UNIVERSIDADE DA CORUÑA

Biodiversity of meiofaunal communities in ecosystem alternative states: ecosystems dominated by erected macroalgae (EMA) or incrusting coralline algae (ECA) in the Mediterranean Sea

Máster en Biología Marina

Autor

Martin Bello Candamio

Tutora

Silvia Biancheli

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1. INTRODUCTION

The increase in the human activities such as fishing, dredging, urbanization and coastal industry negatively affect thereby marine ecosystems characteristics and equilibrium, leading to shifs from one ecosystem state to another (Newell et al. 1998, Mangialajo et al. 2008, Micheli et al. 2005). Marine ecosystems regime shifts have been reported from tropical to polar ecosystems (i.e., coral reefs, salt marshes, Arctic and Antarctic sea ice), acting from ecosystem chemico-physical characteristics to the biota (i.e., thermohaline circulation, hypoxia, standing stocks collapses, marine food webs), leading to the alteration of biodiversity and ecosystem functioning (i.e., eutrophication, transition from an ecosystem state to another as observed for corals, kelp, mangroves) and, ultimately, reducing the ecosystem resilience and impacting on ecosystem services and human well-being (Rocha et al., 2015).

Multiple stressors usually interact in driving regime shifts and, among these, overexploitation of natural populations and overfishing have been recognized worldwide as the major factor driving the shift from ecosystems dominated by macroalgae (EMA) to those dominated by encrusting algae and sea urchins (ECA), also known as barrens (Scheffer et al., 2001; Graham et al. 2015; Möllmann et al., 2015). Indeed, Indeed, fishing target species are usually predators which abundance can affect food web structure and drive interactions among different components (Guidetti et al. 2010).

The shift between EMA and ECA ecosystems has been observed also in the Mediterranean Sea, which represents one of the most important marine biodiversity sources of our Planet (Coll et al., 2010). Due to its peculiar characteristics, as be a semi-enclosed basin and the enormous human impact on its coasts, it is especially susceptible to different types of pressures. Global warming, outbreak of jellyfish populations, habitat destruction and fragmentations, establishment of non-indigenous species and species extinction are actual threats that should be necessarily taken in account for the near future (Boero, 2014).

Erected macroalgae assemblages, especially those belonging to the genus *Cystoseira* form important habitats in the Mediterranean Sea, reaching high biomass values, creating a source of biodiversity and promoting the recruitment of several species of economic importance (Cheminée et al., 2013). On the other hand, barrens are benthic habitats dominated by sea urchins and coralline incrusting algae, where biological richness and complexity are clearly reduced (Filbee-Dexter and Scheibling, 2014). In these habitats, overgrowing of sea urchins, especially those from *Paracentrotus lividus*, may lead to the collapse of canopy algae, as observed in several Mediterranean regions (Agnetta et al., 2015).

During the last years several studies have been dedicated to understand the possible causes (as heavy metal pollution, high sediment loads, sea urchin overgrazing and local anthropogenic

stressors) underlying the shift from EMA to ECA ecosystem (Bulleri and Benedetti-Cecchi, 2006, Bulleri, Bertocci and Micheli, 2002, Konar and Estes, 2003, Filbee-Dexter and Scheibling, 2014, Agnetta et al. 2015, Micheli et al. 2005, Strain et al. 2014). However, these studies were focused on macroalgae, macro- and megafaunal biodiversity or on their population dynamics (Ceccherelli et al., 2006; Bonaviri et al., 2012; Tamburello et al., 2012; Cardona et al., 2013), while information about the effect of regime shift on small benthic components, as meiobenthic communities, are inexistent.

Within coastal marine ecosystems, metazoan meiofauna are considered a significant component in both rocky and soft bottoms where they represent an important trophic source for macrofauna, small fishes, juveniles of large fishes, and other epibenthic predators, thereby changes in their community may affect the relationship between different trophic levels (Danovaro et al., 2007). Moreover, meiofauna, feeding on prokaryotes and detritus, play essential roles in modulating nutrient cycling, secondary production, sediment transport and detritus remineralization (Danovaro et al., 2008). Due to their life cycle characteristics (as small size, high turnover and lack of pelagic larval dispersion), these organisms are highly sensitive to environmental disturbance and respond rapidly to changes in trophic availability (Fraschetti et al., 2006; Danovaro, 1996).Commonly nematodes and harpacticoid copepods are the most abundant groups within the meiofauna community. Typically, copepods are assumed to feed on micro-phytobenthos (Leduc et al., 2009) while nematodes are characterized by high trophic diversity, being detritus feeders, bacterivorous, herbivorous or predators (Gambi et al., 2003).

Due to their trophic characteristics and their role in linking detritus, prokaryotic compart and higher trophic levels, meiofauna could be a sensitive component to regime shifts and have a prominent role in driving the ecosystem functioning in different ecosystem alternative states (Pusceddu et al., 2014b).

2. OBJECTIVE

To assess if ecosystem regime shifts affect meiofaunal communities, we tested the null hypothesis of no differences between EMA and ECA in the abundance, biomass and diversity of meiofaunal communities, as well as in the benthic trophic status, in terms of organic matter content.

To achieve this objective we analyzed the abundance, biomass and biodiversity of metazoan meiofaunal communities from the Mediterranean rocky infralitoral characterized by the presence of EMA and ECA ecosystems in two regions, located at Minorca (Spain) and Montenegro. We also analyzed the benthic organic matter loads, in terms of concentration of biochemical compounds (i.e., protein, carbohydrate and lipid), biochemical composition and nutritional quality.

3. MATERIALS AND METHODS

Sampling was carried out in coastal waters of two Mediterranean regions, located at Minorca (Spain, Western Mediterranean Sea) and Montenegro (Southern Adriatic Sea, Central Mediterranean Sea). Each region was characterized by rocky substrates and the presence of ECA and EMA. In both regions, samples were collected from two sites randomly selected within ECA (ECA Site 1 and ECA Site 2) and EMA (EMA Site 1 and EMA Site 2), in 5 replicates for meiofaunal analyses and 3 replicates for organic matter analyses.

