos e, tendo sido lida e achada conforme, a presente ata foi assinada pelo presidente da sessão, em nome dos avaliadores presentes por videoconferência, pelo mestrando e pela secretária do PPGCA.



Dr. Sérgio Antonio Netto

Presidente da Sessão

Em nome da Comissão Avaliadora presente por videoconferência



João Martins Neto

Mestrando do Programa de Pós-Graduação em Ciências Ambientais



Marieli Bugança

Secretária do Programa de Pós-Graduação em Ciências Ambientais



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This dissertation project is part of the Sustainable Development Goal / SDG 14 "Conserve and sustainably use the oceans, seas and marine resources for sustainable development", of the 2030 Agenda of the United Nations (UN).

The project contributes to Target 14.2 "By 2020, sustainably manage and protect marine and coastal ecosystems to avoid significant adverse impacts, including by strengthening their resilience, and take action for their restoration in order to achieve healthy and productive oceans". For that, we used the Indicator 14.2.1 "the proportion of national exclusive economic zones managed using ecosystem-based approaches".

RESUMO

Os bancos de rodolitos aumentam a complexidade do leito marinho e são hotspots de biodiversidade. Apesar dos diversos e cruciais serviços ecossistêmicos fornecidos pelos bancos de rodolitos, eles ainda são ameaçados pelas mudanças globais e impactos humanos locais. Neste estudo, realizado em um dos maiores bancos de algas calcáreas do mundo localizado na plataforma continental do estado do Espírito Santo, leste do Brasil, nós testamos se a maior complexidade do leito marinho poderia explicar padrões espaciais de biodiversidade da meiofauna e Nematoda marinhos. Nossos resultados mostraram que o habitat sedimentar dos sítios contendo alta densidade de rodolitos apresentou maiores concentrações de fração lábil de matéria orgânica disponível aos consumidores por meio da remineralização. A maior retenção e qualidade da matéria orgânica, evidenciada pelo carbono biopolimérico e pela concentração de proteína, proporcionou maior diversidade, abundância e biomassa dos Nematoda de vida livre, dando suporte a hipótese de estudo. Os resultados deste estudo mostraram que a perda dos bancos de rodolitos ou mesmo a diminuição da densidade dos nódulos calcáreos resultará em uma perda significativa da biodiversidade e, portanto, em uma variedade de serviços ecossistêmicos fornecidos por estes ecossistemas.

Palavras-chave: Bancos de rodolitos; Meiofauna; Nematoda; Biodiversidade bêntica; Biopolímeros; Complexidade de hábitat.

ABSTRACT

Rhodolith beds increase complexity of the seabed and are hotspot of biodiversity. Despite the several crucial ecosystem services provided by the rhodoliths, they are still threatened by global change and local anthropogenic impacts. In this study, conducted on one of the largest beds of calcareous algae in the world located on the continental shelf of Espírito Santo state, eastern Brazil, we test whether higher complexity of seabed could explain spatial biodiversity patterns of marine meiofauna and Nematoda. Our results showed that the sedimentary habitat of the sites containing high density of rhodoliths presented higher concentrations of labile fraction of organic matter available to benthic consumers through remineralization. The higher retention and quality of the organic material, as showed by biopolymeric carbon and concentration of protein, supported higher diversity, abundance, and biomass of free-living nematodes supporting the study hypothesis. The results of this study showed that the loss rhodolith beds or even decrease in density of calcareous nodules by human impacts will result in a significant loss of biodiversity and, this a range of ecosystem services they provide.

Keywords: Rhodolith beds; Meiofauna; Nematodes; Benthic biodiversity; Biopolymers; Habitat complexity.

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1 INTRODUCTION

Rhodoliths are calcareous nodules of red algae (Rhodophyta) that grow unattached over the seafloor in continental shelves (Foster, 2001). They may be composed of a single coralline species or by multi-taxonomic boxwork organisms from the fragmentation of corals, reef rock, bivalve, and gastropod shells or coralline algae alone (Bosence, 1983; Dulin et al, 2020). Formed over the millennia, the rhodolith beds (RBs) are vast ecosystems with global distribution, ranging from tens of square meters to more than 20,000 km² (Amado-Filho et al., 2012; Riosmena-Rodríguez et al., 2017). The largest reported RBs in the world are located on the Brazilian continental shelf (Amado-Filho et al., 2017).

Rhodolith beds are important in providing ecosystem services such as carbon sequestration and calcium carbonate production (van der Heijden and Kamenos, 2015; Schubert et al., 2019). They are also hotspots of marine biodiversity and act as a foundation species and ecosystem engineers (Steller et al., 2007; Cavalcanti et al., 2014; Neill et al., 2015). Due to their rigid three-dimensional structures, RBs increase the seafloor substrate complexity (Riosmena-Rodríguez et al., 2017), providing colonization areas and habitat for other algae, invertebrates and fish, many of them with economic importance (e.g., Steller et al., 2003; Cerqueira Veras et al., 2020). In addition, rhodoliths can affect nutrient availability (Gabara, 2020), due to biogeochemical changes related to their metabolism (Burdett et al., 2018; Carvalho et al., 2019) and its architecture, which can increase the retention of particulate organic matter including microphytobenthos (Grall et al., 2006) and possibly the quality of the organic matter. These organic particles are then largely used by benthic detritivores and transferred to consumers at higher trophic levels (Kelly et al., 2012; Millar and Gagnon, 2018).

Although their importance has been increasingly recognized, RBs are still threatened by factors such as trawling (e.g., Foster et al., 2013), carbonate mining (e.g., Pinheiro et al., 2019), oil spills (e.g., Fredericq et al., 2014), ocean acidification and global warming (e.g., McCoy and Kamenos, 2015; Sordo et al., 2019). The threats to RBs jeopardize a range of ecosystem processes and can be particularly critical for associated benthic biodiversity (e.g., Steller et al., 2003; Cavalcanti et al., 2018). Despite these threats, critical ecological information from RBs on the Brazilian continental shelf remain poorly studied but are crucial for management purposes.

Most studies investigating the biology and ecology in RBs around the world have focused on general biodiversity census of macroalgae, macro and megafauna (e.g. Ling et al., 2015; Fredericq et al., 2019; Carvalho et al., 2020). However, to our knowledge, there are no published studies evaluating processes driving spatial biodiversity changes within the benthic meiofauna in RBs, particularly nematodes. Meiofauna, including free-living nematodes, are key to marine benthic ecosystems due to their life cycles (e.g., lack of larval dispersion), a key role in trophic webs by linking detritus and prokaryotic components to higher trophic levels (Liu et al., 2014), and because they represent the most abundant metazoans on Earth (Heip et al., 1985; Vanreusel et al., 2010; Semprucci et al., 2015; Egres et al., 2019).

As RBs markedly change the overall seafloor physical structure along continental shelves, here we test whether the physical complexity RBs influence the bioavailability of sedimentary organic matter (e.g. biochemical composition and nutritional quality), with consequent effects on meiofaunal assemblages (abundance and richness), trophic diversity and biomass. This study was carried in the eastern Brazilian continental shelf within a Marine Protected Area (APA Costa das Algas; Mazzuco et al., 2019) that harbour RBs with varied density at depths between 10 and 100m. We found RB density, a proxy

for seafloor complexity, increased sedimentary organic matter quality and positively influenced meiofaunal assemblages on the continental shelf.

