Spatio-temporal benthic biodiversity patterns and pollution pressure in three Mediterranean touristic ports

Eva Chatzinikolaou[1], Manolis Mandalakis[1], Panagiotis Damianidis[2], Thanos Dailianis[1], Simone Gambineri[3], Claudia Rossano[3], Felicita Scapini[3], Alessandra Carucci[4], Christos Arvanitidis[1]

[1] Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research (HCMR), Heraklion, 71003, Crete, Greece
[2] Department of Zoology, School of Biology, Aristotle University of Thessaloniki, P.O. Box 134, 54124 Thessaloniki, Greece
[3] Department of Biology, University of Florence (UNIFI), via Romana 17, 50125 Firenze, Italy
[4] Department of Civil-Environmental Engineering and Architecture (DICAAR), University of Cagliari (UNICA), Via Marengo 2, 09123 Cagliari, Italy

Corresponding author

Dr Eva Chatzinikolaou
Institute of Marine Biology, Biotechnology and Aquaculture,
Hellenic Center for Marine Research, P.O. Box 2214,
Thalassokosmos, Former US Base at Gournes, Heraklion, 71003, Crete, Greece
Tel. +30-2810-337741
Email address: evachatz@hcmr.gr

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Abstract

The Mediterranean Sea is one of the busiest areas worldwide in terms of maritime activity, facing considerable anthropogenic disturbance, such as pollution by hydrocarbons and heavy metals. The present study has evaluated the environmental and benthic biodiversity characteristics of three touristic ports, Cagliari (Sardinia, Italy), Heraklion (Crete, Greece) and El Kantaoui (Tunisia), based on the combined assessment of physical parameters, chemical variables (i.e. nutrients, pigments), sediment pollution and macrobenthic biodiversity. Different port sectors (leisure, fishing, passenger, cargo, shipyard) and different seasons (winter, before touristic period, after touristic period) were compared. Salinity and sediment concentration of copper and antimony were the three environmental parameters most highly correlated with benthic species composition and diversity. Both the environmental variables and the benthic biodiversity patterns were significantly different between the three ports (i.e. different geographical locations). Heraklion port was heavily polluted by AHs in surface and anoxic sediments and had the highest percentage of opportunistic species, while Cagliari had the highest levels of PAHs and UCM and low species richness. El Kantaoui port was less polluted and characterised by a richer biodiversity. The shipyard sector in Heraklion port was significantly different from all other sectors in terms of abiotic and biotic parameters. Physico-chemical and pollution variables recorded during the period after tourism (late summer) were significantly different from the ones recorded in winter. Seasonal differences were not significant between benthic species diversity patterns, but were revealed when the patterns derived from the aggregation of higher taxonomic levels were compared. The present study indicates that a regular-basis monitoring plan including evaluation of environmental health based on benthic biodiversity, can provide a basis for

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perceiving changes and reveal the degree of anthropogenic disturbance in port environments.

1. Introduction

The Mediterranean Sea is connected to the Atlantic Ocean on the west through the Straits of Gibraltar, and on the east to the Red Sea and to the Black Sea through the Suez Canal and the Bosporus Strait, respectively. The Mediterranean Sea covers approximately 2.5 million km^2, hosts about 480 ports and terminals and is amongst the world's busiest areas in terms of maritime activity (REMPEC, 2008). Almost 70,000 vessels cross the Straits of Gibraltar every year, 55,000 the Bosporus Strait and 16,000 the Suez Canal, without including ferries and non-merchant vessels (REMPEC, 2008). The shipping of goods between the main EU ports and ports located in the Mediterranean reached 598 million tonnes in 2015, which was equal to 29% of the total EU short sea shipping (Eurostat, 2015). Tankers accounted for 16-19% of the total transits within the Mediterranean and for 24-46% of the total vessel DWT (deadweight tonnage) capacity (values concern ranges between the three access/exit points, i.e. Gibraltar, Bosporus and Suez Canal; REMPEC, 2008). Crude oil shipped through the Mediterranean Sea in 2006 amounted to 421 million tonnes, from which 220 million tonnes were loaded within Mediterranean ports (REMPEC, 2008). A significant amount of this oil (about 400,000 tonnes; UNEP 2006) are dumped annually into the Mediterranean during routine ship operations, which often take place inside ports or oil terminals (e.g. deballasting, tank washing, dry-docking, bunkering) (Abdulla & Linden, 2008).

Ports can be the recipient and the source of considerable anthropogenic disturbance, both for marine and adjacent land habitats, since they centralize a range of environmental problems, such as emission of air pollutants, noise, sediment dredging and transport, industrial installations, jetties construction, wastewater discharges, oil spill accidents, leaks of petroleum derivatives and antifouling coatings, storage and spillage of hazardous materials, as well as introduction of invasive species (Darbra et al., 2005). The most ubiquitous and long-lived petroleum contaminants in the marine sediments are the polycyclic aromatic hydrocarbons (PAHs), since their hydrophobic character makes them easily adsorbed on suspended particles which are eventually deposited in marine sediments (Abdulla & Linden, 2008). The concentration of PAHs in sediments depends on the distance of an area from the pollution source, while their overall degree of toxicity and bioavailability depends on the physico-chemical properties of particular PAH members (i.e. number of aromatic rings, molecular weight) (Abdulla & Linden, 2008). Abdulla & Linden (2008) highlighted that while oil pollution in marine sediments has been extensively investigated in the north-western part of the Mediterranean Sea, there is a significant gap for such data in other parts of the region. Marine sediments contaminated by weathered and biodegraded oils may represent a persistent and ongoing threat for benthic organisms (Reddy et al., 2002). Raman (1995) indicated that in harbour environments the total number of polychaete individuals was decreased and the number of species was increased when moving away from a pollution source. Disturbance on marine biodiversity may initially result in recruitment by opportunistic taxa, which are gradually replaced by slower-growing ''equilibrium species'' leading to a re-adjustment phase which might, however, be prohibited if repeated disturbance such as pollution or dredging occur (see Blanchard & Feder, 2003). The recovery potential of benthic macrofaunal communities is primarily determined by the substrate type and the hydrodynamically mediated nutrient availability (Gutperlet et al., 2015).

Port management is not an easy task, as it entails balancing out requirements and conflicting uses by residents, visitors, industry, shipping and other users. Ports are complex systems requiring the involvement of multi-disciplinary authorities and stakeholders from different sectors (e.g. engineers, ecologists, economists, governmental bodies) in order to identify, understand and manage conflicts within their limits (Pearson et al., 2016). Mediterranean ports are sites of significant economic activity, crucial for local and national economic development, which at the same time are located near coastal areas hosting a high number of residents and tourists; therefore, their effective management is essential for their sustainable use and the protection of adjacent habitats. Ports are classified as "Heavily Modified Water Bodies" (HMWBs) since they constitute water bodies substantially altered in character due to human activities, which cannot meet the common good ecological status criteria (WFD 2000/60/EC). Ports are often characterised by low hydrodynamism, reduced oxygen concentration, increased organic content and pollution caused by maritime activities and uncontrolled discharge of effluents. Quality assessment approaches developed for natural water bodies may not be feasible for HMWBs, thus managing authorities need to define more appropriate and customized methodologies for port environments (Ondiviela et al., 2013). Existing sediment quality assessment tools include chemical analyses combined with ecotoxicological and ecological approaches (Moreira et al., 2017). The Sediment Quality Triad (SQT) integrates evaluations of benthic community structure with sediment chemistry and sediment toxicity in order to provide a better assessment of pollution-induced degradation (McPherson et al., 2008). The Strategic Overview of Significant Environmental Aspects (SOSEA) is another port management tool applied to several EU ports, which includes identification of the Significant Environmental Aspects (SEA), evaluation of their significance and assessment of the respective management procedures (Darbra et al., 2005). Macrobenthic communities are an important component of the port biota since they have an active role in biomineralization, bioturbation, oxygen and nutrient cycling, and due to their reduced mobility and short life cycles they are commonly used as indicators in biomonitoring studies (Gray & Elliot, 2010). The establishment of a baseline database regarding benthic biota present in ports can offer valuable background information when port management activities are required for the identification of biological risks, such as pollution events or invasion by alien species (Mandal & Harkantra, 2013).

The negative effects of maritime activities (i.e. pollution, anchoring, noise) in the Mediterranean Sea have been extensively documented for marine mammals, tortoises and marine birds, whereas when it comes to benthic organisms and habitats only consequences on *Posidonia oceanica* beds, algae, coralligenous reefs and maerl beds have been studied (Abdulla & Linden, 2008). The present study attempts to cover this gap by recording and evaluating the environmental status of three touristic ports located along the Mediterranean Sea, based on the combined assessment of physico-chemical conditions, pollution and macrobenthic species composition and diversity. The selected ports besides differences in geographical location (Sardinia, Crete, Tunisia), also host a range of activities (e.g. leisure, fishing, shipyard, passenger and cargo vessels). Therefore, differences and similarities between ports (location), sectors (type of use) and seasons (proximity to the touristic period) were examined. The present study also investigated the hypothesis that a set of environmental and pollution variables was associated with the observed macrobenthic patterns for all ports, sectors and seasons. The degree of environmental disturbance based on the hierarchic-response-to-stress hypothesis (Olsgard et al., 1998) was evaluated through the divergence between the biodiversity patterns produced as the information from species abundance was aggregated to higher taxonomic levels. Under the framework of the ENPI CBCMED project MAPMED, a common evaluation methodology was established for the three ports, which included a detailed seasonal and spatial protocol. Suggestions for a simplified and cost-effective management plan were indicated aiming to improve the environmental quality in the Mediterranean touristic ports.

2. Materials and Methods

2.1 Study area and collection of samples

The three Mediterranean touristic ports selected as study sites are presented in Figure 1. The port of Cagliari is a large port (2.07 km^2) located on the southern coast of Sardinia (Italy) and represents one of the key points for the trans-shipment activities in western Mediterranean. The medium-sized port of Heraklion (0.87 km^2) is located on the northern coast of Crete (Greece) and is one of the most important and active national ports in the Eastern Mediterranean. El Kantaoui port is a small touristic artificial marina (0.04 km^2) on the eastern Tunisian coast. The ports of Heraklion and Cagliari are both characterised by a significant maritime traffic including large passenger, cruise and cargo vessels, while El Kantaoui port offers moorings for smaller fishing boats, luxury yachts and boats for sporting activities (Anastasiou et al., 2016; Chatzinikolaou & Arvanitidis, 2016). Sampling stations were selected in order to achieve good spatial coverage of the whole area in each port and to represent a range of different sector uses (Figure 1, Table 1). Three seasonal sampling campaigns were carried out during 2012, one in winter (February), one in spring before the beginning of the touristic season (May) and one in late summer after the touristic season (September).

