



# A multimetric approach to evaluate offshore mussel aquaculture effects on the taxonomical and functional diversity of macrobenthic communities

A.Z. Lacson<sup>a,b</sup>, D. Piló<sup>a,c,\*</sup>, F. Pereira<sup>a</sup>, A.N. Carvalho<sup>a</sup>, J. Cúrdia<sup>d</sup>, M. Caetano<sup>e</sup>, T. Drago<sup>f,g</sup>, M.N. Santos<sup>a,1</sup>, M.B. Gaspar<sup>a,c</sup>

<sup>a</sup> Portuguese Institute for the Sea and Atmosphere (IPMA), Avenida 5 de Outubro, 8700-305, Olhão, Portugal

<sup>b</sup> Ghent University, Department of Biology, Krijgslaan 281/S8, 9000, Ghent, Belgium

<sup>c</sup> Center of Marine Sciences (CCMAR), University of Algarve (UAlg), Campus de Gambelas, 8005-139, Faro, Portugal

<sup>d</sup> King Abdullah University of Science and Technology (KAUST), Red Sea Research Center (RSRC), Thuwal, 23955-6900, Saudi Arabia

<sup>e</sup> Portuguese Institute for Sea and Atmosphere (IPMA), Division of Oceanography and Marine Environment, Rua Alfredo Magalhães Ramalho, 61495-165, Algés, Portugal

<sup>f</sup> Portuguese Institute for Sea and Atmosphere (IPMA), Division of Marine Geology and Georesources, Rua Alfredo Magalhães Ramalho, 61449-006, Lisboa, Portugal

<sup>g</sup> Instituto Dom Luiz (IDL) Faculdade de Ciências da Universidade de Lisboa, Campo Grande Edifício C1, Piso 1, 1749-016, Lisboa, Portugal

## ARTICLE INFO

### Keywords:

*Mytilus galloprovincialis*  
Mussel farm  
Biodeposition  
Functional diversity  
Biological traits analysis  
Beta diversity  
Turnover  
Nestedness

## ABSTRACT

A multimetric approach was used to detect structural, compositional, and functional shifts in the underlying macrobenthic communities of an offshore mussel (*Mytilus galloprovincialis*) farm in a Portuguese Aquaculture Production Area. Sampling stations distributed inside and outside this area were used to evaluate sediment descriptors and macrobenthic samples collected before (April and September 2010) and after (June and September 2014) the initiation of mussel farming. Sediment fine fraction, organic matter content, and trace element concentrations were found to increase with depth, independently from the mussel farm. Moreover, the structure and composition of the macrobenthic communities were likewise structured by depth. Turnover was the dominant temporal and spatial pattern of beta diversity for all communities. Furthermore, the functional diversity of these communities was unaffected by the mussel farm. These results suggested that an offshore profile allowed hydrodynamic conditions to weaken the impact of mussel farming and highlighted the importance of conducting an integrative multimetric analysis when studying aquaculture impacts on benthic communities.

## 1. Introduction

Aquaculture has been identified as one of the key contributors to future food security (Anderson et al., 2017). Indeed, more than 3 billion people rely on it as a major source of dietary protein (Fulton et al., 2018), requiring its rapid development to meet the ever-increasing demand for food (Salin and Ataguba, 2018). Yet, due to a growing concern for the well-being of the marine environment, its expansion has pushed for the need to evaluate and reduce the impact of conventional production activities (Cao et al., 2007; Ellis et al., 2016; Ottinger et al., 2016). At present, environmental concerns focus on the efficiency of natural resource use and the risks that aquaculture byproducts pose towards biodiversity (Valenti et al., 2018). These risks are progressively addressed by the advancement of technology, which has paved the way for aquaculture activities to take place increasingly away from calm coastal zones (Bostock et al., 2010; Shainee et al., 2012) where

environmental impacts and competition with other uses may be heightened (Gentry et al., 2016).

Offshore aquaculture is rapidly gaining acceptance (Froehlich et al., 2017; Jansen et al., 2016; Thomas et al., 2019) as a form of food production aligned with the environmental, economic, and social objectives of many coastal nations (Gentry et al., 2017). However, its effects remain to be a subject of investigation as choice of location (Silva et al., 2011), hydrodynamic forces (Henderson et al., 2001; Pérez et al., 2003), species characteristics (Gentry et al., 2017; Oyinlola et al., 2018), and spatial conflicts influence the suitability of offshore sites and their capacity for impact neutralization (Sanchez-Jerez et al., 2016). Principal concerns for offshore farms are associated with organic enrichment as a result of farm additives and biological waste (Cancemi et al., 2003; Holmer, 2010), the spread of diseases via interaction with wild stocks (Lafferty et al., 2015), and the genetic impact of species escapees (Jackson et al., 2015; Naylor et al., 2005). Taking into account

\* Corresponding author. Portuguese Institute for Sea and Atmosphere (IPMA), Avenida 5 de Outubro, 8700-305, Olhão, Portugal.

E-mail address: [david.pilo@ipma.pt](mailto:david.pilo@ipma.pt) (D. Piló).

<sup>1</sup> Currently at the International Commission for the Conservation of Atlantic Tunas (ICCAT), Calle Corazón de Maria 8, planta 6, 28002 Madrid, Spain.

these concerns, offshore mussel farming may present a reduced ecological footprint, particularly because mussels do not rely on artificial additives or feed pellets as a source of nutrition (Hixson, 2014; Shumway et al., 2003).

Globally, more than  $18 \times 10^5$  tons of mussels have been produced annually since 2010, with the total yield increasing every year (FAO, 2018). Mussels are high in protein and provide vital fatty acids that cannot be synthesized by humans (Cherifi et al., 2018; Venugopal and Gopakumar, 2017). Aside from their rich nutritional value, their ecosystem services, such as nutrient remediation, are also known to be beneficial to the environment and to society (van der Schatte Olivier et al., 2018), modifying their habitats in a manner that increases physical heterogeneity and habitat diversity, and reinforcing the reputation of mussels as ecosystem engineers (Borthagaray and Carranza, 2007). Furthermore, by filter feeding at high rates, mussels remove considerable amounts of particulate matter and sequester nitrogen from the water column by converting this material into their own tissue mass (Lüskow and Riisgård, 2018; Petersen et al., 2014). They also contribute to phytoplankton growth dynamics through the facilitation of ammonia cycling in the water column (Dame, 1996). For this reason, mussel farms have been evaluated for their mitigation potential against eutrophication as stand-alone coastal aquaculture systems (Gren et al., 2009; Petersen et al., 2014; Whitmarsh et al., 2006). Although mussel aquaculture has gained favor as a means to improve water quality (Ferreira et al., 2014; Lindahl et al., 2005), it is associated with its own set of environmental consequences. These generally involve the effect of farming on the carrying capacity of the surrounding environment, considering that cultured bivalves may compete with naturally occurring filter feeders and may contribute to local organic enrichment (Gibbs, 2007; McKindsey, 2013). While some researchers have argued that these effects are relatively minor considering the nutrient remediation services provided by mussels (Petersen et al., 2012; Rose et al., 2012), several recent studies have shown significant yet contrasting effects in underlying benthic communities as a result of mussel farming (e.g., Hartstein and Rowden, 2004; Suplicy, 2018; Ysebaert et al., 2009). In shallow coastal inlets with relatively low dispersal capacity, mussel farms may cause hypoxic and sulfidic sediments (Carlsson et al., 2012; Cranford et al., 2009; Grant et al., 2005) due to bulk biodeposition, inducing adverse changes to the underlying macrobenthic infaunal diversity (Chamberlain et al., 2001; Gallardi, 2014; Newell, 2004). This homogenizing response to organic enrichment can be accompanied by ecological shifts on benthic fauna, with predominantly suspension feeding communities of long-lived bivalves, crustaceans, and infaunal polychaetes giving rise to communities dominated by small sized opportunistic deposit feeders marked by their tolerance for byproducts of anoxic reactions (Danovaro et al., 2003; Gallardi, 2014; McKindsey et al., 2011). On the other hand, several studies have concluded that mussel culture can have little to no negative impact on the underlying benthic communities due to the influence of strong local hydrodynamics on the dispersion of biodeposits (Chamberlain et al., 2001; Crawford et al., 2003; da Costa and Nalesso, 2006; Lacoste et al., 2018). In this respect, dose-dependency of biodeposition, which is influenced by water depth and current speeds (Keeley et al., 2009), appears to account for levels of impact (Robert et al., 2013), with total abundance and species richness decreasing as biodeposition is increased (Callier et al., 2009). Conversely, offshore mussel cultivation has also been related to elevated macrofaunal diversity attributed to fall-off of mussels and their shell-hash, which can increase the heterogeneity of benthic structure (McKindsey et al., 2011; Wilding and Nickell, 2013; Wong and O'Shea, 2011).

The alterations in macrobenthic structure have typically been used to assess the degree of disturbance under mussel farms. Classical methods for detecting these impacts include alpha diversity indices, such as the Shannon-Wiener diversity and Pielou evenness index (da Costa and Nalesso, 2006; Fabi et al., 2009), coupled with multivariate statistical tests (Lacoste et al., 2018; Neofitou et al., 2014; Wilding and

Nickell, 2013). More recently, complementary measures have emerged, aiming to increase knowledge about the ecological impacts of offshore farms. The use of beta diversity metrics, for instance, and its partitioning into turnover and nestedness (Baselga, 2010) may especially be useful as an indicator of shifts in community structure and composition induced by mussel farming and in detecting changes at the community level from before and after the settlement of mussels. Another useful approach to evaluate these changes is founded on the concept of functional diversity, which focuses on the composition of the biological traits of species rather than the taxonomic structure of communities (Bremner et al., 2003). This approach, known as Biological Traits Analysis (BTA), aims to provide clues about ecosystem functioning (Bremner, 2008) and refines the link between traits and ecological processes, with habitat as the template that may be used to predict the organization of communities (Dolédec et al., 1996; Keddy, 1992). The use of a combination of metrics to assess benthic community structure, diversity, and functionality has been shown to be effective at detecting a scenario of disturbance (Dimitriadis and Koutsoubas, 2011; Piló et al., 2019). Such approach would generate both a qualitative and quantitative overview of the relevant communities across time and space, and its application in this study is intended to assess mussel farm effects at the macrobenthic-community level.

The Marine Stewardship Council (MSC) grants Sustainable Fisheries Certification to offshore aquaculture projects based on the following principles: health of fish stock, impact on the ecosystem, and management system (FCI, 2014). More specifically, the second principle states that “fishing operations should allow for the maintenance of the structure, productivity, function, and diversity of the ecosystem ... on which the fishery depends” (MSC, 2014). This principle launched the present study, which intends to evaluate whether or not a mussel Aquaculture Production Area (APA) located in southern Portugal could induce changes in the structure, composition and functional ecology of the underlying benthic communities. Given the potential of aquaculture byproducts to change the environment of the proximal seabed, and considering the typical macrobenthic community responses to these changes, the following objectives were proposed: (a) infer whether concentrations of organic matter and trace elements were higher in APA underlying sediments; (b) detect potential differences on the composition of macrobenthic communities between impact and reference areas; (c) evaluate if alpha and beta diversity patterns differ spatially, mirroring APA effects in receiving communities; (d) understand the functional structure and organization of local macrobenthic communities and analyze possible differences between impact and reference areas; and (e) highlight the importance of natural factors in shaping the local communities beyond the structural effects induced by the APA.

## 2. Materials and methods

### 2.1. Site description

The Aquaculture Production Area (APA), otherwise known as Área Piloto de Produção Aquícola da Armonia (APPAA), is an offshore zone for aquaculture established by the Portuguese government and supported by the technical and scientific expertise of the Portuguese Institute of the Sea and Atmosphere (IPMA). It is located on the Algarve coast of Portugal, outside the Ria Formosa Natural Park, and is approximately 3 km away from Armona Island near the coastal inlet of Olhão. The APA covers an area of 15 km<sup>2</sup> and was the site of a rope grown Mediterranean mussel (*Mytilus galloprovincialis*) culture that was initiated in 2012 under the management of *Companhia de Pescarias do Algarve* and *Molushore – “Empresa de Cultivo de Moluscos Marinhos em Offshore, Lda.”* Aside from mussels, the APA also contains small lots with dismantled fish cages and tuna traps that were established on the eastern section of the area long before the designation of APA. Mussels were grown with semi-submerged longlines, each having an average length of 400 m and consisting of 250 socks homogeneously distributed

along the farming area, each 12 m long, with a distance of 1–1.5 m between each and set about 5 m below the sea surface. A system of buoys and anchors maintained the position and buoyancy of the socks, which each had an average mussel production rate of 100 kg/y (Araújo et al., 2018), contributing to a total estimated production of 3990 tons of mussels per year.

The APA site is influenced by both Atlantic and Mediterranean waters, with predominant currents and waves flowing from the W-SW, and countercurrents and waves from the E-SE (Carvalho et al., 2018). Due to the southeastern orientation of the APA in relation to the cusped shape of the Ria Formosa lagoon system, it is more exposed to the “Levante” conditions coming from the SE (Ahab et al., 2014), although the longshore drift in the area remains to be eastward directed with net values ranging from  $0.4 \times 10^5$  to  $1.5 \times 10^5$  m<sup>3</sup>/yr (Pacheco et al., 2011a). Waves from the SE, reaching wave heights of 1.2 m, are significantly higher than waves from the SW, which reach wave heights of 0.9 m (Costa et al., 2001). Salinity in this region remains at an average of 36, whereas mean seawater temperature varies between 15 °C in the Winter and 22 °C in the Summer, with no significant oscillations with depth. An emergence of cold productive waters due to upwelling events regularly occurs between April and October (Leitão et al., 2005), making this a favorable site for offshore aquaculture production.

## 2.2. Sampling design

In order to evaluate the potential impact of the mussel farm on macrobenthic communities, a before-after/control-impact (BACI) approach (Underwood, 1992) was performed. To evaluate the effects over time and different states of farm activity, sampling was carried out at four different periods: April 2010 (I), September 2010 (II), June 2014 (III), and September 2014 (IV). Sampling periods I and II took place before mussel farming was initiated. Sampling could not take place in 2012, when mussel farming was initiated, due to legal constraints. The latter sampling periods (III and IV) took place when the mussel farm was in full operation. Twelve sampling stations, with three replicates each, were established. A division of these stations established two treatment types: six stations were located within the APA and labeled as “impact” stations, and the other six stations were located outside the borders of the APA and labeled as “control” stations. The control stations were located around the APA in all directions, as the currents and waves in the area are influenced by natural conditions from opposing directions. To account for the wide depth range (13–70 m) over the farm area, these stations were strategically located so that an equal number of “impact” and “control” ones were found in the “shallow” and “deep” areas ranging from 13–24 m and 41–70 m, respectively. A total of four main groups, with three stations each, were distinguished “a priori” accordingly: SC (Shallow-Control), SI (Shallow-Impact), DC (Deep-Control), and DI (Deep-Impact) (Fig. 1). This zonation strategy established whether treatment groups in the “shallow” or “deep” area were similarly affected by the mussel farm. Stations may differentially be affected based on the dispersion potential of biodeposits associated with different depths (Weise et al., 2009), given that the deep stations are beyond the seaward limit of wave effects in this region (Carvalho et al., 2018). This type of zonation also improved the analysis of statistical results across groups.

For the assessment of macrobenthic assemblages, triplicate sediment samples in each of these stations were collected using a Van Veen grab (0.05 m<sup>2</sup>). At the impact stations, samples were collected in areas adjacent to mussel longlines. These were sieved through a 1 mm mesh sieve at each site for each sampling period. Macrobenthic organisms found in the samples were then sorted and identified to the lowest possible taxonomic level at the laboratory. A sub-sample (2.5 × 10 cm corer) of each collected grab was used for the determination of sediment grain size and concentrations of organic matter and trace elements.

## 2.3. Sediment descriptors

Considering the potential of mussel farms to induce modifications in nearby sedimentary environments, various descriptors along the selected areas and sampling periods were measured. Given the relevance of fine sediment to coastal community structure (Carvalho et al., 2012), fine particles (silt and clay fraction, < 63 μm) were used as sediment grain size proxy. Sediment samples were dried in an oven at 40 °C. Grain size analysis was carried out using a sieve shaker (Retsch AS 200) for 10 min, with controlled amplitude. The mechanical separation was performed with a 63 μm sieve, and the dry weight of the fine fraction recorded. Sediment organic matter content was determined through the ash-free dry weight of the sediment samples (200 mg) after 2 h at 450 °C.

The quantification of trace elements was performed according to Caetano et al. (2008). Briefly, sediment samples (approximately 100 mg) were completely digested using 6 cm<sup>3</sup> of HF (40%) and 1 cm<sup>3</sup> of Aqua Regia (HCl—36%: HNO<sub>3</sub>—60%; 3:1) in closed Teflon bombs at 100 °C for 1 h. Obtained residue was evaporated to near dryness in Teflon vials (DigiPrep HotBlock — SCP Science), redissolved with 1 cm<sup>3</sup> of double-distilled HNO<sub>3</sub> and 5 cm<sup>3</sup> of Milli-Q water, heated for 20 min at 75 °C, and then diluted to 50 cm<sup>3</sup> with Milli-Q water. Trace elements were determined using a quadrupole ICP-MS (Thermo Elemental, X-Series) equipped with a Peltier Impact bead spray chamber and a concentric Meinhard nebulizer. The experimental parameters were as follows: forward power 1400 W; peak jumping mode; 150 sweeps per replicate; dwell time: 10 ms; and dead time: 30 ns. Two procedural blanks were prepared using the same analytical procedure and reagents and were included in each batch of 10 samples. The blanks always delivered values of less than 1% of the total concentration of elements studied. The isotopes used for quantification were: <sup>51</sup>V, <sup>52</sup>Cr, <sup>59</sup>Co, <sup>60</sup>Ni, <sup>65</sup>Cu, <sup>66</sup>Zn, <sup>75</sup>As, <sup>111</sup>Cd, and <sup>208</sup>Pb. The internal standard chosen was <sup>115</sup>In. For all the elements analyzed, coefficients of variation for counts ( $n = 5$ ) were lower than 2% and a 7-point calibration within a range of 1–100 μg L<sup>-1</sup> was used for quantification. The precision and accuracy of the analytical procedures was controlled through repeated analysis of the elements studied in certified reference materials (MAG-1, MESS-2 and PACS-2). The results obtained did not differ significantly ( $p < 0.05$ ) from the certified values (data not shown).

## 2.4. Data analysis

### 2.4.1. Environmental parameters

To detect significant differences in each of the sediment descriptors (organic matter, sediment fine fraction, trace elements), a three-way analysis of variance (ANOVA) was carried out among the following fixed factors: “Treatment” (2 levels: *Control* or *Impact*), “Sampling period” (4 levels: *I*, *II*, *III*, and *IV*), and “Depth” (2 levels: *Shallow* or *Deep*). The data were previously checked for normality and homogeneity of variance using Shapiro-Wilk and Levene’s test, respectively. Transformations were made in cases where the data failed to meet assumptions for the ANOVA. For post hoc multiple comparisons, the Tukey test was used.