2 MATERIALS AND METHODS

2.1 Study area

The study was carried out in within the marine protected area APA Costa das Algas on the Espírito Santo continental shelf, eastern Brazil (Mazzuco et al., 2019; Fig. 1). The region is influenced by the Brazil Current with sea surface temperatures ranging from 21 to 27° C (Mazzuco et al., 2019; 2020). Along the continental shelf, RBs extend for more than 1953 km² from the middle shelf to the shelf break, with a predominance of rhodolith beds totaling 1521 km², particularly at depths between 45 and 65 m (Holz et al., 2020). Although recent environmental disasters have occurred in close proximity to that region, there is no evidence for impacts of mine tailings or oil spills over the RBs with the MPA (Gomes et al., 2017; Richard et al., 2020; Sissini et al., 2020; Bernardino, pers. obs).

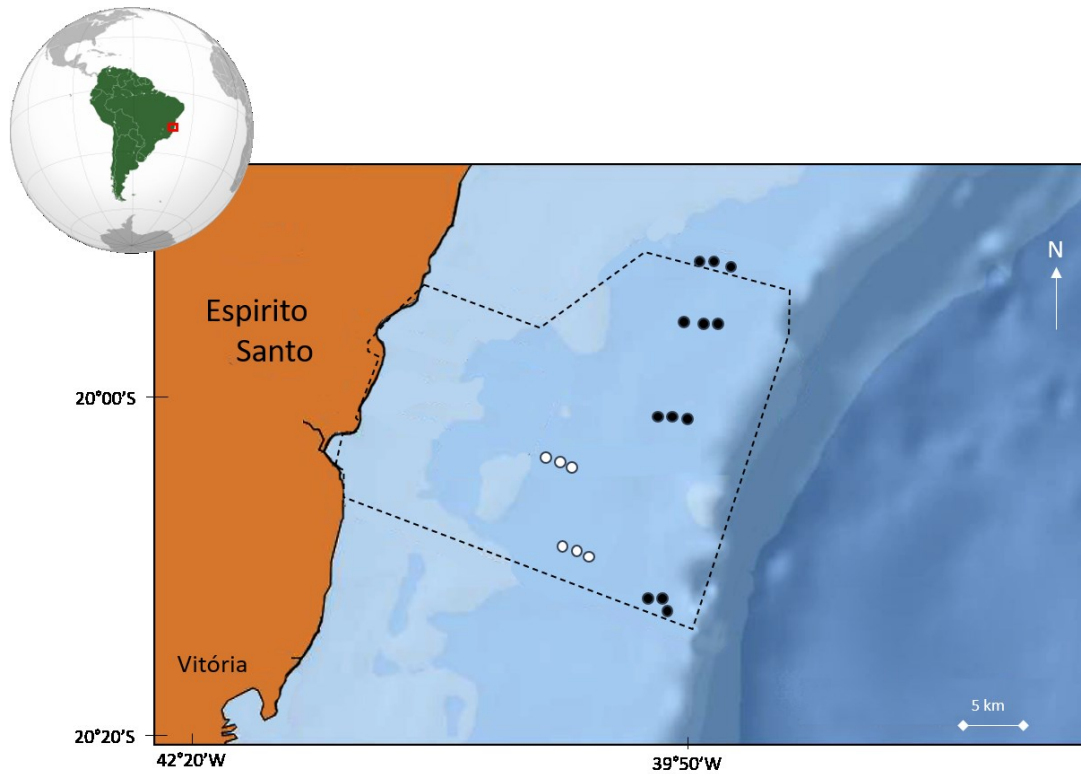


Figure 1. The continental shelf of the Espírito Santo State, in the Eastern Marine Brazilian Ecoregion, contain extensive rhodolith beds, particularly in the Costa das Algas Environmental Protection Area (dashed lines). Samples in the middle and outer shelf were undertaken in sites of high and low density of rhodoliths.

2.2 Sampling

Samplings were performed by scuba diving in the summer (Feb-Mar) of 2019, along random sites within RBs of distinct density (based on divers visual assessments). Due to logistic limitations and rough weather, we sampled two sites with lower density of rhodoliths (mean density of 26 nodules/m²) between 39.7 and 47.4 m depth. Higher density RBs (mean density of 78 of nodules/m²) were sampled at four random sites along depths between 38.6 m and 54.8 m (Fig. 1). In each site, three random replicates were sampled (total of 6 and 12 replicates for low and high density beds, respectively).

At each site, scuba divers recorded images of the seafloor and placed three random 25 x 25 quadrats over the seafloor and manually sampled all rhodoliths within that area. A PVC corer were used to sample sediments within or nearby the sampled quadrat, to avoid

excessive sediment resuspension. Samples were collected for granulometry, carbonate concentration, biopolymers, microphytobenthos and meiofauna. For microphytobenthos (chlorophyll-a and phaeopigments) the samples were taken with a PVC corer of 3.5 cm in diameter and 5 cm high and placed in pots covered with aluminum foil. For meiofauna, a PVC corer of 5 cm in diameter and 5 cm in height was used. All PVC cores were sealed by a cap after sampling during the diving operations to avoid loss of material during the return to the research vessel. Once onboard, samples for the analysis of meiofauna were fixed in 4% formalin and the others were preserved frozen.

2.3 Sediment parameters

Microphytobenthic pigments (chlorophyll-a and phaeopigments) were analyzed photometrically according to the Plante-Cuny method (1978). For pigment extraction, 10 cm³ of acetone at 90% concentration was added to each sample and stored for 24 h in the dark at 4 °C. Subsequently, the sediment was separated from the solution by centrifugation (800 g for 20 min). The pigment contents in the supernatant were determined in a spectrophotometer with absorbances of 665 and 750 nanometers for chlorophyll-a, and after acidification with 0.1 N HCl, for phaeopigments. The pigment concentrations were calculated following the method of Lorenzen (1967).

The analysis of sedimentary organic biopolymers (proteins, carbohydrates, and lipids) followed the procedures described in Danovaro (2010). Total protein analysis (PRT) was conducted after extraction with NaOH (0.5 M, 4 h) and determined according to Hartree (1972) modified by Rice (1982) to compensate for phenol interference. Total carbohydrates (CHO) were analyzed according to Gerchacov and Hatcher (1972). Total lipids (LIP) were extracted from 1 g of homogenized sediment lyophilized by ultrasonication (20 min) in 10 ml of chloroform: methanol (2:0 1 v/v) and analyzed

according to the protocol described in Marsh and Weinstein (1966). Blanks for each analysis were carried out with pre-combusted sediments at 450 and 480° C for 4 h. The concentrations of PRT, CHO and LIP were expressed as bovine serum albumin (BSA), glucose and tripalmitin equivalents, respectively. All analyzes were performed in triplicate. The concentrations of PRT, CHO and LIP were converted into carbon equivalents assuming a conversion factor of 0.49, 0.40 and 0.75, respectively (Fabiano and Danovaro, 1994). The sum of the protein, lipid and carbohydrate carbon equivalents was reported as biopolymeric carbon (BPC) and used as a reliable estimate of the labile fraction of organic carbon (Fabiano et al., 1995). In addition, protein to carbohydrate (PRT: CHO) and carbohydrate to lipid (CHO: LIP) ratios were used to assess the state of biochemical degradation processes (Galois et al., 2000).

