Universidade de Aveiro Departamento de Biologia
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Ana Raquel de Azevedo e Costa

Intertidal benthic macrofauna alterations due to oyster culture

Alterações na comunidade bentónica intertidal resultantes da cultura de ostras

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada – Ramo Biologia Marinha, realizada sob a orientação científica do Doutor Victor Quintino, Professor Auxiliar do Departamento de Biologia da Universidade de Aveiro e co-orientação científica da Doutora Ana Maria Rodrigues, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro.

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palavras-chave

Ria de Aveiro, macrofauna bentónica, ostrícultura, índices bióticos, enriquecimento orgânico

resumo

O presente trabalho foi desenvolvido numa cultura intertidal de ostras, explorada na Ria de Aveiro (Noroeste de Portugal). O objectivo principal deste estudo consistiu em verificar se a referida actividade induz efeitos, na comunidade de macrofauna bentónica, devidos ao enriquecimento orgânico. A cultura de ostras na Ria de Aveiro é efectuada sobre mesas elevadas e o desenho experimental incluiu áreas de cultura sem ostras (com e sem mesas), áreas com ostras juvenis e áreas com espécimes de maiores dimensões. Os resultados obtidos mostraram que nas áreas com ostras, o sedimento superficial localizado sob as mesas apresentou teor em finos e em matéria orgânica mais elevado, com os valores mais altos de ambos os descritores verificados nas áreas com ostras de maiores dimensões. Nas áreas com ostras de menores dimensões foi também demonstrado que o sedimento superficial localizado sob as mesas de cultura apresentou teor em finos e teor em matéria orgânica mais elevados do que nos corredores entre as mesas. Estes resultados mostraram alterações associadas ao enriquecimento orgânico, sob as mesas de cultura. Os descritores da comunidade bentónica foram testados considerando como hipótese nula a não existência de diferenças significativas entre áreas com diferente enriquecimento orgânico. Os resultados obtidos mostraram que as comunidades bentónicas presentes sob as mesas apresentaram menor diversidade, verificada a partir do decréscimo da riqueza específica e abundância, comparativamente às restantes áreas amostradas. Os resultados obtidos revelaram que a comunidade de macrofauna bentónica e os índices riqueza específica (S) e abundância (A) apresentaram diferenças muito significativas entre áreas com e sem cultura de ostras. No entanto, aquando da utilização dos índices bióticos H' (diversidade de Shannon), d (riqueza específica de Margalef), 1-λ' (Simpson), AMBI e M-AMBI, mostrou-se não ser possível evidenciar o empobrecimento da comunidade bentónica, nomeadamente na riqueza específica e abundância, associado ao enriquecimento orgânico resultante da cultura de ostras. Deste modo, a aplicação de índices bióticos deve ser efectuada com precaução em áreas cujo enriquecimento orgânico determina um empobrecimento da macrofauna existente em detrimento de uma alteração ou substituição de espécies.

keywords Ria de Aveiro, Benthic macrofauna, oyster culture, biotic indices, organic enrichment

abstract The present work was developed in an intertidal mudflat oyster farm located in Ria de Aveiro, Northwestern Portugal. The aim of this study was to assess if this practice causes organic enrichment upon the benthic community. The oyster culture in Ria de Aveiro is developed off-bottom, with oysters placed above trestles. The experimental design included farm areas where no oysters were introduced (with or without trestles), areas populated with juvenile oysters and other with larger specimens. The results showed that the superficial sediment located under the trestles in areas with oysters presented both higher fines content and organic matter, with the higher values found in areas with the culture of larger oysters. In the areas with smaller oysters, it was also shown that the superficial sediment under the trestles with oysters had a higher fines content and organic matter, in comparison with the corridors between trestles. These results showed effects associated to organic enrichment, under culture trestles. The benthic community descriptors were tested under the null hypothesis of no significant differences between areas with distinct organic enrichment. The results showed that the benthic community living under the trestles with oysters, presented less diversity, which was verified by the decreasing of both species richness and abundance, comparing with the remaining areas. The results obtained revealed that the benthic macrofauna community and the indices species richness (S) and abundance (N) showed very significant differences between areas with and without oyster culture. However, the biotic indices H' (Shannon diversity), d (Margalef richness), 1-λ' (Simpson), AMBI and M-AMBI failed to reject the null hypothesis revealing that these indices were not able to show the benthic community impoverishment, namely in species richness and abundance, associated to organic enrichment due to oyster culture. Therefore, special attention must be paid when using biotic indices in areas where the organic enrichment results into a macrofauna impoverishment and not into a species replacement.

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1. INTRODUCTION

1.1. Aquaculture

On a worldwide-scale, coastal and transitional waters have been affected by anthropogenic activities (Borja and Dauer, 2008). As consequence, human-induced alterations in natural ecosystems are profound in coastal areas and an essential subject of environmental management is developing policies to balance socio-economic growth and environmental protection. Some of these activities are fisheries, aquaculture, industries and dredging (Aubry and Elliott, 2006).

According to FAO (2006), in response to the overexploitation of natural stocks of living marine resources, aquaculture is a growing and diversified activity which is developed all over the world on coastal zones. Nowadays, it is the fastest growing food-production sector, accounting for nearly 50 percent of the world's food fish (FAO, 2006). This rapid expansion world-wide has been accompanied by increased interest in its environmental effects, concerning fish farming (Hargrave et al., 1997; Mazzola et al., 2000; Kalantzi and Karakassis, 2006; Yucel-Gier et al., 2007, 2008; Papageorgiou et al., 2010), mussel farming (De Casabianca et al., 1997; Danovaro et al., 2004; Callier et al., 2008; Mahmoudi et al., 2008; Dumbauld et al., 2009) and oyster farming (De Casabianca et al., 1997; Dubois et al., 2007; Dumbauld et al., 2009; Forrest et al., 2009).

In the context of oyster production, some studies indicate that this activity may trigger negative ecological impacts, essentially due the production of faecal particles rejected by the oysters (Kaiser et al., 1998; Carvalho et al., 2006). As consequence, the fluxes of organic matter toward the sediments are enhanced, occurring the deposit beneath the cultures (biodeposits). This process is easily noticed when the culture is made in intertidal areas, off-bottom, and the oysters are placed in mesh plastic bags above metal trestles (Kervella et al., 2010).

Biodeposits have several effects on the water column (alteration of the physical and chemical characteristics) (Dame et al., 1989), and the seabed, including increasing of fine particles (Crawford et al., 2003), organic matter content of the sediments (Grant et al., 1995; Chamberlain et al., 2001; Carvalho et al., 2006) and modifications of physical and geochemical properties of the sediment. All of these may affect benthic communities (Forrest and Creese, 2006).

1.2. Oyster Culture

Oyster farming consists in a specific type of aquaculture practice, which involves the production and/or growth of oysters. Several species are cultured around the world, including *Crassostrea gigas*, *Crassostrea virginica*, *Ostrea edulis*, among others.

Oysters naturally grow in estuarine ecosystems. When farmed, several cultivation methods can be used, each showing different impacts (Forrest et al., 2009). One of these methods consists in the distribution of oysters' seeds over intertidal beds (Kervella et al., 2010). In this case, the maturation occurs naturally and the oysters are usually harvested by dredging. Other method includes the disposal of seeds in structures such as racks, bags, cages or vertical ropes held above the bottom. In subtidal cultures, harvesting may include the lifting of the bags or racks to the surface and removal of mature oysters, contrasting with the intertidal culture, where the larger oysters are retrieved when the structures are exposed during low tide. Other technique involves the suspension of oysters in stakes (usually PVC pipes). Seeds can also be placed in artificial maturation tanks, being this technique less susceptible to predators, but most expensive to build and operate. The oysters are fed with water previously prepared for accelerating the growth rate.

The majority of studies related to the benthic impacts of oyster culture are from subtidal cultures (Crawford et al., 2003). However, there are some studies about the benthic impacts of intertidal oyster culture (Sornin et al., 1986; De Grave et al., 1998; Forrest and Creese, 2006; Bouchet and Sauriau, 2008; Macleod et al., 2009), showing an enhancement of sedimentation beneath cultures (Forrest and Creese, 2006; Mallet et al., 2006), higher organic content and a species composition and dominance patterns consistent with a organic enrichment gradient (Forrest and Creese, 2006).

1.3. Organic Enrichment

One of the most common benthic impacts of intertidal oyster culture is sediment organic enrichment. In consequence, the sediment areas with low oxygen concentrations become more widespread, inducing structural changes in the benthos (Rosenberg et al., 2004). Successional alterations in soft-bottom benthic communities in response to organic enrichment have been modelled by Pearson and Rosenberg (1978), showing the relationship between faunal distributions and species-abundance-biomass (SAB) curves along an organic enrichment gradient (Figure 1). As perturbation increases, more sensitive species are replaced by more

tolerant ones. In this context, the occurrence of certain groups of species constitutes an important indicator of marine pollution, in particular associated to organic enrichment (Pearson and Rosenberg, 1978).

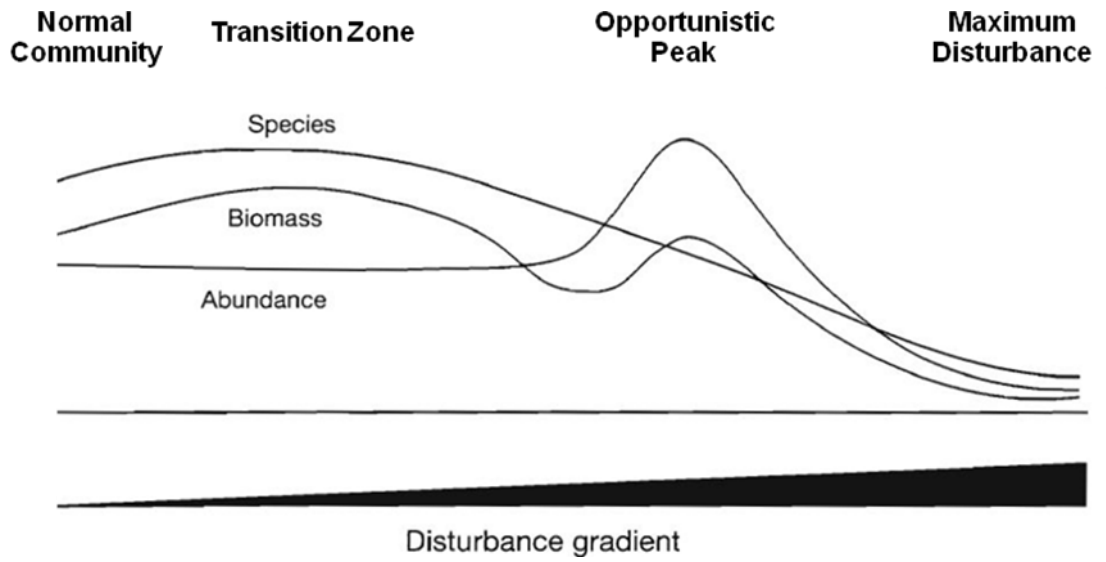

Figure 1: Benthic successional stages along gradient of increasing environmental disturbance (adapted from Nilsson and Rosenberg, 2000)

Therefore, according to the model announced, the macrobenthic community undergoes successive stages from normal community structure with diverse fauna, through transitional community composition with increase in opportunistic species, a peak in arrival of opportunistic species to ultimately an azoic sediment void of macrobenthos (maximum disturbance).

The importance of organic enrichment as an ecological process in marine sediments is connected with the fine fractions of the sediment, which will accumulate organic matter, reduced hydrodynamics and low dissolved oxygen. These environments are characterized by the dominance of deposit feeding organisms using both particulate organic matter and the bacterial biomass associated.

In addition, the Pearson-Rosenberg model has been recently used as the basis of several approaches and indices, applied for the detection and understanding of anthropogenic stress effects, that become from diverse forms of organic enrichment. Allowing to identify the features of anthropogenically-stressed benthic infaunal communities, they are characterized by small organisms, r-strategists and the replacement of k-strategists, high abundances of a restricted number of species, low diversity and low individual biomass organisms, but capable to produce high biomasses (Elliott and Quintino, 2007).

1.4. Biotic Indices

Besides organic enrichment, other activities contribute to increasing pressures in marine waters, which lead many countries to elaborate and implement new legislation to protect these ecosystems. In European Union, the Water Framework Directive (WFD; 2000/60/EC) established measures to protect and improve the transitional and coastal waters, aiming the accomplishment of a good ecological quality status (Muxika et al., 2005; Chainho et al., 2007). To achieve this purpose, the development of new biological indices to distinguish levels of ecological quality and classify coastal areas, have been improved (Salas et al., 2004), namely having their ecological basis on Pearson and Rosenberg's paradigm (Borja and Dauer, 2008). These tools are quite useful in terms of simplifying the complexity of scientific data, such as ecological quality and respective status utilizing several types of holistic information and originating results that form essential basis for management analysis (Quintino et al., 2006; Chainho et al., 2007; Borja et al., 2008; Borja and Tunberg, 2011).

The application and validation of an index should always be achieved, regardless of the geographical area, with the purpose to constitute a useful ecological indicator (Salas et al., 2004). Among the possible limitations concerning spatial application, different biogeographic provinces, differences between intertidal and subtidal habitats and differences between hard and soft bottom, are generally considered (Borja and Dauer, 2008).

Benthic assessment indices can be univariate, such as the Shannon-Wiener diversity (H') (Shannon and Weaver, 1949), Margalef richness (d) (Margalef, 1968), Simpson (1-λ') (Simpson, 1949), Pielou evenness (J') (Pielou, 1966); multimetric, resulting from the combination of several univariate, for instance AMBI (Borja et al., 2000); and multivariate, e.g. M-AMBI (Muxika et al., 2007).