### 2.4.2. Multivariate analyses

In order to assess the patterns of macrobenthic community structure, species abundance data was square root transformed and used to generate a Multidimensional Scaling plot from the Bray-Curtis resemblance matrix. The information provided by this plot was used to visualize potential dissimilarities among samples.

A three-way permutational analysis of variance (PERMANOVA) with the factors “Treatment,” “Sampling period,” and “Depth” was performed on the abundance data. Since the variances across replicates were not homogeneous for each station per sampling period, each replicate of each station sample was then related to its corresponding “Treatment,” “Depth,” and “Sampling period” in order to reduce the

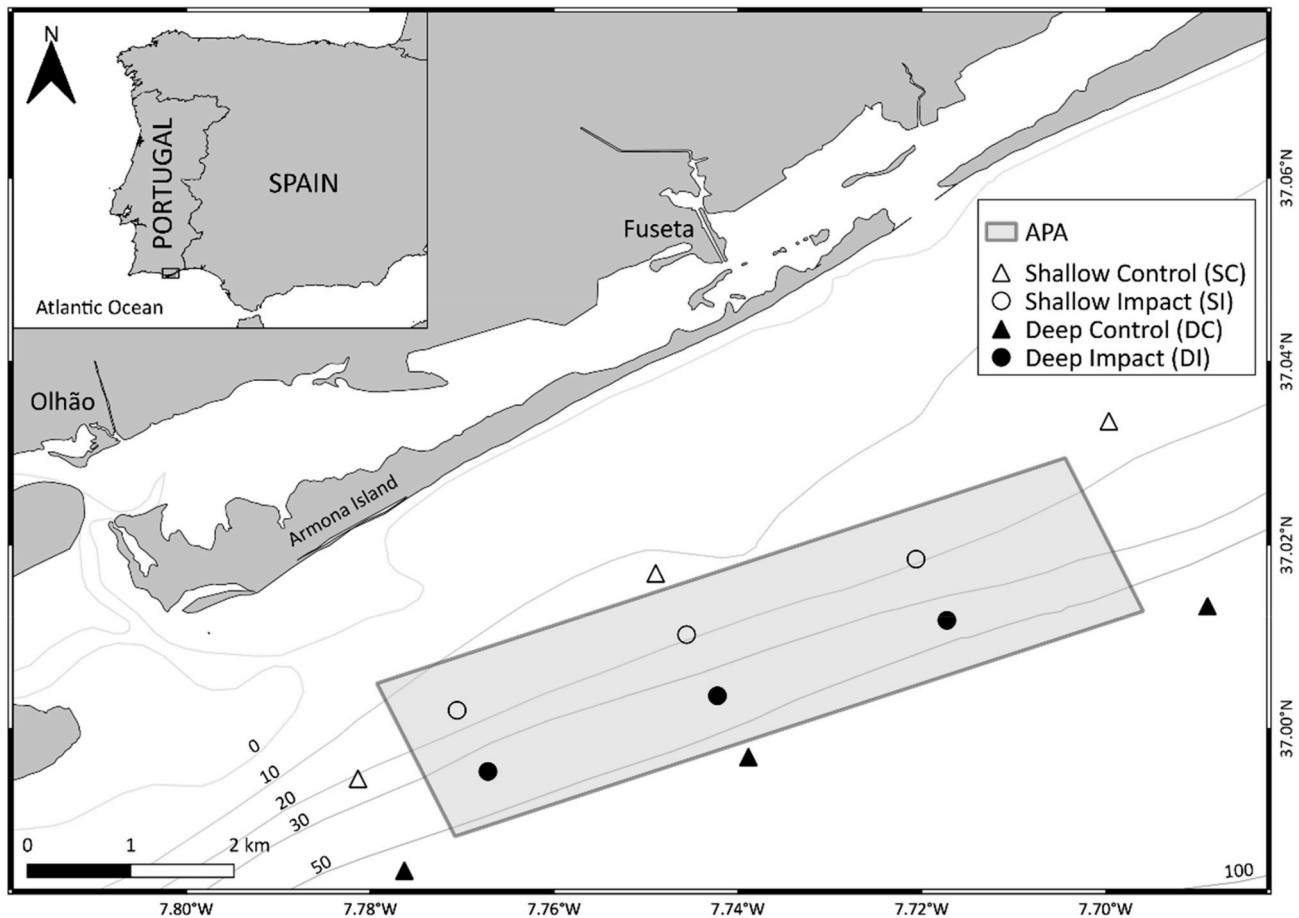


Fig. 1. Sampling stations in the Aquaculture Production Area (APA) situated close to Armona Island (along the coast of southern Portugal).

influence of high intra and inter-station variability on the main test. The statistical test was done with a Bray-Curtis distance matrix and 999 permutations on the residuals of the reduced model. Afterwards, a SIMPER analysis was done to determine the dominant species within each sampling group for every sampling period and to quantify how much these groups differed in terms of species composition. An ANOVA was then used to compare the spatial dissimilarity scores across depth groups. The open source softwares RStudio version 1.0.153 (RStudio Team, 2016) with the “vegan” package (Oksanen et al., 2011) and Primer v6 (Clarke and Gorley, 2006) were used to perform these tests and similar ones described in this study.

#### 2.4.3. Alpha diversity

To describe alpha diversity patterns between the main factors, four indices were determined: the number of individuals per station ( $N$ ), species richness ( $S$  – number of taxa per station), Shannon-Wiener index ( $H'$  - diversity), and Pielou's index ( $J$  – evenness). A three-way ANOVA, followed by a Tukey test as post hoc, was then performed to evaluate the differences between these parameters. Assumptions were tested using Levene's test for homogeneity of variance and the Shapiro-Wilk test for normality. The “vegan” (Oksanen et al., 2011) and “car” (Fox and Weisberg, 2011) packages in RStudio were used to perform these tests.

#### 2.4.4. Beta diversity partitioning

For beta diversity metrics, the abundance data were pooled according to sampling group per sampling period (e.g. SC\_I, SC\_II, SC\_III, SC\_IV). Thus, each sampling group (SC, SI, DC, DI) could be compared to one another both spatially (e.g. SC\_I vs SI\_I) and temporally (e.g. SI\_I vs SI\_IV). This allowed for a more global comparison of the beta

diversity values afterward. The Sorensen dissimilarity index, based on presence-absence data, was used as a measure of general beta diversity. An ANOVA was used to test for differences between the temporal beta diversity scores of each sampling group and period. To understand the underlying patterns influencing similarities or differences in diversity between two sites, beta diversity was partitioned into its spatial turnover (Simpson pair-wise dissimilarities) and nestedness components using the methodology described by Baselga (2010). In order to properly check for differences between beta diversity and its components, a  $t$ -test was used. The use of beta diversity to assess macrobenthic temporal diversity patterns within the same sites has been done before, in the context of disturbances due to dredging (Piló et al., 2019) and seasonal hypoxia (Chu et al., 2018). The “betapart” package (Baselga and Orme, 2012) in RStudio was used to run these tests.

#### 2.4.5. Biological traits analysis

In order to conduct the BTA, a bibliographic survey was performed to assign trait information to the identified species. Because of the limited biological information available for marine invertebrates, a well-known challenge for BTA (Munari, 2013), only the 50 most abundant taxa (out of 601 identified) were surveyed for their traits, taking into account their high contribution (63.3%) to the total abundance. Functional trait information was gathered from a variety of sources: research papers and reports, including their appendices, and web databases such as MarLIN BIOTIC – Biological Traits Information Catalogue: <http://www.marlin.ac.uk/biotic/>, WORMS – World Register of Marine Species: <http://www.marinespecies.org>, Marine Species Identification Portal: <http://species-identification.org/>, SeaLifeBase: <http://www.sealifebase.org>, and ADW - Animal Diversity Web: <http://animaldiversity.org>.



**Table 1**  
Macrobenthic functional traits and their modalities, including codes for each modality. These codes are used in RLQ results (Fig. 7). Trait modalities are derived from Piló et al. (2016).

	Functional Trait Modalities	Code
Feeding Mode	Surface Deposit Feeder	SDF
	Subsurface Deposit Feeder	SSDF
	Carnivore	C
	Herbivore	H
	Suspension Feeder	SF
Sensitivity to disturbance	Sensitive	I
	Indifferent	II
	Tolerant	III
	Second-order Opportunistic	IV
	First-order Opportunistic	V
Lifespan	Short (0–2 years)	LS
	Medium (2–5 years)	LM
	Long (> 5 years)	LL
Body size	Very Small (0–1 cm)	BVS
	Small (1–3 cm)	BS
	Medium (3–10 cm)	BM
	Large (> 10 cm)	BL
Motility	Burrower	Bu
	Crawler	Cr
	Walker	W
	Swimmer	Sw
	Sessile	S
Position	Infaunal	Inf
	Epifaunal	Epi
Larval type	None (Brooding)	Broo
	Benthic	Bent
	Planktonic	Plank

A mix of both qualitative and measurable trait information obtained for the top 50 species included the following: “Feeding mode”, “Life span”, “Body size”, “Motility”, “Position in sediment”, “Larval type”, and “Sensitivity to disturbance”. The main rationales for the selection of these traits are their ability to reflect important responses of species to the environment (Paganelli et al., 2012) and the availability of information for macroinvertebrate taxa in the region of southern Portugal. For each of these traits, a set of modalities based on Piló et al. (2016) were used to categorize the taxa. These traits, and the associated modalities (Table 1), were chosen based on their usefulness in effectively assessing the loss of ecological functioning in the presence or absence of disturbance events, particularly those associated with organic matter input (Dimitriou et al., 2017; Papageorgiou et al., 2009; Sanz-Lázaro and Marín, 2011). The trait “Sensitivity to disturbance” was based on the AMBI index ecological groups. Despite the typical use of this index in the assessment of anthropogenic environmental changes induced by organic matter enrichment, according to the paradigm of Pearson and Rosenberg (1978), the AMBI index proved to be useful in detecting other anthropogenic impacts including trace metal inputs (Borja et al., 2000) and thus may provide important information about mussel farming areas, where these phenomena have been reported. The assignment of the identified species into one of the five ecological groups (I–V) proposed by the AMBI index was based on the list available in AZTI website – <http://www.azti.es>.

Complex combinations of traits can translate to increased survival for some species, thus some species tend to express many facets of a trait given fluctuations of environmental conditions across temporal and spatial scales (Beauchard et al., 2017; Statzner and Bêche, 2010). To account for this level of plasticity, a ‘fuzzy coding’ procedure was employed. This procedure, described by Chevenet et al. (1994), accounts for the use of multiple modalities at different degrees of affinity.

Each modality was given a score of 0–3, with 0 indicating no affinity for the given modality, and 3 indicating total affinity. A score of either 1 or 2 is indicative of partial or facultative affinity towards the given modality. The fuzzy-coded traits matrix is provided in the Supplementary Material (Table S2). A PERMANOVA based on Euclidean resemblance, with 999 permutations, was used to test for differences in trait composition for the three main factors: “Treatment”, “Sampling period”, and “Depth” using the same design described in section 2.4.2. For this test, a new matrix providing trait scores for each sample was created by using the weights of each taxa for each trait (based on the fuzzy-coded trait matrix) according to its mean abundance in each sampling group x sampling period (e.g. I\_SC, IV\_DC). The same matrix was then used to graph the diversity in modalities for each of the respective traits for each sampling group x sampling period.

Following the approach proposed by Dray et al. (2014), an RLQ analysis (Dolédéc et al., 1996) combined with the Fourth Corner method (Legendre et al., 1997) was performed to link environmental conditions with species trait data. This approach has previously been used to assess the effects of a metal concentration gradient on benthic functional diversity (Piló et al., 2016) and the effects of environmental stressors on riverine benthic macroinvertebrate assemblages (Kuzmanovic et al., 2017). The RLQ analysis involves the use of three matrices: an R table, which contains the environmental data for each site; an L table, which contains the species abundance data for each site; and a Q table, which contains the trait data for each species (Dray et al., 2014). Min-max normalization (with 1 being the maximum value, and 0 being the minimum value) was performed on the environmental data, and the abundance data was square root transformed (to minimize the presence of dominant taxa) in preparation for use in the RLQ analysis. The normalized environmental data for the depth, sediment fine fraction, sediment organic matter and trace elements in each replicate per site served as the R matrix, and the fuzzy-coded trait table containing information for the selected traits was used as the Q matrix in the RLQ analysis.

Prior to the RLQ test, separate ordinations were performed on each of the matrices. A correspondence analysis (CA) was performed on the L (abundance) table, a principal component analysis (PCA) was performed on the R (environmental data) table, and a fuzzy correspondence analysis (FCA) was done for the Q table (fuzzy-coded traits matrix). The RLQ test combined these three analyses in order to maximize the covariation between environmental variables and taxonomic traits (Dray et al., 2014). A global Monte-Carlo test with 999 random permutations was then performed on the RLQ values to test relationships under the following models: Model 2 tested for the relationship between the species compositions of the sites and the environmental conditions (R and L), whereas Model 4 tested species distribution as a function of their biological traits rather than their preferences for site conditions (L and Q); Model 6 was a combination of these two tests, and determined if traits were significantly related to environment (Borcard et al., 2018; Dray and Legendre, 2008; ter Braak et al., 2012). To complement RLQ results, a recent and improved version of the Fourth Corner method, with 49999 random permutations, including adjustments for multiple comparisons (Dray et al., 2014), was used. This method combined R, L, and Q results into a single table so that specific traits may be matched to environmental variables (Dray et al., 2014). This analysis was performed using the “ade4” package (Dray and Dufour, 2007) of the open source software RStudio version 1.0.153 (RStudio Team, 2016).

### 3. Results

#### 3.1. Environmental parameters

The sediment fine fraction showed a relation to depth as both interactions “Depth x Treatment” and “Depth x Sampling period” were found significant ( $p < 0.05$ ). Organic matter presented a significant

**Table 2**

Mean  $\pm$  Standard Deviation values of environmental parameters: percentage of the fine fraction (Fines %), OM (%) and trace element concentrations ( $\mu\text{g/g}$ ) in the sediment in each of the sampling groups (SC, SI, DC, DI) before (sampling periods I and II) and after (sampling periods III and IV) mussel settlement in the offshore farm.

Sampling period	Sediment		Trace Elements ( $\mu\text{g/g}$ )									
	Fines (%)	OM (%)	V	Cr	Co	Ni	Cu	Zn	As	Cd	Pb	
SC	I	3.2 $\pm$ 4.7	2.0 $\pm$ 1.5	16.0 $\pm$ 9.0	8.5 $\pm$ 7.3	1.7 $\pm$ 1.2	3.6 $\pm$ 3.1	5.8 $\pm$ 4.7	21.2 $\pm$ 15.6	8.9 $\pm$ 3.3	0.06 $\pm$ 0.01	5.2 $\pm$ 2.7
	II	4.2 $\pm$ 6.0	1.7 $\pm$ 1.0	16.6 $\pm$ 6.6	10.1 $\pm$ 6.7	1.8 $\pm$ 0.8	4.1 $\pm$ 2.4	5.4 $\pm$ 4.2	20.6 $\pm$ 11.9	11.0 $\pm$ 6.4	0.05 $\pm$ 0.02	5.8 $\pm$ 2.1
	III	0.2 $\pm$ 0.4	1.5 $\pm$ 0.6	9.6 $\pm$ 5.9	17.9 $\pm$ 15.0	2.1 $\pm$ 0.7	2.8 $\pm$ 1.5	4.7 $\pm$ 1.7	15.1 $\pm$ 7.5	8.3 $\pm$ 4.9	0.02 $\pm$ 0.01	11.4 $\pm$ 3.8
	IV	4.7 $\pm$ 6.7	2.0 $\pm$ 1.1	14.1 $\pm$ 7.0	12.2 $\pm$ 5.3	1.9 $\pm$ 0.9	3.5 $\pm$ 2.3	5.3 $\pm$ 3.5	19.0 $\pm$ 11.6	9.4 $\pm$ 4.8	0.04 $\pm$ 0.01	7.5 $\pm$ 2.8
SI	I	0.8 $\pm$ 0.7	1.8 $\pm$ 0.7	13.2 $\pm$ 6.0	5.3 $\pm$ 2.3	1.2 $\pm$ 0.6	2.4 $\pm$ 0.5	3.5 $\pm$ 1.7	14.6 $\pm$ 5.2	10.1 $\pm$ 8.4	0.04 $\pm$ 0.01	3.9 $\pm$ 1.3
	II	3.0 $\pm$ 2.3	1.7 $\pm$ 0.3	17.0 $\pm$ 4.6	15.5 $\pm$ 14.7	1.7 $\pm$ 0.2	3.9 $\pm$ 2.2	4.9 $\pm$ 1.6	19.7 $\pm$ 1.3	13.1 $\pm$ 9.0	0.05 $\pm$ 0.01	5.9 $\pm$ 0.9
	III	15.7 $\pm$ 22.5	1.6 $\pm$ 0.6	9.7 $\pm$ 7.1	30.1 $\pm$ 18.0	2.1 $\pm$ 0.8	2.3 $\pm$ 0.7	4.1 $\pm$ 1.2	13.9 $\pm$ 5.7	9.0 $\pm$ 7.0	0.02 $\pm$ 0.01	10.1 $\pm$ 3.5
	IV	1.6 $\pm$ 2.3	1.7 $\pm$ 0.6	13.3 $\pm$ 5.1	17.0 $\pm$ 10.0	1.7 $\pm$ 0.5	2.9 $\pm$ 0.9	4.2 $\pm$ 1.2	16.0 $\pm$ 3.2	10.7 $\pm$ 8.0	0.04 $\pm$ 0.01	6.6 $\pm$ 1.4
DC	I	78.9 $\pm$ 12.4	8.5 $\pm$ 1.2	94.3 $\pm$ 12.9	62.0 $\pm$ 10.0	11.6 $\pm$ 1.4	27.0 $\pm$ 4.3	51.0 $\pm$ 7.1	177 $\pm$ 17.3	24.0 $\pm$ 2.3	0.17 $\pm$ 0.02	55.7 $\pm$ 6.3
	II	77.8 $\pm$ 14.0	7.5 $\pm$ 1.5	86.3 $\pm$ 22.8	56 $\pm$ 14.3	10.6 $\pm$ 2.2	24.0 $\pm$ 5.3	47.7 $\pm$ 11.1	166 $\pm$ 31.5	22.3 $\pm$ 4.8	0.17 $\pm$ 0.05	55.6 $\pm$ 10.7
	III	58.4 $\pm$ 42.3	7.2 $\pm$ 1.1	83.6 $\pm$ 21.6	53.9 $\pm$ 12.2	10.8 $\pm$ 2.1	23.2 $\pm$ 5.4	45.1 $\pm$ 9.1	160 $\pm$ 24.3	21.6 $\pm$ 4.5	0.12 $\pm$ 0.03	53.7 $\pm$ 5.7
	IV	78.6 $\pm$ 14.9	6.5 $\pm$ 1.2	88.1 $\pm$ 18.9	57.3 $\pm$ 11.8	11.0 $\pm$ 1.8	24.7 $\pm$ 4.9	47.9 $\pm$ 8.9	167 $\pm$ 23.9	22.6 $\pm$ 3.8	0.16 $\pm$ 0.03	55.0 $\pm$ 7.3
DI	I	47.6 $\pm$ 18.1	6.3 $\pm$ 2.1	68.3 $\pm$ 33.0	43.3 $\pm$ 20.8	8.5 $\pm$ 4.1	18.5 $\pm$ 9.4	36.3 $\pm$ 19.5	128 $\pm$ 65.0	19.1 $\pm$ 8.6	0.14 $\pm$ 0.05	35.6 $\pm$ 21.3
	II	68.7 $\pm$ 9.0	6.8 $\pm$ 0.5	80.3 $\pm$ 9.2	51.2 $\pm$ 5.5	10.2 $\pm$ 1.3	21.7 $\pm$ 3.0	44.3 $\pm$ 6.4	151 $\pm$ 19.7	20.0 $\pm$ 2.3	0.17 $\pm$ 0.01	48.7 $\pm$ 5.3
	III	58.2 $\pm$ 15.9	6.3 $\pm$ 1.1	67.0 $\pm$ 8.3	42.4 $\pm$ 7.3	8.8 $\pm$ 0.8	18.2 $\pm$ 2.4	35.8 $\pm$ 4.4	125 $\pm$ 16.5	18.2 $\pm$ 2.0	0.12 $\pm$ 0.02	43.3 $\pm$ 5.5
	IV	63.0 $\pm$ 6.0	5.7 $\pm$ 0.7	71.9 $\pm$ 16.3	45.6 $\pm$ 10.5	9.1 $\pm$ 2.0	19.4 $\pm$ 4.8	38.8 $\pm$ 9.9	135 $\pm$ 33.0	19.1 $\pm$ 4.2	0.14 $\pm$ 0.02	42.5 $\pm$ 10.3

“Depth x Treatment” interaction ( $p < 0.05$ ) with differences across Sampling Period ( $p < 0.05$ ). Higher trace element concentrations were found in sediments composed mainly of fine grained particles and with increased organic matter content (DC and DI). Indeed, for almost all the elements (except Cd), a significant “Depth x Treatment” interaction ( $p < 0.05$ ) was found. Overall, the gradient for all analyzed environmental parameters was shown by the Tukey test as  $[\text{DC}] > [\text{DI}] > [\text{SC}] = [\text{SI}]$  (Table 2 and S1).