The levels of microphytobenthic pigments and BPC in sediments reflect the general trophic conditions of coastal sediments, whereas the algal fraction of BPC reflects the food quality of sedimentary detritus (Pusceddu et al., 2009). The percentage of chlorophyll-a and phaeopigments to BPC concentrations, after transformation into C equivalent using 30 as a conversion factor (Danovaro, 2010; Pusceddu et al., 2014), were used as an estimate of organic material of algal origin, including the living (Chl-a) and senescent/detrital (Phaeo). Although the proportion of microalgae pigments can vary from 10 to more than 100 and the use of a constant conversion factor may involve errors (De Jonge, 1980), the use of this conversion factor (30 in this study) allowed us to compare our results with other studies carried on coastal areas (e.g. Pusceddu et al., 1999, 2009, 2011).

The samples for granulometric analysis and carbonate content of sediment were thawed and dried in an oven at 60 °C for 48 hours. Grain size analysis was performed following the sieving methods of Suguio (1973). The dry sediment was macerated and sieved in

mesh openings of -1.5Φ to 4Φ (with 1Φ intervals) in a sieve shaker. The carbonate contents of sediment were determined by muffle combustion at 550°C for 4 h with an additional hour at 800°C .

2.4 Meiofauna and nematodes

Meiofauna samples were washed through sieves with mesh openings of $500\text{ }\mu\text{m}$ and $63\text{ }\mu\text{m}$, extracted by flotation with Ludox TM-50 (gravity of 1.15 g/cm^3). Subsequently, the samples were evaporated in anhydrous glycerol and then permanent slides were made to identify and count the meiofauna (Somerfield et al., 2005). All free-living marine nematodes were counted and identified to genus level (Platt and Warwick, 1983, 1988; Warwick and Clarke, 1998; Nemys Database of Nematodes, Bezerra et al., 2019). Meiofaunal biomass was estimated from sixty nematode individuals (ten individuals per slide) from high and low RBs density, which had their total length and width measured to the nearest 1 mm. Biomass was calculated by the formula: $\text{Wet Weight } (\mu\text{g}) = \text{Length} \times \text{Width}^2 / 1600000$ (Soetaert et al., 2009).

2.5 Data analysis

The spatial patterns in the structural and functional attributes of the meiofaunal communities and nematode assemblages were compared between RBs with high and low density by a permutational analysis of variance (PERMANOVA) based on Bray-Curtis similarity matrices with 9999 permutations (Anderson et al., 2008). The similarity percentages routine (SIMPER) was then applied to define those groups or genera that contributed most to the observed dissimilarities between RBs (Clarke and Gorley, 2006). As meiofaunal assemblage structural descriptor we used total density, richness, and diversity (ES 51; Hulbert, 1971). The index of trophic diversity (ITD) was used as a proxy of functional attributes of the nematode assemblages. The ITD (Heip et al., 1985) is based

on the proportion of each of the four Wieser's (1953) feeding types (selective deposit feeders, nonselective deposit feeders, epigrowth feeders and predators/omnivores). ITD values range from 0.25 (highest trophic diversity with the four trophic groups accounting for 25% each) to 1.0 (lowest trophic diversity when a single feeding type is present). As the highest trophic diversity scores the lowest values, a modified version of this index as 1-ITD was used to better visualize the changes in functional diversity (highest value of trophic diversity is now 0.75 and the lowest trophic diversity is 0). To visualize the similarity of meiofauna and nematode assemblages between the points within the high and low density of rhodoliths, similarity matrices of log-transformed nematode abundances were constructed based on the Bray-Curtis similarity measure. Ordination was done by nMDS and Goodness-of-fit given by the stress value (Clarke, 1993).

The granulometric data (mean grain size, sorting, skewness, kurtosis and percentage of silt, sand, and gravel) were obtained using the SysGran software. Sediment organic and biochemical composition based on concentrations of CHO, PRT, LIP and BPC were determined, in addition to microphytobenthos (Chl-a, Phaeo and the percentage of algal contribution to BPC concentrations). Spatial changes in sediment granulometric data and organic composition between RBs were also tested with a permutational analysis of variance in Euclidian distance similarity matrices with 9999 permutations (Anderson et al., 2008).

In order to identify the contribution of environmental variables to the variability of meiofauna, a distance-based linear model (DistLM, Legendre and Anderson 1999) was applied. DistLM was based on similarity matrices of fauna data and predictors: biochemical composition of organic matter (PRT, CHO, LIP) and contribution (%) of each, biopolymeric carbon, PRT/CHO ratio, CHO / LIP ratio, algae contribution to BPC, concentration of microphytobenthic pigments (Chl-a and Phaeo), carbonate percentage,

grain size, skewness and average depth of the sampling points. We use the selection procedure R² specified and adjusted as a selection criterion for the distance-based redundancy analysis (dbRDA) models, which allows the visualization of the percentage of data variability explained by the axes, and the relative contributions of each of the predictive variables in the fauna structure (Anderson et al., 2008).

3 RESULTS

3.1 Grain size and carbonate content

The sediment carbonate content and sediment skewness were significantly higher in high density RBs (Table 1). The grain size was relatively homogeneous between RBs of different density, with sediments composed of poorly selected medium and coarse sands.

Table 1. Mean values ($\pm$ standard deviation) of sediment proprieties and summary results of the PEMANOVA tests evaluating the effect of rhodoliths density (High x Low). Bold values - significant differences at $p < 0.05$.

	High density Mean ($\pm$ SD)	Low density Mean ($\pm$ SD)	df	SS	MS	Pseudo-F	P (MC)
Carbonate (%)	5.7 ($\pm$ 1.02)	4.6 ($\pm$ 0.83)	1	0.12044	0.12044	5.6118	0.0312
Grain size (mm)	0.6 ($\pm$ 0.17)	0.5 ($\pm$ 0.14)	1	0.03650	0.03650	3.5796	0.0756
Sorting	1.5 ($\pm$ 0.20)	1.6 ($\pm$ 0.07)	1	0.03648	0.03648	1.2377	0.2838
Skewness	0.4 ($\pm$ 0.30)	0.1 ($\pm$ 0.16)	1	0.33356	0.33356	4.7134	0.0444
Kurtosis	0.7 ($\pm$ 0.19)	0.7 ($\pm$ 0.10)	1	0.00299	0.00299	0.1076	0.7424
Gravel (%)	20.3 ($\pm$ 11.05)	12.8 ($\pm$ 6.62)	1	0.69412	0.69412	2.4049	0.1371
Sand (%)	77.2 ($\pm$ 10.46)	85.5 ($\pm$ 6.62)	1	0.04548	0.04548	2.8024	0.1133
Silt+clay (%)	2.4 ($\pm$ 1.17)	1.7 ($\pm$ 0.41)	1	0.14824	0.14824	1.8508	0.1910

3.2 Biochemical composition and nutritional quality of sedimentary organic matter

In general, the biopolymers PRT, CHO, LIP tend to have higher mean concentrations in areas of high rhodoliths density. The greatest difference was observed in the concentrations of PRT (range of 0.44 to 0.86 mg.g⁻¹ and 0.26 to 0.30 mg.g⁻¹; in high and

low density RBs, respectively; $p = 0.0002$, Table A.1). Differences in CHO concentrations (range of 0.30 to 2.42 mg.g⁻¹; 0.75 to 1.28 mg.g⁻¹; in high and low density RBs, respectively; $p = 0.8185$) and LIP (range of 0.02 to 0.30 mg.g⁻¹; 0.01 to 0.25 mg.g⁻¹ in high and low density RBs, respectively; $p = 0.1485$) were not significant (Fig. 2A).