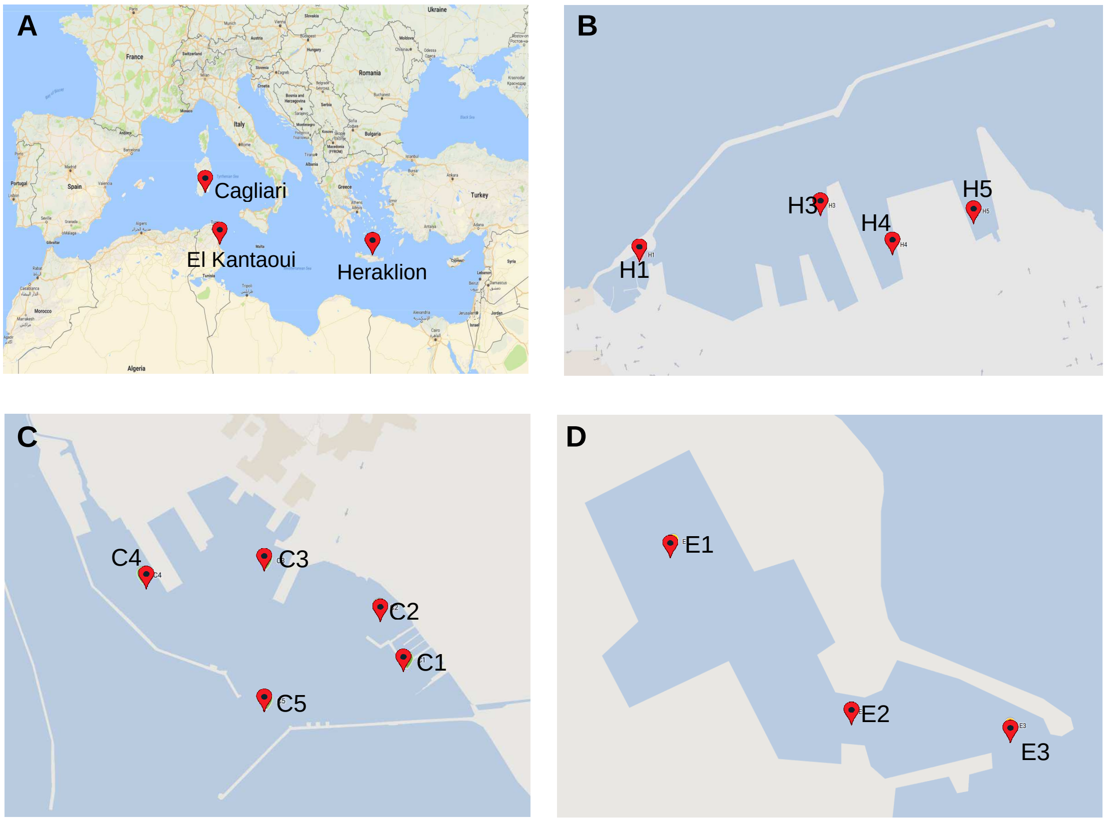


Figure 1. Location of the three studied ports in the Mediterranean Sea (A) and location of the sampling stations in Heraklion (B), Cagliari (C), and El Kantaoui (D) ports.

Three replicates (5 L) of water samples were collected from the sea surface at each station and analysed for inorganic nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, SiO_2), particulate organic carbon (POC), chlorophyll-*a*, phaeopigments, polycyclic aromatic (PAHs) and aliphatic hydrocarbons (AHs), and heavy metals. Five replicates of sediment samples were collected from each station for benthos analysis using a box corer (13.5 x 13.5 x 16 cm). Undisturbed sediment subsamples were collected using small plastic corers (4.4 cm diameter). Three surface sediment subsamples per station were used for the analysis of POC, chlorophyll-*a* and phaeopigments, one was used for granulometric analysis, one for heavy metal analysis and three for hydrocarbon analysis. An additional sample was collected from the anoxic sediment layer (>2 cm below surface) for hydrocarbon analysis only during the third sampling campaign.

Table 1. Depth and sectors of all sampling stations in each port. Sectors identified following the Water Management Units in the MAPMED Action Plans (MAPMED, 2015).

Port	Station	Depth (m)	Port sector	Coordinates
Heraklion	H1	3.7	Leisure/fishing	025°08'12.4''E; 35°20'38.7''N
	H3	19.5	Passenger ships	025°08'43.3''E; 35°20'45.6''N
	H4	10.5	Cargo ships	025°08'55.5''E; 35°20'39.5''N
	H5	19.0	Shipyard	025°09'08.6''E; 35°20'42.9''N
Cagliari	C1	7.8	Leisure/fishing	009°07'25.1''E; 39°12'12.2''N
	C2	4.5	Leisure/fishing	009°07'16.7''E; 39°12'23.0''N
	C3	8.3	Leisure/fishing	009°06'45.9''E; 39°12'34.0''N
	C4	13.5	Passenger/cargo ships	009°06'14.8''E; 39°12'29.7''N
	C5	11.4	Intense maritime traffic	009°06'45.2''E; 39°12'01.7''N
El Kantaoui	E1	2.5	Leisure/fishing	010°35'52.1''E; 35°53'39.9''N
	E2	4.0	Leisure/fishing	010°35'58.9''E; 35°53'34.6''N
	E3	3.2	Leisure/fishing	010°36'05.2''E; 35°53'34.1''N

A Garmin 60 CS portable GPS and a depth meter were available on board to record the exact position and depth of each station respectively. Seawater physical properties (surface temperature, salinity, oxygen, pH) were measured on board using a WTW 3420 multi-meter, whereas turbidity was measured using a Secchi disk. The redox potential (Eh) of the sediment samples was measured immediately after collection using a WTW SenTix ORP 900 calibrated electrode immersed at 1 cm below sediment surface. Sediment temperature was measured using a digital sediment thermometer on the sediment surface (<1 cm) and at a depth of 2 cm below surface (anoxic sediment).

2.2 Chemical analysis of water and sediment samples

A 1-L volume from each replicate of seawater sample was filtered using pre-combusted (300°C for 1.5 hours) Whatman GF/F filters (47mm diameter). The filtrate (500 ml) was used for inorganic nutrient analysis, while the filter was used for the determination of chlorophyll-*α*, phaeopigments and POC. A 2-cm slice collected from the surface of each sediment replicate was analysed for chlorophyll-*α*, phaeopigments and POC. The analyses of inorganic nutrients and POC were performed according to the Strickland & Parsons (1972) method, while NH_4^+ was determined according to Ivančić & Degobbis (1984). Chlorophyll-*α* and phaeopigments were measured using the fluorometric method of Yentsch & Menzel (1963) and Arar & Collins (1992). Grain size analysis by dry sieving and estimation of the silt-clay ratio (% silt-clay) using the pipette method were performed in a 4-cm slice of surface sediment sample from each station according to Buchanan (1984) and the granulometric classification followed the Wentworth chart.

The analysis of hydrocarbons was based on the previously described method by Mandalakis et al. (2014). In brief, the sediment samples (10 g) were solvent extracted in an ultrasonic bath (three times with 40 mL of hexane:acetone 3:2), while water samples (1 L) were subjected to liquid-liquid extraction (three times with 70 mL of hexane). Prior to extraction, all samples were spiked with a mixture of AHs and PAHs surrogate standards. The extracts were concentrated, fractionated on silica gel columns and analysis of individual AH and PAH members was performed on a Hewlett–Packard HP 6890 gas chromatograph coupled to a HP 5972 mass spectrometer (GC-MS) operated in the electron impact ionization mode. In addition, the gas chromatograms derived from the sediment samples were evaluated for the presence of an Unresolved Complex Mixture (UCM).

For the heavy metal analysis, a water sample of 100 ml was acidified and filtered through 0.45 μm Whatman filters. The analysis of Al, As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, V and Zn was performed by Inductively Coupled Plasma optical emission spectrometry (ICP-OES, Perkin Elmer Optima DV 7000). In addition, a 50 g sediment sample dried at 100°C until constant weight was analysed for Al, As, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, V and Zn through metal acid solubilisation and subsequent analysis by ICP-OES.

2.3 Processing of benthic samples

Each of the five replicate sediment samples collected from each station was sieved on site through a 0.5 mm sieve and then fixed and preserved in 5% formaldehyde buffered with seawater and stained with Rose Bengal. The benthic organisms were sorted out of the sediment under a stereoscope, counted and identified down to species level.

2.4 Statistical analysis

A species-by-station abundance matrix for all taxonomic levels (species, genus, family, order, class, phylum) and an environmental variables-by-station matrix were constructed and imported to PRIMER software v.6 for statistical analysis. The number of species, the number of individuals per species, as well as the indices of diversity (d: Margalef's richness index; H': Shannon with an *e* log base) and evenness (J': Pielou's) were calculated for all samples of the three Mediterranean ports. The Bray-Curtis similarity coefficient was applied on the macrobenthic data set matrices, after abundance values had been standardised and fourth-root transformed (Clarke and Warwick, 1994). Multivariate patterns of species distribution were derived by applying the iterative algorithm of the non-metric multidimensional scaling (nMDS) on the corresponding similarity matrices (Kruskal & Wish, 1978; Clarke, 1993). The samples were classified according to three factors: 1) port (Cagliari, Heraklion, El Kantaoui), 2) sector (leisure, cargo, passenger, shipyard) and 3) sampling season (winter, before the touristic season, after the touristic season). Analysis of similarity (ANOSIM) was performed on the corresponding similarity matrices (Clarke, 1993) using the same three factors. The same analysis was also performed on the environmental data, but in this case normalised data were used without applying any transformation and a Euclidean distance coefficient was calculated to produce the corresponding similarity matrix. A SIMPER analysis was performed in order to test the contribution of benthic species to the similarity and dissimilarity among different seasons, sectors and ports. In addition, the percentage of opportunistic species was calculated for each station, as the number of species belonging to the Annelida families Capitellidae, Cirratulidae and Spionidae, as well as to the subclass Oligochaeta (Pearson and Rosenberg, 1978; Munari et al., 2005).

The macrobenthic community patterns derived from the six different taxonomic levels (species, genus, family, order, class, phylum) were compared using a second-stage MDS, by computing a weighted Spearman rank correlation coefficient (ρ) between the corresponding elements of each pair of stations from the respective similarity matrices (Somerfield & Clarke, 1995).

A PCA analysis was performed including all the environmental variables in order to identify which of them contributed the most to the description of the abiotic environment. The BIOENV analysis was applied using the weighted Spearman's coefficient to assess which environmental variables correlated best with the multivariate patterns derived from the faunal similarity matrices (only for the species level) (Clarke & Ainsworth, 1993). A Draftsman plot was used in order to avoid simultaneous inclusion of all the inter-correlated variables in the BIOENV (i.e. sets of variables with Spearman's test values higher than 0.8 were omitted). The RELATE routine (Clarke & Gorley, 2006) was finally applied in order to test for the significance of the correlated patterns between the species similarity matrices and those produced by the subset of the environmental parameters as identified from the BIOENV analysis. The comparison of biodiversity and environmental parameters (e.g. PAHs, AHs, % opportunists, Shannon index, etc.) between the three ports was based on the non-parametric Kruskal-Wallis test (Kruskal & Wallis, 1952) and the respective post-hoc test (Siegel & Castellan, 1988) using STATISTICA v. 8.0, as the data were not normally distributed (Anderson-Darling test $p<0.05$) but had equal variances (Levene's test $p>0.05$).

3. Results

3.1 Environmental parameters

The main sediment environmental parameters of the three Mediterranean ports are presented in Table 2. The PCA analysis (Appendix A, Figure A1) did not indicate any prevailing sediment parameters, while the first two PC axes explained only 48.6% of the variability between samples. However, the BIOENV analysis which combined patterns of environmental variables with the benthic species distribution, indicated that the most highly correlated parameters were salinity and the concentration of the heavy metals copper (Cu) and antimony (Sb) in the sediment, as was also confirmed by the RELATE analysis ($R = 0.503$, $p< 0.001$).