### 3.2. Composition and structure of macrobenthic communities

A total of 17868 macrobenthic organisms, belonging to 601 taxa, were collected and identified during the study period. Identified taxonomic groups included Polychaeta (46.8%), Bivalvia (18.7%), Amphipoda (14.6%), Echinodermata (3.4%), Nemertea (3.1%), Decapoda (2.6%), Gastropoda (2.3%), and others (8.5%). The most abundant species found was the bivalve *Spisula solida*, which comprised 7.9% of the total species count, followed by the polychaetes *Lumbrineris lusitanica* (4.8%) and *Ampharete lindstroemi* (3.8%) (Table 3). The remaining 598 taxa each contributed to less than approximately 3% of the total species count. A total of 122 taxa were singletons (found only once in the whole study). A fair distribution of the abundance was verified throughout the species list with low levels of dominance. The full list of identified taxa is included in the Supplementary Material (Table S2).

The nMDS plot showed that community structure and composition were noticeably related to depth, whereas treatment did not structure the communities considerably (Fig. 2). A temporal consistency was noticeable along the different sampling periods. These results were partially corroborated by the PERMANOVA test, which revealed significant interactions between depth with both treatment and sampling period (“Depth x Treatment”  $p = 0.001$  and “Depth x Sampling period”  $p = 0.001$ ). For the first interaction, the post hoc pairwise tests revealed that the shallow-control (SC) and shallow-impact (SI) communities were similar to one another ( $p = 0.73$ ) while the other relations differ among them ( $p = 0.001$ ). The pairwise test for the second interaction revealed a high temporal variation of community structure and composition not only between different depths (Shallow and Deep) at the same sampling period but also for the same depths at different periods. (See Table S3 for PERMANOVA complete results).

### 3.3. Species contribution across sampling areas

SIMPER results grouped communities by depth: SC-SI and DC-DI – were regularly more similar to one another than treatment regimes: SC-DC and SI-DI differed consistently along the different sampling periods (I-IV). Spatial dissimilarity tended to be higher between control and impact sites found in the shallow area than sites in the deep area ( $p = 0.02$ ). As shown in the nMDS plot and in the spatial SIMPER results (Table 4), species communities from the deep stations were more similar to one another as a whole, compared to shallow communities. Dissimilarity tended to increase over time, with larger dissimilarity scores between the first (I) and final (IV) sampling periods. The trend in temporal dissimilarity scores for both control and impact sites of each depth regime did not differ considerably.

Generally, the sampling areas within depth groups showed different patterns of community structure and composition over time, even prior to mussel settlement. The most common species for each sampling period in the shallow areas did not show consistent levels of dominance, whereas the more common species in the deep areas remained fairly consistent (Table 5). Prior to mussel settlement, the five highest contributing taxa in the shallow area included nemerteans, the polychaetes *Mediomastus* sp., *Nephtys cirrosa*, and *Spiophanes bombyx*, and the amphipod *Ampelisca* spp. Post-mussel settlement, the tanaid *Apeudopsis* spp. and another polychaete *Ampharete lindstroemi* generally increased in abundance. The five highest contributing taxa in the deep area prior to mussel settlement were mostly polychaetes and included the following taxa: *Lumbrineris lusitanica*, *Ampharete lindstroemi*, *Abyssoninoe hibernica*, *Notomastus* sp., and Nemertea. Both the DC and DI communities in the post-mussel settlement scenario were marked by a rise in occurrence of the amphipod *Ampelisca* spp. Of the highest contributing species across sampling periods in each group, the only species considered to be opportunistic (belonging to AMBI groups IV or V) were *Corbula gibba*, a bivalve found in SC, and the polychaetes *Heteromastus filiformis* and *Lagis koreni* in DC and DI, respectively.

### 3.4. Alpha diversity

The mean number of individuals (N) found per station was consistently higher in the shallow areas than in the deep areas (Fig. 3-A). This varied similarly in SC and SI, peaking at sampling period III. For

**Table 3**

List of 50 most abundant taxa, their taxonomic groups, and their total and cumulative contribution to abundance. Labels are included as a guide to Fig. 8. Trait information for each of these taxa are included in the Supplementary Material (Table S6).

Species	Label	Taxonomic Group	A (%)	A (cum%)
<i>Spisula solida</i>	Sso	Bivalvia	7.9	7.9
<i>Lumbrineris lusitanica</i>	Llu	Polychaeta	4.8	12.7
<i>Ampharete lindstroemi</i>	Ali	Polychaeta	3.8	16.5
<i>Ampelisca</i> spp.	Amp	Amphipoda	3.1	19.7
Nemertea	Nem	Nemertea	3.1	22.7
<i>Apseudopsis</i> spp.	Aps	Tanaidacea	2.3	25.0
<i>Modiolus</i>	Mmo	Bivalvia	2.2	27.2
<i>Mediomastus</i> sp.	Med	Polychaeta	1.9	29.1
<i>Corbula gibba</i>	Cgi	Bivalvia	1.7	30.8
<i>Spiophanes bombyx</i>	Sbo	Polychaeta	1.6	32.4
<i>Notomastus</i> sp.	Not	Polychaeta	1.5	33.9
<i>Lagis koreni</i>	Lko	Polychaeta	1.4	35.3
<i>Bathyporeia</i> spp.	Bat	Amphipoda	1.3	36.6
<i>Nephtys cirrosa</i>	Nci	Polychaeta	1.2	37.8
Phoronida	Pho	Phoronida	1.2	39.0
<i>Lygdamis murata</i>	Lmu	Polychaeta	1.2	40.2
<i>Websterinereis glauca</i>	Wgl	Polychaeta	1.1	41.3
<i>Aspidosiphon muelleri</i>	Amu	Sipuncula	1.0	42.4
<i>Magelona minuta</i>	Mmi	Polychaeta	1.0	43.3
<i>Leptocheirus hirsutimanus</i>	Lhi	Amphipoda	1.0	44.3
<i>Abyssoninoe hibernica</i>	Ahi	Polychaeta	0.9	45.2
<i>Pisione remota</i>	Pre	Polychaeta	0.9	46.1
<i>Branchiostoma lanceolatum</i>	Bla	Cephalochordata	0.9	46.9
<i>Photis longicaudata</i>	Plo	Amphipoda	0.9	47.8
<i>Thracia phaseolina</i>	Tph	Bivalvia	0.8	48.6
<i>Photis</i> sp.	Pht	Amphipoda	0.8	49.4
<i>Paralacydonia paradoxa</i>	Ppa	Polychaeta	0.8	50.2
<i>Ophiura grubei</i>	Ogr	Echinodermata	0.8	50.9
<i>Eunice vittata</i>	Evi	Polychaeta	0.7	51.7
<i>Urothoe pulchella</i>	Upu	Amphipoda	0.7	52.4
<i>Prionospio dayi</i>	Pda	Polychaeta	0.7	53.1
<i>Urothoe hesperiae</i>	Uhe	Amphipoda	0.7	53.9
<i>Chamelea striatula</i>	Cst	Bivalvia	0.7	54.6
<i>Melinna palmata</i>	Mpa	Polychaeta	0.7	55.3
<i>Heteromastus filiformis</i>	Hfi	Polychaeta	0.7	56.0
<i>Euclymene oerstedii</i>	Eoe	Polychaeta	0.6	56.6
<i>Iphinoe armata</i>	Iar	Cumacea	0.6	57.1
<i>Magelona alleni</i>	Mal	Polychaeta	0.6	57.7
<i>Sphaerosyllis bulbosa</i>	Sbu	Polychaeta	0.5	58.2
<i>Diogenes pugilator</i>	Dpu	Decapoda	0.5	58.8
<i>Aponuphis bilineata</i>	Abi	Polychaeta	0.5	59.3
<i>Goniadella bobrezkii</i>	Gbo	Polychaeta	0.5	59.8
<i>Harpinia antennaria</i>	Han	Amphipoda	0.5	60.2
<i>Malacoceros</i> sp.	Maa	Polychaeta	0.5	60.7
<i>Glycera unicornis</i>	Gun	Polychaeta	0.4	61.1
<i>Ophiura albida</i>	Oal	Echinodermata	0.4	61.6
<i>Abra prismatica</i>	Apr	Bivalvia	0.4	62.0
<i>Nototropis vedlomensis</i>	Ave	Amphipoda	0.4	62.5
<i>Cheirocratus assimilis</i>	Cas	Amphipoda	0.4	62.9
<i>Nephtys</i> sp.	Nep	Polychaeta	0.4	63.3

DC and DI, N scores were more consistent, but a peak during the post-mussel settlement periods was apparent for DI. The same trends in the scores of species richness (S) was observed for all sampling areas (Fig. 3-B). The ANOVA and post hoc tests confirmed that depth and sampling period significantly (Depth x Sampling period;  $p = 0.001$ ) influenced N and S, with the shallow sites having higher mean scores. The post hoc test did not consider the scores at sampling periods III and IV of DI to be significantly higher than the scores obtained for the earlier sampling periods. Furthermore, S was found to be significantly higher for Impact (Treatment;  $p = 0.04$ ).

Shannon-Wiener diversity scores ( $H'$ , Fig. 3-C) were significantly related to both depth and treatment ( $p < 0.01$ ), but not to sampling period ( $df = 3$ ,  $p = 0.17$ ). Shallow stations had consistently higher  $H'$  scores than deep stations. Species diversity was higher in the impact stations, compared to the control stations, of each depth regime. The Tukey test, however, deemed that SC and SI had similar mean  $H'$  scores

( $p = 0.104$ ). The same result was found for DC and DI ( $p = 0.056$ ). The Pielou scores for evenness (J) did not show a clear trend (Fig. 3-D), despite the significant differences found for treatment ( $p < 0.05$ ) and for the interaction between depth and sampling period ( $p < 0.05$ ) as revealed by ANOVA (See ANOVAs complete results in Table S4). Generally, the post hoc test showed that the trend for mean J scores followed a gradient which favored the deep sampling areas:  $[DC] = [DI] > [SC] = [SI]$ , contrasting the results of other alpha diversity measures.

### 3.5. Beta diversity

Spatially, a similar trend of mean beta diversity scores was found for each sampling period when comparing the groups: I –  $0.52 \pm 0.09$ , II –  $0.55 \pm 0.15$ , III –  $0.52 \pm 0.13$ , and IV –  $0.55 \pm 0.14$  (Sampling Period;  $p = 0.96$ ). Temporally, an ANOVA test showed that mean beta diversity scores across groups was similar (Group;  $p = 0.46$ ), with each group gaining the following mean scores  $\pm$  SD: SC –  $0.47 \pm 0.08$ , SI –  $0.51 \pm 0.07$ , DC –  $0.54 \pm 0.08$ , and DI –  $0.54 \pm 0.05$ . Considering the temporal change of state per sampling group, the beta diversity scores for the comparisons between I vs II and between I vs IV stand out, with the mean beta diversity for I vs II being the lowest ( $0.39 \pm 0.04$ ), and beta diversity for I vs IV being the highest ( $0.58 \pm 0.02$ ) (Figs. 4 and S5).

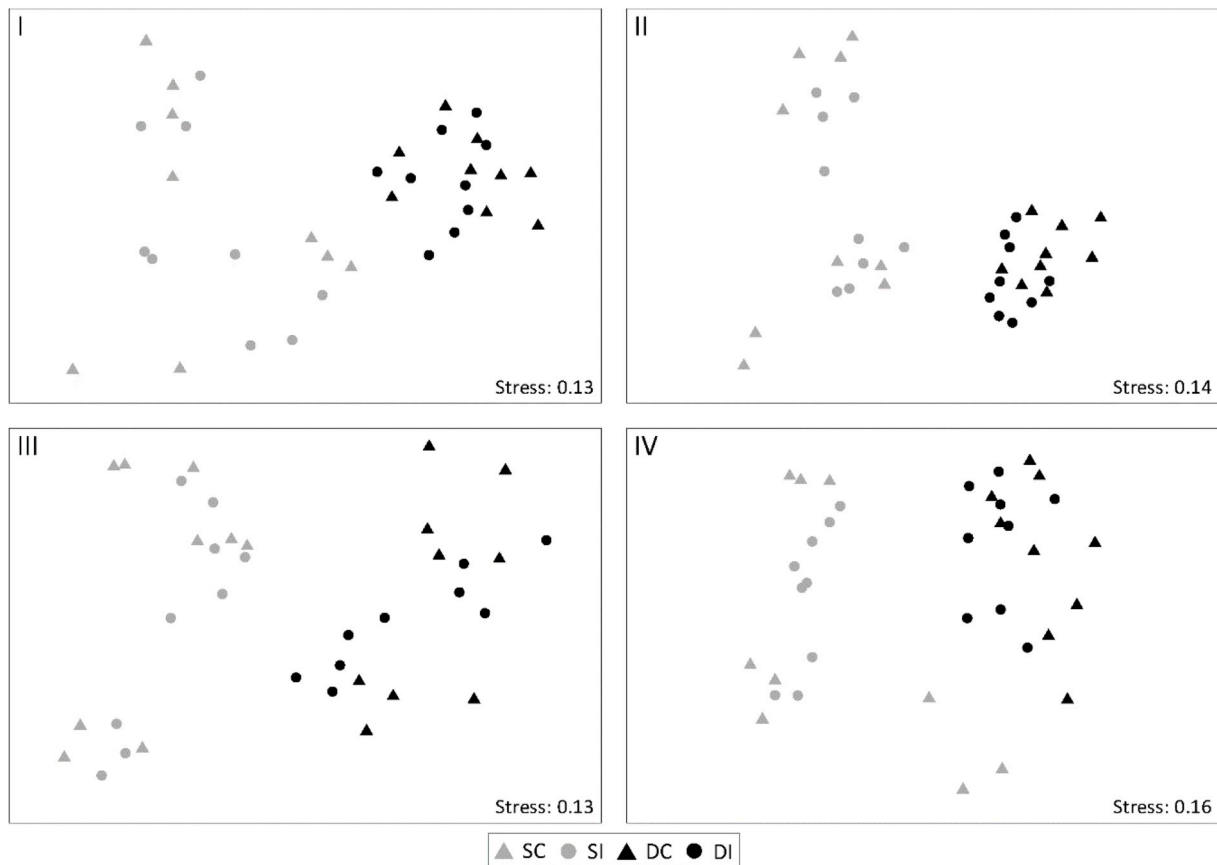
Regarding beta diversity partitioning, a pattern favoring turnover instead of nestedness was detected for both temporal and spatial comparisons in all sampling areas. Nestedness scores were generally higher in impact groups (SI and DI) compared to control groups (SC and DC) (Fig. 4). The  $t$ -test, however, did not detect a significant difference ( $df = 1$ ,  $p > 0.05$ ) between the turnover and nestedness scores before and after mussel settlement. Nestedness scores for I vs II and III vs IV (Pre vs Pre and Post vs Post mussel settlement) were statistically similar ( $p = 0.72$ ) to nestedness scores for Pre vs Post comparisons (I vs III, I vs IV, II vs III, II vs IV) for both impact groups SI and DI.

### 3.6. Functional diversity

The biological traits analysis suggested depth as the strongest factor to influence variations between trait contributions, yet with a relevant temporal variation (Fig. 5). Indeed, PERMANOVA tests revealed that for almost all traits (“Feeding mode”, “Life span”, “Body size”, “Motility”, “Position”, and “Larval type”) these two factors (Depth and Sampling Period) were found significant ( $p < 0.005$ ), not only as single factors but also as interaction (Depth x Sampling Period). The Treatment factor was not significant for the different trait compositions, with the exception of “Sensitivity to disturbance” where this factor was also significant ( $p = 0.02$ ) (Table S7).

The relationship between species distribution (R) and environmental conditions (L) was deemed significant by the global test of RLQ scores (model 2,  $p = 0.001$ ). The relationship between community composition (R) and functional traits (Q), rather than preferences for environmental conditions, was likewise found to be significant (model 4,  $p = 0.006$ ). Thus, model 6, which tests whether traits are significantly related to the environment, was significant as it adopted the higher  $p$  value of the two models ( $p = 0.006$ ). About 98.3% of the total variance was explained by the first RLQ axis, and only 1.1% of the total variance was explained by the second one. Axis eigenvalues for the separate analyses (PCA, CA, FCA) are provided in Table S8. The adjusted Fourth Corner test did not reveal significant relationships between the specific environmental variables and trait modalities ( $p > 0.05$ ).

The RLQ analysis was not able to identify the areas within the impact zones of the mussel farm, instead separating areas based on depth (Fig. 6). A noticeable association between all of the environmental parameters was shown in the negative part of RLQ axis 1 (Fig. 7), where all the DC and DI stations were located. This part of RLQ axis 1



**Fig. 2.** An nMDS plot depicting community structure based on sampling period (I, II, III and IV) and groups regarding depth and treatment (SC- Shallow Control; SI – Shallow Impact; DC – Deep Control; DI – Deep Impact).