The mean values of PRT, CHO, LIP, the contribution of each biopolymers in the organic matter, and the PRT: CHO and CHO: LIP ratios in the points with high and low rhodolith densities are shown in Fig. 2.

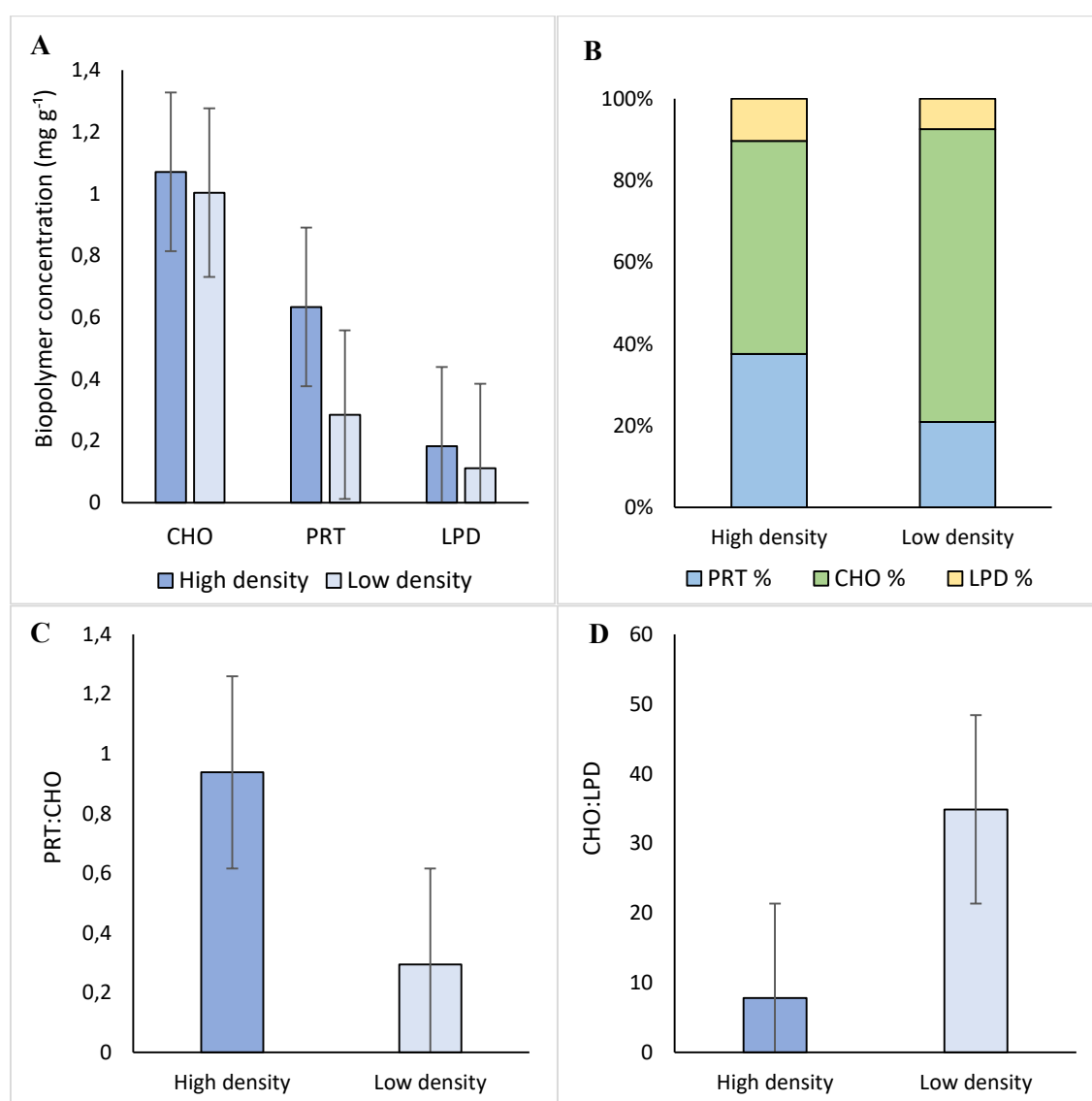


Figure 2. Average values (±SE) of carbohydrate, protein, and lipid concentration (A), the contribution of each biopolymers to the organic matter (B) and the PRT:CHO (C) and CHO:LPD ratios (D) in sediments with high and low densities of rhodoliths.

CHO was the main contributor (52-71%) to the sediment organic matter in RBs, independently of nodule densities (Fig. 2B). However, while the proportion of CHO was higher in sites with low densities of rhodoliths (71.6% and 52.1%; low and high density, respectively; $p = 0.0348$), the proportion of PRT was higher in sites with high rhodolith densities (37.6%), when compared to the low density beds (20.9%; $p = 0.008$). LIP had the lowest proportion in sediment organic matter composition in all areas (7 to 10%; $p = 0.1845$; Fig. 2B). The PRT:CHO ratio was higher in sediments with high-density rhodolith beds (ranging between 0.29 and 2.81), when compared to low-density beds (0.23 and 0.37; $p = 0.0354$; Fig. 2C). On the other hand, the CHO: LIP ratio followed an opposite pattern and was higher (3.0 to 111.4) in low-density rhodoliths beds, when compared to high-density beds (1.6 to 19.4; $p = 0.05$; Fig. 2D).

Sediment chlorophyll-a concentration was similar between sites and ranged from 0.23 to 2.05 $\mu\text{g.g}^{-1}$ at high density and 0.94 and 2.34 $\mu\text{g.g}^{-1}$ at low density beds ($p = 0.0908$; Fig. 3A). The concentration of phaeopigments were higher in low-density beds (2.41 to 3.31 $\mu\text{g.g}^{-1}$) when compared to high-density beds (0.51 to 1.54 $\mu\text{g.g}^{-1}$; $p = 0.0068$).