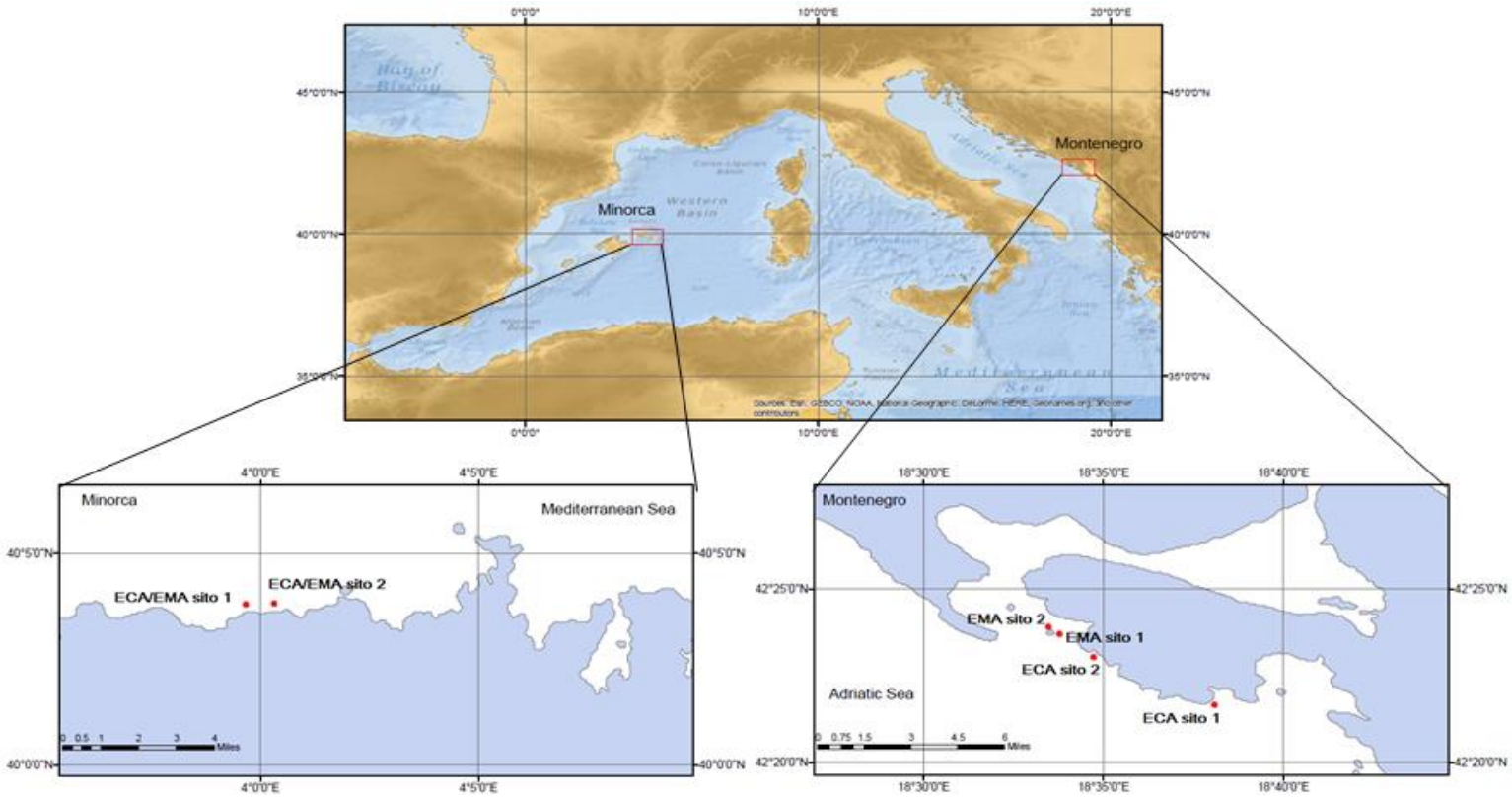

Figure 1: Location of ECA and EMA sampling points in Minorca and Montenegro.

Sampling was carried out by scuba divers using a modified coring system proposed by Danovaro and Fraschetti (2002) which consist on a cylinder (inner diameter: 9 cm; length: 14 cm), made of transparent Plexiglas, closed on top by a plastic bag and open on basal side. The base was covered by rubber ring (1 cm thick) to adapt the sampler to the irregular bottom morphology. The corer had a lateral window 2 cm high, closed hermetically with a plastic bag, which allows the diver to rasp the substrate surface by means of a spatula within the corer. This system allowed efficient removal from both EMA and ECA ecosystems. The material taken away was collected in the plastic bag and taken to the laboratory where it was fixed with buffered formaldehyde (4% final volume) and stained with Rose Bengal (0.5 gL^{-1}) for the subsequent analyse.

The same procedure was applied to collect samples for organic matter analyses, but in this case samples were stored frozen at – 20 ºC until analyses in laboratory.
All the analyses dealing with meiofaunal and organic matter variables were conducted according to Danovaro (2010).

3.1 Meiofauna

Meiofaunal extraction

Meiofaunal extraction was carried out by means of mesh sieves. All samples were passed through a 1000 µm, washed several times with water, and collected in a 2 L beaker. The collected material was then filtered and retained onto a 20 µm mesh. Retained material was placed into 50 mL tubes with 4% formaldehyde solution and a few drops of Rose Bengal (0.5 gL^{-1}).

Meiofaunal abundance and identification

Before the identification, formaldehyde 4% was washed out using fresh water using the 20 µm mesh. In order to facilitate meiofaunal identification and counting, samples were poured into a Delfuss cuvette (subdivided into 200 cells). The counting and identification of individuals was carried out under a stereomicroscope at a minimum of 25X magnification factor. Before starting the sorting, we waited a few minutes to allow the sedimentation of all the organisms in the bottom of the cuvette. At the end of the analysis, the sample is transferred to the 20 µm sieve, and it is transferred again to the 50 mL tube with a wash bottle full of 4% buffered formalin, and stained with a few drops of Rose Bengal (0.5 gL^{-1}).

Meiofaunal biomass

In order to estimate the meiofaunal biomass, during the counting and identification of meiofaunal individuals, individual measures of body length and body width were carried out, using the stereomicroscope equipped with a micrometer scale (for all taxa except for nematodes). The determination of meiofaunal biomass was performed using the volumetric method that consists in indirect estimates of biomass extrapolating organism weight from the biovolume. This method is based on the association of the morphology of meiofaunal organisms to geometric shapes, and the volume estimates can be obtained from body length and body width according to Warwick and Price (1979) formula:

$$V = C \times L \times W^2$$

Where V is the Volume expressed in nL, L is the body length and W is the body width expressed in mm, while C is the conversion factor specific for each taxon.

Nematode biomass

For the estimate of nematode biomass permanent slides were prepared with a drop of glycerine and a ring of paraffin. Each slide contained 10-15 nematodes. For nematodes dehydration first were transferred using a handling needle from the cuvette to a staining block containing a solution of formalin 4% and glycerine adding also a few drops of Rose Bengal (0.5 gL^{-1}). After 12 h, nematodes were transferred to an oven at 36ºC overnight. During the permanence in the oven, a few drops of a solution containing ethanol 95% and glycerine were added in order to prevent the desiccation of the nematodes. After this treatment nematodes are transferred to the slides. For biomass determination we used the same method than for the rest of meiofaunal taxa, but in this case we use a light microscope equipped with a micrometer scale. Measures were taken at 40X magnification.
To assess the nematode biovolume we used the formula

$$V = L \times W^2 \times 0.063 \times 10^5$$

Where V is the Volume expressed in nL, L is the body length and W is the body width expressed in µm.

3.2 Benthic organic matter

Analyses to assess the chlorophyll-a and phaeopigment concentration were carried out according to Lorenzen and Jeffrey (1980). For all the samples, pigments were extracted (12h at 4 in the dark) from triplicate superficial sediment samples (about 1 g) using 5 ml of 90% acetone as the extractant. Extracts were analysed fluorometrically to estimate chlorophyll-a and after acidification with 200 µl 0.1 N HCl, to estimate phaeopigments. The sum of chlorophyll-a and phaeopigment concentration was defined as total phytopigment concentration.

Protein content was determined using Hartree (1972) protocol, after modifications by Rice (1982). This colorimetric method allows the reaction of proteins with rameic tartrate and the Folin-Ciocalteau reactive in basic environment (pH 10). The reaction provides a stable blue coloration whose intensity is proportional to the protein concentrations in the reaction solution. Measures and results were calculated from calibration curves of standard solutions of BSA through a spectrophotometric analyse. Concentrations of total carbohydrates were determined according to Dubois et al. (1956) and optimized for sediments by Gerchacov and Hatcher (1972) after minor modifications. This colorimetric assay is based on the reaction between

sugars and phenol in the presence of concentrated sulphuric acid. The method is nonspecific and allows concentrations of total carbohydrates, cellulose induced, to be determined. Measures and results were calculated from calibration curves of standard solutions of D-glucose through a spectrophotometric analyse. The determination of total lipid concentration was carried out according to Bligh and Dyer (1959) and Marsh and Weinstein (1966), modified to be applied to the sediment matrix. The concentration was calculated from the calibration curves of standard solutions of tripalmitine.

Carbohydrate, protein and lipid sediment contents were then converted into C equivalents using the conversion factors 0.40, 0.49 and 0.75 mgC mg^{-1}, respectively, and their sum defined as the biopolymeric carbon (Fabiano et al., 1995).