**Table 4**

SIMPER results for spatial (within each sampling period I, II, III, IV) and temporal (within each sampling group: SC – Shallow Control, SI – Shallow Impact, DC – Deep Control, DI – Deep Impact) dissimilarities.

Spatial Dissimilarity				Temporal Dissimilarity			
I	SC	SI	DC	SC	I	II	III
	76.09	88.06	87.08	79.10	83.70	81.84	
	86.21	88.84	66.74	86.74	85.22	84.80	
				IV			
II	SC	SI	DC	SI	I	II	III
	74.11	88.25	84.86	76.74	81.66	78.00	
	86.68	82.21	66.57	83.42	79.67	79.98	
				IV			
III	SC	SI	DC	DC	I	II	III
	70.75	87.22	85.88	62.85	72.26	72.10	
	85.41	82.75	71.86	71.36	70.96	71.93	
				IV			
IV	SC	SI	DC	DI	I	II	III
	71.42	84.27	83.00	59.26	72.13	69.78	
	81.03	79.40	65.32	72.25	70.70	70.06	
				IV			

comprised second-order opportunistic infaunal carnivores (IV; Inf; C) and surface deposit feeders with medium body length, medium lifespan, burrowing motility type, and planktonic larvae (SDF; BM; LM; Bu; Plank). Representative species associated with increased depth, OM, and trace elements included the polychaetes *Abyssoninoe hibernica* (Ahi), *Heteromastus filiformis* (Hfi), *Glycera unicornis* (Gun), *Melinna palmata* (Mpa), *Lagis koreni* (Lko), and *Lumbrineris lusitanica* (Llu) (Fig. 8).

In contrast, the positive part of RLQ axis 1 was represented by shallower sites, associated with lower concentrations of OM and trace elements and larger sediments (lower “Fines” values). This component depicted suspension feeders (SF), very small brooders with short life-spans (BVS; Broo; LS), and epibenthic swimmers, crawlers, and walkers (Epi; Sw; Cr and W) (Fig. 7). These depictions match the functional traits of the following taxa, which represent the shallow communities on the positive part of RLQ axis 1: the amphipods *Photis* spp. (Pht) and *Urothoe hesperiae* (Uhe), the bivalve *Spisula solida* (Sso), the tanaidacean *Apsudopsis* spp. (Aps), the hermit crab *Diogenes pugilator* (Dpu), and phoronids (Pho) (Fig. 8).

#### 4. Discussion

The results of this study suggest that the longline mussel culture settled in the Aquaculture Production Area (APA) did not affect the underlying macrobenthic communities in terms of species composition or functional diversity. Since the main trend visible throughout the study was the demarcation of communities according to depth, any influence that the mussel culture had on the surrounding environment may have been superseded by natural conditions. Over time, the underlying communities retained the diversity trends and functional trait profiles observed during the sampling periods prior to mussel settlement.

##### 4.1. Effects of APA on the underlying sediments

Sediment organic matter and elemental concentrations tended to increase with depth while sediment size was notably finer in deeper areas. These results were expected because the physical properties of sediments are linked to particle settling velocity, current strength, and wave height (Seiderer and Newell, 1999), factors which are



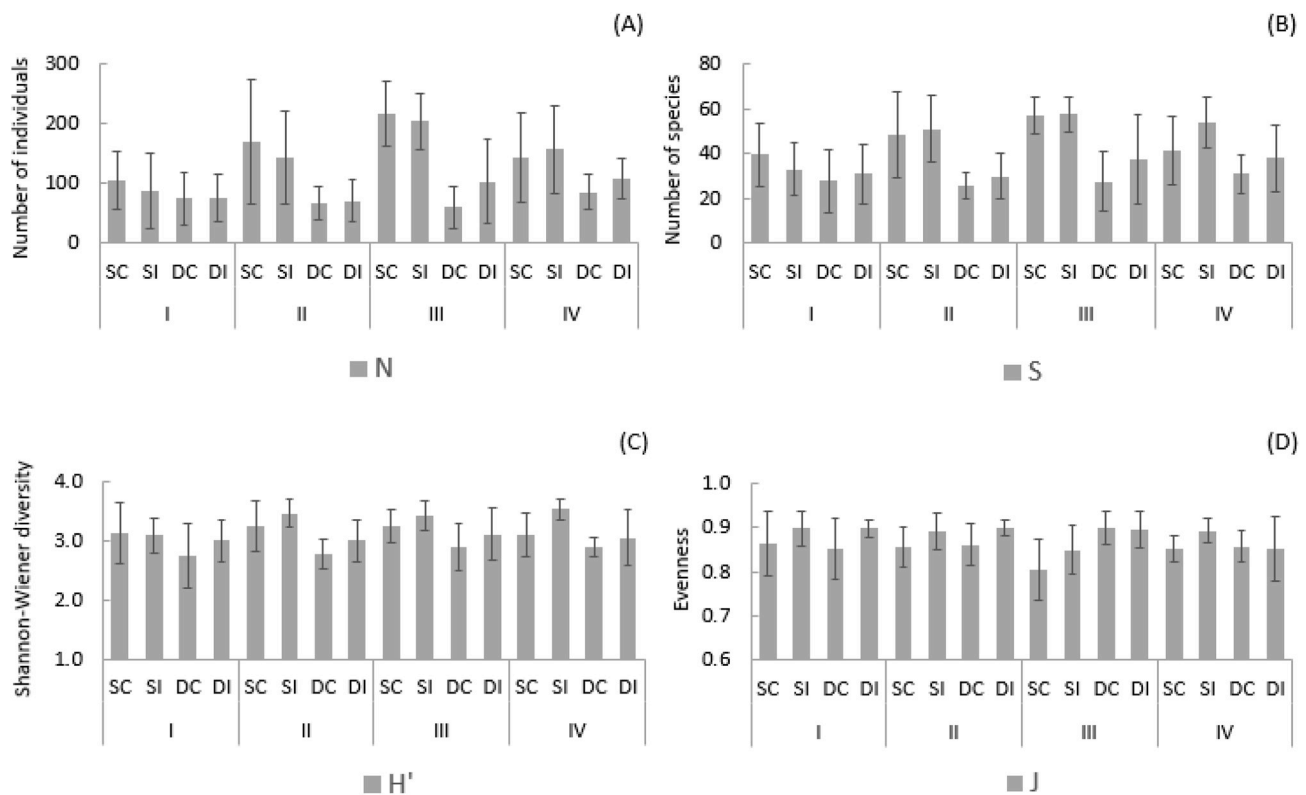
**Table 5**

Top 5 contributing taxa and their mean contribution (%) to the total abundance of each of the sampling groups (SC – Shallow Control, SI – Shallow Impact, DC – Deep Control, DI – Deep Impact) and sampling periods (I – April 2010, II – September 2010 before mussel settlement, III - June 2014, IV - September 2014 after mussel settlement). The corresponding AMBI ecological groups (used as indicator of sensitivity to disturbance) for each taxon are also provided (AG\_I – Sensitive, AG\_II – Indifferent, AG\_III – Tolerant, AG\_IV – Second-order Opportunistic, AG\_V – First-order Opportunistic).

SC	Cont. (%)	AMBI	SI	Cont. (%)	AMBI	DC	Cont. (%)	AMBI	DI	Cont. (%)	AMBI	
I	<i>N. cirrosa</i>	11.35	AG_II	Nemertea	10.36	AG_II	<i>L. lusitana</i>	21.23	AG_II	<i>L. lusitana</i>	15.25	AG_II
	<i>D. pugilator</i>	9.46	AG_II	<i>D. pugilator</i>	9.58	AG_II	<i>A. lindstroemi</i>	19.97	AG_I	<i>Notomastus</i> sp.	9.64	AG_III
	Nemertea	6.46	AG_II	<i>N. cirrosa</i>	8.39	AG_II	<i>H. filiformis</i>	6.49	AG_IV	<i>A. hibernica</i>	8.04	AG_I
	<i>S. bombyx</i>	5.34	AG_III	<i>Ampelisca</i> spp.	6.33	AG_I	<i>A. hibernica</i>	5.29	AG_I	<i>A. lindstroemi</i>	7.43	AG_I
	<i>Mediomastus</i> sp.	5.08	AG_III	<i>Mediomastus</i> sp.	5.17	AG_III	<i>P. paradoxa</i>	4.73	AG_II	Nemertea	6.73	AG_II
II	Nemertea	9.74	AG_II	<i>Mediomastus</i> sp.	7.91	AG_III	<i>L. lusitana</i>	22.49	AG_II	<i>L. lusitana</i>	15.24	AG_II
	<i>Mediomastus</i> sp.	5.89	AG_III	Nemertea	14.65	AG_II	<i>A. lindstroemi</i>	17.38	AG_I	<i>Notomastus</i> sp.	10.75	AG_III
	<i>Ampelisca</i> spp.	5.02	AG_I	<i>S. bombyx</i>	21.29	AG_III	<i>A. hibernica</i>	8.33	AG_I	Nemertea	9.06	AG_II
	<i>B. lanceolatum</i>	3.55	AG_I	<i>E. vittata</i>	26.25	AG_II	Nemertea	8.29	AG_II	<i>A. lindstroemi</i>	8.03	AG_I
	<i>N. cirrosa</i>	3.44	AG_II	<i>A. lindstroemi</i>	31.05	AG_I	<i>M. glebifex</i>	3.88	AG_I	<i>L. koreni</i>	6.54	AG_IV
III	<i>S. solida</i>	11.3	AG_I	Nemertea	8.34	AG_II	<i>Ampelisca</i> spp.	17.19	AG_I	<i>L. lusitana</i>	12.4	AG_II
	<i>C. gibba</i>	6.25	AG_IV	<i>U. hesperiae</i>	6.17	AG_I	<i>L. lusitana</i>	9.85	AG_II	<i>Ampelisca</i> spp.	11.48	AG_I
	Nemertea	5.88	AG_II	<i>S. bombyx</i>	4.58	AG_III	Nemertea	9.64	AG_II	<i>L. koreni</i>	9.7	AG_IV
	<i>Ampelisca</i> spp.	5.17	AG_I	<i>Mediomastus</i> sp.	3.83	AG_III	<i>A. lindstroemi</i>	8.34	AG_I	<i>A. lindstroemi</i>	7.71	AG_I
	<i>T. phaseolina</i>	4.63	AG_I	<i>L. murata</i>	3.29	AG_I	<i>A. prismatica</i>	8.33	AG_I	<i>A. hibernica</i>	4.66	AG_I
IV	Nemertea	9.5	AG_II	<i>U. pulchella</i>	8.37	AG_II	<i>Ampelisca</i> spp.	15.4	AG_I	<i>L. lusitana</i>	12.07	AG_II
	<i>O. albida</i>	8.28	AG_II	Nemertea	6.44	AG_II	<i>A. lindstroemi</i>	14.84	AG_I	<i>Ampelisca</i> spp.	11.79	AG_I
	<i>Ampelisca</i> spp.	7.63	AG_I	<i>Ampelisca</i> spp.	5.67	AG_I	<i>L. lusitana</i>	9.36	AG_II	Nemertea	9.29	AG_II
	<i>U. pulchella</i>	6.67	AG_II	<i>A. lindstroemi</i>	5.43	AG_I	<i>T. flexuosa</i>	5.79	AG_III	<i>A. lindstroemi</i>	8.38	AG_I
	<i>Apeudopsis</i> spp.	6.16	AG_III	<i>Apeudopsis</i> spp.	5.14	AG_III	<i>Acoetes</i> sp.	4.28	AG_I	<i>Apeudopsis</i> spp.	4.78	AG_III

understandably weaker in deep as opposed to shallow areas. Fine suspended particles travel more but start to settle as current speed decreases in deeper areas (Bell and Barnes, 2002). This results from the slopeface topography of the seafloor underlying the mussel longlines, which was possibly subjected to the succession of erosion or

redeposition cycles caused by the downward transport of organic sedimentary material via turbidites and bottom water currents (Arndt et al., 2013). This increasing trend on the concentration of sedimentary organic matter with depth has been observed in the Algarve coastal shelf (Carvalho et al., 2018; Costa e Silva et al., 2008).



**Fig. 3.** (A) Mean number of individuals (“N” ± standard deviation), (B) Species Richness (“S” ± standard deviation), (C) Shannon-Wiener diversity (H’ ± standard deviation) and (D) – Pielou evenness (J ± standard deviation) for each of the sampling groups (SC – Shallow Control, SI – Shallow Impact, DC – Deep Control, DI – Deep Impact) along different sampling periods (I,II,III and IV).

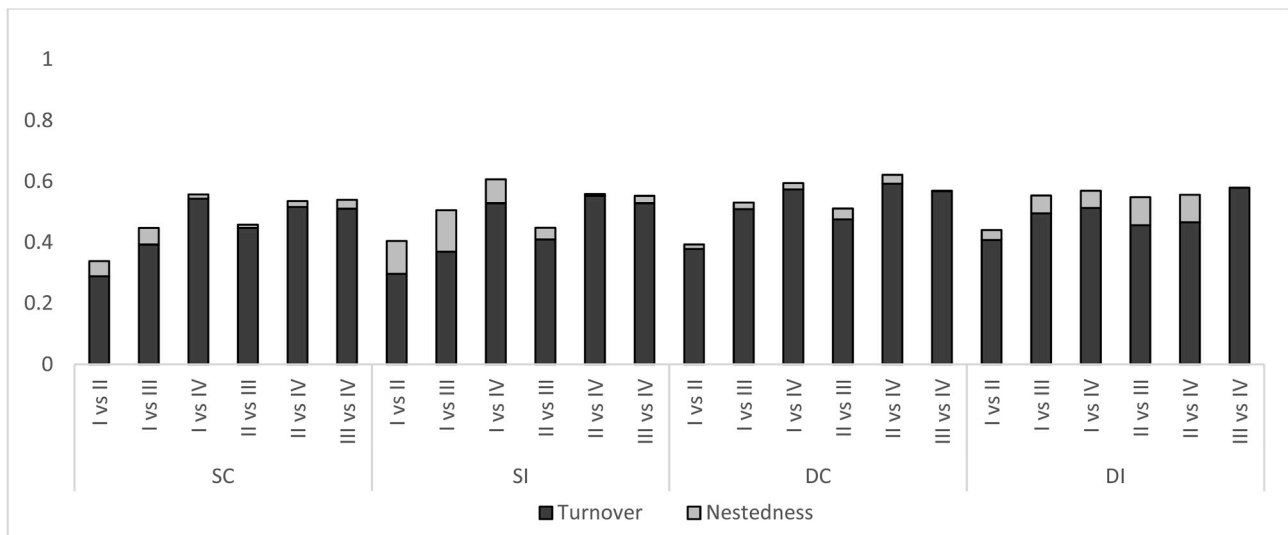


Fig. 4. Beta diversity partitioned into Turnover and Nestedness for temporal comparisons within each of the sampling groups (SC – Shallow Control, SI – Shallow Impact, DC – Deep Control, DI – Deep Impact).

Organic matter has a higher affinity with fine-grained sediments (Keil and Mayer, 2014), that roles out trace element association with particles from both natural and anthropogenic origins. The similarity of trace element concentrations before and after the establishment of the mussel farm points to natural causes, which can lead to strongly elevated concentrations of major and trace elements (van der Veer, 2006), as the cause for their occurrence. A combination of factors, such as the hydrographical conditions and the high degradation rate of mussel pseudofeces (Mahmoudi et al., 2008; Zúñiga et al., 2014), may have minimized the settlement rate of organic matter in areas underlying the APA. Exposed mussel farms have previously been linked to considerably reduced mussel biodeposits on the seabed as sufficient energy can resuspend biodeposits that have settled and disperse these over a wide area (Hartstein and Stevens, 2005), as opposed to sheltered mussel farms where the sedimentation rate can be up to threefold that of nearby reference areas (Dahlbäck and Gunnarsson, 1981). The exposed profile of the offshore APA likely subjected it to favorable dispersion dynamics and eliminated the possibility of bulk biodeposit settlement on the seabed.

#### 4.2. Macrobenthic community composition of the APA seabed

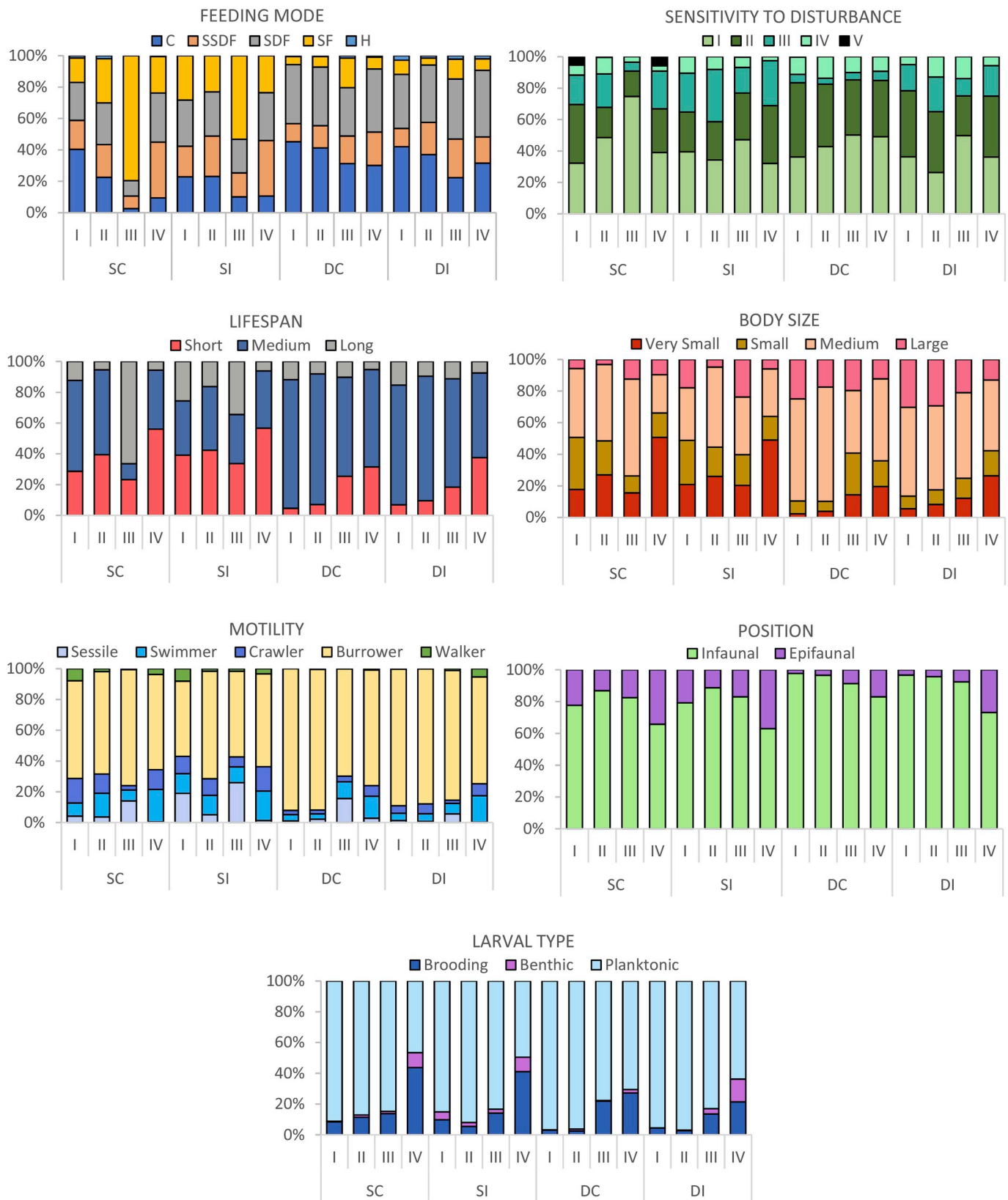
The low level of dominance and the high number of singletons contributed to a high species richness: abundance ratio (Alsaffar et al., 2019). The ratio of major species groups found in the APA seabed followed the pattern for macrobenthic community composition reported in southern Portugal (Carvalho et al., 2018; Martins et al., 2013a) and worldwide (Ellingsen, 2001; Joydas et al., 2015; Sellanes et al., 2007), with polychaetes being the largest group and having the most diverse taxa, followed by crustaceans and molluscs. Multivariate analyses carried out in this study suggested that depth and sampling period shaped the macrobenthic communities under the mussel farm as both the shallow and deep areas had their own unique community compositions which were characterized by a retention of their dominant sensitive fauna even after mussel settlement. The *Urothoe* amphipods, which are considered to have a preference for unpolluted areas because of their sensitivity to organic enrichment (Borja et al., 2000; Dauvin et al., 2017), were among the species which dominated the sites allocated as shallow impact areas after the settlement of mussels in the APA. Furthermore, *Ampelisca*, a genus of amphipods sensitive to common sources of pollution (Larrain et al., 1998), were also among the dominant taxa in both shallow and deep impact sites. In a scenario of bulk sedimentation of biodeposits, species tolerant towards hypoxic or

anoxic conditions would be expected to dominate. Indeed, this was the trend observed in previous studies, where organic matter inputs under offshore farms significantly shaped the associated benthic communities by boosting the occurrence of hypoxia-tolerant species (Lee et al., 2016; Stenton-Dozey et al., 2001; Ysebaert et al., 2009). Only one opportunistic species, the polychaete *Lagis koreni* (AG\_IV), was found in considerable amounts in an impact site. This species has been found to be dominant in areas with organic enrichment due to dredging (Robinson et al., 2005) and sewage disposal (Eleftheriou et al., 1982). In this study, however, the occurrence of *L. koreni* cannot be attributed to the mussel farm as its dominance was already established in the deep area prior to mussel settlement.