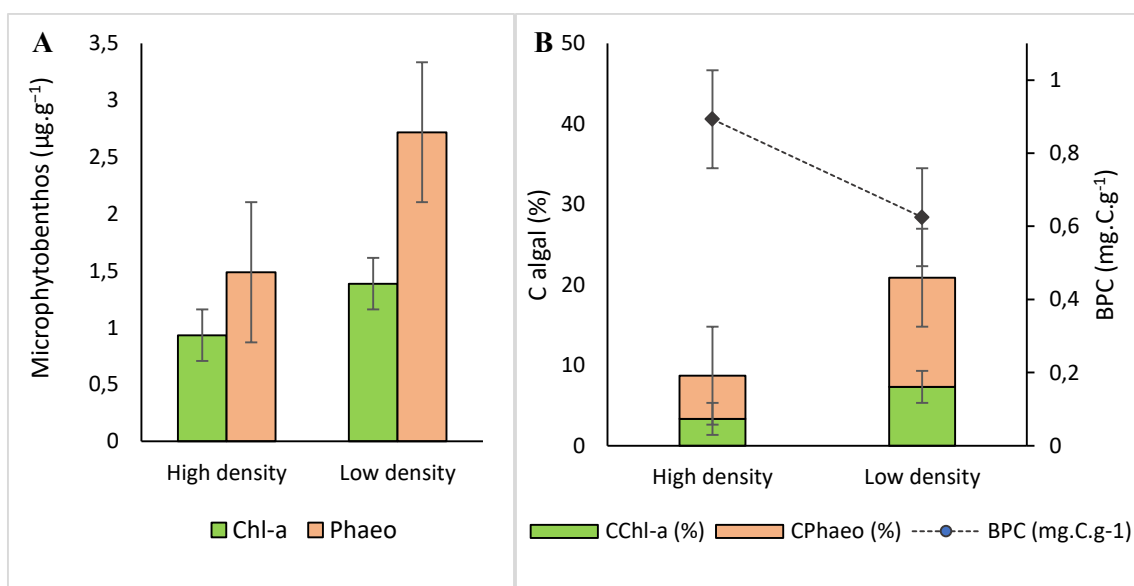


Figure 3. Concentration of chlorophyll-a and phaeopigments (A), and the contribution of carbon derived from the algal fractions (C algal) to the biopolymeric carbon concentrations (BPC) (B). CChl-a - Chlorophyll-a contribution to BPC; CPhaeo - Phaeopigments contribution to BPC.

The algal contribution to biopolymeric carbon was higher low-density rhodolith beds ($p = 0.0033$). The contribution of algae to BPC ranged from 3.7% to 23.1% in high density locations and from 12.2% to 37.5% in low density beds. In both areas, the contribution of algae fractions was mainly due to Phaeopigments, which was higher in low density sites (high density: 2.4% to 14.3%; low density: 8.1% to 21.9%; $p = 0.0021$). In general, a similar trend was observed on the contribution of Chl-a to BPC in both high and low-density beds (high density: 1.1% to 8.8%; low density: 3.4% to 15.5%; $p = 0.0375$; Fig. 3B).

Biopolymeric carbon concentrations exhibited slightly higher concentrations in places with high rhodoliths density (high density: 0.47 to 1.49 mgC.g⁻¹; low density: 0.45 to 0.82 mgC.g⁻¹; $p = 0.0658$; Fig. 3B). Oligotrophic state is suggested for both areas, high and low rhodoliths density, according to the classification of the trophic state based on the concentrations of PRT and CHO (Dell'Anno et al., 2002) and on the BPC and the contributions of algae to the BPC (Pusceddu et al., 2011).

3.3 Meiofauna and nematoda biodiversity

Meiofauna was composed only by nematodes, copepods and polychaetes in both low and high density RBs. Nematodes and copepods, the most abundant groups (65% and 23% of overall relative abundance) were present in all samples. Polychaetes represent 11% of the fauna; while the polychaetes occurred in 83% of the samples in low-density RBs, they occurred only in 40% in high-density RBs. Thus, meiofauna groups number was slightly higher in sites with lower rhodoliths density. Meiofaunal density was higher in high-

density RBs (55.4 ± 28.09 ; $p = 0.004$). The mean values of the meiofauna descriptors (richness, diversity, and density) and the summary results of the PERMANOVA are shown in Table 2.

Table 2. Mean values (max. – min.) and summary results of the PERMANOVA evaluating the effect of rhodoliths density (high x low) on meiofauna and nematodes.

	Rhodoliths high density	Rhodoliths low density	Pseudo-F	P(MC)
Meiofauna				
Richness	2.3 (2-3)	2.8 (2-3)	4.5714	0.0485
Diversity	0.6 (0.2-0.9)	0.8 (0.5-1.1)	3.2912	0.0878
Density (inds.10cm ⁻²)	55.4 (19-108)	17.5 (12-24)	10.482	0.0043
Nematoda				
Richness	12.3 (9-21)	6.2 (3-9)	16.553	0.0014
Diversity	2.2 (1.9-2.7)	1.7 (1.1-2.2)	10.69	0.0045
Density (inds.10cm ⁻²)	34.5 (17-80)	10.7 (9-13)	9.056	0.0087
Biomass (µg)	1.7 (0.26-8.89)	1.1 (0.15-4.26)	13.574	0.0006

In general, Nematode richness was two-fold higher in high-density beds. A total of 49 genera (20 families) of nematodes were registered, of which 44 genera (20 families) were recorded in high-density RBs, whereas only 22 genera (13 families) were present in low-density beds. *Desmodora* (Desmodoridae), *Neochromadora* (Chromadoridae) and *Gammanema* (Selachinematidae) were the most abundant genera at high-density beds (35% of nematodes), whereas *Desmodora* (Desmodoridae), *Halalaimus* (Oxystominidae) and *Sabatieria* (Comesomatidae) (39% of the nematodes) dominated the low-density RBs. Nematodes in high-density RBs were also more diverse and abundant than low-density RBs (Table 2). In addition, nematode biomass within high-density RBs was higher (mean of $1.7 \mu\text{g} \pm 1.38$) when compared to the low-density (mean of $1.1 \mu\text{g} \pm 1.07$; $p = 0.0006$; Table 2).

The nMDS ordination and PERMANOVA derived from meiofauna and nematode abundances suggest a marked distinction between assemblages from high- and low-density RBs (Fig. 4). The results of Simper analysis (Table A.2) confirmed the different

set of dominant taxa between high- and low-density RBs. On the high-density beds, nematode assemblages, were dominated by *Desmodora* and *Halalaimus*.

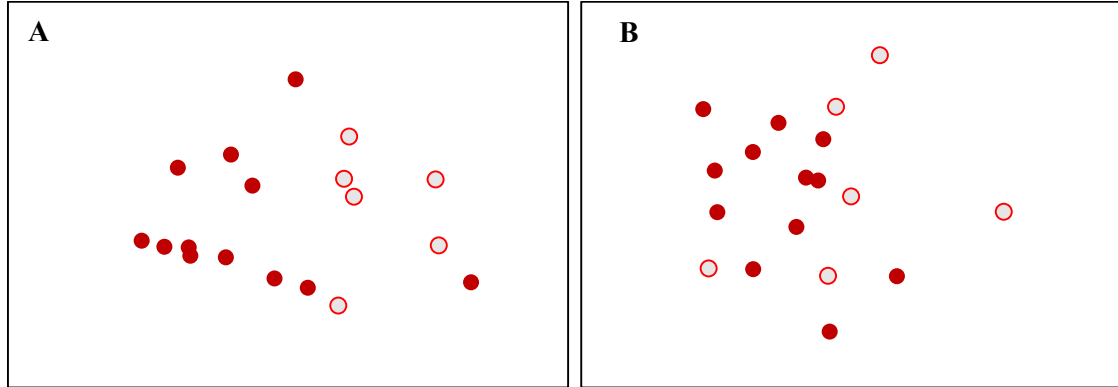


Figure 4. MDS ordination of log-transformed (A) meiofauna (stress 0.08) and (B) nematoda assemblages (stress 0.18) within high (full symbols) and low (empty symbols) rhodoliths density.