AZTI marine biotic indices (AMBI and M-AMBI) developed by the Marine and Food Technological Centre, can be calculated with open access software and they are recently utilized to assess the response of soft-benthic communities to disturbances in coastal (Rosenberg et al., 2004; Muxika et al., 2005; Quintino et al., 2006; Borja et al., 2011) and transitional waters worldwide (Salas et al., 2004; Quintino et al., 2006; Chainho et al., 2007; Borja et al., 2008; Bouchet and Sauriau, 2008; Pinto et al., 2009; Borja et al., 2011; Borja and Tunberg, 2011). AMBI was developed by Borja et al. (2000) with the primary purpose to assess the ecological quality of European waters and M-AMBI, which combines different metric such as AMBI, richness and diversity. The basis of this AMBI is the determination of the proportion of species assigned to five ecological groups, formed according to the

sensitivity or tolerance related to disturbance conditions, from very sensitive to opportunistic species (Borja et al., 2000).

Numerous references in the literature are made to the advantages of benthic community to assess anthropogenic impact, because they allow to integrate impacts over an extensive temporal scale (Muxika et al., 2005); their relatively fast responses to anthropic and natural stress (multiple types of stress, e.g. organic enrichment) (Pearson and Rosenberg, 1978); due to their essential role on sediment processes, predador-prey interactions and capability of building structures in the sediment (bioengineers), usually being characterized by well-defined responses to environmental modifications. Moreover, Dauer (1993) justifies the studies using macrofaunal communities, because they are relatively sedentary animals which makes them incapable of avoiding deteriorating water/sediment quality conditions; have long life-spans, present integrated temporal evolution of quality conditions; are composed by distinct species in terms of tolerance to stress; and have a fundamental role in nutrients and materials cycles, established between sediment and water compartments.

1.5. Objectives

The purpose of this study was to assess if an intertidal off-bottom oyster farm causes detectable organic enrichment effects upon the benthic community. For this, sediment (organic matter content and grain size) and biological descriptors (structure and composition of the macrofauna community) were analysed. In particular, it was of interest to compare the response of synthetic biotic indices (univariate, multimetric and multivariate), to the full, multivariate, species abundance data set.

2. MATERIALS AND METHODS

2.1. Study Area

The present study was undertaken at an intertidal oyster culture located in Ria de Aveiro, Northwestern Portugal (Figure 2).

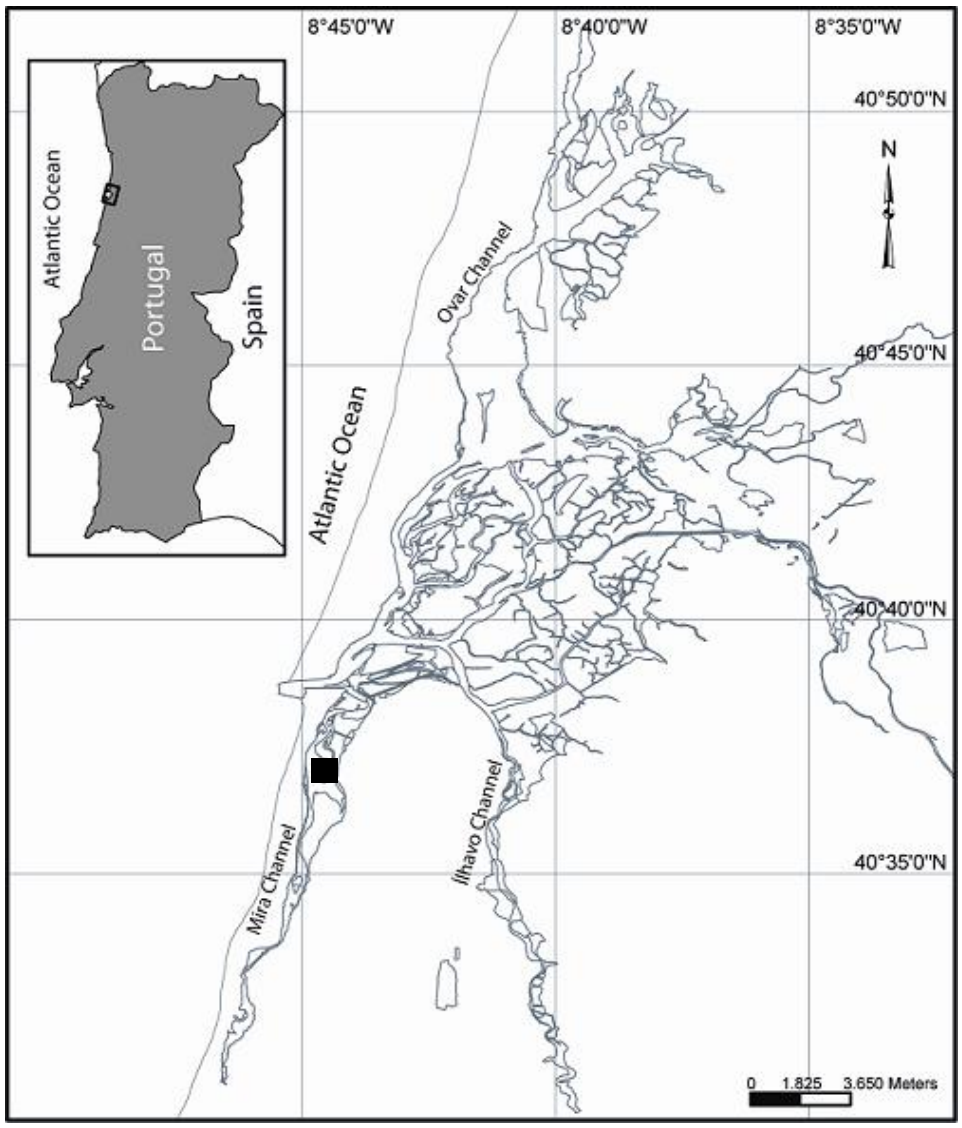

Figure 2: Study area: Ria de Aveiro and location of the oyster culture.

Ria de Aveiro consists of a large number of narrow channels which are characterized by significant intertidal areas, mainly mudflats and salt marshes (Abrantes et al., 1999; Dias et al., 2000; Picado et al., 2010). The bathymetry shows that this is a very shallow lagoon, with an average depth of 1 m (Lopes and Dias, 2007). The tides, semi-diurnal, are responsible for the main forcing of circulation within the lagoon (Lopes and Dias, 2007; Vaz et al., 2007), with a mean tidal range of about 2 m, being the minimum and maximum tidal ranges of 0.6 m

(neap tide) and 3.2 m (spring tide), respectively (Cerejo and Dias, 2007). The area covered by water is 83 km^2 at high tide, contrasting with 66 km^2 at low tide, considering the spring tide (Dias et al., 2001).The most important channels are S. Jacinto, Ovar, Espinheiro, Mira and Ílhavo, which are connected to the Atlantic Ocean by a single tidal channel (Da Silva et al., 2009; Lopes et al., 2010; Plecha et al., 2010). The Mira channel, where the oyster farm presently studied is located, has a length of 20 km and it is characterized by salinities ranged from fully marine to freshwater, at the mouth and head, respectively (Dias et al., 2001; Quintino et al., 2009). In terms of biology, Ria de Aveiro is documented as a very productive environment, providing adequate conditions for the life cycle of several species and an appropriate habitat for commercially important fish and invertebrate species, bringing a special interest for the development of anthropogenic activities, involving resources exploitation (Cerejo and Dias, 2007; Oliveira et al., 2010). The benthic community is formed by bacteria, seaweeds, aquatic plants and fauna. Bivalves are the most important explored resource of the lagoon, including the production on natural banks and anthropologically modified areas leased for the exploitation of cockle, mussel and oyster, having an important economic relevance for the populations surrounding this ecosystem.

This work was performed in an oyster farm with a total area of 8000 m^2 (Figure 3).The production process is initiated with the purchase of very small oysters (*Crassostrea gigas*) with approximately 4 cm from the French market. These oysters are placed inside bags (1 m x 0.50 m) with 4 mm mesh (Figure 4a) during approximately eight months, on top of trestles 0.45 – 0.50 m above the bottom. Following this period, oysters with 6-7 cm long are sorted, which are then transferred to bags with 13 mm mesh (Figure 4b). Oysters with less than 6-7 cm, are placed in the first bags again. After 7-8 months, a second selection is made and oysters with 8-10 cm are marketed (Ribau, Personal Communication).

Figure 3: Overview of the oyster farm studied.

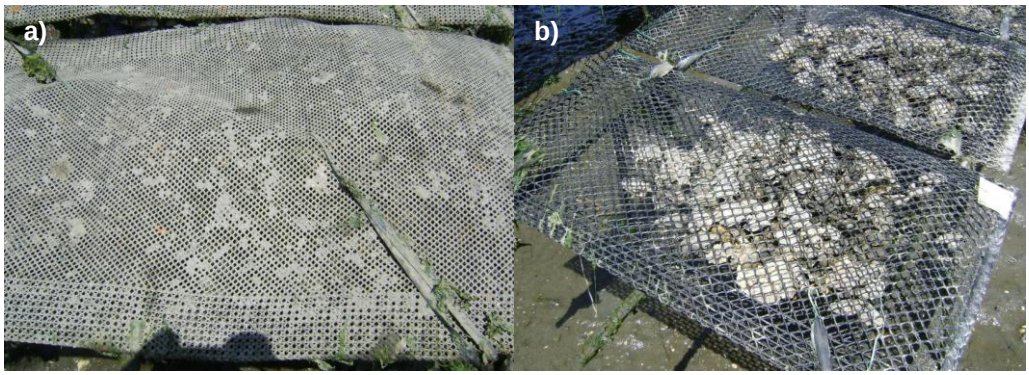

Figure 4: Oyster bags - a) 4 mm mesh; b) 13 mm mesh.

2.2. Sampling

The sampling procedure included the definition of 8 areas (Figure 5), which were named from A to H, taking into account different environmental conditions and culture practices: areas without trestles nor oysters (A, B2, D, G and H), areas with trestles only (E and F) and areas with oysters and so with trestles (B1 and C). Comparing oysters' size, area B1 has smaller specimens than area C. Area B was divided in two subareas: area B1 (under the trestles) and B2 (between trestles) (cf. Figure 5).

Figure 5: Sampling areas. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

In each area, 4 sites were chosen randomly, and at each site, 5 sediment replicates were collected with a handheld corer of 0.01 m^2 unit area (Ø ≈ 11 cm, Figure 6a). Three sediment replicates per site were used for the study of the benthic macrofauna, and two for sediment descriptors: grain size and organic matter content.

The samples for the study of the benthic macrofauna were washed in the field over a 1mm-mesh sieve and the material retained was preserved in 4% formalin in plastic recipients.

The sediment samples for the study of organic matter content were placed in plastic recipients (Figure 6b) and frozen after collection. For the study of grain size, samples were stored at room temperature in 0.5 L plastic recipients (Figure 6c).

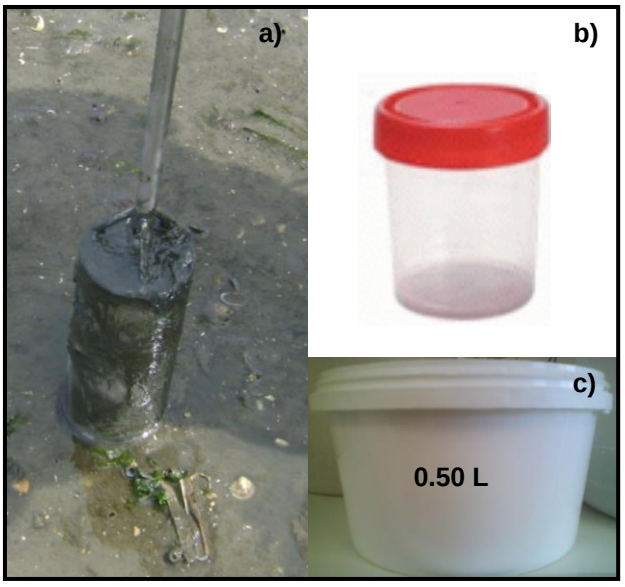

Figure 6: Sampling collectors for – a) Macrofauna; b) organic matter content; c) grain-size.

2.3. Laboratory Analysis

2.3.1. Sediment

The sediment samples collected for the study of grain size were analysed by wet and dry sieving, following the procedure described in Quintino et al. (1989). Initially, the samples were submitted to the chemical destruction of organic matter with H_2O_2, washed and weighted. Following the measurement of total sediment dry weight (P1), tetra-sodium pyrophosphate (30 g/L) is added in order to disperse the sediment. Wet sieving through a 63 µm mesh sieve is performed. With material that remains in the sieve, a second dry weight is measured (P2). The difference between P1 and P2 gives the weight of the fraction < 63 µm

(fines content). The final step involves the dry sieving of the remaining sediment through mesh sieves ranging from 125 µm to 4 mm at 1 ϕ (phi) intervals (ϕ = -$\log_2$ the particle diameter in mm) and the material in each sieve is weighted and expressed as a parameter of P1.

Organic matter content was obtained by weight loss of 1g of dry sediment sample on ignition at 450 ºC during five hours (Byers et al., 1978).

2.3.2. Macrofauna

The samples for the study of macrofauna were stained with rose Bengal. After 1 day, they were washed over a 500 µm mesh sieve, sorted by major taxonomic groups (Annelids, Crustaceans, Molluscs and other) and preserved in 70% ethanol. The identification of macrofauna was made to the lowest possible taxonomic level using a dissecting microscope and an optical microscope, when needed. Following this procedure, the number of specimens of each species was counted, for each replicate. The biomass of the organisms was measured as wet weight after blotting with absorbent paper, for every species, per replicate, with an analytical balance (precision of ± 0.0001 g).