The similarity of within-depth groups (SC-SI and DC-DI) also suggests that the natural conditions associated with depth (such as light, pressure, tidal variation, advective processes, temperature) along with the preferences of each species, regulate which species can thrive (Kröncke et al., 2013; McLachlan and Dorvlo, 2005; Rosenberg, 1995; Snelgrove, 1998). The depth-related patterns of benthic community conditions were similarly affected by temporal changes. Over time, the communities in each of the sampling areas changed at a similar rate, without reverting back to the original compositions at the first period of sampling. Yet, species composition in the deeper areas was more conservative, spatially and temporally. This was possibly due to natural physical conditions being more stable in seaward or deeper benthic areas (Rakocinski et al., 1993; Zacharias and Roff, 2001). Since benthic communities underlying the APA were not affected by the anticipated organic enrichment, as natural conditions overruled as the main structuring element, it followed that the community structure and composition of the macrobenthic communities were not affected.

#### 4.3. Effects of the APA on alpha diversity

The trend for soft-bottom species diversity along nearshore shelves (up to 30 m) is an increase in general abundance, diversity, and species richness with depth (Armonies et al., 2014; Carvalho et al., 2018; Dolbeth et al., 2007; Freitas et al., 2011). The opposite is more typical for offshore shelves, which are beyond the seaward limit of wave effects on the macrobenthos (Armonies et al., 2014; Dauvin et al., 2013; van Hoey et al., 2004) and typically characterized by fine homogeneous sediments (Huang et al., 2013). This was the pattern observed in this study, where the surveyed macrobenthic communities decreased in abundance, diversity, and species richness as the depth increased. Along offshore areas of the Portuguese coastal shelf, the same reduction



**Fig. 5.** Trait modality composition for each sampling group (SC – Shallow Control, SI – Shallow Impact, DC – Deep Control, DI – Deep Impact) per sampling period (I,II,III and IV). Composition is based on each of the studied traits (Feeding mode, Sensitivity to disturbance, Lifespan, Body size, Motility, Position in sediment, Larval type) for the top 50 taxa.

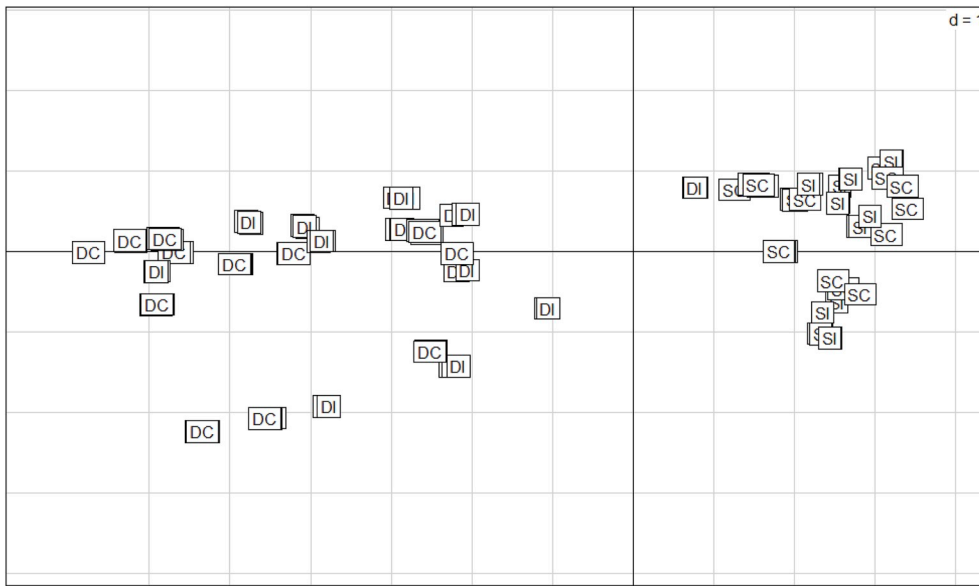


Fig. 6. Projection of sampling groups (SC – Shallow Control, SI – Shallow Impact, DC – Deep Control, DI – Deep Impact) across all periods of sampling. The scale of the graph is depicted by the d value in the upper right-hand corner.

in alpha diversity has been observed for polychaetes, crustaceans, and molluscs in areas without a marked anthropogenic influence (Martins et al., 2013b, 2014; Sampaio et al., 2016). This phenomenon has been observed in offshore coastal shelves of other temperate zones and has been attributed to a reduction in sediment size (Bergen et al., 2001; van Hoey et al., 2004) and variations of sediment organic matter (Coll et al., 2010; Karakassis and Eleftheriou, 1997). Since the deeper areas were associated with muddy sediments, whereas the shallower areas were associated with coarser sediments, it is also possible to attribute the poorer macrobenthic diversity in the deep with the elevated rate of anaerobic processes and intermediate oxygen consumption rates that

naturally take place there (Braeckman et al., 2014).

A secondary pattern of diversity was the increase in Shannon-Wiener diversity ( $H'$ ) scores in the stations underlying the APA. In fact, previous studies have attributed it to the fall-off of shell-hash accompanied by the pre-existence of highly oxygenated sediments (McKindsey et al., 2011; Wilding, 2012; Wilding and Nickell, 2013; Wong and O'Shea, 2011). Although mussel pseudofeces were widely dispersed, it is possible that shell-hash was able to settle in considerable concentrations under the farm (Wilding and Nickell, 2013), given their higher weight. Along with any remaining historical traces of aquaculture projects in the APA, this may have increased the heterogeneity

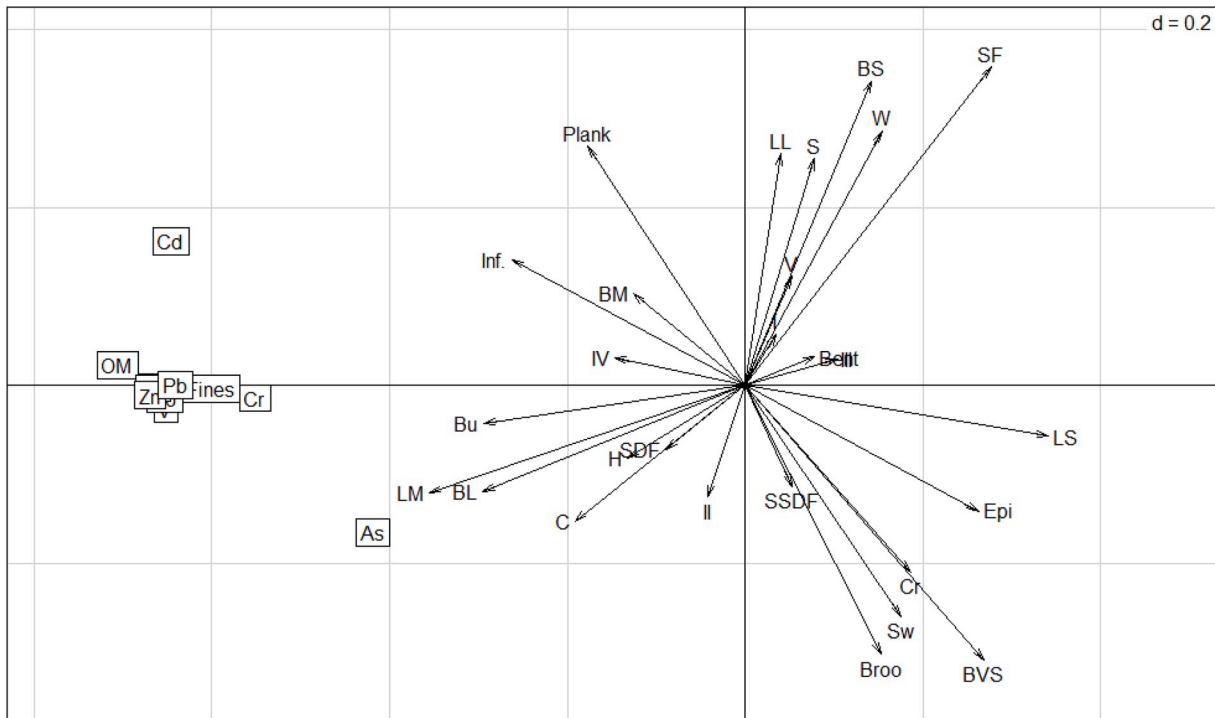


Fig. 7. RLQ projection of functional traits and environmental variables, as defined by the first two axes. Refer Table 1 for the full list of functional trait modalities. Environmental variables located behind Pb include Depth, Fines (percentage of fine particles), V, Ni, Co, Cu, and Zn. The scale of the graph is depicted by the d value in the upper right-hand corner.





possible to infer that, for both control and impact sites, both dominant and rare species were replaced over time. Overall, beta diversity scores also showed that the communities became more and more dissimilar to the original community and that the same degrees of dissimilarity over time were observed for impact sites and their references. This suggests that the drivers promoting biodiversity are similar across depths despite the differences in alpha diversity (Alsaffar et al., 2019).

Although general beta diversity was not sensitive to activities in the APA area, partitioning the beta diversity scores revealed another trend for the impact areas. Species turnover was the dominant pattern in all areas, and nestedness scores, although minimal, were reflected to a greater degree in the impact areas. The sensitivity of partitioning general beta diversity into turnover and nestedness scores to detect anthropogenic activity on macroinvertebrate communities has been noted previously (Dimitriadis and Koutsoubas, 2011; Gutiérrez-Canovas et al., 2013; Piló et al., 2019). Turnover, the main pattern observed in this study, hinted that the natural environmental parameters and neutral processes shaped the communities (Mousing et al., 2016; Socolar et al., 2016) instead of the presence of mussel longlines. The dominance of turnover, which is known to be result of the directional replacement of species, must be highlighted as it can serve as an indication of low anthropogenic disturbance (Baselga, 2010; Medeiros et al., 2016). The replacement of species is a normal process in healthy ecosystems, and suggests that functional roles are conserved in the face of natural changes over space and time (Magurran and Henderson, 2010). Inversely, a greater degree of nestedness is indicative of species loss resulting in a subset of species at richer sites (Baselga, 2010). This can be caused by both natural and anthropogenic stressors that drive the extinction of some species, although this may be followed by the recolonization of others (Barros et al., 2014; Baselga, 2010; Dobrovolski et al., 2012). Nested sites typically have subsets of species in the richer sites, decreasing their species richness in comparison (Flores et al., 2018; Ulrich and Almeida-Neto, 2012). In this study, the species richness in the slightly more nested impact sites was considered similar to that of the control sites. This may be due to the exclusive species found in the impact sites (considering the dominant pattern of turnover) and the species overlap across sites, which is not a requirement for a difference in species richness between sites (Almeida-Neto et al., 2012). Thus, a minimal amount of species may have been lost and replaced by more diverse taxa, resulting in the high rates of turnover observed for all areas.

Since the trend of increased nestedness and species diversity was observed in the first two sampling periods, these results could not be attributed to the mussel farm. Consequently, the appearance of higher nestedness and higher species diversity even in the sampling periods before mussel settlement may be a result of minimal leftover impact, or traces of disturbance history, from the other projects conducted in the area before sampling occurred. The dominant pattern of turnover coupled with the prevailing trend of depth influencing the diversity of the macrobenthic communities is indicative of high heterogeneity and dispersion in the offshore APA. Likewise, the fact that species richness remained the same within depth groups, even if nestedness was slightly higher in impact areas, ruled out the potential of the APA to critically disrupt the underlying species composition. The beta diversity results, coupled with the alpha diversity scores, confirm that alpha and beta diversity patterns would differ spatially, mirroring the effects of the APA in receiving communities.

#### 4.5. Effects of the APA on functional diversity

For all of the traits, temporal fluctuations of the ratios of modality scores was evident, but these fluctuations were largely conserved across treatments for each depth group (e.g. epifaunal taxa increased in sampling period IV for both SC and SI, as did very small taxa with short lifespans), conveying the key influence of depth. The distribution of traits and the environmental conditions in each area were considered to

be significantly related by the RLQ analysis, and the co-structure between species abundance and traits per station was non-random. This was confirmed by the results of the global RLQ test for model 6, which tested for the significance of the traits-environment relationship (Dray et al., 2014), supporting the idea that conditions in the environment determine which functional traits are expressed (Bremner et al., 2006). As all environmental parameters followed the gradient set by depth in this study, the functional traits were likewise suggested to be defined according to depth. The capacity of depth to shift traits in a marine setting has been highlighted before (Bonsdorff and Pearson, 1999; Pacheco et al., 2011b; Tuya et al., 2017), and since the deeper areas were associated with higher concentrations of organic matter and metals, opportunistic species were expected to be more prevalent in these areas. This was true in the cases of some species, although their presence could not be attributed to the APA.

Shallow environmental conditions favored the occurrence of long-lived suspension feeding bivalves and short-lived highly-sensitive amphipods. They also allowed the occurrence of taxa having larvae with a lower dispersion capacity (benthic and brooding), and motility and position which involved more time above the sediment or at the water-sediment interface. Organic matter has previously been shown to shape functional diversity by selecting for small to medium short-lived opportunistic fauna that are typically motile, with limited penetration into the sediment, and capable of surface deposit feeding behavior (Gusmao et al., 2016; Nasi et al., 2018; Ysaebert et al., 2009). Due to the smothering effect of organic debris, the abundance of long-lived burrowing and suspension-feeding bivalves typically increases in sites with less organic matter contamination (Christensen et al., 2003; Kaspar et al., 1985; Stenton-Dozey et al., 1999; Ysaebert et al., 2009). Feeding behavior and lifespan are important functional traits because of their relevance to the short- and long-term structure of invertebrate communities (Pearson and Rosenberg, 1978). Considering the lower concentrations of organic matter in the shallow areas of this study, it followed that a higher number of suspension-feeders, dominated by the bivalve *Spisula solida* and Phoronida, a phylum of typically sessile invertebrates, were found in the shallow stations. It is also highly likely that *S. solida* was restricted to the shallow stations because of its natural depth preference, as specimens are normally found in depths ranging from 5 to 15 m (Gaspar and Monteiro, 1999; Theede et al., 1969) and up to 22 m along the Algarve coastal shelf (Carvalho et al., 2018). Although the presence of sessile macrofauna has also been linked to shallower water depths along a coastal area (Bremner et al., 2003) and coarser sediments (Bolam et al., 2017), their occurrence, represented by Phoronida, may be a strong indication of low organic matter input. Sessile organisms cannot freely relocate, unlike motile organisms which are able to avoid hotspots of contamination (Nasi et al., 2018; Ward et al., 2013).

Amphipods belonging to the genera *Photis* and *Urothoe* were likewise dominant features of shallow stations and are categorized under AMBI groups I and II, which have been shown to decrease in abundance in areas affected by organic enrichment from fish farms (Fernandez-Gonzalez et al., 2013). The restriction of several amphipod species to the shallow areas may, however, also be more a function of depth and sediment type rather than organic matter concentration, as the aforementioned genera have been noted to be abundant in depths ranging from 10 to 25 m and in coarse to fine sediments in Portuguese coastal waters (Carvalho et al., 2012). Interestingly, the amphipod *Ampelisca* spp., known to be more tolerant to pollution (de-la-Ossa-Carretero et al., 2012), was present in the shallow stations even prior to mussel settlement, implying that environmental conditions in the shallow areas may have suited the preferences of AMBI-tolerant species which tend to prefer more enriched sediments (Pinedo et al., 2014; Rabaoui et al., 2015). Since none of the AMBI categories were significantly related to the environmental parameters, it cannot fully be relied upon to confirm that shallow areas had more sensitive species and deep areas had more opportunists. Rather, AMBI groups were spread across all areas

regardless of depth, organic matter, or trace element concentrations.

The decapod *Diogenes pugilator* was not entirely representative of communities in the shallow area, but it had a marked affinity towards conditions in the shallow stations. This species is an epifaunal carnivore that is indifferent to organic enrichment (Borja et al., 2000). The functional traits of this species allow it to forage and freely move across the sediment surface, making it an ideal candidate for food-limiting areas where it can quickly relocate to patches with increased food supply (Grant et al., 2012; Macleod et al., 2008). Small epibenthic predators also appear to be more affected by sediment size and organic carbon concentrations, with abundances increasing in low stressed areas (Papageorgiou et al., 2009). The increase of epibenthic macrofauna linked to mussel aquaculture has been reported before and has been attributed to an increase in habitat heterogeneity and food supply in areas close to and around mussel aquaculture (D'Amours et al., 2008). In this study, organic matter was equally less concentrated across all shallow stations, and it is possible that these stations provided a more heterogeneous habitat, as depicted by the increase in diversity and species richness in all shallow stations.