The patterns of the environmental variables were significantly different between the three ports (Figure 2A; ANOSIM: Global $R = 0.531$, $p<0.001$) as was also indicated by the pairwise tests (C-H: $R= 0.528$, $p<0.001$; C-E: $R= 0.545$, $p<0.001$; H-E: $R= 0.666$, $p<0.001$). Similarly, the samples from different sampling seasons were also significantly different (Figure 2C; ANOSIM: $R = 0.099$, $p=0.008$); more specifically, environmental variables patterns were significantly different between samples collected in winter and samples collected after the touristic period (Pairwise tests: winter - before tourism: $R=0.052$, $p=0.133$; winter - after tourism: $R= 0.236$, $p<0.001$; before tourism - after tourism: $R=0.018$, $p=0.285$). Some port sectors were grouped separately, although significance of differences was marginal (Figure 2B; ANOSIM: Global $R = 0.104$, $p=0.06$). The pairwise comparisons indicated a significant difference in the environmental variables between the shipyard and the passenger sector ($R=0.764$, $p=0.002$), as well as between the shipyard and the cargo sector ($R=0.630$, $p=0.024$), whereas there was no difference between the shipyard and the leisure sectors ($R=0.151$, $p=0.173$). All other sectors had similar patterns (Leisure - Passenger: $R=0.077$, $p= 0.081$; Leisure - Cargo $R=-0.144$, $p=0.939$; Passenger - Cargo $R= 0.199$, $p=0.053$).

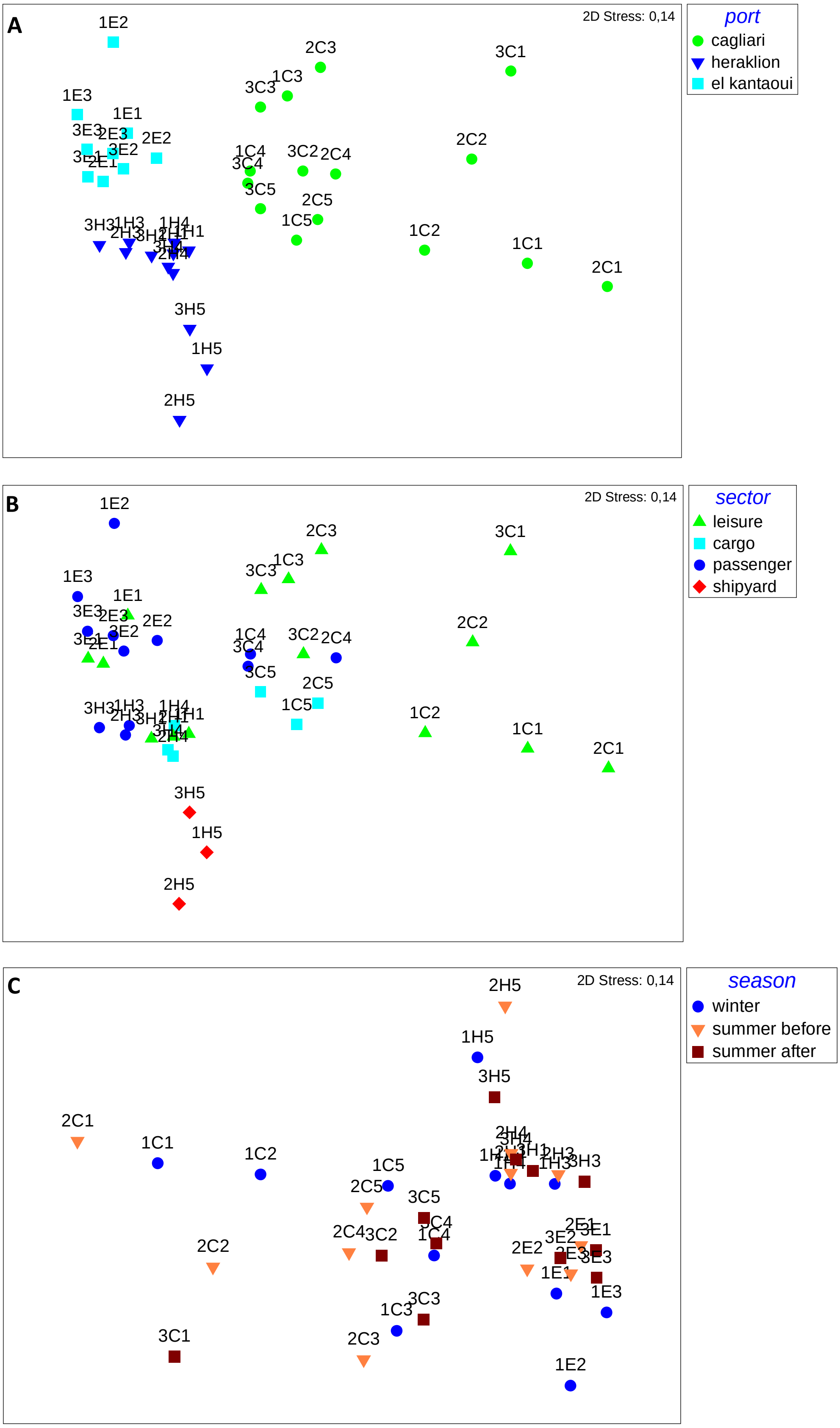
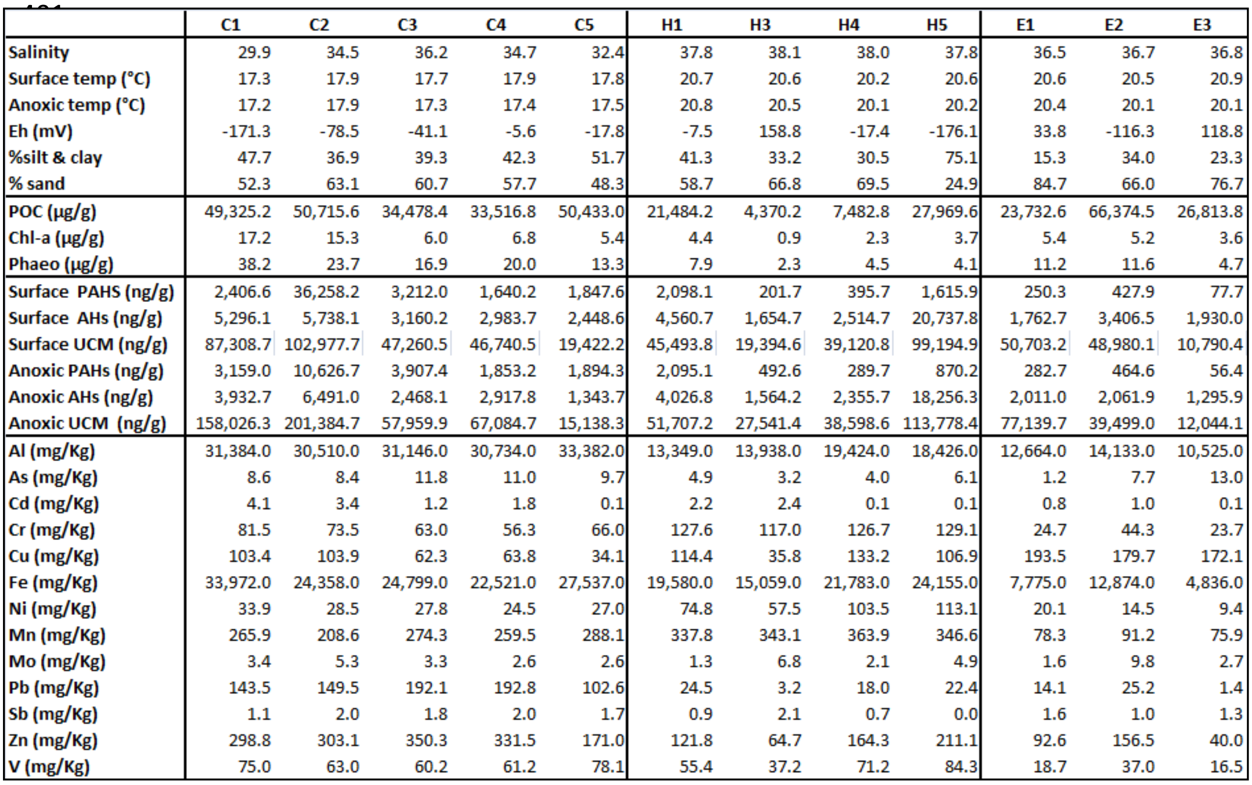


Figure 2. nMDS plots of the environmental variables grouped according to three factors: A) port, B) sector, C) season. Station codes as in Table 1; the preceding number represents sampling season (1: winter, 2: before the touristic period, 3: after the touristic period).

Table 2. Average values for sediment environmental parameters of the three Mediterranean ports (salinity values refer to seawater). Station codes as in Table 1.



	C1	C2	C3	C4	C5	H1	H3	H4	H5	E1	E2	E3
Salinity	29.9	34.5	36.2	34.7	32.4	37.8	38.1	38.0	37.8	36.5	36.7	36.8
Surface temp (°C)	17.3	17.9	17.7	17.9	17.8	20.7	20.6	20.2	20.6	20.6	20.5	20.9
Anoxic temp (°C)	17.2	17.9	17.3	17.4	17.5	20.8	20.5	20.1	20.2	20.4	20.1	20.1
Eh (mV)	-171.3	-78.5	-41.1	-5.6	-17.8	-7.5	158.8	-17.4	-176.1	33.8	-116.3	118.8
%silt & clay	47.7	36.9	39.3	42.3	51.7	41.3	33.2	30.5	75.1	15.3	34.0	23.3
% sand	52.3	63.1	60.7	57.7	48.3	58.7	66.8	69.5	24.9	84.7	66.0	76.7
POC (µg/g)	49,325.2	50,715.6	34,478.4	33,516.8	50,433.0	21,484.2	4,370.2	7,482.8	27,969.6	23,732.6	66,374.5	26,813.8
Chl-a (µg/g)	17.2	15.3	6.0	6.8	5.4	4.4	0.9	2.3	3.7	5.4	5.2	3.6
Phaeo (µg/g)	38.2	23.7	16.9	20.0	13.3	7.9	2.3	4.5	4.1	11.2	11.6	4.7
Surface PAHS (ng/g)	2,406.6	36,258.2	3,212.0	1,640.2	1,847.6	2,098.1	201.7	395.7	1,615.9	250.3	427.9	77.7
Surface AHs (ng/g)	5,296.1	5,738.1	3,160.2	2,983.7	2,448.6	4,560.7	1,654.7	2,514.7	20,737.8	1,762.7	3,406.5	1,930.0
Surface UCM (ng/g)	87,308.7	102,977.7	47,260.5	46,740.5	19,422.2	45,493.8	19,394.6	39,120.8	99,194.9	50,703.2	48,980.1	10,790.4
Anoxic PAHs (ng/g)	3,159.0	10,626.7	3,907.4	1,853.2	1,894.3	2,095.1	492.6	289.7	870.2	282.7	464.6	56.4
Anoxic AHs (ng/g)	3,932.7	6,491.0	2,468.1	2,917.8	1,343.7	4,026.8	1,564.2	2,355.7	18,256.3	2,011.0	2,061.9	1,295.9
Anoxic UCM (ng/g)	158,026.3	201,384.7	57,959.9	67,084.7	15,138.3	51,707.2	27,541.4	38,598.6	113,778.4	77,139.7	39,499.0	12,044.1
Al (mg/Kg)	31,384.0	30,510.0	31,146.0	30,734.0	33,382.0	13,349.0	13,938.0	19,424.0	18,426.0	12,664.0	14,133.0	10,525.0
As (mg/Kg)	8.6	8.4	11.8	11.0	9.7	4.9	3.2	4.0	6.1	1.2	7.7	13.0
Cd (mg/Kg)	4.1	3.4	1.2	1.8	0.1	2.2	2.4	0.1	0.1	0.8	1.0	0.1
Cr (mg/Kg)	81.5	73.5	63.0	56.3	66.0	127.6	117.0	126.7	129.1	24.7	44.3	23.7
Cu (mg/Kg)	103.4	103.9	62.3	63.8	34.1	114.4	35.8	133.2	106.9	193.5	179.7	172.1
Fe (mg/Kg)	33,972.0	24,358.0	24,799.0	22,521.0	27,537.0	19,580.0	15,059.0	21,783.0	24,155.0	7,775.0	12,874.0	4,836.0
Ni (mg/Kg)	33.9	28.5	27.8	24.5	27.0	74.8	57.5	103.5	113.1	20.1	14.5	9.4
Mn (mg/Kg)	265.9	208.6	274.3	259.5	288.1	337.8	343.1	363.9	346.6	78.3	91.2	75.9
Mo (mg/Kg)	3.4	5.3	3.3	2.6	2.6	1.3	6.8	2.1	4.9	1.6	9.8	2.7
Pb (mg/Kg)	143.5	149.5	192.1	192.8	102.6	24.5	3.2	18.0	22.4	14.1	25.2	1.4
Sb (mg/Kg)	1.1	2.0	1.8	2.0	1.7	0.9	2.1	0.7	0.0	1.6	1.0	1.3
Zn (mg/Kg)	298.8	303.1	350.3	331.5	171.0	121.8	64.7	164.3	211.1	92.6	156.5	40.0
V (mg/Kg)	75.0	63.0	60.2	61.2	78.1	55.4	37.2	71.2	84.3	18.7	37.0	16.5