2.4. Data Analysis

2.4.1. Sediment descriptors

The sediment grain-size fractions (>2mm; 1-2; 0.5-1; 0.25-0.5; 0.125-0.25; 0.063-0.125; <0.063mm) were expressed as a percentage of the whole sediment, dry weight. These data were used to calculate the median value, P_{50}, which corresponds to the diameter that has half the grains finer and half coarser (dry weight) and is expressed in phi (ϕ) units. No detailed grain size analysis was performed for the fines fraction (i.e. particles with diameter below 63 µm) and, consequently, for the samples with more than 50% fines content, the median values could not be calculated, being generally classified as mud. The sediments with less than 50% fines content were classified as sands, using the median, and according the Wentworth scale (Doeglas, 1968): gravel [(-2) – (-1) ϕ)]; very coarse sand [(-1) – 0]; coarse sand (0 - 1); medium sand (1 - 2); fine sand (2 - 3); very fine sand (median between 3 - 4). The final sand classification adopted the description “clean“, “silty” or “very silty” when the silt and clay fraction ranged from 0% to 5%, from 5% to 25% and from 25% to 50%, respectively, of the total sediment, dry weight (Doeglas, 1968; Larsonneur, 1977) (Table 1).

Table 1: Sediment classification, *in* Rodrigues et al. (2006)

Median (ϕ)	Sediment calssification		Fines content (%)		
			<5	5-25	25-50
(-1)-0		Very coarse			
0-1		Coarse			
1-2	Sand	Medium	Clean	Silty	Very silty
2-3		Fine			
3-4		Very fine			
≥4	Mud		Above 50%		

Besides the grain size fractions referred, median and organic matter content was also used to characterize the study area. The normalized Euclidean distance was used to produce a resemblance matrix and ordination analysis were performed on grain size fractions data using the software PRIMER v6 (Clarke and Gorley, 2006), submitted to non-metric multidimensional scaling (NMDS) ordination analysis. NMDS diagrams are accompanied by a stress value that quantifies the mismatch between the distances among data points in the resemblance matrix and in the ordination diagram. Stress value below 0.10 are considered to represent very accurately the original resemblance matrix (Clarke and Warwick, 2001). The relationship between sediment fines content and total organic matter content was studied.

The sediment descriptors were submitted to hypothesis testing, under the null hypothesis of no significant differences among sampling areas, using permutation multivariate analysis of variance (Anderson, 2001), with the PERMANOVA+ add-on in PRIMER v6 (Anderson et al., 2008). The sediment data were analyzed following a one-way hierarchical design, with areas as fixed factor. The pseudo-F values in the PERMANOVA main tests were evaluated in terms of significance among different areas. When the main test revealed statistical significant differences ($p < 0.05$), the t-statistic was calculated for pairwise comparisons between the areas.

2.4.2. Macrofauna community descriptors

Macrofauna community descriptors include the species composition and abundance per replicate. The benthic community species data (species abundance for each replicate) were square root transformed and a similarity matrix was obtained by applying the Bray-Curtis coefficient. After the distance among centroids was performed considering the areas, these data were analyzed with the average-clustering algorithm classification analysis and non-metric multidimensional scaling (NMDS) ordination analysis. All analyses were performed with the software PRIMER v6 (Clarke and Gorley, 2006).

Similar to sediment descriptors, the macrofauna descriptors (benthic community species abundance) were submitted to hypothesis testing under the null hypothesis of no significant differences among sampling areas, using permutation multivariate analysis of variance (Anderson, 2001), with the PERMANOVA+ add-on in PRIMER v6 (Anderson et al., 2008). The macrofauna data were analyzed according to a two-way hierarchical design, with sites nested in areas and these as the main fixed factor. The pseudo-F values in the PERMANOVA main tests were evaluated in terms of significance among different areas. When the main test revealed statistical significant differences ($p < 0.05$), the t-statistic was calculated for pairwise comparisons between the areas.

For each site, BIOENV routine was used to determinated the best correlation obtained between the environmental variables that explain the distribution of the biological data (Clarke and Gorley, 2006). The sediment data, grain size fractions and organic matter content, were normalized and the biological data were square root transformed.

Synthesis biotic indices were obtained, per replicate, using the DIVERSE routine, from PRIMER v6 (Clarke and Gorley, 2006): Species richness (S); Abundance (A); Shannon diversity (H') (Shannon and Weaver, 1949); Margalef richness (d) (Margalef, 1968); Simpson index (1-λ') (Simpson, 1949). Index W was also determinated with PRIMER v6. The marine biotic index AMBI (Borja et al., 2000) and the Multivariate-AMBI (Muxika et al., 2007) were also calculated.

AMBI is based upon the percentages of abundance of each ecological group (EG), within each sample, to obtain a continuous index (the Biotic Coefficient, BC), where $BC = [(0 \times \%EG_I) + (1.5 \times \%EG_{II}) + (3 \times \% EG_{III}) + (4.5 \times \%EG_{IV}) + (6 \times \%EG_V)] / 100$. EG represents the relative abundance of soft-bottom species classified according to 5 ecological groups, which represent their sensitivity to increasing organic enrichment (Grall and Glémarec, 1997): EG_I comprises the very sensitive species; EG_{II} indifferent species; EG_{III} tolerant species; EG_{IV} second-order opportunistic species; EG_V first-order opportunistic species. M-AMBI is a combination of the proportion of the AMBI index, the species richness (the total number of species, S) and the diversity through the use of the Shannon diversity index (Muxika et al., 2007). M-AMBI is obtained in the interval from 0 to 1, with the associated ecological quality statement generally considering the default boundaries: Poor / Bad = 0.22; Moderate / Poor = 0.39; Good / Moderate = 0.55 and High /Good = 0.85.AMBI and M-AMBI were calculated using the available free software (AMBI v4.1 http://www.azti.es).

All of these data were also analyzed under the same hierarchical design previously described and null hypothesis, using PERMANOVA+ (Anderson et al., 2008).

3. RESULTS

3.1. Sediment descriptors

The sediment descriptors mean values, across the study areas (A-H), are present in Table 2. Regarding the sediment grain-size and the median values, the study areas were classified as fine sand (considering the number of replicated that in majority verify the sediment type presented), with the median value ranging from 2.26 to 3.49, and the percentage of the fines fraction (silt and clay, particles smaller than 63 μm) always higher than 32%. The areas with oysters (areas B1 and C) and the areas D and H were classified as mud (median value higher than 4ϕ; cf. Table 2). The organic matter content presented the highest values in the areas with oyster culture (B1 and C; cf. Table 2).

Table 2: Sediment baseline descriptors per study area.

	Areas								
	A	**B1**	**B2**	**C**	**D**	**E**	**F**	**G**	**H**
Sediment descriptors									
>4mm	-	-	-	-	-	1.61 ± 3.22	0.03 ± 0.07	-	-
2mm	0.03 ± 0.02	-	0.07 ± 0.06	-	-	0.09 ± 0.07	0.14 ± 0.27	0.01 ± 0.01	0.002 ± 0.005
1.000	0.37 ± 0.13	0.33 ± 0.14	0.59 ± 0.42	0.10 ± 0.04	0.13 ± 0.07	0.73 ± 0.13	0.67 ± 0.91	0.15 ± 0.11	0.17 ± 0.10
0.500	4.35 ± 0.51	2.66 ± 1.21	4.11 ± 2.11	0.57 ± 0.09	0.62 ± 0.15	6.13 ± 0.56	3.92 ± 2.97	1.82 ± 0.95	1.34 ± 1.27
0.250	36.35 ± 6.25	17.72 ± 6.59	28.20 ± 9.15	6.31 ± 2.76	7.62 ± 0.86	37.00 ± 2.97	28.21 ± 6.34	23.44 7.85	12.21 ± 7.34
0.125	17.70 ± 1.91	12.17 ± 4.11	18.17 ± 3.93	9.98 ± 4.86	13.28 ± 1.23	17.35 ± 0.99	17.77 ± 3.84	18.94 ± 2.06	18.62 ± 1.21
0.063	4.90 ± 1.01	7.29 ± 1.42	7.50 ± 2.11	11.97 ± 1.32	18.14 ± 1.47	4.19 ± 0.28	5.76 ± 1.35	7.69 ± 1.93	15.37 ± 4.96
<0,063	36.25 ± 6.69	59.91 ± 10.79	41.19 ± 12.68	71.08 ± 8.68	60.20 ± 1.48	32.78 ± 3.37	43.27 ± 8.54	47.87 ± 9.89	52.25 ± 5.47
Median	2.52 ± 0.41	>4	2.96 ± 0.76	>4	>4	2.26 ± 0.19	3.17 ± 0.84	3.49 ± 0.64	>4
Sediment type	Very silty, fine sand (4/4)*	Mud (4/4)*	Very silty, fine sand (3/4)*	Mud (4/4)*	Mud (4/4)*	Very silty, fine sand (4/4)*	Very silty, fine sand (2/4)*	Mud (2/4)*	Mud (3/4)*
TOM (%)	2.7 ± 0.57	4.9 ± 1.71	3.4 ± 1.38	6.8 ± 0.75	4.7 ± 0.29	3.0 ± 0.54	3.7 ± 0.56	2.9 ± 0.70	4.0 ± 0.14

TOM – Total Organic Matter; Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

*number of replicates that verify the sediment type presented

The results obtained from the sediment grain-size ordination diagram revealed no clear separation of the areas with oysters (B1 and C) from the remaining ones (Figure 7). The areas are interconnected and distributed from the shore to the channel (from left to right on the figure). Areas B1 and C (areas with oysters) are not disposed in clearly separated groups comparing with the remaining areas.

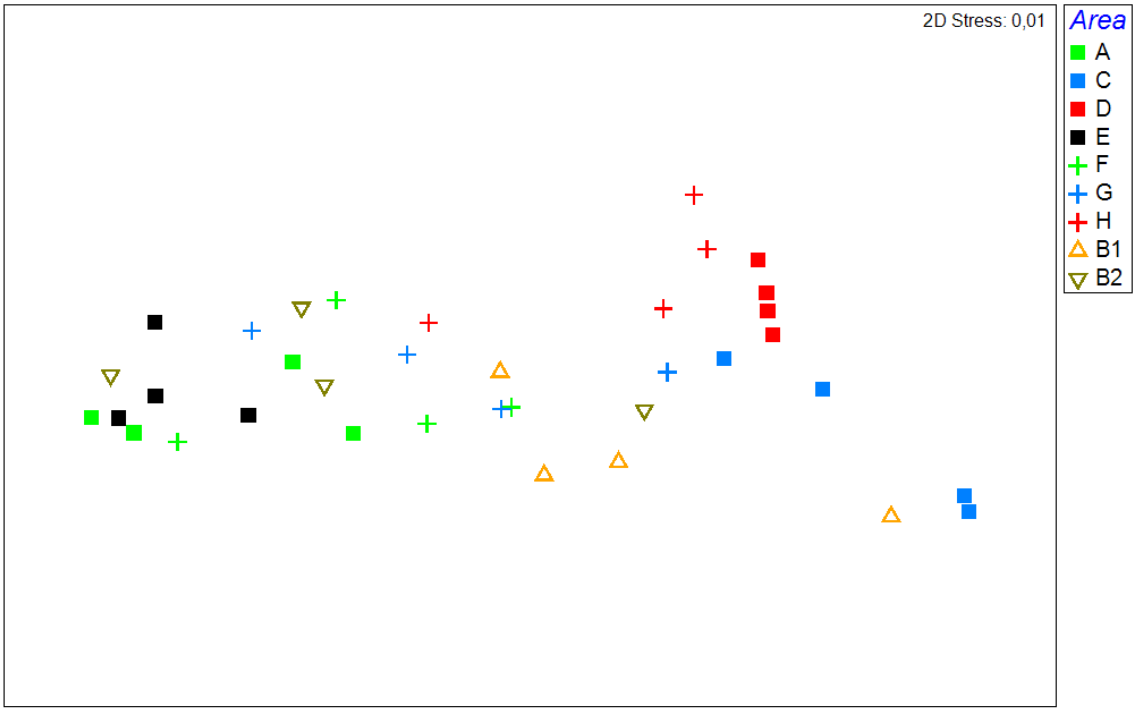

Figure 7: Grain-size showing NMDS ordination over normalized data. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

The distribution of the fines content along the study areas is present in Figure 8. Figure 8a) shows a clearly increasing of the fines content in the areas with oysters (B1 and C). Between the trestles of area B (B2) it was shown a higher fines content comparing with area A, but less than the samples collected under the trestles of area B (B1). The area with larger oysters (area C) presented the higher fines content comparing with the remaining ones. On Figure 8b), the increasing of fines content is also visible from shore to the channel, but it is less abrupt.

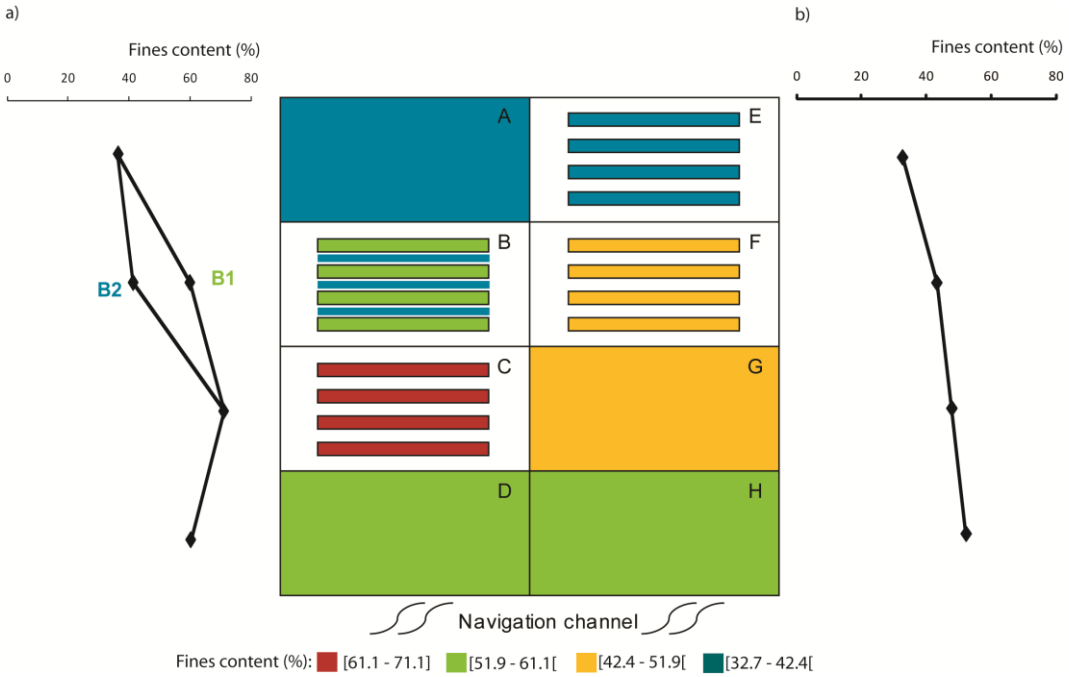

Figure 8: Study areas with fines content characterization: a) areas A-D; b) areas E-H. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

Figure 9 shows that the results obtained for the median characterization. In Figure 9a), an abrupt increase in the areas with oysters and also in areas closer to the navigation channel is presented. Although in Figure 9b) it is also observed an increment of median values, similar to the distribution of fines content, this increase is more gradual than the one reached in Figure 9a).