We also chose the contributions of phytopigment and protein to biopolymeric C concentrations and the values of the protein to carbohydrate ratio as descriptors of the aging and nutritional quality of sediment organic matter (Pusceddu et al., 2000; 2009a). The information about the contribution of total phytopigments to biopolymeric C was used to estimate the freshness of the organic material deposited in the sediment. Photosynthetic pigments and their degradation products are assumed to be labile compounds in a trophodynamic perspective, thereby a low quantity of these compounds within the sediment gives information concerning the organic material age. Moreover, the quantity of organic C associated with phytopigments is also typically associated to compounds that are available for the enzymatic digestion (i.e. promptly available for heterotrophs) (Pusceddu et al., 2003), for this reason, higher values of this fraction will also be indicative of a comparatively higher nutritional quality (Dell'Anno et al., 2002). Since N is the most limiting factor for heterotrophic nutrition and proteins, which are degraded at faster rates than carbohydrates, and are N-rich products, the protein to biopolymeric C and the protein to carbohydrate ratios are indicative of both the aging and the nutritional value of the organic matter (Danovaro et al., 1993, 2001b; Dell'Anno et al., 2002; Tselepides et al., 2000; Pusceddu et al. 2009a).

	MINORCA								MONTENEGRO							
	ECA SITE 1		EMA SITE 1		ECA SITE 2		EMA SITE 2		ECA SITE 1		EMA SITE 1		ECA SITE 2		EMA SITE 2	
Taxa	avg ind10cm-2	SD	avg ind10cm-2	SD	avg ind10cm-2	SD	avg ind10cm-2	SD	avg ind10cm-2	SD	avg ind10cm-2	SD	avg ind10cm-2	SD	avg ind10cm-2	SD
Nematoda	11.20	5.90	31.42	22.70	3.85	2.78	32.07	7.31	3.28	2.00	53.15	19.18	3.98	1.06	37.82	10.90
Copepoda	20.82	12.79	70.31	71.27	9.69	6.54	87.26	32.27	14.30	7.99	103.76	71.65	14.00	4.33	61.95	18.69
Polychaeta	4.53	3.02	13.88	10.02	1.49	1.13	14.72	5.10	1.66	1.51	27.96	17.04	1.28	0.76	16.93	4.43
Bivalvia	0.02	0.05	0.04	0.06	0.00	0.00	0.21	0.25	0.06	0.09	0.36	0.29	0.02	0.05	0.11	0.13
Ostracoda	1.07	0.68	2.11	1.69	0.78	0.37	3.05	1.28	0.63	0.37	1.24	0.42	0.51	0.30	0.11	0.07
Turbellaria	0.21	0.20	1.41	1.00	0.23	0.20	1.31	0.34	0.40	0.16	2.74	2.26	0.46	0.34	2.42	1.01
Gastropoda	0.65	0.40	0.86	1.18	0.57	0.43	1.64	0.62	0.34	0.16	1.96	1.76	0.27	0.28	0.29	0.25
Amphipoda	0.04	0.09	1.12	1.12	0.00	0.00	0.86	0.46	0.02	0.05	0.80	0.51	0.02	0.05	0.46	0.57
Others (rare taxa)	0.06	0.10	1.14	1.25	0.06	0.14	1.92	1.36	0.31	0.59	2.36	2.73	0.15	0.23	2.67	3.38
Total abundance	38.62	21.87	122.27	103.60	16.68	7.71	143.03	41.01	21.02	9.61	194.39	104.61	20.70	4.10	122.80	23.91
Richness of taxa	6.80	0.84	9.60	2.19	6.40	1.52	11.80	1.64	7.80	1.10	12.60	1.52	7.20	1.64	10.00	2.45
Total biomass (µgC .10 cm-2)	7.83	2.90	21.23	6.33	2.98	0.77	29.18	5.85	3.49	0.95	45.85	11.83	4.59	0.52	39.21	9.36

Table1: Meiofaunal abundance, richness of taxa and total biomass in EMA and EMA of each site and area.

	MINORCA								MONTENEGRO							
	ECA SITE 1	SD	EMA SITE 1	SD	ECA SITE 2	SD	EMA SITE 2	SD	ECA SITE 1	SD	EMA SITE 1	SD	ECA SITE 2	SD	EMA SITE 2	SD
Chlorophyll-a (µg g-1)	139.9	54.9	858.7	151.4	18.6	5.4	640.7	181.2	45.0	18.3	38.6	22.2	19.1	14.2	19.9	12.8
Phaeopigment (µg g-1)	383.9	61.4	2542.9	419.8	50.8	9.4	1659.4	711.7	266.4	24.6	30.1	14.0	145.3	229.0	35.2	49.5
Total Phytopigmet (µg g-1)	523.8	116.3	3401.6	555.8	69.3	14.9	2300.0	892.9	311.4	42.9	68.7	8.2	24.5	14.4	74.6	31.9
Protein (mg g-1)	9.3	1.9	5.6	0.3	1.7	0.1	2.7	0.4	5.3	1.3	11.2	2.4	1.6	0.3	4.4	0.3
Carbohydrate (mg g-1)	15.0	5.7	26.3	15.0	14.7	7.9	6.2	0.4	46.5	3.6	10.4	1.9	30.8	4.8	8.8	0.4
Lipid (mg g-1)	0.7	0.0	0.2	0.1	0.3	0.1	0.5	0.0	1.6	0.2	2.2	0.3	3.3	0.4	4.0	0.0
Biopolymeric C (mg g-1)	11.0	2.7	13.4	6.2	6.9	3.2	4.2	0.4	22.4	1.4	11.4	1.2	15.6	2.2	8.7	0.0

Table 2: Concentration of organic compounds in the investigated areas

3.3 Statistical analysis

In order to test significant differences in all the investigated variables among samples, we used uni- and multivariate analysis of variance.

The sampling design included three factors: State (fixed, 2 levels: EMA and ECA), Area (random, 2 levels: Montenegro and Minorca) and Site (random and nested in Area, 2 levels: Site 1 and Site 2). The same sampling design was applied in a univariate contest for meiofaunal abundance, biomass, richness of taxa, for the concentration of all investigated organic matter compounds and the indicators of nutritional quality. The same sampling design was also applied in a multivariate contest for meiofaunal taxonomic composition and for organic matter biochemical composition and nutritional quality. When significant differences were observed, a pairwise test was also applied to ascertain patterns of differences among states, areas and sites. Although post-hoc tests could not be applied on random factors (Underwood, 1997), we forced their use to test differences between the areas and sites, to have more information on variability at different spatial scales. Prior to each analysis, the data was previously transformed (square root) and the PERMANOVA tests based on Bray–Curtis similarity matrices (for meiofaunal variables) or data were normalized and the PERMANOVA tests based on Euclidean distance after normalisation of the data (for organic matter variables).

To visualise differences among states, areas and sites in the meiofaunal taxonomic composition, organic matter biochemical composition and nutritional quality, bi-plots after a Canonical Analysis of Principal Coordinates (CAP) were prepared. CAP analysis was selected as the ordination technique as it allows to find the axis (or axes) in the principal coordinate space that is best at discriminating among the a priori groups. Moreover, this analysis allows identifying the environmental variables which guide the ordination (Anderson and Willis, 2003).

All statistical analyses were performed with the software PRIMER 6+ (Clarke and Gorley, 2006).

MAIN TESTS

Abundance

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	15831	15831	25.663	0.068	699	0.012
Ar	1	23.834	23.834	0.16587	1	6	0.803
Si	1	70.079	70.079	0.48772	0.633	6	0.648
StxAr	1	255.6	255.6	0.33454	0.659	495	0.718
StxSi	1	391.05	391.05	0.51181	0.614	504	0.633
ArxSi	1	143.69	143.69	0.80199	0.368	999	0.382
StxArxSi	1	764.05	764.05	4.2646	0.044	998	0.041
Res	32	5733.2	179.16				
Total	39	23212					

Biomass

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	18210	18210	21.741	0.088	680	0.016
Ar	1	261.65	261.65	1.0687	0.496	6	0.527
Si	1	4.5135	4.5135	1.84E-02	0.82	6	0.977
StxAr	1	629.12	629.12	0.67982	0.558	507	0.596
StxSi	1	251.05	251.05	0.27129	0.702	514	0.749
ArxSi	1	244.83	244.83	0.85377	0.409	999	0.382
StxArxSi	1	925.41	925.41	3.2271	0.061	998	0.066
Res	32	9176.4	286.76				
Total	39	29703					