Deep environmental conditions had considerably higher organic matter and metal concentrations, along with finer sediments. To an extent, the presence of higher trace element concentrations and lower oxygen levels in the sediment may have structured the community by favoring the dominance of some opportunistic subsurface-deposit feeding polychaetes that have a tolerance for metals and anoxia, such as *Heteromastus filiformis* and *Lagis koreni*. Survival via anaerobic metabolic processes and an increase in dominance after disturbance events inducing oxygen-deficiency have been noted in these species (Gogina et al., 2014; Ryu et al., 2011). Furthermore, *H. filiformis* is known to be capable of metal bioaccumulation and can selectively exploit contaminated areas (Ahn et al., 1995; Ward and Hutchings, 1996). Tolerant surface-deposit feeders, such as the polychaetes *Notomastus* sp. and *Melinna palmata*, which are typically found in enriched areas (Al-Farraj Saleh, 2012; Dauvin, 2000), were also linked to the deeper macrobenthic communities. The dominance of deposit feeders in contaminated sediments has been highlighted previously and associated with low hydrodynamism, smaller-sized particles, and elevated metal concentrations (Grant et al., 2012; Lopez and Levinton, 1987; Piló et al., 2016). An increase in the abundance of *M. palmata* has been recorded in areas with muddy sand (Blanchet et al., 2005; Uysal et al., 2002). Additionally, *M. palmata* is capable of integrating copper into its tissues as a defense against predation (Gibbs et al., 1981) and has been shown to have an affinity for the more metal-enriched muddy areas of this study. Despite these findings, it is important to note that this species was found in the deep control and impact stations even prior to mussel settlement and that these stations had similar organic matter and metal concentrations throughout the sampling periods of this study. This hints that some of the deep sampling stations may be naturally hypoxic due to the reduced strength of hydrodynamics and the finer sediments, or they may have previously been affected by aquaculture activities in the APA prior to the first sampling period in 2010. Furthermore, the presence of large infaunal carnivores with medium to long lifespans such as *Abyssoninoe hibernica*, *Glycera unicornis*, and *Lumbrineris lusitanica* suggests that the impact of organic enrichment in deeper areas may not have been severe enough to structure the community towards an opportunism-dominated functional ecology, considering that large macrofauna are usually associated with absent or low inputs of a disturbance gradient (de Juan et al., 2007; Pearson and Rosenberg, 1978; Thrush et al., 2006).

Larval type is relevant in depicting the dispersal of invertebrates as it gives clues about the recovery potential of species in disturbed sites (Pacheco et al., 2013). In this study, the epifaunal tanaid *Apsseudopsis* spp. was one of the few species with multiple stages of benthic larval development (Esquete et al., 2012), and it was dominant in both the shallow control and impact areas. Benthic larvae can be particularly sensitive to pollution because pollutants tend to have higher

concentrations in the sediment than in the water column (Mileikovsky, 1970). However, considering *Apsseudopsis*' tolerant nature towards pollutants (Ateş et al., 2014), its presence in the shallow areas cannot be relied upon to depict lower levels of pollution. On the other hand, a prevalence of species with planktonic larvae were observed in the deeper stations, although this relationship was not considered significant, possibly due to the homogeneous dominance of this modality over other types of larval development (Paganelli et al., 2012). Planktonic larvae may be more resistant to disturbance due to their greater dispersal potential and capacity to feed in the water column, making this a common reproductive strategy in highly variable benthic environments (Marchini et al., 2008; McHugh and Fong, 2002; Paganelli et al., 2012).

Isolating the drivers and effects of each of the environmental parameters is highly complex, especially due to the influence of natural conditions in this study, but it is likely that these natural conditions shaped the functional diversity of the macrobenthic communities more than the APA. That the Fourth Corner test did not strongly relate any traits with the shallow or deep area was another indicator that the stations, across both depths, had similar available niches. The multivariate data analyses provided by the RLQ and Fourth Corner tests integrated the environmental, taxonomic, and functional diversity and successfully related functional ecology to depth-related conditions. Given the results, the functional structure and organization of local macrobenthic communities seem to not differ between impact and reference areas. The functional analysis complemented alpha diversity results by showing that, although the deep stations are less diverse taxonomically, they share the functional diversity found in the more taxonomically diverse shallow areas.

#### 4.6. A multimetric approach to detect aquaculture impact

The use of more than one type of statistical test and the corroboration of their results contributed to the completeness of the overall findings of this study, which detected that variation in macrobenthic communities was largely due to depth rather than impacts from the APA. Both quantitative and qualitative tests must be used to better understand how aquaculture can affect the surrounding communities. This study showed that innovative multivariate approaches are able to not only complement classical statistical tests, but also provide a clearer picture of ecological impact, by both natural and anthropogenic factors. The beta diversity and functional trait analyses aided in determining how the communities changed under various pressures by placing relevance on rare species (based on the Sorensen presence-absence method) and by focusing on dominant species, respectively. They depicted whether the community compositions changed due to species loss or natural species replacement, and they showed how community composition varied due to differences in the functional ecology of stations. Classical statistical tests may not always be sensitive to ecological change in different environments because they are largely based on abundance data. Additionally, the complexity of the offshore system due to varied levels of hydrodynamic impact and bulk water exchange may have induced a degree of patchiness across stations, requiring the need for beta diversity tests which placed more weight on the rare species (Piló et al., 2019). Because taxa understandably differ due to their depth preferences, and communities in shallow and deep areas have different ecological processes due to the differences in natural conditions, it was essential to use biological traits to detect opportunism in this study. Species status may vary geographically and over time, but traits are enduring characteristics of species and/or populations (Costello et al., 2015). However, functional trait analysis remains to be an ecological test in development as detailed fundamental trait data for many species are still lacking (Marchini et al., 2008; Tyler et al., 2012). The same may be said for the use of all functional approaches to understanding the ecological impacts of disturbance. The community-function relationship changes depending on the drivers of

environmental change (Thrush et al., 2017), and considering that a species usually contributes to more than one function (Degen et al., 2018), interpreting the ecological implications of anthropogenic disturbance can be a highly complicated task.

#### 4.7. Implications for offshore aquaculture development and management

Although it was not possible to detect the degree of impact from the APA, the capacity of natural environmental factors to mask any impact from the mussel aquaculture activity was apparent. The results of this study highlighted the importance of location when it comes to the development and management of aquaculture farms. The APA was situated in an offshore area where currents and waves likely provided the forces necessary to thoroughly disperse organic matter before it could settle on and mix with the underlying sediments. This emphasizes the potential of offshore farming as an alternative to nearshore/coastal or lagoon-based aquaculture methods. The methodology presented in this study would aid stakeholders of aquaculture farms in evaluating the degree of resistance or resilience of the marine habitats around their farms. The capacity of natural factors to supersede the effects of biodeposition, as shown in this study, suggest that the APA is located in a highly resistant marine environment. However, continued monitoring of the underlying benthic communities, along with reconsiderations of site selection (e.g. greater distance from the APA) for the control sites of the study, may influence impact detection. Coupled with the expansion of fundamental trait data of macrobenthic species and the integration of other scientific disciplines to assess short- and long-term changes in the chemical and physical profiles under offshore mussel farms, this multimeric approach may reveal more clues about the resistance of these communities and would likewise provide a stronger foundation for interpreting the effects of deposition-related disturbance.

## 5. Conclusion

This study provided a multifaceted investigation into the health of macrobenthic ecosystems and how they can be evaluated as a means to detect a specific aquaculture impact. Beta diversity and functional traits, tests used for their sensitivity to detect anthropogenic disturbance, corroborated the findings of the classical statistical tests, and also provided insights into the ecological relationships of the underlying macrobenthic communities. As the buildup of organic matter and trace element concentrations was not detected in the stations underlying the farm, the main factor which shaped the taxonomic structure and functional diversity of the macrobenthic communities was depth. The APA may have influenced the increase of diversity and species richness in the underlying communities, but not to an extent which exceeded the influence of natural forces. Since the encompassing beta diversity pattern observed was turnover, and the general beta diversity scores were similar across all surveyed groups, it was possible to conclude that the APA did not generate the species loss and habitat homogeneity required to cause a negative shift in the community structure. Moreover, results from the BTA showed that the natural species replacement observed by partitioning beta diversity did not compromise the functional ecology of the macrobenthic communities. These findings suggest the importance of employing ecology-based approaches, in addition to classical statistical tests, to detect the environmental impacts of offshore aquaculture. Furthermore, they draw attention to the importance of hydrodynamic forces in shaping macrobenthic communities in offshore areas, highlighting the sustainability potential of offshore mussel farms.

## Acknowledgements

This work was funded under the FEDER Program through the project IAPAA “Avaliação do impacto da área de produção aquícola da Armona no ecossistema costeiro e comunidades locais” (PROMAR

project 31-03-01-FEP-0014). The authors would like to thank Pedro Lino, Jorge Ramos, João Araújo, Ana Mendes, and Pedro Pousão for providing valuable information about the Armona APA and Maria L. Inácio for her contribution during the chemical analysis. Special thanks are also due to all the colleagues that participated in the sampling campaigns and laboratory procedures that were vital to this project. The authors would also like to thank the editor and the three reviewers for constructive comments that highly contributed to improving the quality of the manuscript.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2019.104774>.

## References

- MSC, 2014. MSC Fisheries Certification – Requirements v2.0. London, United Kingdom.
- Achab, M., Ferreira, Ó., Alveirinho Dias, J.M., 2014. Evaluation of sedimentological and morphological changes induced by the rehabilitation of sandy beaches from the Ria Formosa barrier island system (South Portugal). *Thalassas* 30 (2), 21–31.
- Ahn, I., Kang, Y., Choi, J., 1995. The influence of industrial effluents on intertidal benthic communities in Panweol, Kyeonggi Bay (Yellow Sea) on the west coast of Korea. *Mar. Pollut. Bull.* 30, 200–206.
- Al-Farraj Saleh, A., 2012. Effects of multiple-source pollution on spatial distribution of polychaetes in Saudi Arabia. *Res. J. Environ. Toxicol.* 6, 1–12.
- Almeida-Neto, M., 2012. Rethinking the relationship between nestedness and beta diversity – a comment on Baselga (2010). *Glob. Ecol. Biogeogr.* 21, 772–777.
- Alsaffar, Z., Cúrdia, J., Borja, A., Irigoien, X., Carvalho, S., 2019. Consistent variability in beta-diversity patterns contrasts with changes in alpha-diversity along an onshore to offshore environmental gradient: the case of Red Sea soft bottom macrobenthos. *Mar. Biodivers.* 49, 247–262.
- Anderson, J.L., Asche, F., Garlock, T., Chu, J., 2017. Aquaculture: its role in the future of food. In: Schmitz, A., Kennedy, P.L., Schmitz, T.G. (Eds.), *World Agricultural Resources and Food Security (Frontiers of Economics and Globalization, vol. 17)*. Emerald Publishing Limited, pp. 159–173.
- Araújo, J., Soares, F., Pousão-Ferreira, P., 2018. Offshore production of mediterranean mussels in southern Portugal. *World Aquac.* 49, 55–57.
- Armonies, W., Buschbaum, C., Hellwig-Armonies, M., 2014. The seaward limit of wave effects on coastal macrobenthos. *Helgol. Mar. Res.* 68, 1–16.
- Arndt, S., Jørgensen, B.B., LaRowe, D.E., Middelburg, J.J., Pancost, R.D., Regnier, P., 2013. Quantifying the degradation of organic matter in marine sediments: a review and synthesis. *Earth Sci. Rev.* 123, 53–86.
- Ateş, A.S., Katağan, T., Sezgin, M., Acar, S., 2014. The response of *Apeudopsis latreillii* (Milne-Edwards, 1828) (Crustacea, Tanaidacea) to environmental variables in the Dardanelles. *Turk. J. Fish. Aquat. Sci.* 14, 113–124.
- Barros, F., Blanchet, H., Hammerstrom, K., Sauriau, P.G., Oliver, J., 2014. A framework for investigating general patterns of benthic  $\beta$ -diversity along estuaries. *Estuar. Coast Shelf Sci.* 149, 223–231.
- Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. *Glob. Ecol. Biogeogr.* 19, 134–143.
- Baselga, A., Orme, C.D.L., 2012. betapart: an R package for the study of beta-diversity. *Methods Ecol. Evol.* 3, 808–812.
- Beauchard, O., Verrísimo, H., Queirós, A.M., Herman, P.M.J., 2017. The use of multiple biological traits in marine community ecology and its potential in ecological indicator development. *Ecol. Indic.* 76, 81–96.
- Bell, J.J., Barnes, D.K.A., 2002. The relationship between sedimentation, flow rates, depth and time at Lough Hyne Marine Nature Reserve. *Ir. Nat. J.* 27 (3), 106–116.
- Bergen, M., Weisberg, S.B., Smith, R.W., Cadien, D.B., Dalkey, A., Montagne, D.E., Stull, J.K., Velarde, R.G., Ranasinghe, J.A., 2001. Relationship between depth, sediment, latitude, and the structure of benthic infaunal assemblages on the mainland shelf of southern California. *Mar. Biol.* 138 (3), 637–647.
- Blanchet, H., de Montaudouin, X., Chardy, P., Bachelet, G., 2005. Structuring factors and recent changes in subtidal macrozoobenthic communities of a coastal lagoon, Arcachon Bay (France). *Estuar. Coast Shelf Sci.* 64 (4), 561–576.
- Bolam, S.G., Garcia, C., Eggleton, J., Kenny, A.J., Buhl-Mortensen, L., Gonzalez-Mirelis, G., van Kooten, T., Dinesen, G., Hansen, J., Hiddink, J.G., Sciberras, M., Smith, C., Papadopoulou, N., Gumus, A., Van Hoey, G., Eigaard, O.R., Bastardie, F., Rijnsdorp, A.D., 2017. Differences in biological traits composition of benthic assemblages between unimpacted habitats. *Mar. Environ. Res.* 126, 1–13.
- Bonsdorff, E., Pearson, T.H., 1999. Variation in the sublittoral macrozoobenthos of the Baltic Sea along environmental gradients: a functional group approach. *Aust. J. Ecol.* 24, 312–326.
- Borcard, D., Gillet, F., Legendre, P., 2018. *Numerical Ecology with R*, second ed. Springer Nature, Cham, Switzerland. <https://doi.org/10.1007/978-3-319-71404-2>.
- Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Mar. Pollut. Bull.* 40, 1100–1114.
- Borthagaray, A.I., Carranza, A., 2007. Mussels as ecosystem engineers: their contribution to species richness in a rocky littoral community. *Acta Oecol.* 31, 243–250.



- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handiside, N., Gatward, I., Corner, R., 2010. Aquaculture: global status and trends. *Philos. Trans. R. Soc. Biol. Sci.* 365, 2897–2912.
- Braeckman, U., Yazdani Foshтоми, M., Van Gansbeke, D., Meysman, F., Soetaert, K., Vincx, M., Vanaverbeke, J., 2014. Variable importance of macrofaunal functional biodiversity for biogeochemical cycling in temperate coastal sediments. *Ecosystems* 17, 720–737.
- Bremner, J., 2008. Species' traits and ecological functioning in marine conservation and management. *J. Exp. Mar. Biol. Ecol.* 366, 37–47.
- Bremner, J., Rogers, S.I., Frid, C.L.J., 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. *Mar. Ecol. Prog. Ser.* 254, 11–25.
- Bremner, J., Rogers, S.I., Frid, C.L.J., 2006. Matching biological traits to environmental conditions in marine benthic ecosystems. *J. Mar. Syst.* 60, 302–316.
- Caetano, M., Vale, C., Cesário, R., Fonseca, N., 2008. Evidence for preferential depths of metal retention in roots of salt marsh plants. *Sci. Total Environ.* 390, 466–474.
- Callier, M.D., Richard, M., McKindsey, C.W., Archambault, P., Desrosiers, G., 2009. Responses of benthic macrofauna and biogeochemical fluxes to various levels of mussel biodeposition: an *in situ* “benthocosm” experiment. *Mar. Pollut. Bull.* 58, 1544–1553.
- Cançani, G., De Falco, G., Pergent, G., 2003. Effects of organic matter input from a fish farming facility on a *Posidonia oceanica* meadow. *Estuar. Coast Shelf Sci.* 56, 961–968.
- Cao, L., Wang, W., Yang, Y., Yang, C., Yuan, Z., Xiong, S., Diana, J., 2007. Environmental impact of aquaculture and countermeasures to aquaculture pollution in China. *Environ. Sci. Pollut. Res.* 14, 452–462.
- Carlsson, M.S., Engström, P., Lindahl, O., Ljungqvist, L., Petersen, J.K., Svanberg, L., Holmer, M., 2012. Effects of mussel farms on the benthic nitrogen cycle on the Swedish west coast. *Aquacult. Environ. Interact.* 2, 177–191.
- Carvalho, S., Cunha, M.R., Pereira, F., Pousão-Ferreira, P., Santos, M.N., Gaspar, M.B., 2012. The effect of depth and sediment type on the spatial distribution of shallow soft-bottom amphipods along the southern Portuguese coast. *Helgol. Mar. Res.* 66, 489–501.
- Carvalho, A.N., Pereira, F., Bosnic, I., Taborda, R., Drago, T., Gaspar, M.B., 2018. Sedimentary dynamics and benthic macrofauna distribution: insights from the shoreline in southern Portugal. *J. Sea Res.* 137, 9–25.
- Chamberlain, J., Fernandes, T.F., Read, P., Nickell, T.D., Davies, I.M., 2001. Impacts from biodeposits of suspended mussel (*Mytilus edulis* L.) culture on the surrounding surficial sediments. *ICES J. Mar. Sci.* 58, 411–416.
- Cherifi, H., Chebil Ajjabi, L., Sadok, S., 2018. Nutritional value of the Tunisian mussel *Mytilus galloprovincialis* with a special emphasis on lipid quality. *Food Chem.* 268, 307–314.
- Chevenet, F., Dolédec, S., Chessel, D., 1994. A fuzzy coding approach for the analysis of long-term ecological data. *Freshw. Biol.* 31, 295–309.
- Christensen, P.B., Glud, R.N., Dalsgaard, T., Gillespie, P., 2003. Impacts of longline mussel farming on oxygen and nitrogen dynamics and biological communities of coastal sediments. *Aquaculture* 218, 567–588.
- Chu, J.W.F., Curkan, C., Tunnicliffe, V., 2018. Drivers of temporal beta diversity of a benthic community in a seasonally hypoxic fjord. *Roy. Soc. Open Sci.* 5, 172284.
- Clarke, K.R., Gorley, R.N., 2006. PRIMER V6 User Manual/Tutorial. PRIMER-E, Plymouth, pp. 192.
- Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., Ballesteros, e., Nike Bianchi, C., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Frogliola, C., Galil, B.S., Gasol, J.M., Gertwagen, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., Kitsos, M.S., Koukouras, A., Lampadariou, N., Laxamana, E., López-Fé de la Cuadra, C.M., Lotze, H.K., Martin, D., Mouillot, D., Oro, D., Raicevich, S., Rius-Barile, J., Saiz-Salinas, J.I., San Vicente, C., Somot, S., Templado, J., Turon, X., Vafidis, D., Villanueva, R., Voultsiadou, E., 2010. The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. *PLoS One* 5, e11842.
- Costa e Silva, M., Pereira, P., Falcão, M., Canceleda da Fonseca, L., 2008. Caracterização das comunidades de anelídeos poliquetas ao longo de um gradiente de profundidade na região do Ancão (Algarve - Portugal). *Pan Am. J. Aquat. Sci.* 3, 214–231.
- Costa, M., Silva, R., Vitorino, J., 2001. Contribuição para o estudo de clima de agitação marítima na costa Portuguesa. In: *Proceedings of 2as Jornadas Portuguesas de Engenharia Costeira e Portuária*. International Navigation Association PIANC, Sines, Portugal, pp. 20.
- Costello, M.J., Claus, S., Dekeyser, S., Vandepitte, L., Tuama, É.Ó., Lear, D., Tyler-Walters, H., 2015. Biological and ecological traits of marine species. *PeerJ* 3, e1201.
- Coutinho, M.S., Bernardino, A.F., 2017. Spatial and seasonal changes in benthic macrofauna from two dissipative sandy beaches in Eastern Brazil. *Braz. J. Oceanogr.* 65, 666–677.
- Cranford, P.J., Hargrave, B.T., Doucette, L.I., 2009. Benthic organic enrichment from suspended mussel (*Mytilus edulis*) culture in Prince Edward Island. *Can. Aquacult.* 292, 189–196.
- Crawford, C.M., Macleod, C.K.A., Mitchell, I.M., 2003. Effects of shellfish farming on the benthic environment. *Aquaculture* 224, 117–140.
- da Costa, K.G., Nalesso, R.C., 2006. Effects of mussel farming on macrobenthic community structure in Southeastern Brazil. *Aquaculture* 258, 655–663.
- Dahlbäck, B., Gunnarsson, L.Å., 1981. Sedimentation and sulfate reduction under mussel culture. *Mar. Biol.* 63 (3), 269–275.
- Dame, R.F., 1996. *Ecology of Marine Bivalves: an Ecosystem Approach*. CRC Press.
- Danovaro, R., Corinaldesi, C., La Rosa, T., Mazzola, A., Mirtó, S., Vezzulli, L., Fabiano, M., 2003. Aquaculture impact on benthic microbes and organic matter cycling in coastal Mediterranean sediments: a synthesis. *Chem. Ecol.* 19, 59–65.
- Dauvin, J.-C., 2000. The muddy fine sand *Abra abra* – *Melinna palmata* community of the Bay of Morlaix twenty years after the *Amoco Cadiz* oil spill. *Mar. Pollut. Bull.* 40, 528–536.
- Dauvin, J.-C., Grimes, S., Bakalem, A., 2013. Marine biodiversity on the Algerian continental shelf (Mediterranean Sea). *J. Nat. Hist.* 47, 1745–1765.
- Dauvin, J.-C., Bakalem, A., Baffreau, A., Delecrin, C., Bellan, G., Lardicci, C., Balestri, E., Sarda, R., Grimes, S., 2017. The well sorted fine sand community from the western Mediterranean Sea: a resistant and resilient marine habitat under diverse human pressures. *Environ. Pollut.* 224, 336–351.
- de Juan, S., Thrush, S.F., Demestre, M., 2007. Functional changes as indicators of trawling disturbance on a benthic community located in a fishing ground (NW Mediterranean Sea). *Mar. Ecol. Prog. Ser.* 334, 117–129.
- de-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casaldueiro, F., Sánchez-Lizaso, J.L., Dauvin, J.C., 2012. Sensitivity of amphipods to sewage pollution. *Estuar. Coast Shelf Sci.* 96, 129–138.
- Degen, R., Aune, M., Bluhm, B.A., Cassidy, C., Kedra, M., Kraan, C., Vandepitte, L., Włodarska-Kowalczyk, M., Zhulay, I., Albano, P.G., Bremner, J., Grebmeier, J.M., Link, H., Morata, N., Nordström, M.C., Shojaei, M.G., Sutton, L., Zuschin, M., 2018. Trait-based approaches in rapidly changing ecosystems: a roadmap to the future polar oceans. *Ecol. Indic.* 91, 722–736.
- Dimitriadis, C., Koutsoubas, D., 2011. Functional diversity and species turnover of benthic invertebrates along a local environmental gradient induced by an aquaculture unit: the contribution of species dispersal ability and rarity. *Hydrobiologia* 670, 307–315.
- Dimitriou, P.D., Papageorgiou, N., Karakassis, I., 2017. Response of benthic macrofauna to eutrophication in a mesocosm experiment: ecosystem resilience prevents hypoxic conditions. *Front. Mar. Sci.* 4, 391.
- Dobrovolski, R., Melo, A.S., Cassemiro, F.A.S., Diniz-Filho, J.A.F., 2012. Climatic history and dispersal ability explain the relative importance of turnover and nestedness components of beta-diversity. *Glob. Ecol. Biogeogr.* 21, 191–197.
- Dolbeth, M., Ferreira, O., Teixeira, H., Marques, J.C., Dias, J.A., Pardal, M.A., 2007. Beach morphodynamic impact on a macrobenthic community along a subtidal depth gradient. *Mar. Ecol. Prog. Ser.* 352, 113–124.
- Dolédec, S., Chessel, D., ter Braak, C.J.F., Champely, S., 1996. Matching species traits to environmental variables: a new three-table ordination method. *Environ. Ecol. Stat.* 3, 143–166.
- Dray, S., Dufour, A.B., 2007. The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Softw.* 22, 1–20.
- Dray, S., Legendre, P., 2008. Testing the species traits-environment relationships: the fourth-corner problem revisited. *Ecology* 89, 3400–3412.
- Dray, S., Choler, P., Dolédec, S., Peres-Neto, P.R., Thuiller, W., Pavoine, S., ter Braak, C.J.F., 2014. Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. *Ecology* 14–21.
- D'Amours, O., Archambault, P., McKindsey, C.W., Johnson, L.E., 2008. Local enhancement of epibenthic macrofauna by aquaculture activities. *Mar. Ecol. Prog. Ser.* 371, 73–84.
- Eleftheriou, A., Moore, D.C., Basford, D.J., Robertson, M.R., 1982. Underwater experiments on the effects of sewage sludge on a marine ecosystem. *Neth. J. Sea Res.* 16, 465–473.
- Ellingsen, K.E., 2001. Biodiversity of a continental shelf soft-sediment macrobenthos community. *Mar. Ecol. Prog. Ser.* 218, 1–15.
- Ellis, R.P., Urbina, M.A., Wilson, R.W., 2016. Lessons from two high CO<sub>2</sub> worlds – future oceans and intensive aquaculture. *Glob. Chang. Biol.* 23, 2141–2148.
- Esquete, P., Bamber, R.N., Moreira, J., Troncoso, J.S., 2012. Redescription and post-marsupial development of *Apseudopsis latreillii* (Crustacea: tanaidacea). *J. Mar. Biol. Assoc. U. K.* 92, 1023–1041.
- Fabi, G., Manoukian, S., Spagnolo, A., 2009. Impact of an open-sea suspended mussel culture on macrobenthic community. *Aquaculture* 289, 54–63.
- FAO, 2018. *FAO Yearbook. Fishery and Aquaculture Statistics 2016*. Rome. pp. 104.
- FCI, 2014. *Public Consultation Draft Report: Companhia de Pescarias do Algarve rope grown Mediterranean mussel Fishery*. Inverness. p. 110.
- Fernandez-Gonzalez, V., Aguado-Giménez, F., Gairin, J.I., Sanchez-Jerez, P., 2013. Exploring patterns of variation in amphipod assemblages at multiple spatial scales: natural variability versus coastal aquaculture effect. *Aquacult. Environ. Interact.* 3, 93–105.
- Ferreira, J.G., Saurel, C., Lencart e Silva, J.D., Nunes, J.P., Vazquez, F., 2014. Modelling of interactions between inshore and offshore aquaculture. *Aquaculture* 426–427, 154–164.
- Flores, O., Seoane, J., Hevia, V., Azcárate, F.M., 2018. Spatial patterns of species richness and nestedness in ant assemblages along an elevational gradient in a Mediterranean mountain range. *PLoS One* 13, e0204787.
- Fox, J., Weisberg, S., 2011. *An R Companion to Applied Regression*, second ed. Sage, Thousand Oaks, CA, pp. 449.
- Freitas, R., Ricardo, F., Pereira, F., Sampaio, L., Carvalho, S., Gaspar, M., Quintino, V., Rodrigues, A.M., 2011. Benthic habitat mapping: concerns using a combined approach (acoustic, sediment and biological data). *Estuar. Coast Shelf Sci.* 92, 598–606.
- Froelich, H.E., Smith, A., Gentry, R.R., Halpern, B.S., 2017. Offshore aquaculture: I know it when I see it. *Front. Mar. Sci.* 4, 154.
- Fulton, E.A., Plagányi, É., Cheung, W., Blanchard, J., Watson, R., 2018. *Marine systems, food security, and future earth*. In: Beer, T., Li, J., Alverson, K. (Eds.), *Global Change and Future Earth: the Geoscience Perspective*. Cambridge University Press, UK, pp. 296–311.
- Gallardi, D., 2014. Effects of bivalve aquaculture on the environment and their possible mitigation: a review. *Fish. Aquac.* J. 5, 1000105.
- Gaspar, M.B., Monteiro, C.C., 1999. Gametogenesis and spawning in the subtidal white clam *Spisula solida* in relation to temperature. *J. Mar. Biol. Assoc. U. K.* 79, 753–755.
- Gaston, K.J., Kunin, W.E., 1997. Rare-common differences: an overview. In: Kunin, W.E., Gaston, K.J. (Eds.), *The Biology of Rarity: Causes and Consequences of Rare-Common Differences*. Chapman and Hall, London, pp. 12–29.

- Gentry, R.R., Lester, S.E., Kappel, C.V., White, C., Bell, T.W., Stevens, J., Gaines, S.D., 2016. Offshore aquaculture: spatial planning principles for sustainable development. *Ecol. Evol.* 7, 733–743.
- Gentry, R.R., Froehlich, H.E., Grimm, D., Kareiva, P., Parke, M., Rust, M., Gaines, S.D., Halpern, B.S., 2017. Mapping the global potential for marine aquaculture. *Nat. Ecol. Evol.* 1, 1317–1324.
- Gibbs, M.T., 2007. Sustainability performance indicators for suspended bivalve aquaculture activities. *Ecol. Indic.* 7, 94–107.
- Gibbs, P.E., Bryan, G.W., Ryan, K.P., 1981. Copper accumulation by the polychaete *Melinna palmata*: an antipredation mechanism? *J. Mar. Biol. Assoc. U. K.* 61, 707–725.
- Gogina, M., Darr, A., Zettler, M.L., 2014. Approach to assess consequences of hypoxia disturbance events for benthic ecosystem functioning. *J. Mar. Syst.* 129, 203–213.
- Grant, J., Cranford, P., Hargrave, B., Carreu, M., Schofield, B., Armsworthy, S., Burdett-Coutts, V., Ibarra, D., 2005. A model of aquaculture biodeposition for multiple estuaries and field validation at blue mussel (*Mytilus edulis*) culture sites in eastern Canada. *Can. J. Fish. Aquat. Sci.* 62, 1271–1285.
- Grant, C., Archambault, A., Olivier, F., McKindsey, C.W., 2012. Influence of ‘bouchot’ mussel culture on the benthic environment in a dynamic intertidal system. *Aquacult. Environ. Interact.* 2, 117–131.
- Gren, I.M., Lindahl, O., Lindqvist, M., 2009. Values of mussel farming for combatting eutrophication: an application to the Baltic Sea. *Ecol. Eng.* 35, 935–945.
- Gusmao, J.B., Brauko, K.M., Eriksson, B.K., Lana, P.C., 2016. Functional diversity of macrobenthic assemblages decreases in response to sewage discharges. *Ecol. Indic.* 66, 65–75.
- Gutiérrez-Canovas, C., Millán, A., Velasco, J., Vaughan, I.P., Ormerod, S.J., 2013. Contrasting effects of natural and anthropogenic stressors on beta diversity in river organisms. *Glob. Ecol. Biogeogr.* 22, 796–805.
- Hartstein, N.D., Rowden, A.A., 2004. Effect of biodeposits from mussel culture on macroinvertebrate assemblages at sites of different hydrodynamic regime. *Mar. Environ. Res.* 57, 339–357.
- Hartstein, N.D., Stevens, C.L., 2005. Deposition beneath long-line mussel farms. *Aquacult. Eng.* 33, 192–213.
- Hawkins, C.P., Mykrå, H., Oksanen, J., Vander Laan, J.J., 2015. Environmental disturbance can increase beta diversity of stream macroinvertebrate assemblages. *Glob. Ecol. Biogeogr.* 24, 483–494.
- Henderson, A., Gamito, S., Karakassis, I., Pederson, P., Smaal, A., 2001. Use of hydrodynamic and benthic models for managing environmental impacts of marine aquaculture. *J. Appl. Ichthyol.* 17, 163–172.
- Hixson, S.M., 2014. Fish nutrition and current issues in aquaculture: the balance in providing safe and nutritious seafood, in an environmentally sustainable manner. *J. Aquacult. Res. Dev.* 5, 234.
- Holmer, M., 2010. Environmental issues of fish farming in offshore waters: perspectives, concerns and research needs. *Aquacult. Environ. Interact.* 1, 57–70.
- Huang, Z., MacArthur, M., Przeslawski, R., Siwabessy, J., Nichol, S., Brooke, B., 2013. Predictive mapping of soft-bottom benthic biodiversity using a surrogacy approach. *Mar. Freshw. Res.* 65, 409–424.
- Jackson, D., Drumm, A., McEvoy, S., Jensen, Ø., Mendiola, D., Gabiña, G., Borg, J.A., Papageorgiou, N., Karakassis, Y., Black, K.D., 2015. A pan-European valuation of the extent, causes and cost of escape events from sea cage fish farming. *Aquaculture* 436, 21–26.
- Jansen, H.M., Van Den Burg, S., Bolman, B., Jak, R.G., Kamermans, P., Poelman, M., Stuiver, M., 2016. The feasibility of offshore aquaculture and its potential for multi-use in the North Sea. *Aquacult. Int.* 24, 735–756.
- Joydas, T.V., Qurban, M.A., Manikandan, K.P., Ashraf, T.T.M., Ali, S.M., Al-Abdulkader, K., Qasem, A., Krishnakumar, P.K., 2015. Status of macrobenthic communities in the hypersaline waters of the Gulf of Salwa, Arabian Gulf. *J. Sea Res.* 99, 34–46.
- Karakassis, I., Eleftheriou, A., 1997. The continental shelf of Crete: structure of macrobenthic communities. *Mar. Ecol. Prog. Ser.* 160, 185–196.
- Kaspar, H.F., Gillespie, P.A., Boyer, I.C., MacKenzie, A.L., 1985. Effects of mussel aquaculture on the nitrogen cycle and benthic communities in Keneperu Sound, Marlborough Sounds, New Zealand. *Mar. Biol.* 85, 127–136.
- Keddy, P.A., 1992. Assembly and response rules: two goals for predictive community ecology. *J. Veg. Sci.* 3, 157–164.
- Keeley, N., Forrest, B., Hopkins, G., Gillespie, P., Knight, B., Webb, S., Clement, D., Gardner, J., 2009. Sustainable Aquaculture in New Zealand: Review of the Ecological Effects of Farming Shellfish and Other Non-fish Species. Prepared for the Ministry of Fisheries, pp. 150 Cawthron Report No. 1476.
- Keil, R.G., Mayer, L.M., 2014. Mineral matrices and organic matter. In: Holland, H.D., Turekian, K.K. (Eds.), *Treatise in Geochemistry*, second ed. Elsevier Ltd., Oxford, pp. 337–359.
- Kröncke, I., Reiss, H., Dippner, J.W., 2013. Effects of cold winters and regime shifts on macrofauna communities in shallow coastal regions. *Estuar. Coast Shelf Sci.* 119, 79–90.
- Kuzmanovic, M., Dolédec, S., de Castro-Catala, N., Ginebreda, A., Sabater, S., Muñoz, I., Barceló, D., 2017. Environmental stressors as a driver of the trait composition of benthic macroinvertebrate assemblages in polluted Iberian rivers. *Environ. Res.* 156, 485–493.
- Lacoste, E., Drouin, A., Weise, A.M., Archambault, P., McKindsey, C.W., 2018. Low benthic impact of an offshore mussel farm in Îles-de-la-Madeleine, eastern Canada. *Aquacult. Environ. Interact.* 10, 473–485.
- Lafferty, K.D., Harvell, C.D., Conrad, J.M., Friedman, C.S., Kent, M.L., Kuris, A.M., Powell, E.N., Rondeau, D., Saksida, S.M., 2015. Infectious diseases affect marine fisheries and aquaculture economics. *Annu. Rev. Mar. Sci.* 7, 471–496.
- Larrain, A., Soto, E., Bay-Schmith, E., 1998. Assessment of sediment toxicity in San Vicente Bay, Central Chile, using the amphipod *Ampelisca araucana*. *Bull. Environ. Contam. Toxicol.* 61, 363–369.
- Lee, Y.G., Jeong, D.U., Lee, J.S., Choi, Y.H., Lee, M.O., 2016. Effects of hypoxia caused by mussel farming on benthic foraminifera in semi-closed Gamak Bay, South Korea. *Mar. Pollut. Bull.* 109, 566–581.
- Legendre, P., Galzin, R., Harmelin-Vivien, M., 1997. Relating behavior to habitat: solutions to the fourth-corner problem. *Ecology* 78, 547–562.
- Leitão, P., Coelho, H., Santos, A., Neves, R., 2005. Modeling the main features of the Algarve coastal circulation during July 2004: a down-scaling approach. *J. Atmos. Ocean Sci.* 10, 421–462.
- Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.O., Olrog, L., Rehnstam-Holm, A.S., Svensson, J., Svensson, S., Syversen, U., 2005. Improving marine water quality by mussel farming: a profitable solution for Swedish society. *Ambio* 34, 131–138.
- Lopez, G.R., Levinton, J.S., 1987. Ecology of deposit-feeding animals in marine sediments. *Q. Rev. Biol.* 62, 235–260.
- Lu, L., 2005. Seasonal variation of macrobenthic fauna in the Johor Strait, Singapore. *Aquat. Ecol.* 39, 107–111.
- Lüskow, F., Riisgård, H.U., 2018. In situ filtration rates of blue mussels (*Mytilus edulis*) measured by an open-top chamber method. *Open J. Mar. Sci.* 8, 395–406.
- Macleod, C.K., Moltchanivskyj, N.A., Crawford, C.M., 2008. Ecological and functional changes associated with long-term recovery from organic enrichment. *Mar. Ecol. Prog. Ser.* 365, 17–24.
- Magurran, A.E., Henderson, P.A., 2010. Temporal turnover and the maintenance of diversity in ecological assemblages. *Philos. Trans. R. Soc. Biol. Sci.* 365, 3611–3620.
- Mahmoudi, E., Essid, N., Beyrem, H., Hedfi, A., Boufahja, F., Aïssa, P., Vitiello, P., 2008. Mussel-farming effects of Mediterranean benthic nematode communities. *Nematology* 10, 323–333.
- Marchini, A., Munari, C., Mistri, M., 2008. Functions and ecological status of eight Italian lagoons examined using biological traits analysis (BTA). *Mar. Pollut. Bull.* 56, 1076–1085.
- Martins, R., Quintino, V., Rodrigues, A.M., 2013a. Diversity and spatial distribution patterns of the soft-bottom macrofauna communities on the Portuguese continental shelf. *J. Sea Res.* 83, 173–186.
- Martins, R., Sampaio, L., Rodrigues, A.M., Quintino, V., 2013b. Soft-bottom Portuguese continental shelf polychaetes: diversity and distribution. *J. Mar. Syst.* 123–124, 41–54.
- Martins, R., Sampaio, L., Quintino, V., Rodrigues, A.M., 2014. Diversity, distribution and ecology of benthic molluscan communities on the Portuguese continental shelf. *J. Sea Res.* 93, 75–89.
- McHugh, D., Fong, P.P., 2002. Do life history traits account for diversity of polychaete annelids. *Invertebr. Biol.* 121, 325–338.
- McKindsey, C.W., 2013. Carrying capacity for sustainable bivalve aquaculture. In: Christou, P., Savin, R., Costa-Pierce, B.A., Misztal, I., Whitelaw, C.B.A. (Eds.), *Sustainable Food Production*. Springer, New York.
- McKindsey, C.W., Archambault, P., Callier, M.D., Olivier, F., 2011. Influence of suspended and off-bottom mussel culture on the sea bottom and benthic habitats: a review. *Can. J. Zool.* 89, 622–646.
- McLachlan, A., Dorvlo, A., 2005. Global patterns in sandy beach macrobenthic communities. *J. Coast. Res.* 21, 674–687.
- Medeiros, C.R., Hepp, L.U., Patrício, J., Molozzi, J., 2016. Tropical estuarine macrobenthic communities are structured by turnover rather than nestedness. *PLoS One* 11, e0161082.
- Mileikovsky, S.A., 1970. The influence of pollution on pelagic larvae of bottom invertebrates in marine nearshore and estuarine waters. *Mar. Biol.* 6, 350–356.
- Mousing, E.A., Richardson, K., Bendtsen, J., Cetinić, I., Perry, M.J., 2016. Evidence of small-scale spatial structuring of phytoplankton alpha- and beta-diversity in the open ocean. *J. Ecol.* 104, 1682–1695.
- Munari, C., 2013. Benthic community and biological trait composition in respect to artificial coastal defence structures: a study case in the northern Adriatic Sea. *Mar. Environ. Res.* 90, 47–54.
- Myers, J.A., Chase, J.M., Crandall, R.M., Jiménez, I., 2015. Disturbance alters beta-diversity but not the relative importance of community assembly mechanisms. *J. Ecol.* 103, 1291–1299.
- Nasi, F., Nordström, M.C., Bonsdorff, E., Auriemma, R., Cibic, T., Del Negro, P., 2018. Functional biodiversity of marine soft-sediment polychaetes from two Mediterranean coastal areas in relation to environmental stress. *Mar. Environ. Res.* 137, 121–132.
- Naylor, R., Hindar, K., Fleming, I.A., Goldberg, R., Williams, S., Volpe, J., Whoriskey, F., Eagle, J., Kelso, D., Mangel, M., 2005. Fugitive salmon: assessing the risks of escaped fish from net-pen aquaculture. *Bioscience* 55, 427–437.
- Neofitou, N., Charizopoulos, N., Vafidis, D., Skordas, K., Tziantziou, L., Neofitou, C., 2014. Mussel farming impacts on trophic status and benthic community structure in Maliakos Gulf (Eastern Mediterranean). *Aquacult. Int.* 22, 843–857.
- Newell, R.I.E., 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. *J. Shellfish Res.* 23, 51–61.
- Oksanen, J., Blanchet, F., Kindt, R., Legendre, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, M.H.H., Wagner, H., 2011. *Vegan: Community Ecology Package*. R Package Version 1. 17-6.
- Ottinger, M., Clauss, K., Kuenzer, C., 2016. Aquaculture: relevance, distribution, impacts and spatial assessments – a review. *Ocean Coast Manag.* 119, 244–266.
- Oyinlola, M.A., Reygondeau, G., Wabnitz, C.C.C., Troell, M., Cheung, W.W.L., 2018. Global estimation of areas with suitable environmental conditions for mariculture species. *PLoS One* 13, e0191086.
- Pacheco, A., Ferreria, Ó., Williams, J.J., 2011a. Long-term morphological impacts of the opening of a new inlet on a multiple inlet system. *Earth Surf. Process. Landforms* 36, 1726–1735.
- Pacheco, A.S., González, M.T., Bremner, J., Oliva, M., Heilmayer, O., Laudien, J., Riascos, J.M., 2011b. Functional diversity of marine macrobenthic communities from