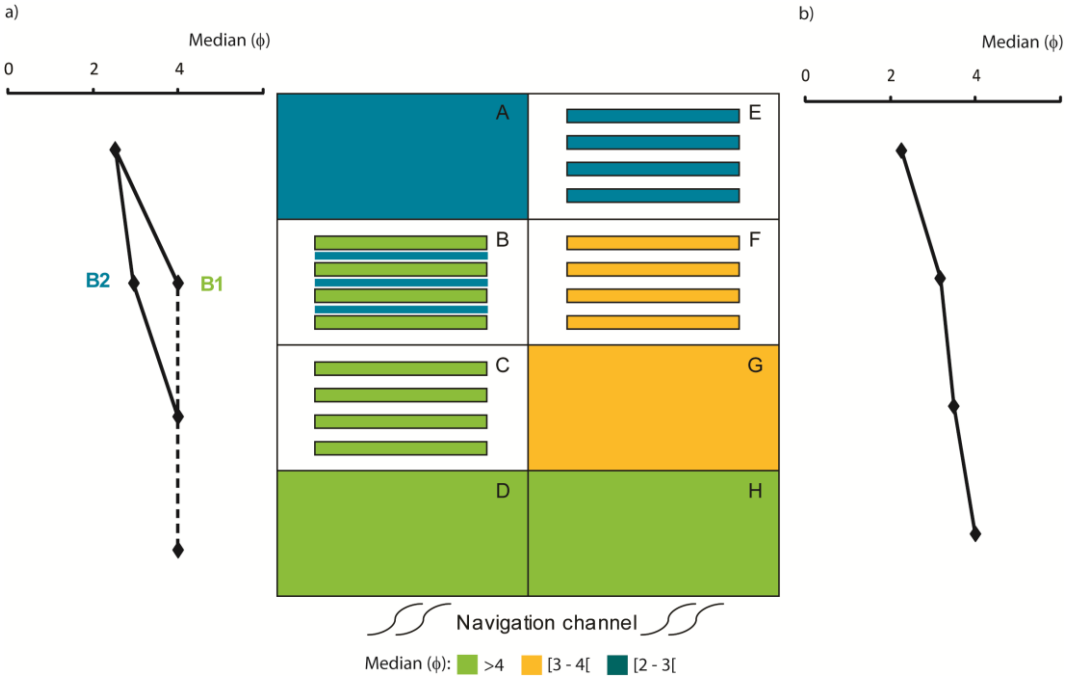

Figure 9: Study areas with median characterization: a) areas A-D; b) areas E-H. The median values higher than 4ϕ are represented with the dashed line. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

Figure 10a) shows the distribution of organic matter content. Similar to the results obtained for fines content and median distribution, the areas with oysters presented higher values of organic matter content. The area with larger oysters showed the highest organic matter contents, comparing with the remaining areas. On the Figure 10b), it is presented a slightly decreasing of organic matter values in area G.

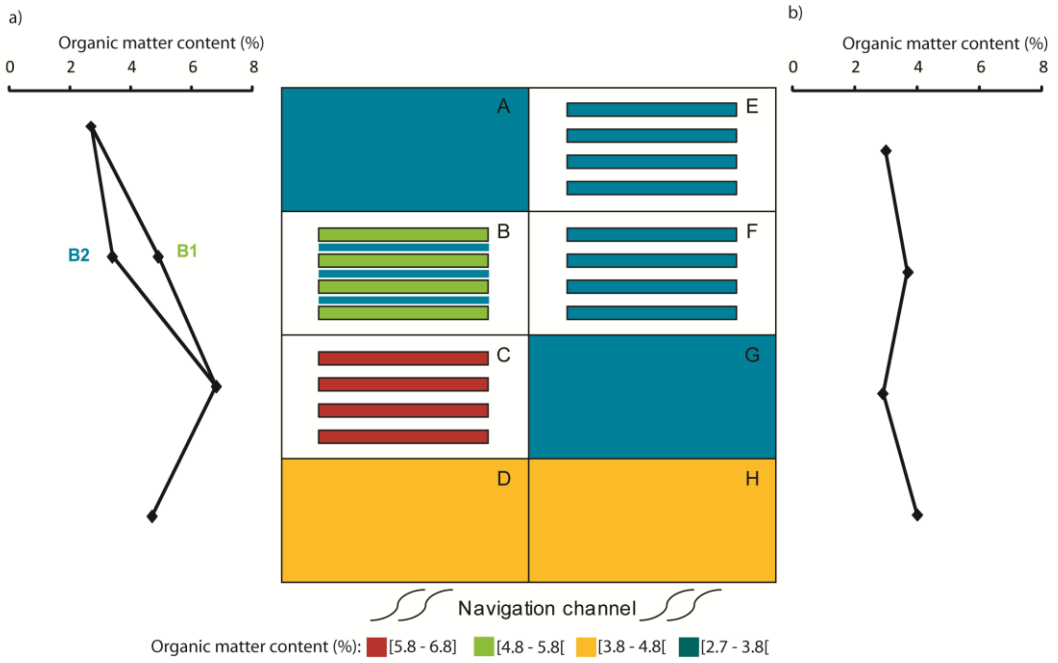

Figure 10: Study areas with organic matter content characterization: a) areas A-D; b) areas E-H. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

Permutation multivariate analysis of variance revealed that the sedimentary descriptors (grain size, median and organic matter content) rejected the null hypothesis of no significant differences among the study areas (Table 3).

Table 3: PERMANOVA main-test results for the sediment descriptors.

Test	df	SS	MS	Pseudo-F	p
Grain size					
Areas	8	10591	1323.80	10.765	0.0001**
Residuals	27	3320	122.98		
Total	35	13911			
Fines content					
Areas	8	5048.1	631.01	9.2867	0.0001**
Residuals	27	1834.6	67.948		
Total	35	6882.7			
Median					
Areas	8	14.456	1.81	8.233	0.0002**
Residuals	27	5.926	0.22		
Total	35	20.381			
Organic Matter Content					
Areas	8	52.94	6.6175	8.5919	0.0001**
Residuals	27	20.795	0.7702		
Total	35	73.735			

df – dregrees of freedom; SS – sums of squares; MS – mean square; p – significance level (significance values: ** $p<0.01$).

The relationship between the total organic matter and fines content (Figure 11a) showed that the area with larger oysters (area C) is characterized by higher fines content which was accompanied by higher values of organic matter. For the same relationship, considering the mean values of the study areas (Figure 11b), it is clearly visible that to a low fines content corresponds a low organic matter content and that higher fines content is associated with a visible increment of organic matter content.

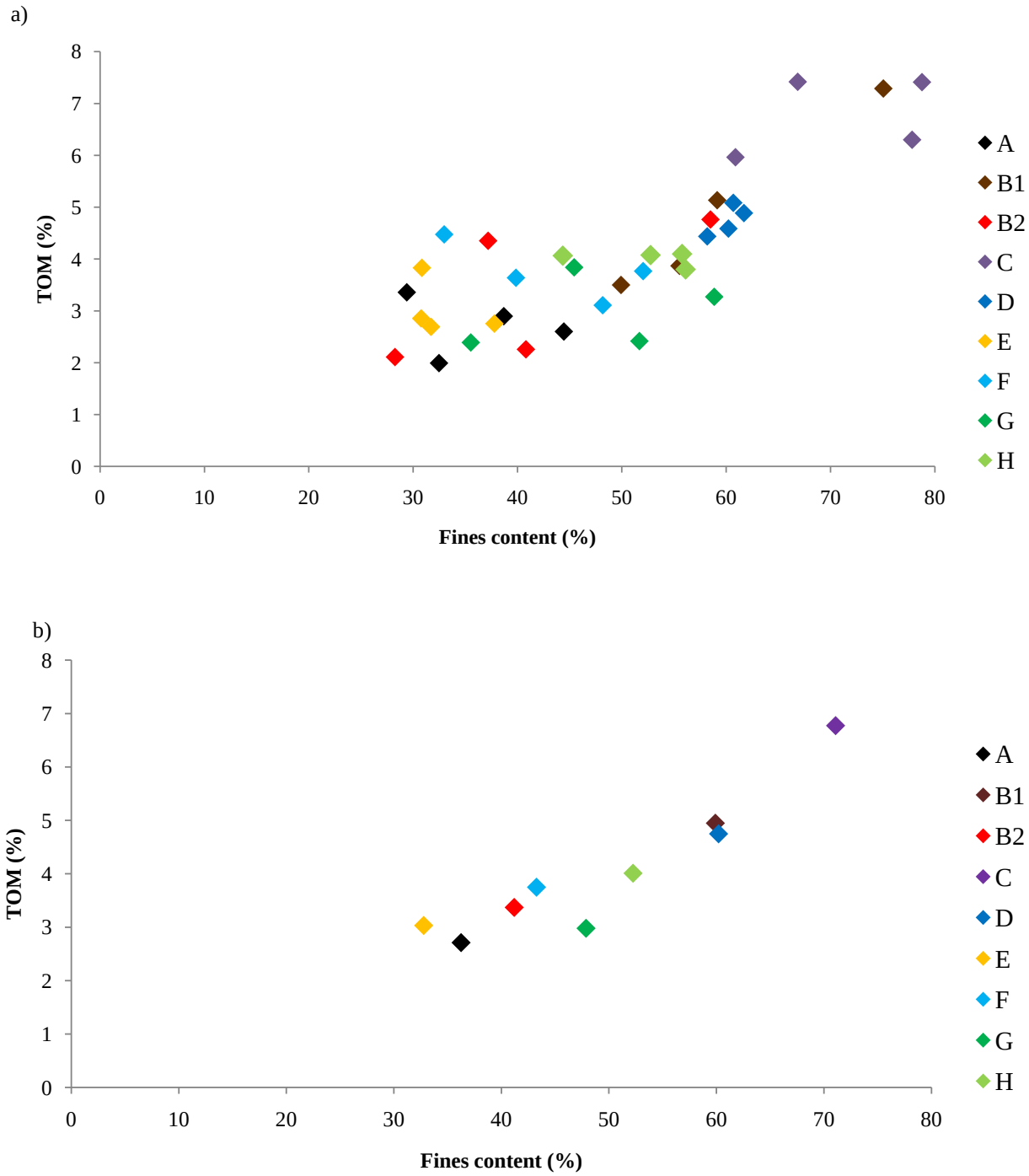

Figure 11: Relationship between total organic matter and fines content: a) distribution per sites within the areas; b) distribution per area, considering the mean values. Areas without trestles nor oysters - A, D, G and H; Area with large oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

The join analysis of grain-size and total organic matter results shows that there is a site of area B1 (subarea B with oysters), integrated in the distribution of the sites of area C (Figure 12). These results also show that, within area B, the subareas B1 and B2, were not distributed in clearly separated groups.

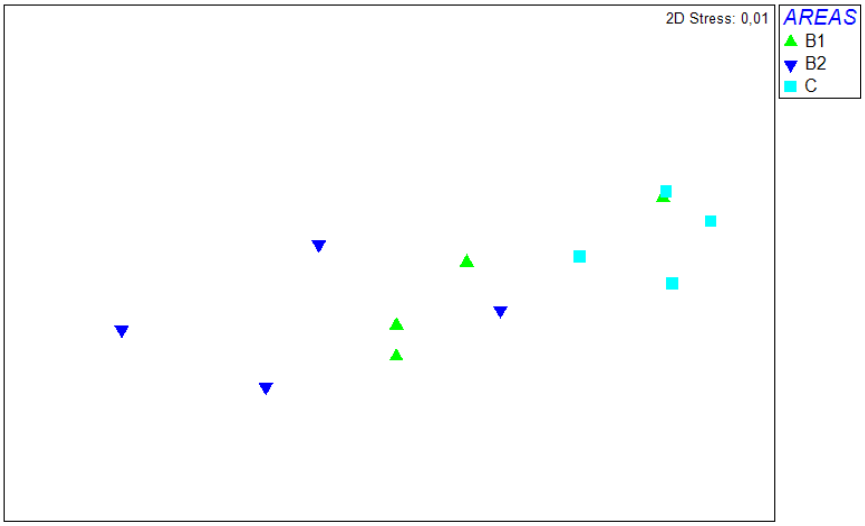

Figure 12: NMDS ordination of grain-size normalized data and total organic matter content of areas B and C. Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters. C - Area with larger oysters.

The pairwise comparisons between area B and C for sediment descriptors are summarized in Table 4. Within area B, no significant differences were observed between the areas under the trestles with oysters (B1) and the corridors between them (B2). Comparing the study areas characterized by the oyster culture (B1 and C), also no significant differences were obtained. However, the area between the corridors of the area B (B2) and the area under the oyster culture of area C, high significant differences were found. These results are consistent with those obtained in ordination analysis (cf. Figure 12).