Richness of taxa

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	4397.9	4397.9	54.712	0.021	705	0.006
Ar	1	178.5	178.5	0.50518	0.677	6	0.615
Si	1	52.346	52.346	0.14814	1	6	0.818
StxAr	1	44.659	44.659	0.15242	0.744	506	0.796
StxSi	1	41.08	41.08	0.1402	0.751	506	0.79
ArxSi	1	353.34	353.34	3.831	0.04	998	0.069
StxArxSi	1	293	293	3.1768	0.079	999	0.076
Res	32	2951.4	92.232				
Total	39	8312.3					

Composition PA

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	5669.3	5669.3	156.34	0.002	689	0.001
Ar	1	1299.5	1299.5	2.1553	0.482	6	0.28
Si	1	169.76	169.76	0.28157	0.822	6	0.7665
StxAr	1	7.4229	7.4229	2.40E-02	0.91	503	0.9272
StxSi	1	30.819	30.819	9.96E-02	0.833	505	0.8477
ArxSi	1	602.91	602.91	3.5999	0.039	999	0.022
StxArxSi	1	309.48	309.48	1.8479	0.145	999	0.172
Res	32	5359.3	167.48				
Total	39	13448					

Composition SQ

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	18158	18158	18.295	0.036	695	0.004
Ar	1	919.82	919.82	2.1443	0.504	6	0.3133
Si	1	181.81	181.81	0.42385	0.856	6	0.7538
StxAr	1	488.75	488.75	0.7211	0.591	494	0.594
StxSi	1	540.83	540.83	0.79794	0.558	494	0.5601
ArxSi	1	428.96	428.96	1.4235	0.222	998	0.235
StxArxSi	1	677.79	677.79	2.2493	0.113	998	0.116
Res	32	9642.7	301.33				
Total	39	31039					

PAIR-WISE TESTS

Abundance

Montenegro					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	6.1743	0.013	126	0.001
Sito2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	13.477	0.009	126	0.001
Minorca					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	1.6098	0.12	126	0.131
Sito 2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	7.6892	0.007	126	0.001

Biomass

Montenegro					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	5.3151	0.011	126	0.001
Sito2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	7.2428	0.015	126	0.001
Minorca					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	1.4574	0.145	126	0.168
Sito 2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	5.2436	0.008	126	0.001

Richness of taxa

Montenegro					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	5.8145	0.009	30	0.002
Sito2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	2.0166	0.102	41	0.069
Minorca					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	2.5093	0.054	19	0.033
Sito 2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	5.1611	0.005	19	0.001

Composition PA

Montenegro					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	3.0374	0.008	126	0.002
Sito2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	2.5899	0.008	126	0.011
Minorca					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	1.6794	0.113	66	0.07
Sito 2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	5.0837	0.006	41	0.001

Composition SQ

Montenegro					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	4.8312	0.004	126	0.001
Sito2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	6.2024	0.009	126	0.001
Minorca					
Sito 1	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	1.6631	0.126	126	0.108
Sito 2	Groups	t	P(perm)	perms	P(MC)
	ECA, EMA	4.9079	0.005	126	0.001

Figure 2: Results of meiofaunal statistical analysis.

MAIN TESTS

Chlo-a

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	6.2846	6.2846	0.98526	0.515	677	0.518
Ar	1	8.3064	8.3064	27.112	0.143	6	0.116
Si	1	0.51965	0.51965	1.6961	0.5	6	0.401
StxAr	1	6.3891	6.3891	167.63	0.084	507	0.042
StxSi	1	2.82E-02	2.82E-02	0.73971	0.566	513	0.556
ArxSi	1	0.30637	0.30637	4.3475	0.058	999	0.057
StxArxSi	1	3.81E-02	3.81E-02	0.54086	0.464	997	0.461
Res	16	1.1275	7.05E-02				
Total	23	23					

Phaopygments

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	5.0303	5.0303	0.71107	0.75	686	0.537
Ar	1	7.437	7.437	14.286	0.165	6	0.191
Si	1	0.76336	0.76336	1.4664	0.493	6	0.428
StxAr	1	7.2735	7.2735	36.979	0.083	491	0.104
StxSi	1	7.74E-02	7.74E-02	0.39336	0.668	492	0.611
ArxSi	1	0.52056	0.52056	4.8957	0.028	999	0.037
StxArxSi	1	0.19669	0.19669	1.8498	0.2	998	0.193
Res	16	1.7013	0.10633				
Total	23	23					

Total phytopigments

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	5.6667	5.6667	0.88745	0.57	691	0.531
Ar	1	7.9303	7.9303	20.801	0.183	6	0.149
Si	1	0.79135	0.79135	2.0757	0.491	6	0.391
StxAr	1	6.5893	6.5893	31.813	0.134	507	0.099
StxSi	1	2.95E-02	2.95E-02	0.1422	0.67	483	0.75
ArxSi	1	0.38125	0.38125	4.343	0.059	999	0.054
StxArxSi	1	0.20713	0.20713	2.3595	0.152	998	0.144
Res	16	1.4045	8.78E-02				
Total	23	23					

Proteins

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	1.1444	1.1444	0.74664	0.556	686	0.599
Ar	1	0.33476	0.33476	2471.3	0.361	6	0.013
Si	1	13.703	13.703	1.01E+05	0.163	6	0.002
StxAr	1	3.9747	3.9747	2.1061	0.405	511	0.398
StxSi	1	8.57E-02	8.57E-02	4.54E-02	0.803	494	0.854
ArxSi	1	1.35E-04	1.35E-04	1.16E-03	0.982	995	0.978
StxArxSi	1	1.8872	1.8872	16.146	0.002	996	0.002
Res	16	1.8702	0.11689				
Total	23	23					

Lipids

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	0.22199	0.22199	0.50064	0.62	689	0.636
Ar	1	17.291	17.291	7.5253	0.33	6	0.19
Si	1	2.2003	2.2003	0.95763	0.647	6	0.509
StxAr	1	0.4648	0.4648	6.2932	0.293	491	0.274
StxSi	1	0.12613	0.12613	1.7078	0.434	520	0.41
ArxSi	1	2.2977	2.2977	113.36	0.001	994	0.001
StxArxSi	1	7.39E-02	7.39E-02	3.644	0.074	998	0.062
Res	16	0.3243	2.03E-02				
Total	23	23					

Carbohydrates

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	5.6224	5.6224	1.1252	0.487	687	0.542
Ar	1	2.1816	2.1816	125.83	0.29	6	0.056
Si	1	2.6183	2.6183	151.01	0.155	6	0.051
StxAr	1	6.8217	6.8217	3.218	0.332	500	0.338
StxSi	1	5.88E-02	5.88E-02	2.77E-02	0.863	491	0.884
ArxSi	1	1.73E-02	1.73E-02	7.79E-02	0.778	996	0.777
StxArxSi	1	2.1199	2.1199	9.5276	0.005	998	0.011
Res	16	3.56	0.2225				
Total	23	23					

BPC

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	3.6367	3.6367	1.3553	0.458	681	0.493
Ar	1	5.5082	5.5082	35.19	0.335	6	0.089
Si	1	5.6878	5.6878	36.338	0.152	6	0.118
StxAr	1	3.3633	3.3633	3.6099	0.305	496	0.306
StxSi	1	7.50E-03	7.50E-03	8.05E-03	0.945	487	0.95
ArxSi	1	0.15653	0.15653	0.67537	0.436	997	0.435
StxArxSi	1	0.93169	0.93169	4.02	0.068	994	0.06
Res	16	3.7082	0.23176				
Total	23	23					

PAIR-WISE TESTS

Chlo-a

ECA						Montenegro					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		2.8417	0.115	10	0.046		ECA, EMA	0.38485	0.706	10	0.714
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		5.85E-02	1	10	0.955		ECA, EMA	7.91E-02	1	10	0.943
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		9.2852	0.102	10	0.001		ECA, EMA	7.7331	0.109	10	0.003
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		5.9177	0.102	10	0.005		ECA, EMA	5.943	0.082	10	0.005