3.2 Benthic communities

A total of 46,187 individuals were identified down to species level, from which 15,535 were found in Cagliari port, 11,571 in Heraklion port and 19,081 in El Kantaoui port. A total of 284 macrofaunal species were identified in the three ports, from which 33 were present only in Cagliari port, 23 were found only in Heraklion port and 58 were found exclusively in El Kantaoui port (Figure 3). Out of the 284 species, 96 were common between all three Mediterranean ports. El Kantaoui port hosted the highest number of species in total (211), while Cagliari and Heraklion ports had 174 and 165 species in total, respectively. The different taxonomic phyla found in all ports and their percentage distribution are presented in Figure 4. Mollusca were the most abundant group (34%), Annelida (28%) and Arthropoda (24%) were also highly represented, while each of the remaining groups contributed less than 5% in the benthic assemblages studied. One Foraminifera (0.4%) and four Nematoda (1.4%) species were also identified, which were, however, excluded from the analysis since these groups commonly constitute part of the meiofauna.

The number of species, the number of individuals and the different indices of diversity and evenness are presented per station and season for each port in Table 3. The leisure boat station C1 had a significantly lower species richness (S, d, H') within Cagliari port in all seasons and the lowest evenness (J') in winter and after the touristic season since these samples were dominated by the bivalve *Corbula gibba* (Kruskal-Wallis, S: H=59.90, p<0.001; d: H=57.40, p<0.001; H': H=54.78, p<0.001; J': H=39.29, p<0.001). The highest species richness (S, d) in Cagliari port during all seasons was found at the other leisure boat station (C2). However, the highest species diversity (H') and evenness (J') were found at stations C5 (cargo ships) before the touristic season and at C2 (leisure boats) after the touristic season.

In Heraklion port, the shipyard station (H5) had the lowest species richness (S, d, H') during all seasons and the lowest evenness (J') before the touristic season when it was dominated by the Capitellidae *Notomastus latericeus* and *Capitella capitata* (Kruskal-Wallis, S: H=49.84, p<0.001; d: H=42.76, p<0.001; H': H=50.24, p<0.001; J': H=23.95, p=0.02). The highest species diversity (H') was found at station H1 (leisure boat) in winter and before the touristic season, whereas the highest evenness was found at the shipyard station (H5) after the touristic season when the numbers of species and individuals were extremely low. In El Kantaoui the lowest species diversity (d, H'), as well as the lowest evenness (J') were found at station E1 during all seasons but mainly after the touristic season (Kruskal-Wallis, d: H=16.09, p=0.041; H': H=23.32, p<0.001; J': H=19.87, p=0.011). The dominating species here were the gastropods *Bittium latreillii* and *Cerithium scabridum*. The highest diversity and evenness values were observed at station E3 which was located near the port entrance.

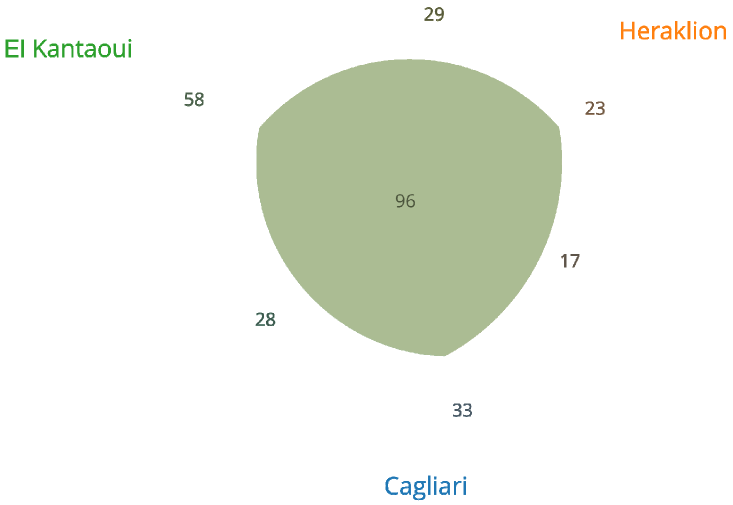


Figure 3. Venn chart showing the overlap of species, as well as the number of unique species in the three Mediterranean ports (Cagliari, Heraklion, El Kantaoui).

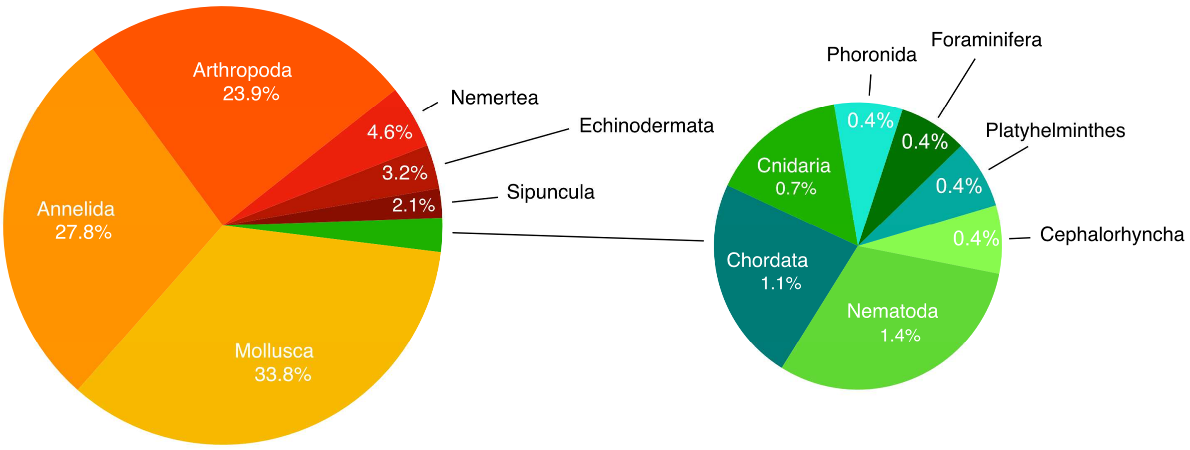


Figure 4. Distribution of the different phyla collected from the three Mediterranean ports.

Table 3. Mean numbers of species (S) and individuals (N), Margalef's (d), Pielou's evenness (J') and Shannon (H') indices for all stations and seasons in each port. Also the percentage of opportunistic species at each station (i.e. species of Capitellidae, Cirratulidae, Spionidae, Oligochaeta) is reported. Station codes as in Table 1; the preceding number represents sampling season (1: winter, 2: before the touristic period, 3: after the touristic period).

Sample	S	N	d	J'	H'	% opportunistic
1C1	15	18	4.86	0.497	1.345	9.7
1C2	87	344	14.73	0.557	2.488	24.8
1C3	54	139	10.73	0.643	2.566	22.7
1C4	49	230	8.83	0.583	2.269	29.8
1C5	32	68	7.35	0.615	2.131	17.4
2C1	20	46	4.97	0.484	1.450	22.4
2C2	91	845	13.35	0.312	1.406	8.6
2C3	69	294	11.97	0.619	2.622	31.3
2C4	56	236	10.07	0.487	1.961	18.0
2C5	55	152	10.75	0.746	2.990	32.1
3C1	17	60	3.91	0.315	0.893	14.8
3C2	58	93	12.59	0.805	3.269	29.4
3C3	54	121	11.05	0.673	2.685	32.2
3C4	36	213	6.53	0.579	2.075	18.6
3C5	55	251	9.78	0.658	2.638	33.1
1H1	58	283	10.10	0.699	2.840	26.6
1H3	55	325	9.33	0.596	2.387	58.0
1H4	57	175	10.85	0.634	2.563	44.5
1H5	10	13	3.49	0.645	1.485	59.4
2H1	98	918	14.22	0.618	2.836	19.9
2H3	46	184	8.63	0.695	2.659	51.8
2H4	52	141	10.31	0.664	2.624	31.8
2H5	12	45	2.90	0.502	1.247	89.3
3H1	46	144	9.05	0.638	2.444	43.6
3H3	25	32	6.90	0.648	2.086	54.1
3H4	45	54	11.04	0.665	2.532	41.4
3H5	6	3	5.23	0.787	1.411	12.2
1E1	67	557	10.44	0.561	2.359	43.4
1E2	76	348	12.82	0.695	3.008	20.1
1E3	75	618	11.51	0.609	2.628	30.2
2E1	54	202	9.98	0.670	2.672	57.3
2E2	78	368	13.03	0.545	2.376	7.5
2E3	108	926	15.66	0.620	2.901	15.9
3E1	67	452	10.79	0.466	1.959	5.5
3E2	65	122	13.33	0.662	2.763	11.7
3E3	65	222	11.84	0.740	3.089	36.1

Significantly increased percentages (>50%) of opportunistic species (i.e. short-lived species often characterising disturbed or stressed habitats) were found in Heraklion port at stations H3 (passenger ships) during all seasons and at H5 (shipyard) in winter and before the touristic season (Kruskal-Wallis, H=34.06, p<0.001), as well as at El Kantaoui station E1 (leisure boats) before the touristic season (Kruskal-Wallis, H=35.19, p<0.001) (Table 3). The lowest presence of opportunists was recorded in El Kantaoui and more specifically at stations E1 and E2, after and before the touristic season respectively. Significant differences were observed when the presence of opportunistic species was examined in relation to hydrocarbon pollution levels in each port (Figure 5) (Kruskal-Wallis, % opportunists: H=28.37, p<0.001; PAHs surface: H=60.96, p<0.001; PAHs anoxic: H=23.91, p<0.001; AHs surface: H=13.39, p<0.001; AHs anoxic: H=8.23, p=0.016). Heraklion port was the one hosting a significantly higher percentage of opportunistic species and at the same time the highest concentration of AHs both in its surface and anoxic sediment samples. El Kantaoui had significantly lower levels of PAHs and AHs pollution both for surface and anoxic sediments, a low percentage of opportunists and was characterised by a significantly richer biodiversity (Kruskal-Wallis, H': H=22.8, p<0.001). Cagliari, which had significantly higher levels of PAHs in surface sediments and higher UCM both in surface and anoxic sediments (Table 2), was characterised by a significantly lower percentage of opportunistic species, but a low Shannon index as well.