Epi-growth feeder nematodes were the most abundant in RBs, with 34% and 33.8% of relative abundance in high-density and low-density beds, respectively. However, in high-density beds, the selective deposit feeders were the second most abundant group (29.2%). On the other hand, non-selective deposit feeders were the second most abundant group in low-density RBs (28.8%; Fig. 5). The ITD did not differ between RBs of high and low-density ($p = 0.5247$), with values typically higher than 0.62. The mean ITD value in the high-density beds (0.67 ± 0.07 , range of 0.52 to 0.73), was similar to the low-density beds (0.64 ± 0.08 , range of 0.49 to 0.74).

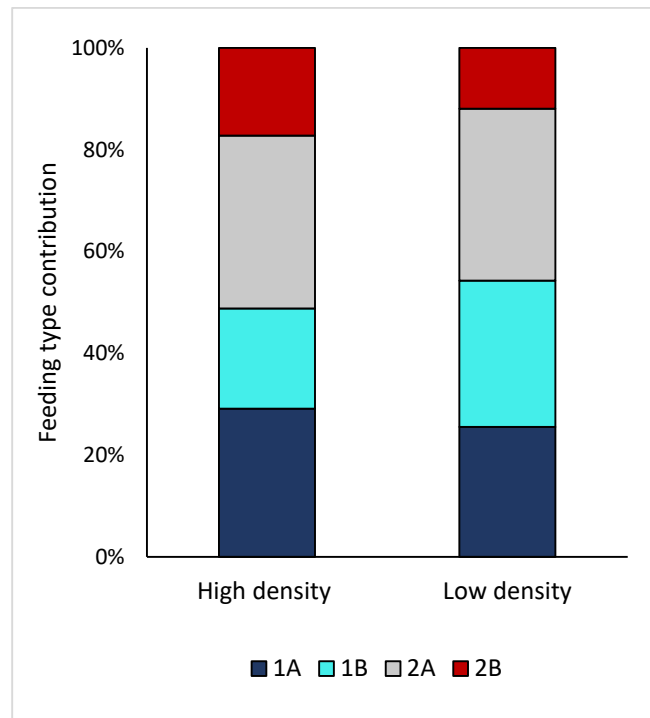


Figure 5. Contribution of each feeding type of nematodes (1A - selective deposit feeders; 1B - non-selective deposit feeders; 2A - epi-growth feeders; 2B - predators/omnivores).

3.4 Relationships between sediments, nutritional quality of sedimentary organic matter and biodiversity

The DistLM analysis detected significant correlations between meiofauna and nematode assemblages with some of the predictor variables, in particular, carbonate content, algal contribution to BPC and PRT (Table 3). The dbRDA based on meiofaunal data (Fig. 6A) showed that the first two axes explained 80.2% of the variability of meiofaunal assemblages and 95.6% of the relationship between the meiofauna groups and environmental variables. The first axis, represented by the RBs densities and responsible for 61.6% of the adjusted model, showed that the meiofauna of high-density RBs (positive portion of the axis) were mainly related to higher concentrations of proteins in organic matter and a higher percentage of carbonate in the sediment. On the other hand, meiofauna in RBs with low density (negative portion of the axis), was related to microphytobenthic pigments and sedimentary organic matter with a greater algae

contribution. The second axis, responsible for 34% of the adjusted model, was mainly represented by the internal variability of the RBs environment, with meiofauna related to sediment granulometry and CHO percentage.

The dbRDA of the nematode assemblages (Fig. 6B) showed that the first two axes explained 38.1% of the variability in the fauna data set and 48.7% of the relationship between the nematoda genera and environmental variables. The first axis, in general, represents the RBs of different densities and is responsible for 26.7% of the adjusted model. It is observed that the nematode assemblages in high-density RBs (negative portion of the axis) are mainly related to a higher percentage of carbonate, while in low-density beds (positive portion of the axis), meiofauna was related to microphytobenthic pigments and algae contribution to organic matter. The second axis, responsible for 22% of the adjusted model, as well as in the meiofauna, represented an internal variability within the RBs, being related to CHO descriptors.

Table 3. Results of the marginal test and sequential tests performed by Distance-based linear model of meiofauna and nematode assemblages against environmental drivers in RBs, eastern Brazil. The percentage of variance in faunal data explained by the variable is abbreviated as “% var.” Values in boldface indicate significant correlation ($P < 0.05$). PRT - protein; CHO - carbohydrate; LPD - lipid; %PRT, %CHO and %LPD - protein, carbohydrate and lipid contribution in organic matter; BPC – biopolymeric carbon; Calgal, CChl-a and CPhaeo – algal, chlorophyll-a and phaeopigments contribution to BPC.

Meiofauna				Nematoda			
Variable	Pseudo-F	P	% var	Variable	Pseudo-F	P	% var
PRT	69.749	0.0013	30.36	Carbonate	2.698	0.0012	14.43
Carbonate	57.271	0.003	26.36	Calgal	20.855	0.0183	11.53
%LPD	27.114	0.0725	14.49	Phaeo	17.242	0.0634	9.73
Grain size	2.521	0.0838	13.61	Chl-a	1.667	0.0788	9.44
Calgal	23.401	0.1012	12.76	Grain size	16.444	0.0826	9.32
Skewness	22.472	0.1017	12.32	PRT	14.417	0.1479	8.27
Phaeo	21.891	0.1165	12.04	PRT:CHO	1.424	0.167	8.17
BPC	21.114	0.1283	11.66	BPC	13.351	0.2042	7.7
CHO:LPD	19.546	0.1286	10.89	Skewness	13.303	0.2108	7.68

Sequential tests				Sequential tests			
PRT	69.749	0.0013	30.4	Calgal	20.798	0.0419	11.33
LPD	30.472	0.052	11.8	PRT	14.417	0.1517	8.27
Carbonate	33.319	0.0845	9.5	Carbonate	15.262	0.1914	8.43
CHO:LPD	23.605	0.1199	8.5	Depth	14.442	0.2664	7.08
Phaeo	14.174	0.2852	5.4	LPD	11.829	0.2964	6.7
%PRT	11.381	0.3456	4.4	PRT:CHO	10.901	0.3995	6.4
Depth	0.9999	0.4255	4	Skewness	11.204	0.3746	6.07
CHO	0.8702	0.4677	3.8	CHO	0.99588	0.4547	5.71
%CHO	0.746	0.5277	2.9	Grain size	0.97092	0.4607	5.29