Phaeopygments

ECA						Montenegro					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		3.0764	0.097	10	0.036		ECA, EMA	14.446	0.104	10	0.002
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		0.71444	1	10	0.523		ECA, EMA	0.81424	0.703	10	0.475
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		10.361	0.072	10	0.001		ECA, EMA	8.8138	0.088	10	0.003
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		3.9433	0.089	10	0.018		ECA, EMA	3.9146	0.106	10	0.016

Total phytopigments

ECA						Montenegro					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		2.9682	0.113	10	0.033		ECA, EMA	9.6144	0.098	10	0.002
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		3.75E+00	0.113	10	0.024		ECA, EMA	2.48E+00	0.102	10	0.08
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		10.384	0.104	10	0.001		ECA, EMA	8.7776	0.108	10	0.002
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		4.3141	0.102	10	0.013		ECA, EMA	4.3265	0.113	10	0.016

Proteins

ECA						Montenegro					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		2.9847	0.106	10	0.049		ECA, EMA	3.7634	0.106	10	0.022
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		0.40459	0.83	10	0.693		ECA, EMA	11.561	0.097	10	0.002
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		4.0747	0.097	10	0.017		ECA, EMA	3.3543	0.103	10	0.026
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		5.6649	0.106	10	0.01		ECA, EMA	4.177	0.088	10	0.014

Lipids

ECA						Montenegro					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		6.6951	0.11	10	0.006		ECA, EMA	2.6218	0.093	10	0.057
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		13.597	0.092	10	0.001		ECA, EMA	3.3729	0.091	10	0.035
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		10.812	0.097	10	0.002		ECA, EMA	9.46	0.12	10	0.003
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		465.96	0.111	10	0.001		ECA, EMA	3.3225	0.093	10	0.028

Carbohydrates

ECA						Montenegro					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		8.0339	0.103	10	0.005		ECA, EMA	15.301	0.106	10	0.001
Sito2	roups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		3.0079	0.106	10	0.041		ECA, EMA	7.8243	0.122	10	0.005
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		1.8117	0.293	10	0.152		ECA, EMA	1.2147	0.323	10	0.32
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		8.2284	0.082	10	0.001		ECA, EMA	1.8578	0.086	10	0.14

BPC

ECA						Montenegro					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		6.465	0.101	10	0.002		ECA, EMA	10.712	0.109	10	0.002
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		3.8454	0.11	10	0.026		ECA, EMA	5.4532	0.104	10	0.004
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		0.56173	0.684	10	0.592		ECA, EMA	0.6033	0.622	10	0.574
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		20.661	0.116	10	0.001		ECA, EMA	1.4349	0.4	10	0.225

Chlo-a:BPC

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	4.9857	4.9857	1.6651	0.401	693	0.435
Ar	1	0.76847	0.76847	6.113	0.351	6	0.243
Si	1	4.1233	4.1233	32.8	0.171	6	0.124
StxAr	1	5.0287	5.0287	1.1685	0.483	514	0.493
StxSi	1	0.55	0.55	0.1278	0.8	501	0.779
ArxSi	1	0.12571	0.12571	0.64578	0.415	994	0.461
StxArxSi	1	4.3035	4.3035	22.107	0.001	997	0.001
Res	16	3.1146	0.19466				
Total	23	23					

Chlo-a:BPC

ECA						**Montenegro**					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		5.2309	0.108	10	0.005		ECA, EMA	8.0196	0.101	10	0.003
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		2.2627	0.092	10	0.093		ECA, EMA	18.549	0.095	10	0.002
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		2.8174	0.101	10	0.048		ECA, EMA	1.9291	0.198	10	0.108
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		4.2625	0.106	10	0.016		ECA, EMA	4.3519	0.091	10	0.018

Proteins:CHO

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	4.6527	4.6527	1.3785	0.436	697	0.535
Ar	1	0.13397	0.13397	0.6769	0.656	6	0.564
Si	1	3.055	3.055	15.435	0.354	6	0.16
StxAr	1	6.2579	6.2579	1.5472	0.404	494	0.411
StxSi	1	5.13E-02	5.13E-02	1.27E-02	0.935	509	0.923
ArxSi	1	0.19792	0.19792	0.68746	0.438	997	0.418
StxArxSi	1	4.0448	4.0448	14.049	0.002	998	0.003
Res	16	4.6065	0.2879				
Total	23	23					

Proteins:BPC

ECA						**Montenegro**					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		3.0654	0.104	10	0.046		ECA, EMA	4.1376	0.106	10	0.016
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		1.9087	0.088	10	0.13		ECA, EMA	14.093	0.1	10	0.001
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		3.0752	0.097	10	0.057		ECA, EMA	1.8122	0.218	10	0.139
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		1.6493	0.204	10	0.189		ECA, EMA	5.7172	0.097	10	0.003

OM Bioquemical composition

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	18.304	18.304	0.89405	0.563	677	0.565
Ar	1	35.551	35.551	11.314	0.162	6	0.024
Si	1	19.804	19.804	6.3029	0.301	6	0.07
StxAr	1	24.924	24.924	5.775	0.059	502	0.071
StxSi	1	0.37619	0.37619	8.72E-02	0.969	495	0.977
ArxSi	1	3.1421	3.1421	5.8571	0.003	999	0.004
StxArxSi	1	4.3158	4.3158	8.045	0.001	999	0.001
Res	16	8.5833	0.53646				
Total	23	115					

OM Biochemical composition

ECA						**Montenegro**					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		5.2715	0.096	10	0.002		ECA, EMA	6.049	0.094	10	0.001
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		5.5825	0.084	10	0.002		ECA, EMA	6.1133	0.092	10	0.002
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		5.3134	0.107	10	0.002		ECA, EMA	4.2257	0.1	10	0.012
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		6.5421	0.094	10	0.003		ECA, EMA	4.1701	0.099	10	0.007

OM Nutritional quality

Source	df	SS	MS	Pseudo-F	P(perm)	perms	P(MC)
St	1	9.6384	9.6384	1.513	0.402	689	0.47
Ar	1	0.90245	0.90245	2.7885	0.493	6	0.316
Si	1	7.1784	7.1784	22.181	0.181	6	0.079
StxAr	1	11.287	11.287	1.352	0.461	501	0.431
StxSi	1	0.60128	0.60128	7.20E-02	0.859	499	0.871
ArxSi	1	0.32363	0.32363	0.67065	0.431	999	0.429
StxArxSi	1	8.3482	8.3482	17.3	0.001	997	0.002
Res	16	7.7211	0.48257				
Total	23	46					

OM Nutritional quality

ECA						**Montenegro**					
Site 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		4.005	0.106	10	0.016		ECA, EMA	5.051	0.112	10	0.008
Sito2	Groups	t	P(perm)	perms	P(MC)	Sito2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		2.2002	0.122	10	0.106		ECA, EMA	16.063	0.105	10	0.001
EMA						**Minorca**					
Sito 1	Groups	t	P(perm)	perms	P(MC)	Sito 1	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		2.9806	0.097	10	0.03		ECA, EMA	1.8732	0.206	10	0.157
Sito 2	Groups	t	P(perm)	perms	P(MC)	Sito 2	Groups	t	P(perm)	perms	P(MC)
Minorca, Montenegro		3.2313	0.111	10	0.02		ECA, EMA	4.6698	0.111	10	0.011

Figure 3: Results of organic matter statistical analysis

4. RESULTS

4.1 Organic matter

The results of organic matter analysis are reported in Table 3. It is important to mention that in all organic matter and meiofauna data we have found a dissimilarity in one sampling point, Minorca site 1, in this point the ECA state was smaller and less differentiated from EMA that those from the other sites both in Minorca and Montenegro, and this is possibly the reason for some abnormal results.

The total phytopigment concentrations in Minorca ranged from 69.3 µg g^{-1} ± 3.4 mg.g^{-1} and 311.4 ± 24.5 µg g^{-1} in Montenegro. The carbohydrate concentrations ranged from 26.3 ± 6.2 mg g^{-1} in Minorca and 46.5 ± 8.8 mg g^{-1} in Montenegro. Protein concentrations ranged from 9.3 ± 2.7 mg g^{-1} in Minorca and 11.2 ± 1.6 mg g^{-1}. The lipid concentration ranged from 0.7 ± 0.2 mg g^{-1} in Minorca and 4.0 ± 1.6 in Montenegro. Total biopolymeric C ranged from 13.4 ± 4.2 mg g^{-1} in Minorca and 22.4 ± 8.7 mg g^{-1} in Montenegro.