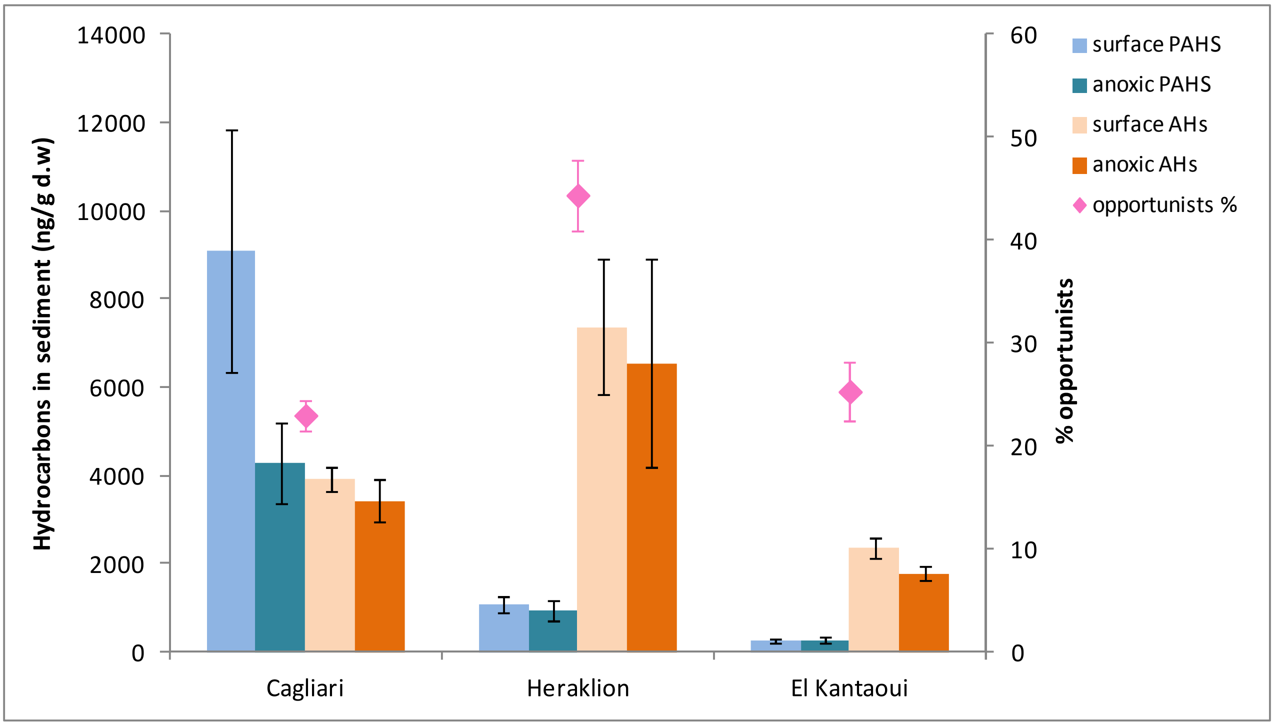


Figure 5. Average concentration (ng/g d.w.) (±SE) of polycyclic aromatic (PAHs) and aliphatic hydrocarbons (AHs) in the surface and anoxic sediment samples from the three Mediterranean ports (Cagliari, Heraklion, El Kantaoui). Average values were calculated for all stations and all seasons in each port. Percentage of opportunists includes the % of species belonging to Capitellidae, Cirratulidae, Spionidae and Oligochaeta.

The multivariate analysis of the samples based on species composition indicated significant differences between the three Mediterranean ports (Figure 6A; ANOSIM: Global R = 0.631, p<0.001), as was also confirmed by the pairwise tests (C-H: R= 0.526, p<0.001; C-E: R= 0.87, p<0.001; H-E: R= 0.516, p<0.001). The species mainly contributing to the dissimilarity between Cagliari and Heraklion ports (68.2%) were the polychaetes *Levinsenia gracilis* and *Aphelochaeta filiformis*, and the bivalve *Corbula gibba*. The dissimilarity between Cagliari and El Kantaoui port was even higher (70.8%) and could be attributed mainly to the polychaetes *Levinsenia gracilis* and *Dorvillea atlantica*, and the bivalve *Corbula gibba*. The polychaetes *Dorvillea atlantica* and *Praxillella gracilis* were mainly responsible for the high dissimilarity between Heraklion and El Kantaoui ports (70.6%).

The shipyard port sector (H5) was characterised by a significantly different pattern of species composition, while all other sectors were grouped together (Figure 6B; ANOSIM: Global R = 0.172, p=0.013). This finding was also confirmed by a pairwise comparison of the different sectors (Leisure - Passenger: R=0.013, p= 0.339; Leisure - Cargo: R=-0.118, p=0.812; Leisure - Shipyard: R=0.760, p<0.001; Passenger - Cargo: R= 0.082, p=0.165; Passenger - Shipyard: R=0.808, p=0.002; Cargo - Shipyard: R=0.889, p=0.012). Dissimilarities between the shipyard and leisure sectors (83.6%) can be mainly attributed to the bivalve *Corbula gibba* and the polychaete *Notomastus latericeus*. Dissimilarities between the shipyard and passenger sectors (83.1%) were mainly the result of the polychaetes *Notomastus latericeus* and *Aphelochaeta filiformis*. Finally, dissimilarities between the shipyard and cargo sectors (82.8%) can be mainly attributed to abundance differences of the bivalve *Corbula gibba* and the polychaete *Levinsenia gracilis*.

The multivariate patterns of benthic species were not significantly different between the different seasons (Figure 6C; ANOSIM: Global R = 0.012, p=0.322), with the polychaete *Notomastus latericeus* being one of the species mainly responsible for these similarities. However, when a second-stage MDS was performed on all taxonomic levels (not only on species) comparing the similarity matrices of all the three seasons, a significant separation of the seasonal patterns was observed (Figure 7; ANOSIM Global R = 0.429, p<0.001), which was also confirmed by the pairwise tests (winter – before touristic period: R= 0.367, p= 0.004; winter – after touristic period: R= 0.719, p=0.002; before touristic period – after touristic period: R=0.320, p=0.004), thus indicating signs of disturbance between seasons.

A second-stage MDS was also performed using the macrobenthic community patterns derived from the six different taxonomic levels (279 species, 229 genera, 145 families, 69 orders, 30 classes, 11 phyla) and by combining data from all the three Mediterranean ports. Figure 8 demonstrates that the biodiversity patterns derived from the species/genus levels are very similar with the patterns derived from the family level, while they are all different from those derived from the higher taxonomic levels (phylum, class, order). The same pattern was observed when the second-stage MDS analysis was performed for each port separately or for each season separately (graphs not shown).

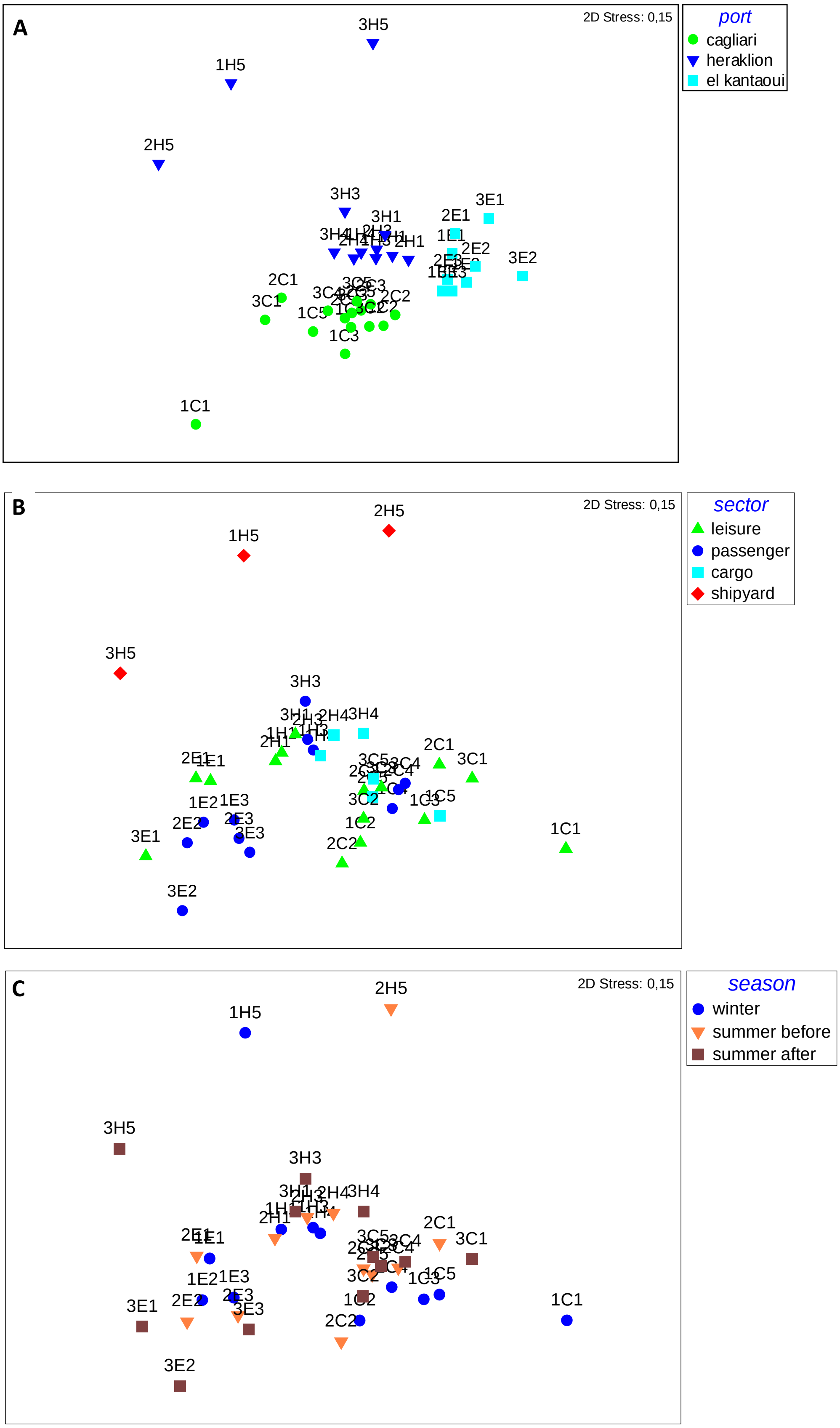


Figure 6. nMDS plots of the benthic samples grouped according to three factors: A) port, B) sector, C) season. Station codes as in Table 1; the preceding number represents sampling season (1: winter, 2: before touristic period, 3: after touristic period).