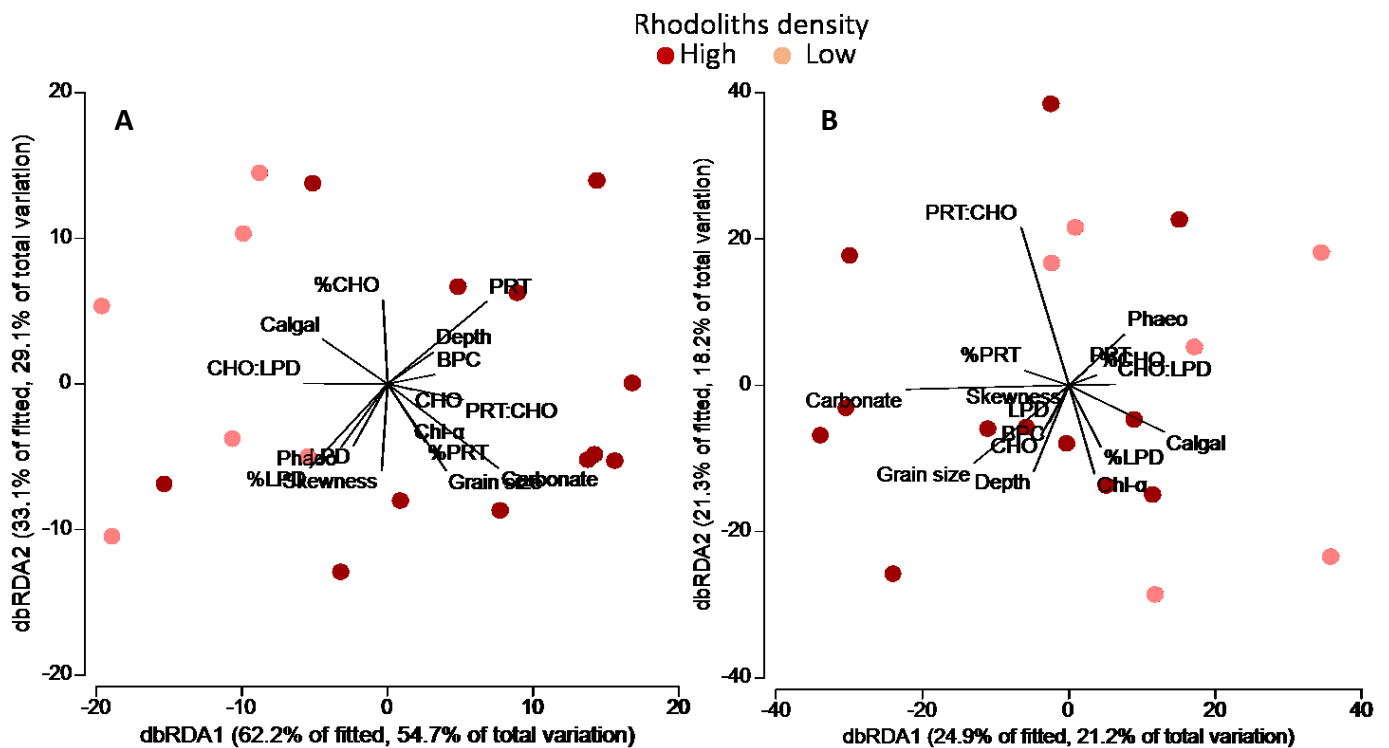


Figure 6. Distance-based redundancy analyses relating environmental variables and (A) Meiofauna and (B) Nematoda of rhodolith beds.

4 DISCUSSION

Habitat complexity is an integrative concept that encapsulates multidimensional habitat traits (e.g. Gratwicke and Speight, 2005; Otero-Ferrer et al., 2019). Complexity is accepted as a general mechanism for increasing the abundance of organisms, though the

generality of complexity as a mechanism to explain increased abundance and richness has been questioned (e.g. Bracewell et al., 2018). This is because effects of habitat complexity may depend on the scale (Williams et al., 2002), degree of disturbance (McAfee et al., 2016) and the taxonomic or functional group (Scharf et al., 2006). In this study we tested if the density of rhodoliths nodules on the seafloor, which increase physical complexity, would affect the bioavailability of sedimentary organic matter and support higher meiofaunal and nematode biodiversity. Our results showed that the sedimentary habitat of the high-density RBs presented higher concentrations of the labile fraction of organic matter available to benthic consumers through remineralization. The higher retention and quality of the organic material, as showed by increased concentration of PRT, supported higher richness (2-fold higher), diversity (30% higher), abundance (3-fold higher), and biomass (54% higher) of free-living nematodes. However, higher habitat complexity (greater number of rhodoliths per square meter) was not related to nematode trophic diversity neither the diversity of meiofauna higher taxa, although some sedimentary parameters were distinct between those areas.

The concentration of biopolymers (proteins, carbohydrates, and lipids) is normally lower on continental shelf sediments when compared to estuaries and coastal bays (Pusceddu et al., 2009, Misic and Harriague, 2013; Hadlich et al., 2018). Biogeochemical indicators suggest the RBs located on the shelf were typically oligotrophic environments with relatively low biopolymer contents. However, due to the rigid three-dimensional structure, the rhodoliths beds can act as a trap for organic material, retaining and also aggregating particulate organic matter from the water column and/or from adjacent areas. This is supported by a higher biopolymeric protein content in areas of high-density RBs, which exhibited PRT: CHO ratios over two-fold higher (2.81), when compared to the low-density beds (PRT: CHO ratios <1). These differences in the PRT:CHO ratio also

represents a change in the nature of organic matter (Danovaro et al. 1993), suggesting an aged organic detritus in areas of low-density beds and a fresh organic material in areas of higher densities. This can be translated into a more nutritious organic matter, since proteins are more labile than carbohydrates (Dell'Anno et al., 2002; Pusceddu et al., 2009). Moreover, even though the contribution of Chl-a to BPC had been similar between sites, the proportion of refractory organic matter in the low-density RBs was significantly higher as showed by the contribution of phaeopigments to BPC. Besides, the higher algal contribution to BPC indicated a higher contribution of autochthonous organic matter at low-density beds (Pusceddu et al., 2009; 2011).

The meiofauna of the sedimentary habitat of RBs was numerically dominated by nematodes, corresponding to over 60% of the meiofauna in RBs. The nematode assemblages at the high-density RBs showed a higher biodiversity (number of genera, diversity) and higher abundance, and higher individuals biomass when compared to similar assemblages at the low-density RBs. Of the 20 nematode families sampled in the RBs, all of them were present in the high-density beds and 35% were exclusive to these locations. The greater number of nodules and therefore a higher habitat complexity likely increases the interstitial spaces within and between rhodoliths, and the retention of more abundant and labile fraction of organic material with clear effects on both the secondary production (i.e. individual biomass) and community structure of the RBs (Grall et al., 2006; Smith, 2006; Gabara, 2020). In addition, the microhabitats created by higher rhodolith density may directly influence niche partitioning (Citadin et al., 2018), competitive and predator-prey interactions and provide refuge from physical stressors (Scheffers et al. 2014), favouring the biodiversity in calcareous algae systems (Rastelli et al., 2020). On the other hand, it is evident that lower density of rhodoliths support

meiofaunal and nematode assemblages with lower abundance and diversity, in a similar way to macrobenthic communities (Gabara et al. 2018).