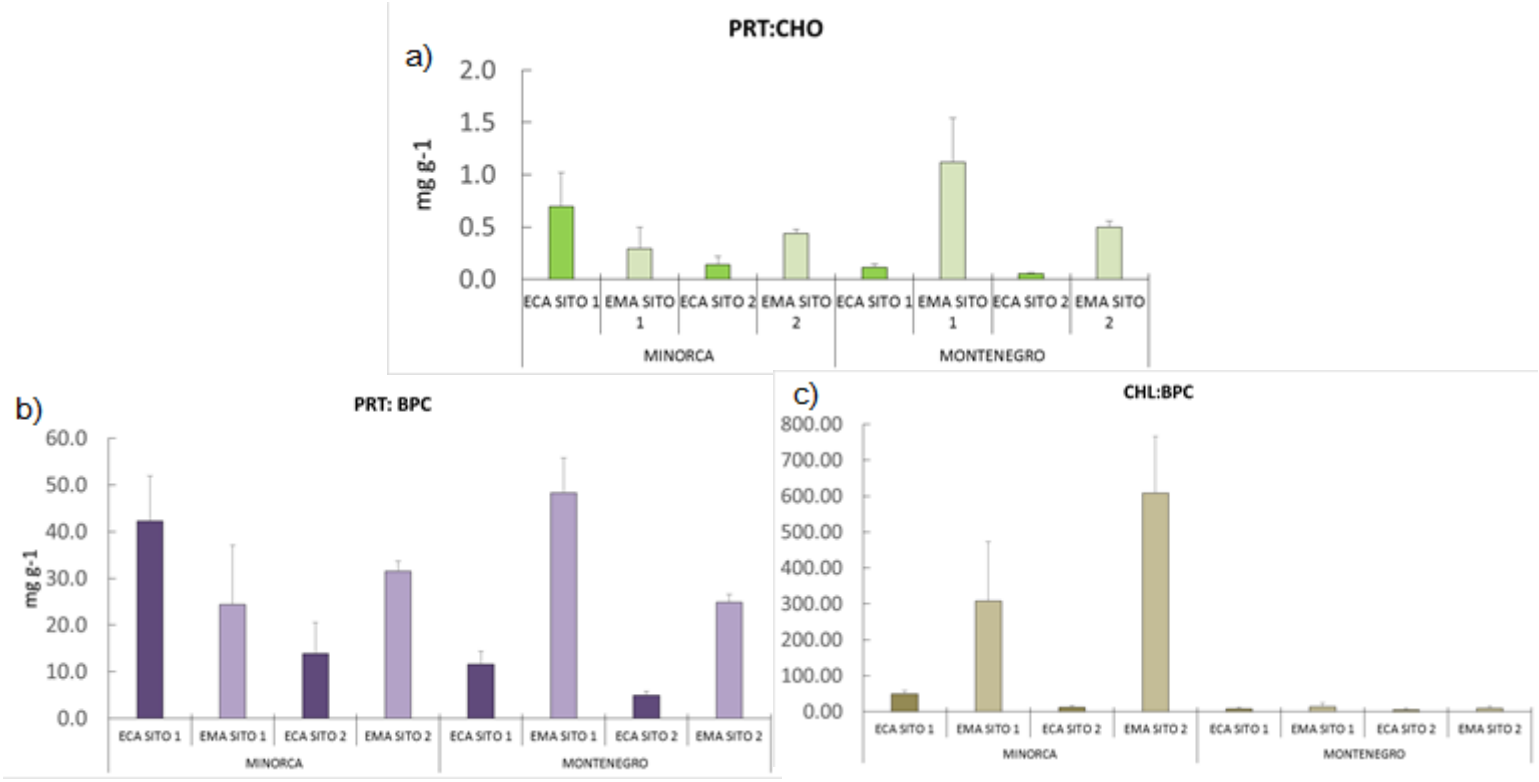

Figure 4: a) Protein/ carbohydrate relation within the sediment. b) quantity of protein within the biopolymeric carbon. c) quantity of carbohydrate within the biopolymeric carbon.

The results of PERMANOVA tests were carried out separately showing differences in quantity, quality and aged of organic matter. The PERMANOVA main tests revealed a significant difference between ECA and EMA of all sites and areas only in terms of biopolymeric C, contribution of phytopigments to BPC and OM nutritional quality. But the PAIR-WAISE confrontation of all factors (Site, Area and State) with the site revealed some significant differences; an increase in chlorophyll-a in EMAs was found respect from the ECAs in Minorca, whereas Montenegro did not show statistical differences. The test also revealed higher phaeopigment concentrations in EMAs than ECAs of Minorca, however, for Montenegro phaeopigments were significantly elevated in ECAs of site 1 and no significant in site 2. Statistical results of total phytopigments showed a significant high quantity in EMA than in ECA with the exception of Montenegro site 1 where ECA reach higher values than EMA. Protein concentration showed a significant difference being more elevated in EMA than in ECA with the exception of Minorca site 1. Carbohydrates only were significant in Montenegro where ECAs showed higher values. Lipid concentrations were significant in all sites except Montenegro site 1, and showed higher values in EMAs.

The CAP revealed that organic matter composition of EMA was more affected for the area of study than ECA states. Total phytopigments, proteins and lipids were the parameters that affected more the difference.

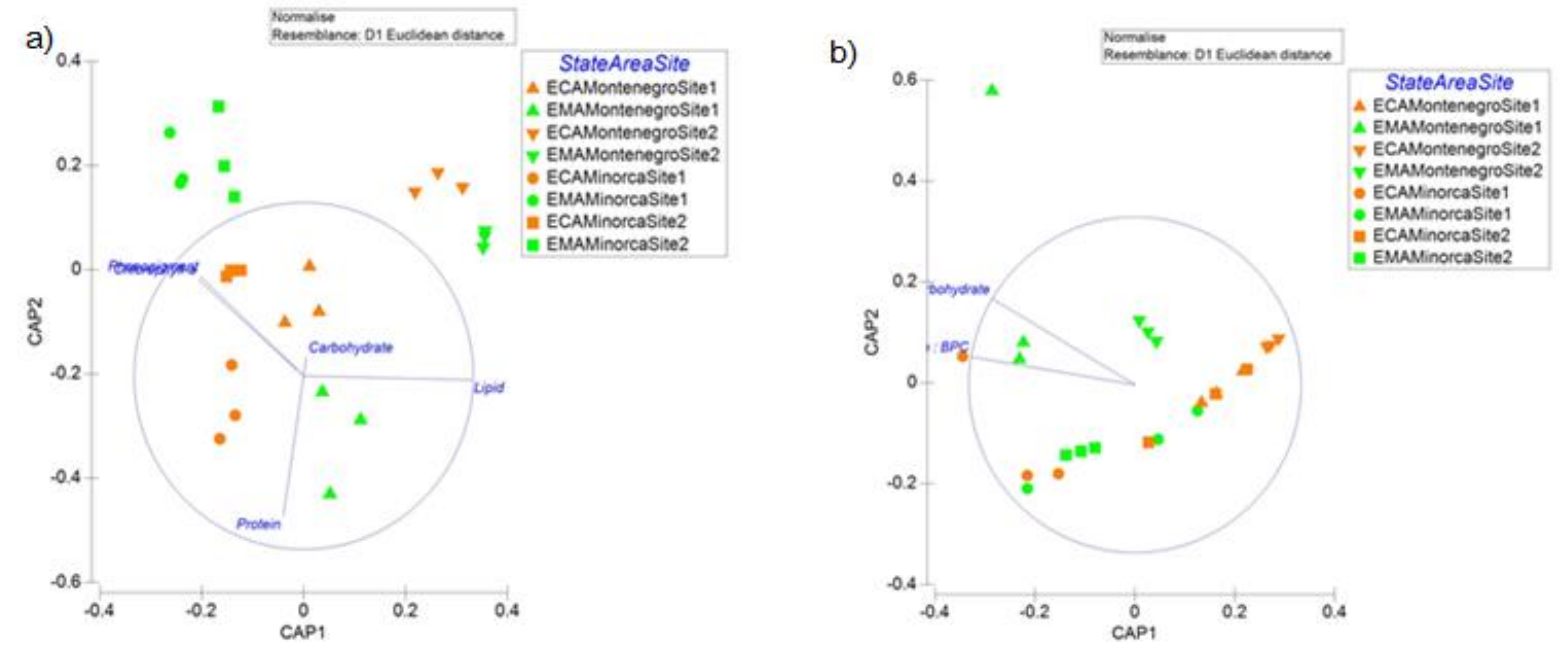

Figure 5: Results of the CAP for every state, area and site. a) resemblance of organic matter composition between EMA(green) and ECA (orange). b) resemblance of organica mater in terms of nutritional quality between EMA and ECA.