Table 4: Values for the t-statistic and associated significance in the pairwise comparisons between area B and C, for sediment descriptors.

Areas	Organic matter content	Grain size	Fine content
B1 vs B2	1.4349(ns)	2.1061(ns)	2.2484(ns)
B1 vs C	1.9531(ns)	1.9919(ns)	1.6144(ns)
B2 vs C	4.3246**	3.9506**	3.8904**

Significance values: ns – non significant; ** p<0.01; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters; C – Area with larger oysters.

3.2. Macrofauna community descriptors

Considering the study areas, a total of 65 taxa were found with an abundance of 3268 specimens. Species richness and abundance of the major taxonomic groups is presented in Table 5.

Table 5: Species Richness, Abundance and Biomass of the major taxonomic groups.

	S	**%**	**A**	**%**	**B**	**%**
ANNELIDA	32	49.2	2759	84.4	24,7266	17,5
CRUSTACEA	16	24.6	322	9.9	0,9629	0,7
MOLLUSCA	12	18.5	104	3.2	114,9133	81,4
OTHER	5	7.7	83	2.5	0,6462	0,5
TOTAL	65	100	3268	100	141,2490	100

Annelids are the most representative group regarding species richness (49.2%) and abundance (84.4%), followed by Crustacea and Mollusca. In all areas, annelids contribute the most for both species richness (Table 6) and abundance (Table 7). Higher species richness values were found in area B2 (area between trestles; Table 6) and the lowest values occurred in area B1. Table 7 shows that areas E and F (areas with trestles but without oyster culture) present higher total abundance values, when comparing with other areas, contrasting with the areas B1 and C (areas with oysters), which present the lowest abundance.

Table 6: Species richness per taxonomic group along the study areas (0.01 m^2, n=12).

	A	**B1**	**B2**	**C**	**D**	**E**	**F**	**G**	**H**
ANNELIDA	12	15	22	18	17	17	15	17	17
CRUSTACEA	9	4	8	7	9	6	5	4	8
MOLLUSCA	7	3	5	4	4	6	4	5	5
OTHER	2	1	2	2	2	4	2	2	3
TOTAL	30	23	37	31	32	33	26	28	33

Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

Table 7: Abundance per taxonomic group along the study areas (0.01 m^2, n=12).

	A	**B1**	**B2**	**C**	**D**	**E**	**F**	**G**	**H**
ANNELIDA	258	129	340	78	168	350	867	339	230
CRUSTACEA	42	12	17	43	34	33	62	32	47
MOLLUSCA	23	4	15	5	12	15	8	13	9
OTHER	4	2	7	2	30	4	9	8	17
TOTAL	327	147	379	128	244	402	946	392	303

Areas without trestles nor oysters - A, D, G and H; Area with large oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

The biomass values ranged from 0.7227 to 34.3482 g. Table 8 shows that the highest value of biomass were found in area A. Areas with oysters, B1 and C, and area G present the lowest values of biomass.

Table 8: Biomass per taxonomic group along the study areas (0.01 m^2, n=12).

	A	**B1**	**B2**	**C**	**D**	**E**	**F**	**G**	**H**
ANNELIDA	1.6298	0.7181	4.1534	0.5233	2.7276	2.0951	4.9629	0.3716	7.5447
CRUSTACEA	0.0732	0.3579	0.0177	0.0590	0.0747	0.0638	0.1468	0.0592	0.1106
MOLLUSCA	32.6424	4.7248	16.9623	0.0406	12.9239	10.1112	18.8797	0.5938	18.0346
OTHER	0.0028	0.1833	0.0005	0.0998	0.2023	0.1068	0.0033	0.0031	0.0444
TOTAL	34.3482	5.9841	21.1338	0.7227	15.9286	12.3769	23.9927	1.0277	25.7343

Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

Figure 13a) shows that the areas with oysters (B1 and C) presented a lower species richness mean, contrasting with the remaining areas. On Figure 13b) the species richness increases from the shore to the channel, with a slightly reduction in area G.

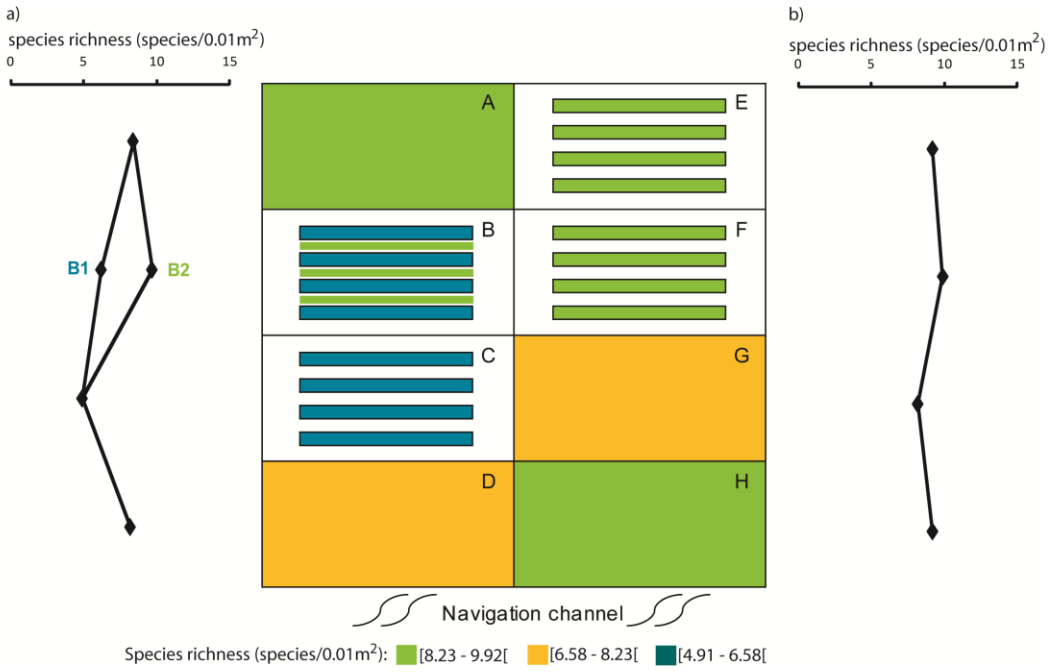

Figure 13: Species richness mean along study areas (0.01 m^2, n=12): a) areas A-D; b) areas E-H. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

Similar to species richness mean values along study areas, Figure 14 shows that areas B1 and C (areas with trestles and oysters) present low abundance mean values (Figure 14a)). On the other side, exceptionally high values were found in area F (area with trestles only; Figure 14b)).

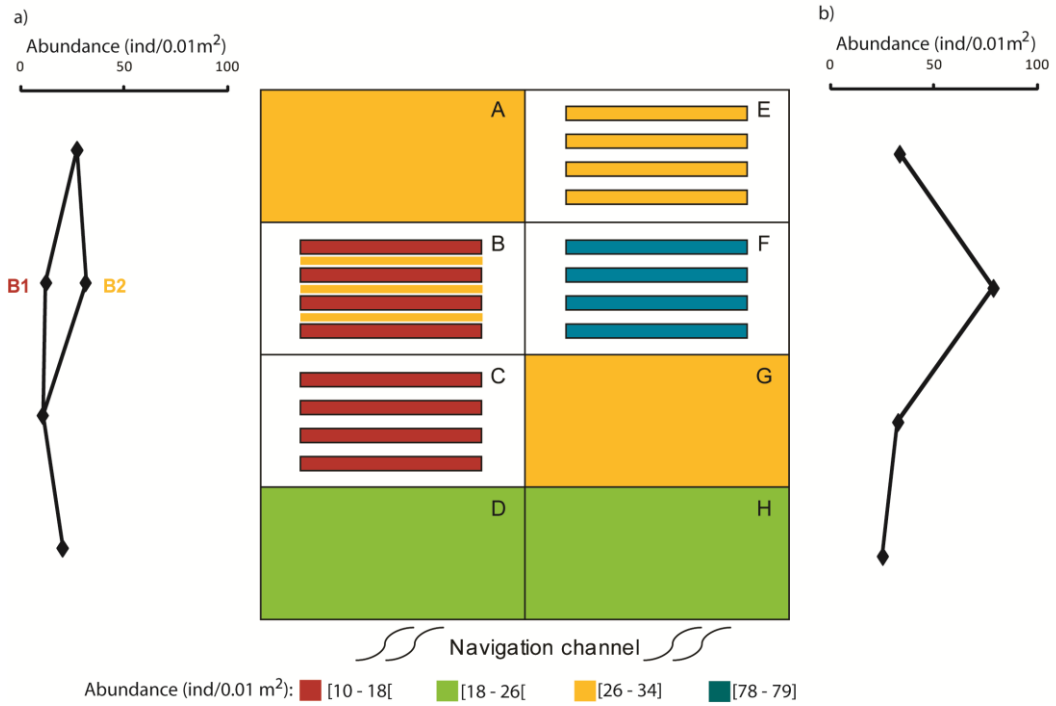

Figure 14: Abundance mean along study areas (0.01 m^2, n=12): a) areas A-D; b) areas E-H. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

The results of biomass illustrated in Figure 15a) show an accentuated reduction on areas with oysters (B1 and C). This variable has a huge increasing in area F followed by a decreasing in area G and a new increment in area H Figure 15b).

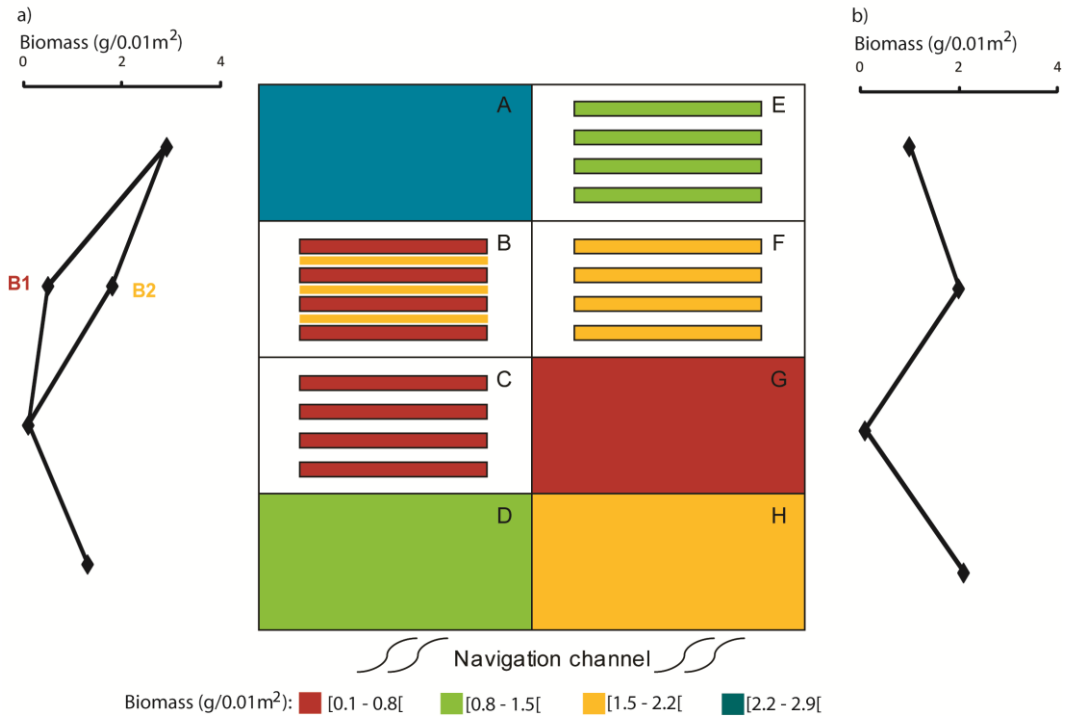

Figure 15: Biomass mean along the study areas (0.01 m^2, n=12): a) areas A-D; b) areas E-H. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

The ordination diagram obtained for the species abundance transformed data is present in Figure 16. The distribution of the sites within the area C (area with larger oysters) shows that they are less similar between them, comparing with the remaining sites within the other areas. Even so, the distribution of the sites within the areas is random.

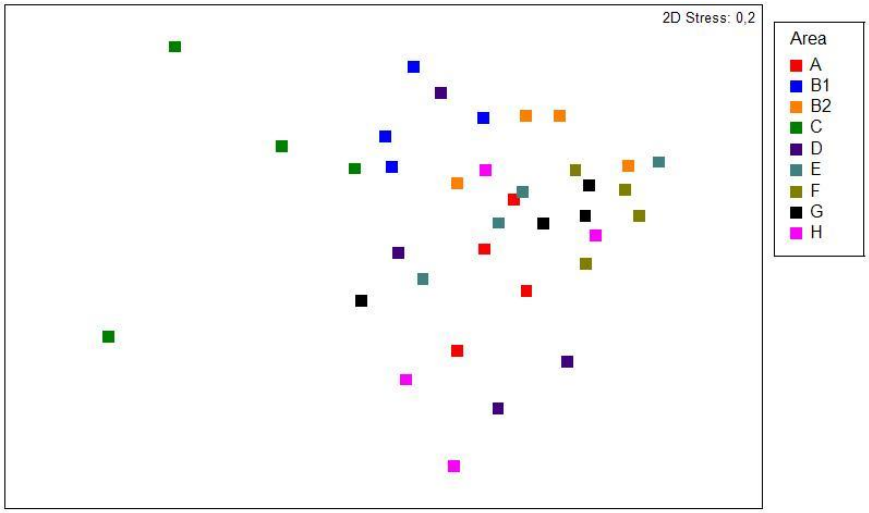

Figure 16: NMDS ordination over species abundance transformed data, using distance among centroids, per site. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

The classification and ordination diagrams obtained, over the transformed species abundance data set, using the distance among centroids, are presented in Figure 17. These diagrams illustrate the separation of areas B1 and C from the remaining areas, highlighting the difference between the areas with oyster culture and the areas where no oysters are present.