The meiofauna in low-density RBs showed a higher taxa diversity. Although unexpected, this was only a result of the low dominance of nematodes and not the presence of different or a higher number of meiofaunal taxonomic groups (i.e. higher richness). The higher number of copepods on the low-density RBs were associated to the higher microphytobenthos concentration (Todaro et al., 2006), the higher algal input to sedimentary organic matter, and a tendency of sediments becoming finer (low values of skew). Our data suggests that RBs may sustain higher diversity of meiofaunal groups even in low densities, which need further investigations and comparisons with sites with no RBs to ascertain the full effect of rhodoliths on meiofaunal biodiversity.

The functional attributes of the nematodes showed clear signs of the influence of rhodoliths. In both areas (high and low density) the nematodes epi-growth feeders were the most abundant, including the genus *Desmodora*, the numerically dominant taxon in both RBs, and *Neochromadora*, the second dominant genus in high densities. The epi-growth feeders Desmodoridae and Chromadoridae have already been cited as dominant families in carbonate sediments (e.g., Netto et al., 1999; Santos and Venekey, 2018) where these nematodes, similar to macrofauna grazers, scrape food off the rhodoliths. Besides, selective nematode deposit-feeders, the second most representative trophic guild, benefits from a rich microbiome of bacterial guilds on RBs (Cavalcanti et al., 2014; Fredericq et al., 2019).

In summary, this study, which represents an initial step in the knowledge about the meiofaunal and nematode biodiversity in RBs, showed that the rhodoliths have a positive effect on faunal richness, biodiversity, abundance and individual biomass. Although the unbalanced and limited number of samples, our study further supports that rhodolith

density and therefore habitat complexity can positively influence organic matter nutritional quality, provide new ecological niches and change the trophic support to nematode assemblages. Based on the worldwide occurrence and importance of RBs over continental shelves, our study supports that these ecosystems support a rich and unique marine benthic biodiversity that would not be encountered in rhodolith-free continental shelves. In a scenario of anthropogenic pressures, climate change and ocean acidification, the loss, or even degradation of RBs would result in a significant loss of biodiversity and ecosystem services they may provide.

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APPENDICES

Table A.1 - Average values (max. – min.) and summary results of the Permanova evaluating the effect of rhodoliths density (High x Low) in biochemical composition, microphytobenthos and nutritional quality of sedimentary organic matter.

	High density	Low density	df	SS	MS	Pseudo-F	P (MC)
PRT	0.6 (0.44-0.86)	0.28 (0.26-0.30)	1	0.47331	0.47331	33.71	0.0002
CHO	1.07 (0.30-2.42)	1.00 (0.75-1.28)	1	0.01768	0.01768	0.05471	0.8185
LPD	0.18 (0.02-0.30)	0.11 (0.01-0.25)	1	0.01947	0.01947	2.2473	0.1485
%PRT	37.6 (19.9-65.7)	20.9 (16.5-25.7)	1	0.98715	0.98715	8.9921	0.008
%CHO	52.1 (22.5-71.3)	71.6 (77.4-58.8)	1	0.51242	0.51242	5.677	0.0348
%LPD	10.3 (19.6-1.68)	7.47 (0.69-19.4)	1	1.0968	1.0968	1.873	0.1845
PRT:CHO	0.94 (0.29-2.81)	0.29 (0.23-0.37)	1	0.44951	0.44951	5.2241	0.0354
CHO:LPD	7.83 (1.61-19.4)	34.9 (3.03-111.4)	1	3.8624	3.8624	4.334	0.0559
BPC	0.88 (0.47-1.49)	0.62 (0.45-0.82)	1	0.02084	0.02084	4.1371	0.0658
Calgal	9.03 (3.71-23.1)	20.9 (12.2-37.5)	1	10.027	10.027	11.953	0.0033
C Chl-a	3.53 (1.11-8.76)	7.31 (3.42-15.5)	1	55.27	55.27	5.0601	0.0375
C Phaeo	5.50 (2.39-14.3)	13.6 (8.11-21.9)	1	253.51	253.51	13.941	0.0021
Chl-a	0.93 (0.22-2.19)	1.39 (0.94-2.34)	1	0.06245	0.06245	3.1735	0.0908
Phaeo	1.49 (0.46-3.27)	2.72 (1.34-3.31)	1	5.8768	5.8768	9.3859	0.0068

Table A.2 Results from Similarity Percentage analysis (SIMPER) indicating the meiofaunal taxon and nematode genera contributed most to the similarity within each rhodoliths density (high and low). Average abundance, average similarity and the % contribution to the similarity made by each characterizing genus area shown.

Rhodolith density	Meiofauna taxon	Average abundance	Average similarity	Sim/SD	Contrib%	Cum.%	
High	Nematoda	3.46	49.12	5.86	63.06	63.06	
	Copepoda	2.4	26.47	1.95	33.99	97.05	
Low	Nematoda	2.45	47.55	7.03	58.19	58.19	
	Copepoda	1.38	20.25	2.72	24.79	82.98	
	Polychaeta	1.23	13.91	1.25	17.02	100	
Average abundance							
		Group High	Group Low	Average similarity	Sim/SD	Contrib%	Cum.%
High x Low	Copepoda	2.4	1.38	11.3	1.77	37.69	37.69
	Polychaeta	0.82	1.23	10.09	1.43	33.63	71.31
	Nematoda	3.46	2.45	8.6	2.49	28.69	100
Rhodolith density	Nematoda genus	Average abundance	Average similarity	Sim/SD	Contrib%	Cum.%	

High	<i>Desmodora</i>	1.61	8.52	1.74	21.68	21.68
	<i>Halalaimus</i>	1.19	6.16	1.58	15.66	37.34
	<i>Axonolaimus</i>	0.86	3.46	0.79	8.82	46.16
	<i>Gammanema</i>	1.01	3.36	0.78	8.56	54.72
	<i>Terschellingia</i>	0.91	3.24	0.79	8.25	62.97
Low	<i>Halalaimus</i>	0.71	6.18	0.74	28.01	28.01
	<i>Desmodora</i>	0.78	5.54	0.76	25.08	53.09
	<i>Sabatieira</i>	0.57	2.79	0.47	12.64	65.73
	<i>Richtersia</i>	0.48	2.54	0.47	11.53	77.26

Average abundance							
		Group High	Group Low	Average similarity	Sim/SD	Contrib%	Cum.%
High x Low	<i>Desmodora</i>	1.61	0.78	5.43	1.43	7.59	7.59
	<i>Neochromadora</i>	1.07	0.45	5.04	1.12	7.06	14.65
	<i>Gammanema</i>	1.01	0.12	4.66	1.31	6.51	21.16
	<i>Axonolaimus</i>	0.86	0.23	4.65	1.11	6.5	27.66
	<i>Terschellingia</i>	0.91	0.41	4.35	1.26	6.08	33.74
	<i>Tricoma</i>	0.88	0	4.05	1.49	5.66	39.41
	<i>Halalaimus</i>	1.19	0.71	3.9	1.26	5.45	44.86
	<i>Richtersia</i>	0.84	0.48	3.36	1.36	4.7	49.56
	<i>Sabatieira</i>	0.38	0.57	3.22	1.08	4.51	54.07
	Density	34.50	10.67	19.45	2.49	34.60	34.60
	Richness	12.25	6.17	5.56	2.01	9.89	44.50