4.1 Meiofaunal assemblages

Meiofaunal abundance for each taxa, richness of taxa, total biomass and total abundance of all sampling points are reported in Table 1 and Table 2. The results of meiofaunal abundance are shown in terms of number of individuals per 10 cm^2. Meiofaunal density ranged from 363 to 94 ind. Per 10 cm^2 in Montenegro EMAs and 33 to 9 idv. per 10 cm^2 in ECAs. In Minorca density ranged from 291 to 14 ind. per 10 cm^2 in EMAs and from 63 to 8 ind. per 10 cm^2 in ECAs.

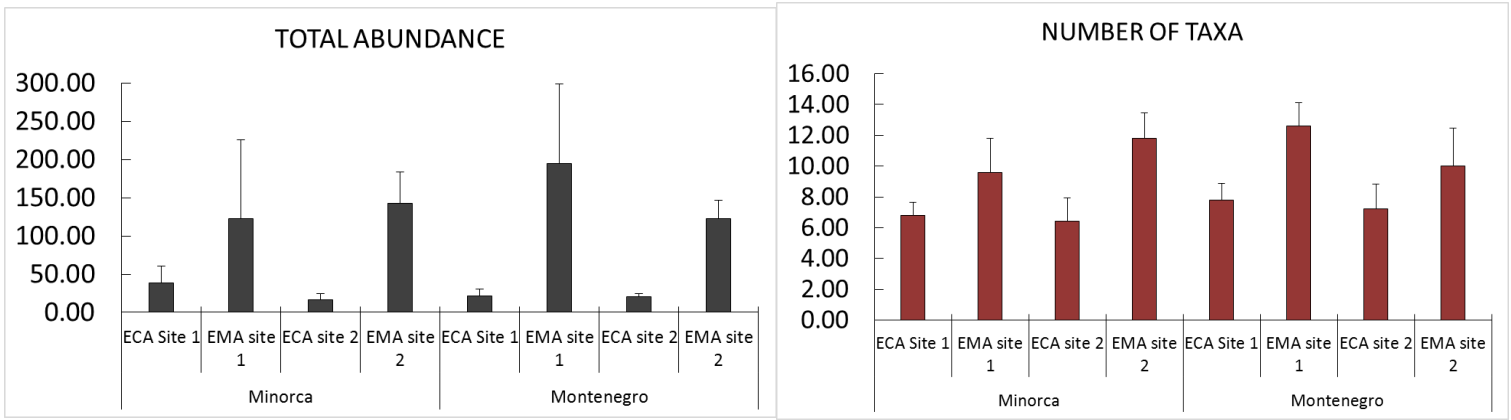

Figure 5: Meiofaunal total abundance (number of individuals) and number of taxa in the different areas.

We collected a total of 16 different taxa, meiofaunal composition is illustrated in Figure 5. For all cases we found more different taxas in EMA than in ECA. Harpacticoid copepods were the most abundant ranging from 50 to 68% of the total community. In Minorca copepods accounted for 54 to 61% (mean 58%) of meiofaunal community. Nematodes were the second most abundant group ranging from 22 to 29 % (mean 24%) of total meiofaunal density. Polychetes accounted for 8 to 11 % (mean 10%). Ostracoda ranged from 1 to 4% of the community (mean 3%). Other taxa (included Bivalvia, Kinorhyncha, oligochaete, tardigrade, cumaceae, amphipoda, isopoda, tanaidacea, halaroidea and peracarida) accounted for 0.3 to 2 % (mean 1%) of total community and gastropods reached from 1 to 3% (mean 2%). In Montenegro copepods ranged from 50 to 68% of total density (mean 60%). Nematodes accounted for 19 to 30% (mean 23%). Polychaetes reached from 6 to 14% (mean 10%). Ostracodes accounted for 2 to 5% (mean 3%). Turbellarian ranged from 1 to 2% (mean 2%) and other taxa ranged from 1 to 3% of total density (mean 2%).

Total Meiofaunal biomass results are reported in Table X, in Minorca values ranged from a mean of 5 µgC.10 cm^2 in ECAs to 25 µgC.10 cm^2 in EMA state. In Montenegro biomass ranged from a mean of 4 µgC.10 cm^2 in ECA to 43 µgC.10 cm^2 in EMA state.

The results from the comparison between permanent and temporary meiofauna are shown in Figure 6. In Minorca permanent meiofauna ranged ± 86% whereas temporary meiofauna reached ± 14% of total community. In Montenegro EMAs showed 83% and 17% of permanent and temporary meiofauna respectively, and 90% and 10% in ECAs.

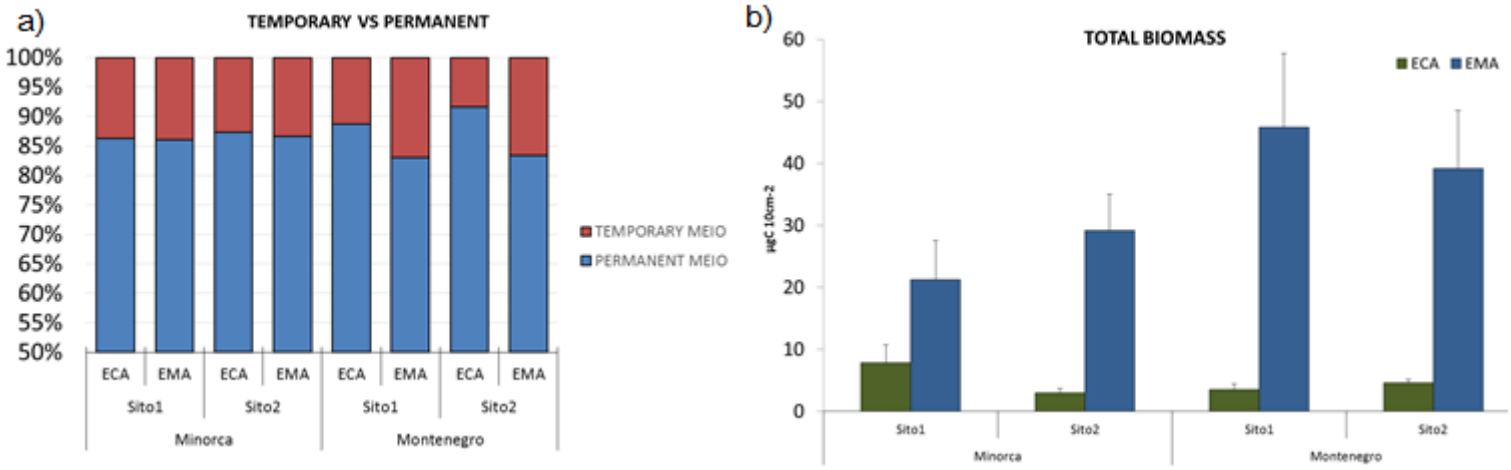

Figure 6: a) percentage of temporary and permanent meifauna. b) Total biomass (µg.C.10cm^2) in the different areas.