- sublittoral soft-sediment habitats off northern Chile. *Helgol. Mar. Res.* 65, 413–424.
- Pacheco, A.S., Uribe, R.A., Thiel, M., Oliva, M.E., Riascos, J.M., 2013. Dispersal of post-larval macrobenthos in subtidal sedimentary habitats: roles of vertical diel migration, water column, bedload transport and biological traits' expression. *J. Sea Res.* 77, 79–92.
- Paganelli, D., Marchini, A., Occhipinti-Ambrogi, A., 2012. Functional structure of marine benthic assemblages using biological traits analysis (BTA): a study along the Emilia-Romagna coastline (Italy, north-west Adriatic sea). *Estuar. Coast Shelf Sci.* 96, 245–256.
- Papageorgiou, N., Sigala, K., Karakassis, I., 2009. Changes of macrofaunal functional composition at sedimentary habitats in the vicinity of fish farms. *Estuar. Coast Shelf Sci.* 83, 561–568.
- Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanogr. Mar. Biol. Annu. Rev.* 16, 229–311.
- Pérez, O.M., Telfer, T.C., Ross, L.G., 2003. On the calculation of wave climate for offshore cage culture site selection: a case study in Tenerife (Canary Islands). *Aquacult. Eng.* 29, 1–21.
- Petersen, J.K., Timmermann, K., Carlsson, M., Holmer, M., Maar, M., Lindahl, O., 2012. Mussel farming can be used as a mitigation tool – a reply. *Mar. Pollut. Bull.* 64, 452–454.
- Petersen, J.K., Hasler, B., Timmermann, K., Nielsen, P., Tørring, D.B., Larsen, M.M., Holmer, M., 2014. Mussels as a tool for mitigation of nutrients in the marine environment. *Mar. Pollut. Bull.* 82, 137–143.
- Piló, D., Ben-Hamadou, R., Pereira, F., Carriço, A., Pereira, A., Corzo, A., Gaspar, M.B., Carvalho, S., 2016. How functional traits of estuarine macrobenthic assemblages respond to metal contamination? *Ecol. Indic.* 71, 645–659.
- Piló, D., Carvalho, A.N., Pereira, F., Coelho, H.E., Gaspar, M.B., 2019. Evaluation of macrobenthic community responses to dredging through a multimetric approach: effective or apparent recovery? *Ecol. Indic.* 96, 656–688.
- Pinedo, S., Jordana, E., Ballesteros, E., 2014. A critical analysis on the response of macroinvertebrate communities along disturbance gradients: description of MEDOCC (MEDiterranean Occidental) index. *Mar. Ecol.* 36, 141–154.
- Rabaoui, L., El Zrelli, R., Mansour, M.B., Balti, R., Mansour, L., Tlig-Zouari, S., Guerfel, M., 2015. On the relationship between the diversity and structure of benthic macroinvertebrate communities and sediment enrichment with heavy metals in Gabes Gulf, Tunisia. *J. Mar. Biol. Assoc. U. K.* 95, 233–245.
- Rakocinski, C.F., Heard, R.W., LeCroy, S.E., McLelland, J.A., Simons, T., 1993. Seaward change and zonation of the sandy-shore macrofauna at Perdido Key, Florida, U.S.A. *Estuar. Coast. Shelf Sci.* 36, 81–104.
- Robert, P., McKindsey, C.W., Chailou, G., Archambault, P., 2013. Dose-dependent response of a benthic system to biodeposition from suspended blue mussel (*Mytilus edulis*) culture. *Mar. Pollut. Bull.* 66, 92–104.
- Robinson, J.E., Newell, R.C., Seiderer, L.J., Simpson, N.M., 2005. Impacts of aggregate dredging on sediment composition and associated benthic fauna at an offshore dredge site in the southern North Sea. *Mar. Environ. Res.* 60, 51–68.
- Rose, J.M., Ferreira, J.G., Stephenson, K., Bricker, S.B., Tedesco, M., Wikfors, G.H., 2012. Comment on Stadmark and Conley (2011) "Mussel farming as a nutrient reduction measure in the Baltic Sea: consideration of nutrient biogeochemical cycles". *Mar. Pollut. Bull.* 64, 449–451.
- Rosenberg, R., 1995. Benthic marine fauna structured by hydrodynamic processes and food availability. *Neth. J. Sea Res.* 34, 303–317.
- RStudio Team, 2016. RStudio. Integrated Development for R. RStudio, Inc., Boston, MA. <http://www.rstudio.com/>.
- Ryu, J., Khim, J.S., Kang, S., Kang, D., Lee, C., Koh, C., 2011. The impact of heavy metal pollution gradients in sediments on benthic macrofauna at population community levels. *Environ. Pollut.* 159, 2622–2629.
- Salin, K.R., Ataguba, G.A., 2018. Aquaculture and the environment: towards sustainability. In: Hai, F., Visvanathan, C., Boopathy, R. (Eds.), *Sustainable Aquaculture*. Applied Environmental Science for the Future. Springer, Cham, pp. 60.
- Sampaio, L., Mamede, R., Ricardo, F., Magalhães, L., Rocha, H., Martins, R., Dauvin, J.C., Rodrigues, A.M., Quintino, V., 2016. Soft-sediment crustacean diversity and distribution along the Portuguese continental shelf. *J. Mar. Syst.* 163, 43–60.
- Sanchez-Jerez, P., Karakassis, I., Massa, F., Fezzardi, D., Aguilar-Manjarrez, J., Soto, D., Chapela, R., Avila, P., Macias, J.C., Tomassetti, P., Marino, G., Borg, J.A., Franičević, V., Yucel-Gier, G., Fleming, I.A., Biao, X., Nhhala, H., Hamza, H., Forcada, A., Dempster, T., 2016. Aquaculture's struggle for space: the need for coastal spatial planning and the potential benefits of Allocated Zones for Aquaculture (AZAs) to avoid conflict and promote sustainability. *Aquacult. Environ. Interact.* 8, 41–54.
- Sanz-Lázaro, C., Marín, A., 2011. Diversity patterns of benthic macrofauna caused by marine fish farming. *Diversity* 3, 176–199.
- Seiderer, L.J., Newell, R.C., 1999. Analysis of the relationship between sediment composition and benthic community structure in coastal deposits: implications for marine aggregate dredging. *ICES J. Mar. Sci.* 56, 757–765.
- Sellanes, J., Qurioga, E., Neira, C., Gutiérrez, D., 2007. Changes of macrobenthic composition under different ENSO cycle conditions on the continental shelf off central Chile. *Cont. Shelf Res.* 27, 1002–1016.
- Shainee, M., Haskins, C., Ellingsen, H., Leira, B.J., 2012. Designing offshore fish cages using systems engineering principles. *Syst. Eng.* 15, 396–406.
- Shumway, S.E., Davis, C., Downey, R., Karney, R., Kraeuter, J., Parsons, J., Rheault, R., Wikfors, G., 2003. Shellfish aquaculture – in praise of sustainable economies and environments. *World Aquac.* 34, 15–17.
- Silva, C., Ferreira, J.G., Bricker, S.B., DelValls, T.A., Martín-Díaz, M.L., Yáñez, E., 2011. Site selection for shellfish aquaculture by means of GIS and farm-scale models, with an emphasis on data-poor environments. *Aquaculture* 318, 444–457.
- Snelgrove, P.V.R., 1998. The biodiversity of macrofaunal organisms in marine sediments. *Biodivers. Conserv.* 7, 1123–1132.
- Socolar, J.B., Gilroy, J.J., Kunin, W.E., Edwards, D.P., 2016. How should beta-diversity inform biodiversity conservation? *Trends Ecol. Evol.* 31, 67–80.
- Statzner, B., Bêche, L.A., 2010. Can biological invertebrate traits resolve effects of multiple stressors on running water ecosystems? *Freshw. Biol.* 55, 80–119.
- Stenton-Dozey, J.M.E., Jackson, L.F., Busby, A.J., 1999. Impact of mussel culture on macrobenthic community structure in Saldanha Bay, South Africa. *Mar. Pollut. Bull.* 39, 357–366.
- Stenton-Dozey, J., Probyn, T., Busby, A., 2001. Impact of mussel (*Mytilus galloprovincialis*) raft-culture on benthic macrofauna, in situ oxygen uptake, and nutrient fluxes in Saldanha Bay, South Africa. *Can. J. Fish. Aquat. Sci.* 58, 1021–1031.
- Suplicy, F.M., 2018. A review of the multiple benefits of mussel farming. *Rev. Aquac.* <https://doi.org/10.1111/raq.12313>.
- ter Braak, C., Cormont, A., Dray, S., 2012. Improved testing of species traits-environment relationships in the fourth corner problem. *Ecology* 93, 1525–1526.
- Theede, H., Ponat, A., Hiroki, K., Schlieper, C., 1969. Studies on the resistance of marine bottom invertebrates to oxygen-deficiency and hydrogen sulphide. *Mar. Biol.* 2, 325–337.
- Thomas, L.R., Clavelle, T., Klinger, D.H., Lester, S.E., 2019. The ecological and economic potential for offshore mariculture in the Caribbean. *Nat. Sustain.* 2, 62–70.
- Thrush, S.F., Hewitt, J.E., Gibbs, M., Lundquist, C., Norrko, A., 2006. Functional role of large organisms in intertidal communities: community effects and ecosystem function. *Ecosystems* 9, 1029–1040.
- Thrush, S.F., Hewitt, J.E., Kraan, C., Lohrer, A.M., Pilditch, C.A., Douglas, E., 2017. Changes in the location of biodiversity – ecosystem functioning hot spots across the seafloor landscape with increasing sediment nutrient loading. *Proc. R. Soc. Ser. B* 284, 20162861.
- Tuya, F., Herrero-Barrencia, A., Bosch, N.E., Abreu, A.D., Haroun, R., 2017. Reef fish at a remote tropical island (Príncipe Island, Gulf of Guinea): disentangling taxonomic, functional and phylogenetic diversity patterns with depth. *Mar. Freshw. Res.* 69, 395–402.
- Tyler, E.H.M., Somerfield, P.J., Vanden Berghe, E., Bremner, J., Jackson, E., Langmead, O., Palomares, M.L.D., Webb, T.J., 2012. Extensive gaps and biases in our knowledge of a well-known fauna: implications for integrating biological traits into macroecology. *Glob. Ecol. Biogeogr.* 21, 922–934.
- Ulrich, W., Almeida-Neto, M., 2012. On the meanings of nestedness: back to the basics. *Ecography* 35, 001–007.
- Underwood, A.J., 1992. Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. *J. Exp. Mar. Biol. Ecol.* 161, 145–178.
- Uysal, A., Yüsek, A., Okuş, E., Yılmaz, N., 2002. Benthic community structure of the Bosphorus and surrounding area. *Water Sci. Technol.* 46, 37–44.
- Valenti, W.C., Kimpara, J.M., Preto, B.D.L., Moraes-Valenti, P., 2018. Indicators of sustainability to assess aquaculture systems. *Ecol. Indic.* 88, 402–413.
- van der Schatte Olivier, A., Jones, L., Le Vay, L., Christie, M., Wilson, J., Malham, S.K., 2018. A global review of the ecosystem services provided by bivalve aquaculture. *Rev. Aquac.* 1–23.
- van der Veer, G., 2006. *Geochemical Soil Survey of The Netherlands*. NG. 347. pp. 157–174.
- van Hoey, G., Degraer, S., Vincx, M., 2004. Macrobenthic community structure of soft-bottom sediments at the Belgian Continental Shelf. *Estuar. Coast Shelf Sci.* 59, 599–613.
- Vellend, M., 2010. Conceptual synthesis in community ecology. *Q. Rev. Biol.* 85, 183–206.
- Venugopal, V., Gopakumar, K., 2017. Shellfish: nutritive value, health benefits, and consumer safety. *Compr. Rev. Food Sci. Food Saf.* 16, 1219–1242.
- Ward, T.J., Hutchings, P.A., 1996. Effects of trace metals on infaunal species composition in polluted intertidal and subtidal marine sediments near a lead smelter, Spencer Gulf, South Australia. *Mar. Ecol. Prog. Ser.* 135, 123–135.
- Ward, D.J., Simpson, S., Jolley, D.F., 2013. Slow avoidance response to contaminated sediments elicits sublethal toxicity to benthic invertebrates. *Environ. Sci. Technol.* 47, 5947–5953.
- Weise, A.M., Cromey, C.J., Callier, M.D., Archambault, P., Chamberlain, J., McKindsey, C., 2009. Shellfish-DEPOMOD: modelling the biodeposition from suspended shellfish aquaculture and assessing benthic effects. *Aquaculture* 288, 239–253.
- Whitmarsh, D.J., Cook, E.J., Black, K.D., 2006. Searching for sustainability in aquaculture: an investigation into the economic prospects for an integrated salmon-mussel production system. *Mar. Policy* 30, 293–298.
- Wilding, T.A., 2012. Changes in sedimentary redox associated with mussel (*Mytilus edulis* L.) farms on the west-coast of Scotland. *PLoS One* 7, e45159.
- Wilding, T.A., Nickell, T.D., 2013. Changes in benthos associated with mussel (*Mytilus edulis* L.) farms on the west-coast of Scotland. *PLoS One* 8, e68313.
- Wong, K.L.C., O'Shea, S., 2011. The effects of a mussel farm on benthic macrofaunal communities in Hauraki Gulf, New Zealand. *N. Z. J. Mar. Freshw. Res.* 45, 187–212.
- Ysebaert, T., Hart, M., Herman, P.M.J., 2009. Impacts of bottom and suspended cultures of mussels *Mytilus* spp. on the surrounding sedimentary environment and macrobenthic diversity. *Helgol. Mar. Res.* 63, 59–74.
- Zacharias, M.A., Roff, J.C., 2001. Explanations of patterns of intertidal diversity at regional scales. *J. Biogeogr.* 28, 471–483.
- Zúñiga, D., Castro, C.G., Aguiar, E., Figueiras, F.G., Fernández-Reiriz, M.J., 2014. Biodeposit contribution to natural sedimentation in a suspended *Mytilus galloprovincialis* Lmk mussel farm in a Galician Ría (NW Iberian Peninsula). *Aquaculture* 432, 311–320.