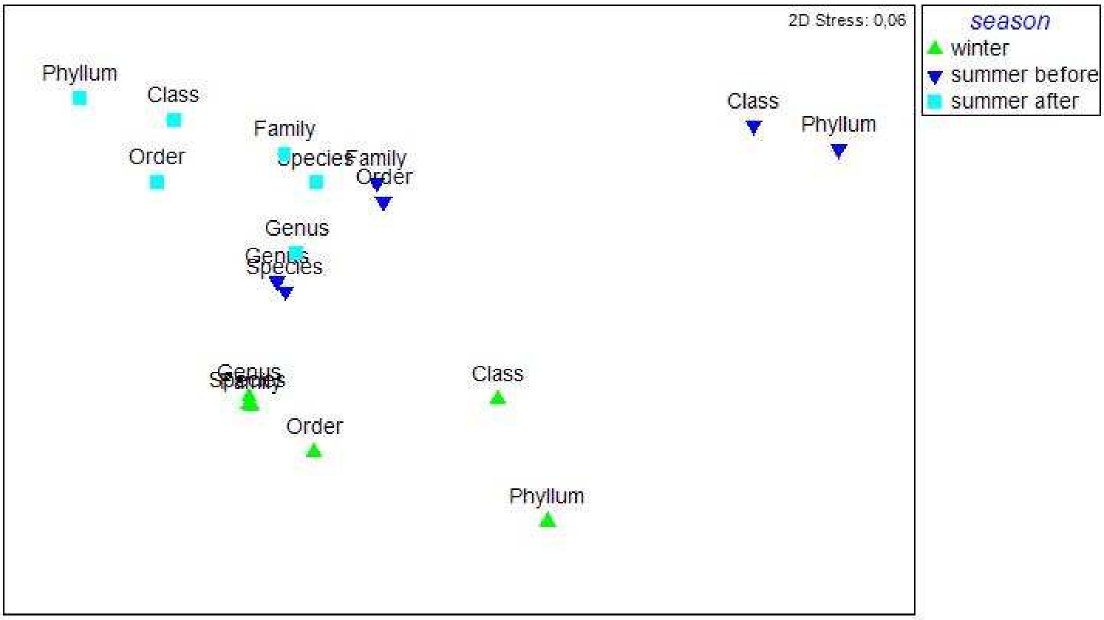


Figure 7. Second-stage MDS for all taxonomic levels using macrobenthic community patterns from the three different sampling seasons (winter, before touristic season, after touristic season).

Figure 8. Second stage MDS for all taxonomic levels using macrobenthic community patterns from the three Mediterranean ports.

4. Discussion

4.1 Environmental variables

The spatial and seasonal assessment of the three Mediterranean ports in Cagliari (Sardinia), El Kantaoui (Tunisia) and Heraklion (Crete) revealed significant differences in terms of their environmental variables. More specifically, the environmental parameters were significantly different between the three ports indicating, as expected, a strong geographical variation. Cagliari is the most north-western port in the Mediterranean between the ones studied and has lower temperature and salinity values, while Heraklion port, located in the south-eastern Mediterranean, is characterized by higher seawater temperature and salinity levels. The temperature and salinity ranges observed between the different ports are also confirmed by several historical studies published for the Mediterranean Sea (Zavatarelli & Mellor, 1994; Brasseur et al., 1996). In addition, Cagliari is a "port-canal" connected to a wide network of inland canals, which are increasing freshwater inputs and thus reducing seawater salinity. Environmental parameters at the shipyard sector were significantly different from those at the passenger and the cargo sectors, thus revealing a significantly degraded profile. The shipyard in Heraklion port is a small-scale, land based enterprise devoted mainly to the construction and maintenance of traditional wooden fishing boats. Even if activities at this sector are relatively restricted and definitely less intensive than those typically occurring at large vessel shipyards, it is quite likely that disposal of land wastes and reduced water circulation in this area are still capable of inducing marine pollution and highly anoxic conditions in sediments. When season was taken into consideration, significant differences regarding environmental variables were observed only between samples collected after the touristic period and in winter. Samples collected after the touristic period can be considered as records of stressful conditions, since maritime traffic is increased during that period. Furthermore, after the touristic period (i.e. September) temperatures are still high in the Mediterranean and wave action is not very intense in comparison to winter, thus weather conditions do not facilitate dispersal of pollutants.

Previous studies in ports have indicated depth and pollution (i.e. hydrocarbons) as significant environmental factors associated with the clustering of sampling stations regarding benthos abundance (Blanchard et al., 2002). In the present study, one of the environmental parameters mainly affecting benthic species distribution in the three Mediterranean ports was salinity. It is commonly accepted that the diversity and species composition of benthic macroinvertebrate assemblages are largely controlled by salinity and its fluctuations (Fürsich, 1981; Rosenberg et al., 2004). Even tolerant species such as *Capitella capitata* dominating disturbed areas can be replaced by others, such as the bivalve *Macoma balthica* and oligochaetes, under low salinities (Pearson & Rosenberg, 1978). Areas with different salinities or different depth halocline levels (salinity stratification) host different benthic species depending on their resistance.

The multivariate pattern of the benthic communities in the present study was also strongly affected by heavy metals concentration in sediments, and more specifically copper (Cu) and antimony (Sb). Copper may enter in the marine environment both from natural (e.g. mineral soils, volcanic action, thermal vents) and anthropogenic sources (mine works, metal constructions, antifouling paints, impregnated aquaculture nets). It has been used as a biocide in antifouling paints for more than 100 years and its concentration is therefore increased in areas where ships are anchored, moored or maintained (e.g. marinas, ports, quays, shipyards). In the present study, copper concentration in sediments of all ports (besides stations C5 and H3) was higher than the threshold effect level (35.7 mg/kg) indicated by the Canadian Sediment Quality Guidelines (SQGs) (Burton, 2002), thus demonstrating a heavily polluted status according to the US EPA guidelines (Pekey et al., 2004). The highest concentrations of copper were recorded at station H4 (cargo ships) in Heraklion port and at all stations of El Kantaoui port (leisure and fishing boats). Copper was considerably higher (2-10 times) in sediments of all the three Mediterranean ports in comparison to other large ports where values ranged between 19-20 mg/kg (Sanger et al., 2004; Moreira et al., 2017). Copper is probably more toxic than cadmium or zinc for some benthic species, such as the shrimp *Callianassa australiensis* and the polychaetes *Arenicola marina*, *Neanthes arenaceodentata*, *Capitella capitata* and *Nereis diversicolor* (Bryan, 1976; Reish et al., 1976; Ahsanullah et al., 1981; Bat & Raffaelli, 1998). According to Weis & Weis (1994), copper leaks from treated boat-wood caused a decrease in the number of benthic species and diversity, as well as in the number of individuals in Pensacola Beach (Florida). Exposure of sea scallops to environmentally realistic concentrations of seawater copper (10-20 μg/L), similar to the ones recorded in Heraklion and El Kantaoui ports during the present study (11-27 μg/L and 12-16 μg/L, respectively; MAPMED, 2013), decreased their sperm and oocyte production (Solomon, 2008). Morrisey et al. (1996) conducted a toxicity field study and indicated that copper concentrations ranging between 140 - 1200 mg/kg reduced the number of individuals of several benthic taxa. All the three stations within the El Kantaoui port had copper levels higher than 140 mg/kg. According to Bat & Raffaelli (1998), the LC_{50} (lethal concentration 50%) and EC_{50} (effective concentration 50%) copper levels for the amphipod *Corophium volutator* are 36.85 and 31.66 mg/kg respectively, while the values recorded at all ports during the present study (except stations C5 and H3) were much higher.

Antimony is not considered to be a highly reactive element in oceans and can be introduced in the aquatic environment due to rock weathering, soil runoff or anthropogenic activities (Filella et al., 2002). It is commonly found in ores of copper, silver and lead, as well as a component of coal and petroleum. Typical concentrations of dissolved Sb in unpolluted waters are less than 1 μg/L, while according to the Environmental Quality Standard of the Italian Government (DM 260/2010) the accepted "good chemical status" limit may reach up to 5 μg/L. The Sb in all water samples collected during the present study was below the detection limit of the analytical method (<5 μg/L, MAPMED, 2013). Antimony concentrations in sediments higher than a few μg/g are related to anthropogenic sources (e.g. sewage outfall, smelting plants) (Filella et al., 2002), but no such levels were found in any of the three ports examined. Not much information is available regarding the toxicity of antimony. Mori et al. (1999) indicated that increased concentrations of Sb had a negative impact on taxonomic diversity, but did not influence abundance of benthic communities. However, the fact that Sb was found to be highly correlated with the macrobenthic communities in the present study suggests that considerations regarding the toxicity of this heavy metal should be revised, thus more evidence will come to light.

Zinc is associated with pollution from antifouling paints in ports and marinas (Moreira et al., 2017) and can cause sublethal effects on benthic organisms. Both the amphipod *Corophium volutator* and the lugworm *Arenicola marina* preferred to emerge out of contaminated sediments but were able to rebury once removed from the source of contamination (Bat & Raffaelli, 1998). Most stations in all the three Mediterranean ports studied, exceeded by far the zinc LC_{50} levels reported for *C. volutator* and *A. marina* (31.87 μg/g and 50 μg/g respectively) (Bat & Raffaelli, 1998), as well as the threshold effect level (123 mg/kg) indicated by the Canadian SQGs (Burton, 2002). However, the maximum zinc concentrations in Heraklion (211.1 μg/g), Cagliari (350.3 μg/g) and El Kantaoui (156.5 μg/g) ports were by far lower than values recorded in other ports (e.g. Pecém Harbor, Brazil 639 μg/g; Moreira et al., 2017). Cadmium (Cd) is more toxic for *C. volutator* than zinc or copper (Bat & Raffaelli (1998), but the Cd levels recorded in all the three Mediterranean ports (<4 μg/g) were lower than both the EL_{50} and LC_{50} values indicated for this amphipod (>9 μg/g). However, all the three ports had higher Cd levels than those recorded in other large ports (≤1.4 μg/g)

(Moreira et al., 2017) and also higher than the threshold effect level (0.6 μg/g) indicated by the Canadian SQGs (Burton, 2002).