PERMANOVA test rejected the null hypothesis that EMA and ECA are similar in terms of abundance, richness of taxa and biomass. Pair-wise test showed that significant differences occurred also in every site and area in relation to the ecosystem state with the exception of Minorca site 1. Richness of taxa PAIR-WISE test showed no significant difference in

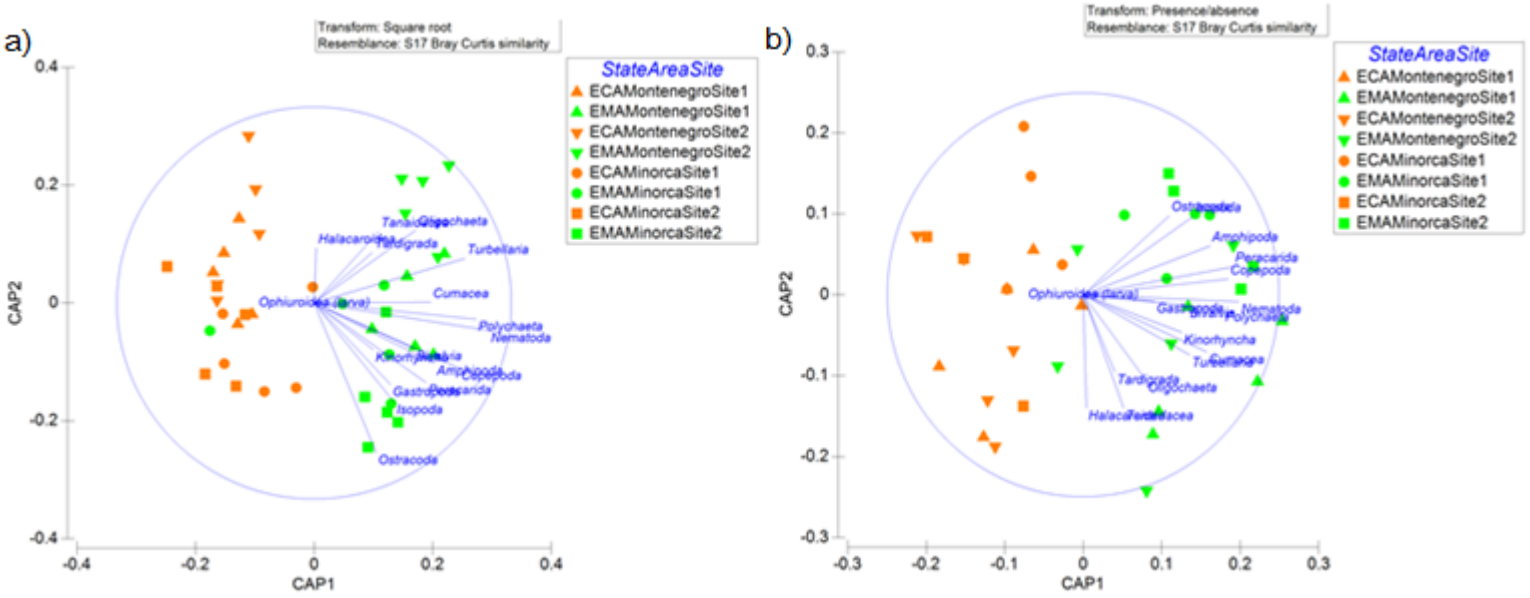

Figure 7: a) resemblance in meiofaunal composition between EMA (green) and ECA (orange) states in terms of abundance of each taxa. b) resemblance in meiofaunal composition between EMA and ECA states in terms of presence/absence of each taxa.

Montenegro site 2. The multivariate PERMANOVA test of meiofaunal composition was also significant showing a difference between ECA and EMA state. Also with the exception of Minorca site 1, pair-wise test showed significant difference between state for every site and area.

The results of the CAP are reported in Figure 7, meiofaunal composition is clearly divided based on the ecosystem state. The CAP for meiofaunal composition in terms of presence/absence also reported results clearly differentiated for this factor.

3 DISCUSSION

Meiofaunal community in ECA and EMA

Little information is available about meiofaunal communities and organic compounds in hard substrates of sublitoral coastal waters, and even less comparing EMA and ECA states in the Mediterranean Sea. Most of studies have centred its work in understanding the effects of sea urchins, coralline algaes and anthropogenic stressors on macroalgae assemblages.

Danovaro et al. 2007 revealed that a significant quantity of energy from metazoan meiofauna is transferred to higher trophic levels, being an important food source in benthic systems. The areas where EMA and ECA appeared are coastal zones and for this reason are sensitive to sewage discharge and contaminants, previous studies have also revealed that this anthropogenic factor may affect meiofaunal communities in terms of taxon richness and community structure (Fraschetti et al. 2006).

The results presented in this study suggest that benthic meiofauna found in sediments from erected algae assemblages reach significant higher levels of biomass ($p<0.016$) richness of

taxa ($p<0.006$), and abundance ($p<0.012$) than those sediments from incrusting coralline assemblages (barrens). No significant differences were found comparing the two areas of study or the two sites in each one between them. Although other studies on meiofaunal communities associated with macroalgae assemblages revealed a dominance of crustaceans and a minor presence of nematodes (Danovaro and Fraschetti 2002), in this study we found that nematodes were the second most abundant taxa within the sediments.

Previous studies demonstrated that the presence of biogenic structures as macroalgae assemblages may difficult predation over metazoan meiofauna and create more refuges and protection (Danovaro et al. 2007). Copepods and other meiofaunal organisms are important preys for shrimps and demersal fishes (Walters et al. 1996). It is possible that after the increase in sea urchin number and the subsequent grazing rate, a reduced number of erected macroalgae leave meiofaunal community exposed to predators affecting also its abundance.

The canonical analysis of principal coordinates (CAP) carried out in order to test meiofaunal composition ($p<0.004$) also revealed significant differences between ECA and EMA states in terms of abundance of each taxon. Nematodes and harpacticoid copepods were the dominant taxon in all areas, sites and states but in EMAs had a great abundance in contrast with ECAs. In Montenegro nematodes abundance was from 89% to 93% lower in ECAs than in EMAs. Copepods abundance was also from 77% to 86% lower in ECAs.

Taxons in which permanent meiofaunal forms exist where more abundant in ECA state with the exception of Minorca site 2. Permanent meiofauna is constituted by some predators especially some turbellarians and nematodes that feed on juvenile states of some macrofauna organisms (Danovaro et al. 1995). Thereby this fact may affect the macrofaunal community reducing also juvenile survival.

Organic matter in ECA and EMA

The presence of macroalgae such as *Cystoseira* in rocky substrates of the sublittoral area allow a higher level of photosynthetic primary production, this increments also the input of organic compounds that reach the sediment (Sales and Ballesteros 2012). The increase in these organic compounds coming from algae production may also stimulate bacterial community being a factor that might increase meiofaunal biomass and abundance (Danovaro 1996).

Our study reveals that the sediment concentrations of biochemical components (proteins, phytopigments, carbohydrates and lipids) and biopolimeric C have not significant differences between EMA and ECA according to the main PERMANOVA test. However we observed significant differences in the organic matter biochemical composition between the sediments of the two areas of study. Pair-waise test also reveals that in every site and area separately there was a significant difference between the ecosystem states. Always with the exception of Minorca site 1, proteins and lipids were more abundant in EMA while biopolimeric C and carbohydrates were higher in ECA.

The analysis revealed that in all ECA carbohydrates dominated the organic matter pool. Therefore results show a protein to carbohydrate ratio always <1. Such results are indicative of detrital-heterotrophic environments, similar results were also found in oligotrophic environments (Danovaro 1996). Since proteins are typically compounds enzymatically accessible for organisms the low quantity in sediments from ECA may be indicative of aged organic detritus (Pusceddu et al. 2010).

Another factor that should be taken in account is the lack of biogenic structures constituted by macroalgae assemblages in ECA states. The organic matter composition in sediments is also dependent of hydrodynamic fluxes, some studies revealed that macroalgae assemblages may play a role in turbidity of some estuarine and lagoon ecosystems (Canal-vergés et al. 2010). It may also be an important factor in this case.

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