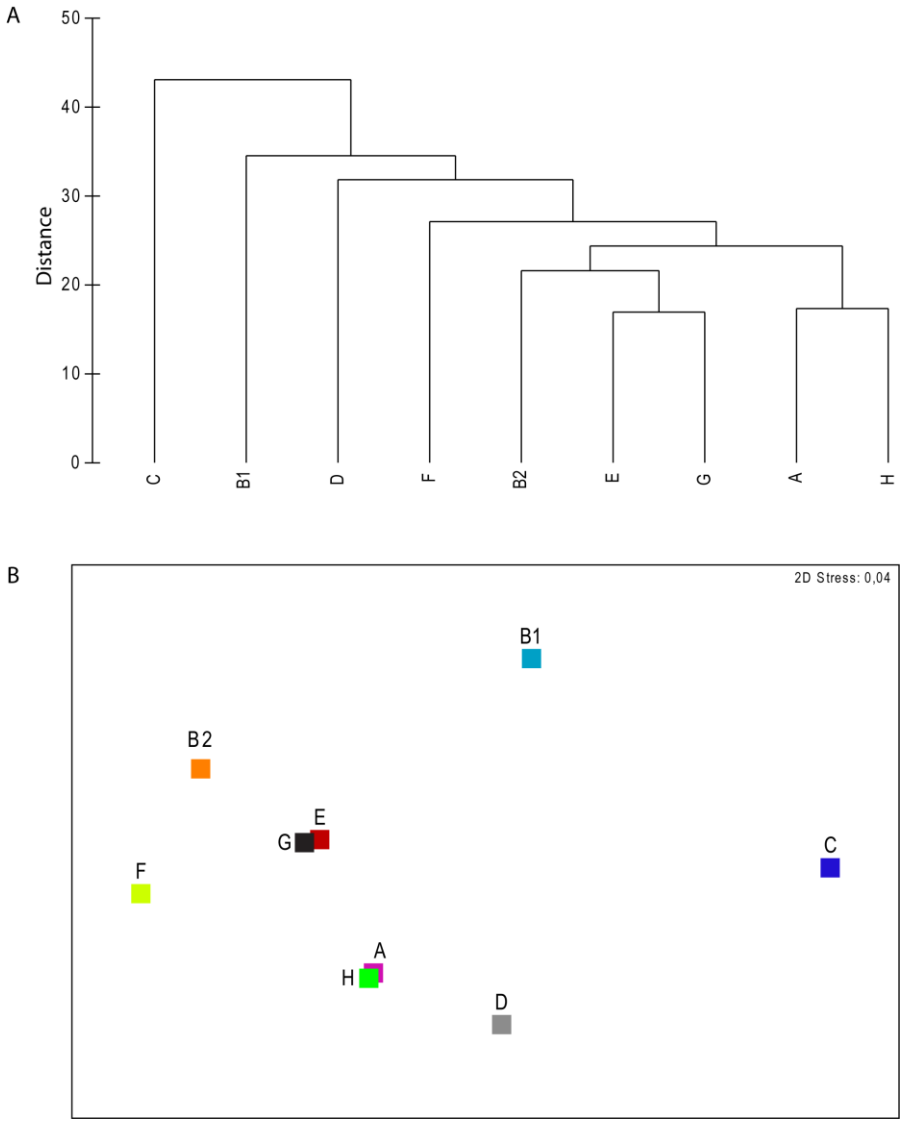

Figure 17: Macrofauna analysis, using distances among centroids, shown in A) classification diagram, B) NMDS ordination. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

BIOENV routine was used to evaluate the relationship between sedimentary (grain size fractions and total organic matter content) and biological data (species abundance data set). The best correlation between the datasets, with a value of 0.445, was obtained for the variables: grain-size fractions of 0.125 mm and 0.063 mm and the total organic matter content. The correlation that involved the participation of more variables (five, which included all the sediment grain size fractions lower than 0.25 mm and organic matter content) was 0.392.

The total macrobenthic species abundance per area is presented in Annex 1, showing the faunal impoverishment, both in species richness and abundance, in areas with higher organic content (areas B1 and C, cf. Table 9). This Table presents the total taxa identified showing

that all species appear randomly spread over the study areas but areas with oysters, and so with higher organic content, presented less species and lower abundance per species. The representation of the 5-most abundant species along the study areas showed that the annelid *Tharyx marioni* contributed with the highest relative abundance in all the study areas (Figure 18). It also showed that, only in the areas with larger oysters (area C) and area D, some of the 5-most abundant species were not among the top 5 abundant species of at least one remaining area (cf. Figure 18).

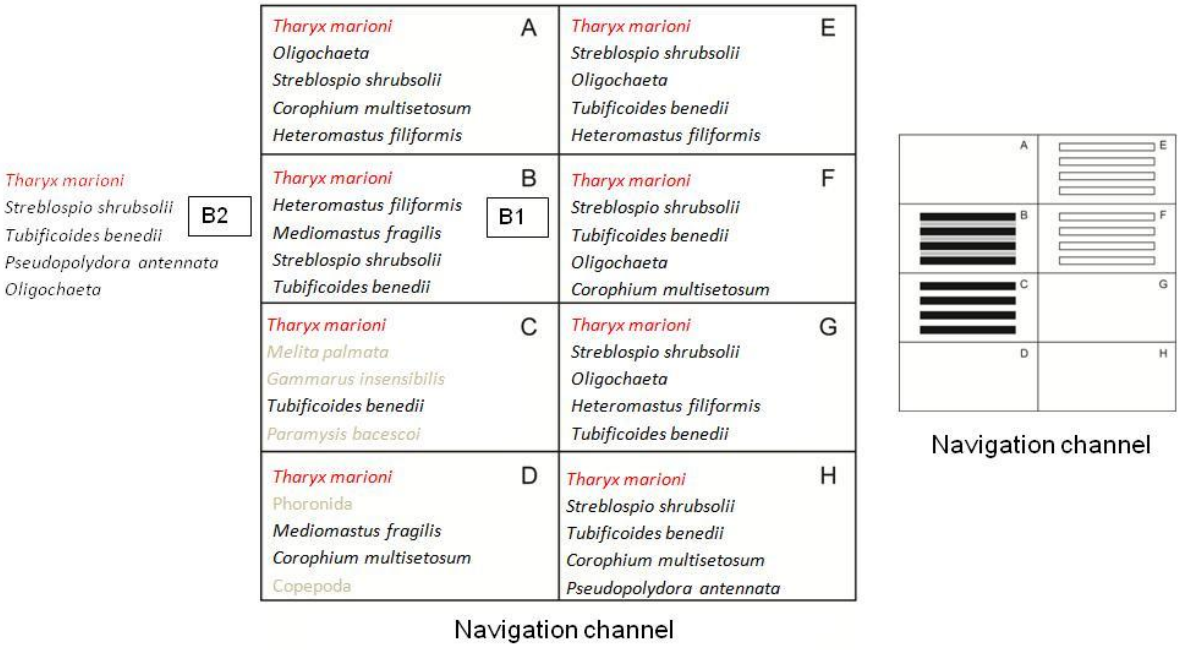

Figure 18: Representation of the 5-most abundant species along the study areas: in red is present the most abundant species common to all areas; in black are present the most abundant species common at least to one more study area; and grey species are the species more abundant not common to the remaining areas. Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

Table 9 presents the benthic macrofauna descriptors mean values across the study areas. The analysis of these data indicated that areas B1 and C, with oyster culture, presented the lowest Species richness (S), Abundance (A) and Biomass (B) values. Although the ratio between abundance and species richness (A/S) was lower in the areas with oysters, the ratio between biomass and abundance (B/A) reached lower values in area G followed by the area with larger oysters. The areas F and A presented the highest A/S and B/A ratios, respectively. With the exception of area F, the Margalef richness (d) index also presented lower values in the areas with oysters, which is similar to the Shannon diversity (H'), without considering the areas F and G. These results reveal a strong impoverishment of the benthic community, in the areas with oyster culture. Nevertheless, the Simpson (1-λ'), AMBI and M-AMBI indices have not shown a clearly variation between all areas (cf. Table 9).

Table 9: Benthic macrofauna biotic indices along the study areas (0.01 m^2, n=12).

	Areas								
	A	**B1**	**B2**	**C**	**D**	**E**	**F**	**G**	**H**
Biotic indices									
S	8.4 ± 3.60	6.2 ± 2.41	9.7 ± 3.20	4.9 ± 2.81	8.2 ± 3.10	9.2 ±2.12	9.9 ± 2.81	8.2 ± 2.82	9.2 ± 2.33
A	27.3 ± 24.25	12.3 ± 7.00	31.6 ± 14.72	10.7 ± 10.92	20.3 ± 11.11	33.5 ± 25.24	78.8 ± 51.92	32.7 ± 21.36	25.3 ± 15.53
B	2.9 ± 4.40	0.5 ± 1.33	1.8 ± 2.50	0.1 ± 0.09	1.3 ± 2.04	1.0 ± 1.80	2.0 ± 3.51	0.1 ± 0.15	2.1 ± 3.88
A/S	2,8405 ± 1,8406	1,9418 ± 0,6633	3,2089 ± 1,3600	1,8440 ± 0,9285	2,6301 ± 1,8321	3,5834 ± 2,5962	7,5136 ± 3,7698	3,7236 ± 2,2016	2,6758 ± 1,4085
B/A	0,3106 ± 0,6812	0,0473 ± 0,1336	0,0966 ± 0,1374	0,0050 ± 0,0056	0,0490 ± 0,0678	0,0445 ± 0,0903	0,0213 ± 0,0307	0,0030 ± 0,0036	0,0691 ± 0,0908
W	0,4476	0,5680	0,3845	0,3582	0,4106	0,2983	0,1734	0,1948	0,5047
H' (log_2)	2.4 ± 0.64	2.3 ± 0.69	2.6 ± 0.63	1.8 ± 0.78	2.5 ± 0.70	2.5 ± 0.53	1.9 ± 0.47	2.1 ± 0.87	2.7 ± 0.39
d	2.5 ± 0.68	2.1 ± 0.70	2.6 ± 0.71	1.9 ± 0.63	2.5 ± 0.77	2.5 ± 0.59	2.1 ± 0.47	2.3 ± 0.46	2.7 ± 0.59
1-λ'	0.8 ± 0.13	0.8 ± 0.12	0.8 ± 0.12	0.8 ± 0.12	0.8 ± 0.19	0.8 ± 0.14	0.6 ± 0.15	0.7 ± 0.18	0.9 ± 0.09
AMBI	3.4 ± 1.05	3.9 ± 0.49	3.6 ± 1.15	3.0 ± 1.00	3.1 ± 0.94	3.8 ± 0.47	4.1 ± 0.37	3.8 ± 0.63	3.2 ± 0.66
M-AMBI	0.6 ± 0.12	0.5 ± 0.13	0.6 ± 0.10	0.5 ± 0.16	0.6 ± 0.13	0.6 ± 0.10	0.5 ± 0.09	0.5 ± 0.13	0.6 ± 0.10

S – Species richness; A – abundance; B – biomass; H' – Shanon diversity; d – Margalef richness; 1-λ' – Simpson index; AMBI – AZTI Marine Biotic Index; M-AMBI – Multimetric AMBI; Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

Table 10 shows the results for the PERMANOVA main test for the macrofauna community data, considering the species abundance and the biotic indices. The benthic community data, species richness and abundance strongly rejected the null hypothesis of no significant differences between areas. The remaining indices (except AMBI) were not as effective in rejecting the null hypothesis, showing an overall significance value sometimes very close to 0.05. The AMBI index related to the sensitivity/tolerance of species to organic enrichment, failed to reject the null hypothesis, although the significance level was very close to the threshold value (p=0.0549).

Table 10: PERMANOVA main test results for the macrofauna community (square-root transformed data) and biotic indices (non-tranformed data).

Test	df	SS	MS	Pseudo-F	p
Macrofauna community					
Areas	8	51359	6419.9	2.5623	0.0001**
Sites (areas)	27	67649	2505.5	1.1628	0.0509
Residuals	72	155140	2154.7		
Total	107	274140			
Species richness (S)					
Areas	8	263.17	32.896	4.9049	0.0009**
Sites (areas)	27	181.08	6.7068	0.78561	0.7502
Residuals	72	614.67	8.537		
Total	107	1058.9			
Abundance (A)					
Areas	8	38620	4827.6	9.5158	0.0001**
Sites (areas)	27	13698	507.32	0.85546	0.6616
Residuals	72	42699	593.04		
Total	107	95017			
Shannon diversity (H')					
Areas	8	9.0658	1.1332	2.8061	0.0215*
Sites (areas)	27	10.904	0.40384	0.94112	0.5559
Residuals	72	30.896	0.42911		
Total	107	50.865			
Margalef richness (d)					
Areas	8	6.5236	0.81546	2.3355	0.0431*
Sites (areas)	27	9.418	0.34882	0.8316	0.6973
Residuals	70	29.362	0.41945		
Total	105	45.168			
Simpson index (1-λ')					
Areas	8	0.64416	0.0852	2.7196	0.0261*
Sites (areas)	27	0.80115	0.029672	1.825	0.0229
Residuals	70	1.1381	0.016258		
Total	105	2.6115			
AMBI					
Areas	8	15.971	1.9964	2.2617	0.0549(ns)
Sites (areas)	27	23.834	0.88273	1.6187	0.0533
Residuals	72	39.264	0.54533		
Total	107	79.069			
M-AMBI					
Areas	8	0.32582	0.040727	2.8579	0.0213*
Sites (areas)	27	0.38477	0.014251	0.99714	0.494
Residuals	72	1.029	0.014292		
Total	107	1.7396			

df – dregrees of freedom; SS – sums of squares; MS – mean square; p – significance level (significance values: * p<0.05; ** p<0.01; ns = non significant).