4.2 Macrobenthos biodiversity patterns

A higher number of species was identified in the three Mediterranean ports examined (El Kantaoui: 211 sp, Cagliari: 174 sp, Heraklion: 165 sp), in comparison to other harbours around the world (e.g. Valdez, Alaska: 109 sp, Mumbai, India: 43 sp, JadeWeser, North Sea: 57sp, Pecèm, Brazil: 27 sp, Mucuripe, Brazil: 39 sp) (Blanchard et al., 2002; Mandal & Harkantra, 2013; Gutperlet et al., 2015; Moreira et al., 2017). The three Mediterranean ports had higher percentages of Mollusca (34%, from which 15.2% were Bivalvia) in comparison to Annelida (27%), while usually a predominance of Polychaetes (72.09%) has been observed in other ports (e.g. 57% in Valdez port, Alaska; 72.09% in Mumbai port, India; 22-100% in Mucuripe port, Brazil) (Blanchard et al 2002; Mandal & Harkantra, 2013; Moreira et al., 2017;). According to Blanchard et al. (2002) Echinodermata and Amphipoda are not expected to thrive in port environments since they are sensitive to hydrocarbon pollution, as was the case for Pecèm port where Echinodermata were present at a very low abundance (2%) and only at one station (Moreira et al., 2017). However, in the three Mediterranean ports examined during the present study, Amphipoda constituted 15.1% and Echinodermata 3.2% of the total fauna identified, thus representing much higher percentages in comparison to port Valdez (<1.5% and <1% respectively) where such communities were limited due to high sedimentation rates and low food resources (Blanchard et al., 2002).

Benthic species composition was not significantly different between seasons; however, seasonal differences were revealed regarding the composition of benthic taxa when the patterns from all the different taxonomic levels were combined. Alternating degradation and recovery of benthic communities between different seasons may be the result of seasonal oxygen deficiency (Villnas & Norkko, 2011) or salinity fluctuations (Kundu et al., 2010). Benthic diversity was also significantly affected by the different geographical locations within the Mediterranean Sea and by the port sector type. Mandal & Harkantra (2013) did not find any differences regarding biodiversity between the different stations in Mumbai port, whereas they also indicated that seasonal variability was significant. The species mainly contributing to the dissimilarities between the three selected Mediterranean ports were the polychaetes *Levinsenia gracilis*, *Aphelochaeta filiformis*, *Dorvillea atlantica* and *Praxillella gracilis*, as well as the bivalve *Corbula gibba*, which belonged to the most abundant orders within the populations (Orbiniida 19.7%, Capitellida 27.9% and Terebellida 21.5%).. The shipyard sector had a significantly different distribution of benthic species in comparison to all other port sectors. Species contributing the most to these dissimilarities were more or less the same as the ones responsible for dissimilarities between the three Mediterranean ports: the polychaetes *Notomastus latericeus*, *Aphelochaeta filiformis* and *Levinsenia gracilis,* and the bivalve *Corbula gibba*.

4.3 Comparisons within and between ports

Sediments provide an ideally homogenized natural archive of pollution, integrating the input over the several past years, as a result of the combined processes of deposition, runoff, water mixing, and sedimentation of pollutants. PAHs in the marine environment originate from anthropogenic pollution, such as urban runoffs, shipping, oil discharges, etc. Prolonged traffic of leisure and touristic boats combined with intrinsic hydrological patterns may have caused over the years a more severe pollution in Cagliari, since this port had the highest level of PAHs pollution in comparison to all the Mediterranean ports examined. PAHs in Cagliari port were almost 1000 times higher than the respective values recorded in two Brazilian ports (Moreira et al., 2017), 21 times higher than Barcelona harbour in Spain and 5 times higher than Port Vendres in France (Baumard et al., 1998). PAHs in Cagliari were higher than the threshold effect level (870 ng/g) indicated by the Canadian SQGs (Burton, 2002). In addition, the threshold reported for heavily modified waters by Ondiviela et al. (2012) (40,000 ng/g) was also very close to the levels recorded at the most polluted Cagliari station (C2). Cagliari port was characterised by a low percentage of opportunistic species, as well as a low Shannon index. The lowest species richness and the lowest evenness in Cagliari port were found almost in all seasons at the leisure boat station C1 which was the most anoxic one. Benthic samples of C1 in winter and after the touristic period were dominated by *Corbula gibba*. This bivalve is considered as an indicator of environmental instability caused by pollution, low oxygen content or increased turbidity (Hrs-Brenko, 2006), conditions which are typically encountered in port environments. *Corbula gibba* thrives in degraded or stress-recovering low diversity communities, since it is a short-lived and fast-growing species, with high fecundity and wide larval dispersal (Hrs-Brenko, 2006). The highest richness and diversity of benthic species within Cagliari port was found at the shallower leisure boat station C2 after the touristic season, which was notably the most polluted station regarding PAHs and AHs. Also high diversity and evenness were found before the touristic season at station C5 which was closer to the port entrance and was characterised by intense maritime traffic. Station C5 had the highest POC in sediments but was one of the less polluted stations in terms of PAHs, AHs and heavy metals.

In addition, Cagliari had the highest levels of Unresolved Complex Mixture (UCM), especially in anoxic sediments. The UCM fraction includes the chemical components of petroleum that are more resistant to weathering and bacteria degradation, thus representing the highly degraded oil residues which can remain largely unchanged in sediments for many years (Killops & Al-Juboori, 1990). The presence of UCMs in chromatograms indicates a chronic pollution by petroleum or relevant products (lubricating oils, etc.) (Melbye et al., 2009). All stations in all the ports investigated during the present study had a relatively low UCM in surface sediments ranging from 10.8 to 103 μg/g, in comparison, for example, with mussel samples collected near an Australian oil refinery outfall (5.8-55.5 times higher) (Smith & Burns, 1978) or from the east UK coast (2.6 - 25 times higher) (Rowland et al., 2001). Rowland & Volkman (1982) indicated a more similar UCM range in mussels collected near a North Sea oil production platform (range 52-77 μg/g). Monoaromatic components of UCMs may be responsible for sublethal toxic responses (70% reduction of feeding rate) and impaired health in *Mytilus edulis* (Rowland et al., 2001; Donkin et al., 2003). According to Boyd et al. (2002) mechanisms of toxicity affecting the burrowing behaviour of benthic organisms may include epithelial damage to sensory structures and neurotoxic effects on the nervous system (i.e. decreased conduction velocity in nerve fibres) affecting general coordination. Scarlett et al. (2007) indicated that reduced reproductive success and reduced growth rate associated with abnormal amphipod behaviour, such as failure to burrow rapidly and re-emerge from the sediment, were observed after exposure to 500 μg/g of weathered oils, which is 5 times higher than the UCM concentration of the most polluted surface sediment samples of the present study.

El Kantaoui was the smallest port surveyed (50 times smaller than Cagliari and 20 times smaller than Heraklion) used only as a touristic marina for fishing and leisure boats. El Kantaoui port had the lowest levels of PAHs and AHs both for surface and anoxic sediments and was characterised by a rich biodiversity. This port had the highest number of species in total, as well as the highest number of unique species in comparison to Cagliari and Heraklion port. Although a peak of opportunistic species was recorded at station E1 (leisure boats) before the touristic season, these were extremely reduced three months later (after the touristic season). When a high percentage of opportunistic taxa is observed only during spring at stressful or degraded sites, this indicates that the recruitment of these taxa fails to survive beyond the initial settlement of their tolerant larvae (Alden et al., 1997; Rosenberg et al., 2004). Within El Kantaoui port, the lowest species diversity was observed at the inner station (E1), while the port entrance station (E3) hosted the highest diversity. The dominating species at station E1 after the touristic period were the gastropods *Bittium latreillii* and *Cerithium scabridum*. *Bittium latreillii* is among the most dominant species in *Posidonia oceanica* beds which are found mainly in deep waters (>25 m) (Russo et al., 2002), while El Kantaoui is a very shallow port (<4.5 m). However, mapping of the area just outside the port revealed the presence of shallow *P. oceanica* meadows (MAPMED, 2013), which could therefore explain the significant presence of this gastropod even in shallow depths inside the port. *Cerithium scabridum* is an invasive Lessepsian immigrant usually found in shallow depths (1-2 m) and reported for the first time in Tunisia in 2000 (CIESM, 2000) probably as the result of shipping activities from the Eastern Mediterranean (Garilli & Caruso, 2003). Established populations have been recorded within harbours on soft or hard substrata associated with seagrasses or algal mats (Garilli & Caruso, 2003; Albano & Trono, 2008). This species seems to be very resistant to oil pollution (e.g. fuels, motor lubricants), since it has thrived inside Otranto harbour (Italy) which is characterised by heavy boat traffic (Albano & Trono, 2008). *Cerithium scabridum* is a strong coloniser having a planktotrophic, long-lived larval stage (45-60 days) and a wide dispersion range (Garilli & Caruso, 2003).

Between the three ports examined, Heraklion had the highest concentration of AHs in both surface and anoxic sediments (2-12 times higher than the respective PAHs). AHs do not only originate from anthropogenic pollution, but they may also originate from natural sources, such as the biomass of marine microorganisms (phytoplankton, algae and bacteria) and the transfer of terrestrial plants detritus from the land into the sea (Mandalakis et al., 2014). The increased AHs in both surface and anoxic sediments of Heraklion were also accompanied by the highest number of opportunistic species. In contrast with El Kantaoui, where opportunists fail to survive following their initial settlement in spring, opportunists in Heraklion port thrived in all seasons at the passenger station (H3), as well as in winter and before the touristic season at the shipyard station (H5). According to Gray (1989), reduced diversity of benthic fauna and retrogression to opportunistic species are indicators of natural and anthropogenic stress in marine environments.

Station H5 was the most anoxic one within Heraklion port, had a high PAHs level, the highest POC and AHs concentration, as well as the highest percentage of silt and clay. Fine grain sediments absorb and store a higher concentration of pollutants thus acting as sinks and secondary sources of chemicals (Luoma and Davis, 1983). However, even the highest AHs concentration recorded at H5 (0.021 mg/g) was 14 times lower than the lowest value recorded in the Brazilian port Mucuripe (0.3 mg/g; Moreira et al., 2017) and 5 times lower than values recorded in Patagonia ports (Commendatore et al., 2000). The Heraklion shipyard station had the lowest species richness in all seasons and the lowest evenness before the touristic season when it was dominated by the Capitellidae *Notomastus latericeus* and *Capitella capitata.* In addition, station H5 had the highest evenness after the touristic season when the number of species and individuals was extremely low. *Capitella capitata* has been indicated as a pollution resistant species and has been often recorded in ports, harbours, estuaries and organically enriched or polluted areas (Pearson & Rosenberg, 1978; Raman & Ganapati, 1983; Tsutsumi, 1987; Raman, 1995; Blanchard et al., 2002). *Capitella* species can also respond to non-toxic fractions of crude oil as they would to other forms of organic enrichment (Spies et al., 1988; Blanchard et al., 2002). *Notomastus latericeus* is also an opportunistic species characterised by fast growth, rapid increase in density and heavy adult mortality (Giangrande & Fraschetti, 1993). The ability of the opportunistic Capitellidae species to thrive in unpredictable habitats is enhanced by their short life cycle, continuous reproduction, production of planktonic larvae with great dispersal ability and fast population growth (Tsutsumi, 1987).