The pairwise comparisons between areas for the descriptors which rejected the main test null hypothesis are summarized in Table 11. When considering the community data, areas B1

and C (areas with oyster culture) were always significantly different from the remaining areas ($p<0.05$), but some of the areas without oysters are also significantly different from each other. Species richness was also very effective in differentiating the oyster culture areas from all the others, which between themselves showed significant differences. This descriptor was the best to distinguish areas with oysters (B1 and C) from all the others, although without showing significant differences between B1 and C. Abundance was less effective to distinguish these two areas from the remaining. All other indices presented only a small number of significant differences between areas, without isolating any particular one.

Table 11: Values for the t-statistic and associated significance in the pairwise comparisons between areas, for the descriptors that rejected the main test null hypothesis.

Areas	Macrofauna community	Species richness (S)	Abundance (A)	Margalef richness (d)	Shannon diversity (H')	Simpson index (1-λ')	M-AMBI
A *vs* B1	1.9816**	3.1894*	3.3498*	1.8102(ns)	1.3120(ns)	0.0450(ns)	3.0418*
A *vs* B2	1.5755*	1.4639(ns)	0.7446(ns)	0.5287(ns)	0.6807(ns)	0.3644(ns)	0.9393(ns)
A *vs* C	1.8017**	3.1963*	3.5085*	1.8367(ns)	1.8964(ns)	0.1006(ns)	1.4542(ns)
A *vs* D	1.3889(ns)	0.2480(ns)	1.2761(ns)	0.0392(ns)	0.2774(ns)	0.5271(ns)	0.4583(ns)
A *vs* E	1.1047(ns)	0.6547(ns)	0.4808(ns)	0.2486(ns)	0.2439(ns)	0.7348(ns)	0.0055(ns)
A *vs* F	1.9627**	1.6665(ns)	6.4990**	1.6352(ns)	2.4804*	3.4446*	1.7821(ns)
A *vs* G	1.1847(ns)	0.1761(ns)	0.5502(ns)	0.6433(ns)	1.1436(ns)	1.1238(ns)	1.3505(ns)
A *vs* H	0.8027(ns)	0.8050(ns)	0.2509(ns)	0.8101(ns)	1.7915(ns)	0.5568(ns)	1.6660(ns)
B1 *vs* B2	1.9910**	7.0000**	4.2899**	2.9428*	1.5597(ns)	0.4312(ns)	5.2789**
B1 *vs* C	1.7369*	1.4732(ns)	0.5342(ns)	0.8398(ns)	1.4577(ns)	0.1371(ns)	0.3108(ns)
B1 *vs* D	1.7491**	2.7292*	2.0322(ns)	1.7380(ns)	1.4138(ns)	0.5885(ns)	2.9100*
B1 *vs* E	1.6318*	3.2863*	1.7044(ns)	1.9815(ns)	1.0805(ns)	0.7949(ns)	2.3168(ns)
B1 *vs* F	2.9148**	6.5179**	9.4693**	0.1136(ns)	1.9300(ns)	3.5922*	0.7971(ns)
B1 *vs* G	1.6789*	1.6132(ns)	2.2359(ns)	1.7077(ns)	0.5831(ns)	1.1849(ns)	0.8213(ns)
B1 *vs* H	1.6025*	4.8107**	1.8386(ns)	3.2440*	3.1423*	0.5512(ns)	5.0494**
B2 *vs* C	1.8664**	4.8698**	4.3999**	2.4171*	2.1243(ns)	0.1802(ns)	1.9514(ns)
B2 *vs* D	1.8073**	1.7111(ns)	2.0665(ns)	0.4619(ns)	0.4298(ns)	0.2044(ns)	0.2855(ns)
B2 *vs* E	0.9536(ns)	0.4845(ns)	0.1473(ns)	0.2291(ns)	0.3588(ns)	0.4386(ns)	0.7813(ns)
B2 *vs* F	1.7055*	0.3333(ns)	5.9408**	2.6157*	2.5476*	3.0865*	3.0160*
B2 *vs* G	1.2199(ns)	1.1279(ns)	0.1099(ns)	1.4073(ns)	1.4719(ns)	0.8571(ns)	2.3006(ns)
B2 *vs* H	1.3219(ns)	0.6348(ns)	0.7928(ns)	0.3234(ns)	0.7340(ns)	0.8587(ns)	0.9437(ns)
C *vs* D	1.6902*	2.9204*	2.2715(ns)	1.8118(ns)	1.9836(ns)	0.3337(ns)	1.6547(ns)
C *vs* E	1.8136**	3.4306*	1.8181(ns)	1.9854(ns)	1.8517(ns)	0.5278(ns)	1.3804(ns)
C *vs* F	2.3853**	4.9209**	9.4775**	0.8540(ns)	0.2590(ns)	2.8256*	0.5723(ns)
C *vs* G	1.7845**	2.1723(ns)	2.3769*	1.5728(ns)	0.7609(ns)	0.8878(ns)	0.6459(ns)
C *vs* H	1.6017*	4.0702**	2.0168(ns)	2.6353*	2.8153*	0.5266(ns)	2.3402(ns)
D *vs* E	1.5405*	0.8601(ns)	1.0257(ns)	0.2025(ns)	0.0198(ns)	0.2326(ns)	0.4105(ns)
D *vs* F	2.3270**	1.8987(ns)	7.6310**	1.5847(ns)	2.5175*	2.6899*	1.9810(ns)
D *vs* G	1.5374*		1.2809(ns)	0.6514(ns)	1.2669(ns)	0.6461(ns)	1.6110(ns)
D *vs* H	1.0573(ns)	1.0498(ns)	0.6384(ns)	0.7325(ns)	1.3864(ns)	0.9554(ns)	0.9969(ns)
E *vs* F	1.3662(ns)	0.7007(ns)	3.2185*	1.8188(ns)	2.1808(ns)	2.3108(ns)	1.4901(ns)
E *vs* G	0.8216(ns)	0.6521(ns)	0.0547(ns)	0.8910(ns)	1.1614(ns)	0.4151(ns)	1.1762(ns)
E *vs* H	0.9090(ns)		0.5849(ns)	0.5006(ns)	1.1422(ns)	1.1147(ns)	1.4399(ns)
F *vs* G	1.3173(ns)	1.2868(ns)	4.1076**	1.3918(ns)	0.6783(ns)	1.6896(ns)	0.1819(ns)
F *vs* H	1.5533*	0.8955(ns)	5.5581**	2.9130*	3.6986*	3.6582**	3.4559*
G *vs* H	1.0244(ns)	0.7241(ns)	0.6584(ns)	1.7325(ns)	2.2356(ns)	1.4437(ns)	2.8216*

p – significance level (significance values: * $p<0.05$; ** $p<0.01$; ns = non significant). Areas without trestles nor oysters - A, D, G and H; Area with larger oysters - C; Areas with trestles and without oysters - E and F; Subarea B: B1 – Area with oysters; B2 – Corridor between trestles with oysters.

4. DISCUSSION

The overexploitation of marine resources has urging the need of developing new strategies for food production, being aquaculture the most emergent alternative. Several studies are known concerning the assessment of the impacts induced by shellfish aquaculture, namely oyster culture, on the benthic environment and associated macrofauna communities. The majority of these studies focuses on subtidal environments (e.g., Crawford et al., 2003), but a few have been recently dedicated to intertidal areas (De Grave et al., 1998; Forrest and Creese, 2006; Bouchet and Sauriau, 2008). As showed in the present work, the majority of studies demonstrated that seabed sediments beneath oyster cultures can become organically enriched and fine-textured, when compared to the surrounding areas, which will produce impacts into the benthic macrofauna (Forrest and Creese, 2006). In fact, the present study showed a significant enrichment of seabed sediments with organic matter and fine particles content in the areas with oysters, when compared to areas with trestles but no oysters or areas without trestles. Bouchet and Sauriau (2008) also showed that the mean grain size was finer in sites affected by oyster culture. Furthermore, the presence of oyster farming structures, namely trestles, are regarded as artificial obstacles to water circulation that disturb tidal flow and wave propagation, inducing modifications namely in sedimentation patterns (Kervella et al., 2010). This however was not observed in the present study, since the areas with trestles but without oysters have not shown clearly this effect.

Several works demonstrate negative effects of aquaculture practices namely concerning organic matter loads on benthic environments. Considering the biological descriptors, in the present work, indices, S (species richness) and A (abundance), allowed rejecting the null hypothesis, showing that significant differences occurred in areas with and without oyster culture. In the present work, along different organic enriched areas, although macrofauna community impoverishment was detected, a species replacement was not visible since the most representative species are generally the same in all areas. These results were not in agreement with those found by Forrest and Creese (2006), which have not showed a clearly trend in macrofaunal species richness, although the species composition and dominance patterns were consistent with the disturbance gradient. However, the results of the present work are consistent with the findings that revealed impacts in the macrobenthic community, namely the decrease of species richness, by Ritz et al. (1989), Weston (1990), Mirto et al. (2002) and Bouchet and Sauriau (2008), as well as a decrease in the species abundance and biomass, showed by Weston (1990) and Mirto et al. (2002). Therefore, due to the presence of

oysters, a biodeposition increment occurs, as well as subsequent higher organic enrichment, enhancing the negative effects on macrobenthic community, namely the dominance of opportunistic species (Bouchet and Sauriau, 2008). The results from the present work are in agreement with such findings, showing a significant decrease in both species richness and abundance as a consequence of organic enrichment. The benthic community composition and abundance data revealed significant differences between areas with and without oyster cultures, with the areas beneath trestles with oysters presenting the lowest species richness and abundance values, however without the replacement of different species in the most enriched areas.

In this context, considering the SAB model presented by Pearson and Rosenberg (1978), in forward succession stages occurs the reduction on species richness, abundance and biomass, slightly offset due to abundance increase of opportunistic species. Considering this, species richness and abundance results obtained in the present work showed that the areas with oysters should be beyond the opportunistic species peak. However, the biomass results obtained in this work, do not show a very clear trend when compared with the disturbance gradient increase. This can be observed in the case of area G (area without trestles), where a similar mean value of biomass is obtained relatively to areas B1 and C (areas with oyster culture), but contrasting in terms of organic matter content (note that *Diopatra neapolitana* occurred in all areas, except G). A possible explanation for this could be the presence of individuals such as *Diopatra neapolitana*, which is a large species (Rodrigues et al., 2009) that may mask the difference on biomass results.

In terms of macrofauna, analogous results are obtained by Bouchet and Sauriau (2008) for intertidal off-bottom oyster parks, where opportunistic polychaetes families, such as Cirratulidae, Capitellidae and Spionidae (Pearson and Rosenberg, 1978), are present. In fact, among the most abundant polychaete species found in all sampling areas are *Tharyx marioni* (Cirratulidae family), *Heteromastus filiformis* (Capitellidae family), *Streblospio shrubsolii* (Spionidae family) and *Pseudopolydora antennata* (Spionidae family).

Regarding the results obtained with BIOENV routine, the low Spearman rank correlation coefficient was obtained for the sediment grain-size of 0.125 mm and 0.063 mm and the total organic matter content (with a value of 0.445). These results revealed that the sediment descriptors considered in the present work were not able to explain the species abundance data distribution along the study area. Studies conducted by Rodrigues et al. (2006) referred that, on Portuguese coastal areas, the best relationship between environmental and benthic macrofauna data ranged from 0.605 on the lagoon of Óbidos, 0.717 on the shelf off Aveiro

and 0.851 on the shelf of Tagus estuary, suggesting that this correlation could be less remarkable when natural disturbance increases. The study referred showed that this relationship was stronger for the fines content, which was not observed in the present study. However, studies recently conducted in Ria de Aveiro by Rodrigues et al. (*in press*) showed that the correlation between biological and sediment grain-size descriptors was particularly low, which highlights why these were never selected as part of the best combination of environmental variables.

In this work, the biotic indices were not able to show the benthic community impoverishment indicated by species richness (S) and abundance (A), despite some of their determination involving the utilization of species richness and diversity. In this context, the remaining biotic indices utilized, such as Shannon diversity (H'), Margalef richness (d); Simpson index (1-λ'), AMBI and M-AMBI, were not able to show a clear impact on benthic macrofauna communities resulting from organic enrichment effects. In terms of Shannon-Wiener index, the results obtained by Bouchet and Sauriau (2008) also failed to distinguish the area with and without oyster culture.

In the case of marine biotic index (AMBI) several works, developed in different European coastal environments (e.g., Atlantic, Baltic, North Sea and Mediterranean European coasts) and under numerous pollution sources (e.g., drill cutting discharges, outfalls, heavy metals, industrial and mining wastes, sand extraction, hypoxia processes, oil platform impacts, among others), showed its ability in the detection of benthic macrofauna responses to organic enrichment, namely resulting from aquaculture (Muxika et al., 2005; Bouchet and Sauriau, 2008; Callier et al., 2008). However, Muxika et al. (2005) warned that AMBI has not been shown to be useful in naturally-stressed and poor communities, e.g. high hydrodynamic energy areas, subtidal sandbanks and the inner part of estuaries. Additionally, Carvalho et al. (2006) developed studies, in Ria Formosa (southern Portugal), about the distribution patterns of macrobenthic species in relation to organic enrichment within aquaculture earthen ponds, involved the application of the marine biotic index (AMBI) and showed that this index is sufficiently robust to discriminate, within a small area, differences in macrobenthic communities due to organic enrichment. Nevertheless, the authors advised caution when applying this index or others based on ecological group's assignment, as the classification of a certain area may differ when allocating a certain species to an unsuitable group, being this particularly evident when common species are involved. Chainho et al. (2007) also concluded that AMBI index seems to be adequate in diverse coastal environments, although its

application to systems with strong seasonality and/or naturally high levels of stress, such as transitional or estuarine systems, could be problematic.

Some studies have already shown a feeble performance of AMBI index (e.g., Muniz et al., 2005; Albayrak et al., 2006; Labrune et al., 2006), essentially when the disturbance is not related with organic enrichment. Recently, a study developed by Sampaio et al. (2011), in a subtidal area, showed the incapacity of this index in the detections of benthic community responses due to a mild organic enrichment. However, although Forchino et al. (2011) showed benthic impacts resulting from offshore fish aquaculture using AMBI, in the present work, this index was unable to reflect the differences of the benthic community in the areas with and without oysters. Concerning M-AMBI, this index is based on species richness and Shannon-Wiener diversity. Even so, the detection of macrofauna impoverishment in areas with oysters and consequently high organic matter content was not obtained. Although regarding the effects of aquaculture, it was already shown that M-AMBI is a useful tool to detect benthic community effects (Bouchet and Sauriau, 2008; Forchino et al., 2011), this work revealed that this index was not sensitive enough to recognize benthic macrofauna community alterations in intertidal off-bottom culture, between areas with and without oysters.

5. CONCLUSIONS

This work showed that intertidal off-bottom oyster culture induces alterations respecting to abundance and species richness of benthic macrofauna, revealing an organic enrichment effect.

Concerning sediment descriptors, higher fines content, as well as higher organic matter content, was observed in result of oyster culture in the study areas. This effect on the benthic habitat resulted in a generalized impoverishment of the community in the areas with oyster culture, but not a species replacement. In this context, the best biological descriptors to diagnose the effects associated with the organic enrichment were macrofauna community and their species richness and abundance.

Some biotic indices used in this work were not able to detect a clear difference between areas with and without oyster culture, and so were not able to identify the impacts of organic enrichment on benthic macrofauna. These results show that special attention must be paid when using biotic indices in areas where organic enrichment induces an impoverishment of the benthic community but not necessarily a species replacement.

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ANNEXES

Annex 1: Species abundance, relative abundance and taxonomic group of species identified in the study areas (0.01 m2, n=12). Taxonomic group: A – Annelids; C – Crustaceans; M – Molluscs; O – Other. Subarea B: B1 – Area with oysters; B2 – Corridor between trestles.

Area A	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	137	41.9	A
Oligochaeta	26	8.0	A
Streblospio shrubsolii	26	8.0	A
Corophium multisetosum	17	5.2	C
Heteromastus filiformis	14	4.3	A
Mediomastus fragilis	14	4.3	A
Tubificoides benedii	14	4.3	A
Pseudopolydora antennata	13	4.0	A
Melita palmata	9	2.8	C
Cerastoderma edule	7	2.1	M
Cyathura carinata	6	1.8	C
Galathowenia oculata	5	1.5	A
Abra tenuis	4	1.2	M
Solen marginatus	4	1.2	M
Euclymene oerstedi	3	0.9	A
Nemertea	3	0.9	O
Notomastus latericeus	3	0.9	A
Paramysis bacescoi	3	0.9	C
Ruditapes decussatus	3	0.9	M
Scrobicularia plana	3	0.9	M
Copepoda	2	0.6	C
Crangon crangon	2	0.6	C
Diopatra neapolitana	2	0.6	A
Insecta	1	0.3	O
Jassa falcata	1	0.3	C
Lutraria lutraria	1	0.3	M
Microdeutopus gryllotalpa	1	0.3	C
Microprotopus longimanus	1	0.3	C
Nassarius reticulatus	1	0.3	M
Owenia fusiformis	1	0.3	A

Area B – subarea B1	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	31	21.1	A
Heteromastus filiformis	22	15.0	A
Mediomastus fragilis	17	11.6	A
Streblospio shrubsolii	17	11.6	A
Tubificoides benedii	16	10.9	A
Pseudopolydora antennata	9	6.1	A
Corophium multisetosum	6	4.1	C
Oligochaeta	4	2.7	A
Galathowenia oculata	3	2.0	A
Melita palmata	3	2.0	C
Cnidaria	2	1.4	O
Diopatra neapolitana	2	1.4	A
Hediste diversicolor	2	1.4	A
Owenia fusiformis	2	1.4	A
Paramysis bacescoi	2	1.4	C
Scrobicularia plana	2	1.4	M
Capitomastus sp.	1	0.7	A
Carcinus maenas	1	0.7	C
Cerastoderma edule	1	0.7	M
Glycera convoluta	1	0.7	A
Lagis koreni	1	0.7	A
Mysta picta	1	0.7	A
Mytilus galloprovincialis	1	0.7	M

Area B – Subarea B2	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	134	35.4	A
Streblospio shrubsolii	68	17.9	A
Tubificoides benedii	31	8.2	A
Pseudopolydora antennata	29	7.7	A
Oligochaeta	17	4.5	A
Heteromastus filiformis	13	3.4	A
Galathowenia oculata	8	2.1	A
Mediomastus fragilis	8	2.1	A
Capitella sp.	6	1.6	A
Hediste diversicolor	6	1.6	A
Abra tenuis	5	1.3	M
Paramysis bacescoi	5	1.3	C
Polydora cornuta	5	1.3	A
Solen marginatus	5	1.3	M
Melita palmata	4	1.1	C
Nemertea	4	1.1	O
Diopatra neapolitana	3	0.8	A
Nematoda	3	0.8	O
Crangon crangon	2	0.5	C
Lagis koreni	2	0.5	A
Microdeutopus gryllotalpa	2	0.5	C
Nassarius reticulatus	2	0.5	M
Owenia fusiformis	2	0.5	A
Scrobicularia plana	2	0.5	M
Ampithoe sp.	1	0.3	C
Calyptraea chinensis	1	0.3	M
Cirriformia tentaculata	1	0.3	A
Corophium multisetosum	1	0.3	C

Area B – Subarea B2 (continuation)	Abundance	Relative Abundance(%)	Taxonomic group
Cossura sp.	1	0.3	A
Cyathura carinata	1	0.3	C
Glycera convoluta	1	0.3	A
Monocorophium ascherusicum	1	0.3	C
Mysta picta	1	0.3	A
Nephtys hombergii	1	0.3	A
Notomastus latericeus	1	0.3	A
Polydora ciliata	1	0.3	A
Spirobranchus triqueter	1	0.3	A

Area C	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	28	21.9	A
Melita palmata	17	13.3	C
Gammarus insensibilis	14	10.9	C
Tubificoides benedii	9	7.0	A
Paramysis bacescoi	8	6.3	C
Pseudopolydora antennata	7	5.5	A
Heteromastus filiformis	6	4.7	A
Oligochaeta	4	3.1	A
Polydora ciliata	4	3.1	A
Streblospio shrubsolii	4	3.1	A
Capitella sp.	3	2.3	A
Notomastus latericeus	3	2.3	A
Abra tenuis	2	1.6	M
Mediomastus fragilis	2	1.6	A
Calyptraea chinensis	1	0.8	M
Cerastoderma edule	1	0.8	M
Chamelea striatula	1	0.8	M
Cirriformia tentaculata	1	0.8	A
Copepoda	1	0.8	C
Corophium multisetosum	1	0.8	C
Crangon crangon	1	0.8	C
Diopatra micrura	1	0.8	A
Diopatra neapolitana	1	0.8	A
Euclymene oerstedi	1	0.8	A
Galathowenia oculata	1	0.8	A
Lagis koreni	1	0.8	A
Monocorophium insidiosum	1	0.8	C
Nematoda	1	0.8	O
Nemertea	1	0.8	O
Nephtys hombergii	1	0.8	A
Owenia fusiformis	1	0.8	A

Area D	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	84	34.4	A
Phoronida	27	11.1	O
Mediomastus fragilis	17	7.0	A
Corophium multisetosum	13	5.3	C
Copepoda	12	4.9	C
Heteromastus filiformis	11	4.5	A
Tubificoides benedii	10	4.1	A
Euclymene oerstedi	8	3.3	A
Cerastoderma edule	7	2.9	M
Owenia fusiformis	7	2.9	A
Streblospio shrubsolii	7	2.9	A
Diopatra neapolitana	5	2.0	A
Pseudopolydora antennata	4	1.6	A
Diopatra micrura	3	1.2	A
Galathowenia oculata	3	1.2	A
Nemertea	3	1.2	O
Oligochaeta	3	1.2	A
Solen marginatus	3	1.2	M
Crangon crangon	2	0.8	C
Gammarus insensibilis	2	0.8	C
Pygospio elegans	2	0.8	A
Abra tenuis	1	0.4	M
Calyptraea chinensis	1	0.4	M
Capitella sp.	1	0.4	A
Cirriformia tentaculata	1	0.4	A
Idotea chelipes	1	0.4	C
Lagis koreni	1	0.4	A
Melita palmata	1	0.4	C

Area D (continuation)	Abundance	Relative Abundance(%)	Taxonomic group
Monocorophium ascherusicum	1	0.4	C
Monocorophium insidiosum	1	0.4	C
Nephtys hombergii	1	0.4	A
Paramysis bacescoi	1	0.4	C

Area E	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	200	49.8	A
Streblospio shrubsolii	47	11.7	A
Oligochaeta	21	5.2	A
Tubificoides benedii	20	5.0	A
Heteromastus filiformis	18	4.5	A
Pseudopolydora antennata	16	4.0	A
Corophium multisetosum	11	2.7	C
Paramysis bacescoi	10	2.5	C
Mediomastus fragilis	7	1.7	A
Hediste diversicolor	6	1.5	A
Solen marginatus	5	1.2	M
Cyathura carinata	4	1.0	C
Euclymene oerstedi	4	1.0	A
Melita palmata	4	1.0	C
Scrobicularia plana	4	1.0	M
Cerastoderma edule	3	0.7	M
Owenia fusiformis	3	0.7	A
Capitella sp.	2	0.5	A
Copepoda	2	0.5	C

Area E (continuation)	Abundance	Relative Abundance(%)	Taxonomic group
Crangon crangon	2	0.5	C
Abra alba	1	0.2	M
Abra segmentum	1	0.2	M
Abra tenuis	1	0.2	M
Aonides oxycephala	1	0.2	A
Cnidaria	1	0.2	O
Diopatra micrura	1	0.2	A
Diopatra neapolitana	1	0.2	A
Malacoceros sp.	1	0.2	A
Nematoda	1	0.2	O
Nemertea	1	0.2	O
Phoronida	1	0.2	O
Polydora cornuta	1	0.2	A
Pygospio elegans	1	0.2	A

Area F	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	569	60.1	A
Streblospio shrubsolii	98	10.4	A
Tubificoides benedii	74	7.8	A
Oligochaeta	51	5.4	A
Corophium multisetosum	28	3.0	A
Paramysis bacescoi	24	2.5	C
Heteromastus filiformis	23	2.4	A
Pseudopolydora antennata	18	1.9	A
Mediomastus fragilis	12	1.3	A
Nemertea	8	0.8	O
Cyathura carinata	6	0.6	C
Diopatra neapolitana	6	0.6	A
Galathowenia oculata	5	0.5	A
Solen marginatus	4	0.4	M
Crangon crangon	3	0.3	C
Hediste diversicolor	3	0.3	A
Notomastus latericeus	3	0.3	A
Cerastoderma edule	2	0.2	M
Owenia fusiformis	2	0.2	A
Abra tenuis	1	0.1	M
Capitella sp.	1	0.1	A
Diopatra micrura	1	0.1	A
Melita palmata	1	0.1	C
Nematoda	1	0.1	O
Scrobicularia plana	1	0.1	M
Spio martinensis	1	0.1	A

Area G	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	204	52.0	A
Streblospio shrubsolii	44	11.2	A
Oligochaeta	26	6.6	A
Heteromastus filiformis	19	4.8	A
Tubificoides benedii	13	3.3	A
Corophium multisetosum	12	3.1	C
Paramysis bacescoi	12	3.1	C
Mediomastus fragilis	8	2.0	A
Cyathura carinata	6	1.5	C
Pseudopolydora antennata	6	1.5	A
Abra segmentum	5	1.3	M
Abra tenuis	5	1.3	M
Galathowenia oculata	5	1.3	A
Nemertea	4	1.0	O
Phoronida	4	1.0	O
Capitella sp.	3	0.8	A
Crangon crangon	2	0.5	C
Glycera convoluta	2	0.5	A
Notomastus latericeus	2	0.5	A
Owenia fusiformis	2	0.5	A
Abra alba	1	0.3	M
Alkmaria romijni	1	0.3	A
Dipolydora coeca	1	0.3	A
Hediste diversicolor	1	0.3	A
Nassarius reticulatus	1	0.3	M
Phyllodoce mucosa	1	0.3	A
Polydora ciliata	1	0.3	A
Solen marginatus	1	0.3	M

Area H	Abundance	Relative Abundance(%)	Taxonomic group
Tharyx marioni	94	31.0	A
Streblospio shrubsolii	26	8.6	A
Tubificoides benedii	22	7.3	A
Corophium multisetosum	17	5.6	C
Pseudopolydora antennata	16	5.3	A
Heteromastus filiformis	13	4.3	A
Paramysis bacescoi	13	4.3	C
Diopatra neapolitana	10	3.3	A
Oligochaeta	10	3.3	A
Owenia fusiformis	10	3.3	A
Mediomastus fragilis	9	3.0	A
Phoronida	9	3.0	O
Nemertea	7	2.3	O
Crangon crangon	5	1.7	C
Euclymene oerstedi	5	1.7	A
Melita palmata	5	1.7	C
Cyathura carinata	4	1.3	C
Galathowenia oculata	4	1.3	A
Glycera convoluta	4	1.3	A
Cerastoderma edule	3	1.0	M
Dipolydora coeca	2	0.7	A
Notomastus latericeus	2	0.7	A
Scrobicularia plana	2	0.7	M
Solen marginatus	2	0.7	M
Abra tenuis	1	0.3	M
Ampithoe sp.	1	0.3	C
Capitella sp.	1	0.3	A
Caprella sp.	1	0.3	C
Hediste diversicolor	1	0.3	A
Monocorophium ascherusicum	1	0.3	C
Nematoda	1	0.3	O
Ruditapes decussatus	1	0.3	M
Spirobranchus triqueter	1	0.3	A