Sediments from the leisure boat station in Heraklion (H1) had the highest PAHs content, increased AHs and POC, and exceeded by over 50% the chemical limit level regarding Cd and Cu. However, this station was characterised by the highest species diversity within Heraklion port in winter and before the touristic season. The same pattern was observed at the leisure boat station (C2) of Cagliari port; the highest species richness and diversity were recorded at the most polluted station regarding AHs and PAHs. Although several studies have indicated that species richness and diversity in ports were low due to high pollution levels and reduced oxygen (Tsutsumi, 1987; Estacio et al., 1997; Dhainaut-Courtois et al., 2000), the current study suggests that benthic diversity was not severely affected by oil pollution, increased organic content and heavy metals, at least regarding the levels recorded here. Guerra-Garcia & Garcia-Gomez (2004) also indicated that species richness and diversity was not significantly different between polluted and unpolluted stations in Ceuta harbour in the Strait of Gibraltar. The toxic effects of oil pollution on the mysid shrimp *Mysidopsis bahia* are not correlated with PAH concentration (Barron et al., 1999), since PAHs are characterised by a lower toxicity in comparison to other petroleum compounds (Melbye et al., 2009). The increased PAHs levels at station H1 indicate a more recent pollution by petroleum and oil discharges from leisure and fishing boats, whereas the high levels of AHs and UCMs recorded at station H5 imply that shipyard activities during the past in this area have caused a more severe, but localized, accumulation of the specific pollutants over the years. Ports are definitely disturbed and heavily modified environments. However, the three Mediterranean touristic ports examined during the present study are not characterized as severely impacted since the biodiversity patterns of the lower taxonomic levels (species to family) were not similar to those derived from the higher levels. According to the hierarchic-response-to-stress hypothesis (Olsgard et al., 1998), when the fauna patterns of the lower taxonomic levels become similar to the ones derived from the higher levels, an increased degree of disturbance is indicated.

4.4 Port management approaches

Although ports are environments where anthropogenic disturbance is considered to be a fact, spatial and temporal biodiversity changes are not being monitored thoroughly or regularly. For example, even though benthic fauna in port Valdez (Alaska) has been sampled since 1971, long term biota responses to oil-terminal activities have been rarely identified since only deep stations were monitored for almost 20 years (Blanchard et al., 2002). When a regular and detailed monitoring plan was later on adopted in that area, accumulation of undissolved hydrocarbon fractions exiting the ballast-water treatment settling ponds was revealed and the situation was effectively confronted (Blanchard et al., 2002). None of the three Mediterranean ports investigated during the present study is yet implementing a regular-basis monitoring plan including evaluation of environmental health based on benthic biodiversity. However, benthic species are useful as bioindicators since they have a wide geographic distribution, are available all year round and are continuously exposed to contaminants in the sediment (Bat & Raffaelli, 1998). They can be used in the development of bioassays protocols as they constitute important links in coastal food chains and play an important role in sediment community organisation. One-off sampling surveys can capture only a segment of a community at temporally or spatially discrete intervals, therefore often missing the critical transition points in between (Blanchard et al., 2002). On the contrary, long-term monitoring is a useful management tool which can be used to evaluate the health status of the marine environment, provide a basis for perceiving changes and reveal anthropogenic disturbance which might be masked by natural sources of stress. The results of the present study indicate that seasonal differences are not a significant differentiating factor, especially regarding benthic species composition, thus an annual sampling campaign would be sufficient considering the monetary, time and labor costs of such activities. However, monitoring of the different sectors of a port and adequate dispersal of the sampling stations is essential for the identification of disturbance applying locally. Macrobenthic community pattern assessment before and after port operations (e.g. dredging activities, construction of new sectors) may offer additional information regarding the degree of habitat restoration and environmental health status. Furthermore, identification of organisms to the lowest taxonomic level may not be necessary for the evaluation of biodiversity patterns in environmental monitoring routines (Olsgard et al., 1998). Indeed, the present study indicated that the biodiversity information derived from the family level was similar to the information derived from the genus and species levels, thus indicating that identification of specimens down to the species level is not necessary during a regular monitoring programme in port environments. Similarly, Hilsenhoff (1988) indicated that a family-level biotic index for arthropods was sufficient for the evaluation of organic pollution in streams. Taxonomic identification to species level - a highly demanding procedure regarding time resources and expertise - may therefore be omitted, thus making more feasible and cost-effective the implementation of a regular monitoring programme and the fast and simplified evaluation of biodiversity status in ports under the supervision of the local management authorities.

5. Conclusions

The present study presents a valuable baseline study of environmental variables (including hydrocarbon and heavy metal pollution) and benthic diversity covering three touristic ports of the still insufficiently studied eastern Mediterranean Sea. The environmental parameters mainly affecting benthic species distribution in the three ports were salinity, copper and antimony in sediments. Patterns of environmental variables and benthic species diversity were significantly different between ports indicating a strong geographical variation. The shipyard sector was significantly degraded in terms of its abiotic and biotic profile. Seasonal variability in the environmental parameters was observed only between late summer (after the touristic period) and winter. Seasonal differences in benthic species composition were revealed when the patterns from all the different taxonomic levels were combined. El Kantaoui had the lowest levels of hydrocarbons in sediments, the highest number of species in total, as well as the highest number of unique species. Recruitment of opportunistic species in El Kantaoui failed to survive beyond their initial settlement. Cagliari had the highest levels of PAHs and UCM pollution, it was characterised by a low percentage of opportunistic species, but a low species diversity as well. Heraklion had the highest concentration of AHs in sediments and the highest percentage of opportunists, which thrived during all seasons, thus indicating increased environmental stress in this port. The Heraklion shipyard sector was the most anoxic and polluted station (PAHs, AHs, POC) and had the lowest species richness. However, in general terms, the three Mediterranean touristic ports were not severely impacted since the biodiversity patterns of the lower taxonomic levels were not similar to those derived from the higher levels. The levels of pollution recorded at some stations, although considered to be comparatively high, had not negatively affected benthic diversity. A port monitoring plan should include evaluation of benthic biodiversity, especially before and after heavy port operations, in order to assess the degree of habitat restoration and the environmental health status. A regular annual sampling campaign, including monitoring of the different sectors in a port and adequate dispersal of the sampling stations, would be essential for the identification of disturbance applying locally. Identification of organisms to the lowest taxonomic level (i.e species), which is a highly demanding procedure regarding time, labour and expertise, is not necessary. Local port management authorities could take a great advantage following the above suggestions in order to organise and implement a feasible, simple, fast and cost-effective monitoring plan.

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References

Abdulla A, Linden O (2008) Maritime traffic effects on biodiversity in the Mediterranean Sea: Review of impacts, priority areas and mitigation measures. Malaga, Spain: IUCN Centre for Mediterranean Cooperation. p. 184.

Ahsanullah M, Negilski D S, Mobley M C (1981) Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis*. I. Effect of individual metals. Marine Biology. 64: 299–304.

Albano P G, Trono D (2008) Record of the alien species *Cerithium scabridum* Philippi, 1848 (Gastropoda: Cerithiidae) from Otranto, southern Adriatic Sea. Boll. Malacol., 44 (1-4): 1-4.

Alden R W, Weisberg S B, Ranasinghe J A, Dauer, D M (1997) Optimizing temporal sampling strategies for benthic environmental monitoring programs. Marine Pollution Bulletin. 34(11): 913-922.

Anastasiou T I, Chatzinikolaou E, Mandalakis M, Arvanitidis C (2016) Imposex and organotin compounds in ports of the Mediterranean and the Atlantic: Is the story over? Science of the Total Environment. 569–570: 1315–1329.

Arar E J, Collins G B (1992) In vitro determination of Chlorophyll-a and Pheophytin in Marine and Freshwater Phytoplankton by Fluorescence. Environmental Monitoring and Support Laboratory. U.S.EPA.

Barron M G, Podrabsky T, Ogle S, Ricker R W (1999) Are aromatic hydrocarbons the primary determinant of petroleum toxicity to aquatic organisms. Aquatic Toxicology. 46: 253-268.

Bat L, Raffaelli D (1998) Sediment toxicity testing: a bioassay approach using the amphipod *Corophium volutator* and the polychaete *Arenicola marina*. Journal of Experimental Marine Biology and Ecology. 226: 217–239.

Baumard P, Budzinski H, Michon Q, Garrigues P, Burgeot T, Bellocq J (1998) Origin and Bioavailability of PAHs in the Mediterranean Sea from Mussel and Sediment Records. Estuarine, Coastal and Shelf Science. 47: 77–90.

Blanchard A L, Feder H M (2003) Adjustment of benthic fauna following sediment disposal at a site with multiple stressors in Port Valdez, Alaska. Marine Pollution Bulletin. 46: 1590–1599.

Blanchard A L, Feder H M, Shaw D G (2002) Long-term investigation of benthic fauna and the influence of treated ballast water disposal in Port Valdez, Alaska. Marine Pollution Bulletin. 44: 367–382.

Boyd W A, Brewer S K, Williams P L (2002) Altered behaviour of invertebrates living in polluted environments. In: Behavioural Ecotoxicology (eds: Dell'Omo G). John Wiley & Sons, Chichester, UK.

Brasseur P, Beckers J M, Brankart J M, Schoenauen R (1996) Seasonal temperature and salinity fields in the Mediterranean Sea: Climatological analyses of a historical data set. Deep-Sea Research. 43 (2): 159-192.

Bryan G W (1976) Heavy metal contamination in the sea. Marine Pollution (ed. R. Johnston). Academic Press, London.

Buchanan J B (1984) Sediment analysis. In: Holme, N. A. and McIntyre, A. D. (eds), Methods for the Study of Marine Benthos. Blackwell Scientific Publishers, Boston, pp. 41–65.

Burton G A (2002) Sediment quality criteria in use around the world. Limnology 3 (2): 65-76.

Chatzinikolaou E, Arvanitidis C (2016) Status, values and present threats in Heraklion harbour (Crete, Greece). Regional Studies in Marine Science. doi:10.1016/j.rsma.2016.01.010

CIESM (2000) Illustrated atlas of the exotic species in the Mediterranean Sea covering Fishes, Molluscs and Crustaceans. Http://www.Ciesm.org/atlas/index.html.

Clarke K R (1993) Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology. 18: 117–143.

Clarke K R, Ainsworth M (1993) A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series. 92: 205–219.

Clarke K R, Gorley R N (2006) User manual/tutorial. PRIMER-E Ltd., Plymouth, p. 93.

Clarke K R, Warwick R M (1994) Similarity-based testing for community pattern: the two-way layout with no replication. Marine Biology. 118(1): 167–176.

Commendatore M G, Esteves J L, Colombo J C (2000) Hydrocarbons in Coastal Sediments of Patagonia, Argentina: Levels and Probable Sources. Marine Pollution Bulletin. 40 (11): 989-998.

Darbra R M, Ronza A, Stojanovic T A, Wooldridge C, Casal J (2005) A procedure for identifying significant environmental aspects in sea ports. Marine Pollution Bulletin. 50: 866-874.

Dhainaut-Courtois N, Pruvot C, Empis A, Baudet K (2000) Les peuplements macrozoobenthiques indicateurs des qualités physico-chimiques et chimiques des sédiments portuaires—exemple du Port de Boulogne-sur-mer (Manche). Bull Soc Zool Fr. 125:49–62.

Donkin P, Smith E L, Rowland S J (2003) Toxic effects of Unresolved Complex Mixtures of aromatic hydrocarbons accumulated by mussels, *Mytilus edulis*, from contaminated field sites. Environmental Science and Technology. 37 (21): 4825-4830.

Estacio F J, García-Adiego E M, Fa D A, García-Gómez J C, Daza J L, Hortas F, Gómez-Ariza J L (1997) Ecological analysis in a polluted area of Algeciras Bay (Southern Spain): external "versus" internal outfalls and environmental implications. Marine Pollution Bulletin. 34:780–793.

Eurostat (2015) Maritime transport statistics - short sea shipping of goods (http://ec.europa.eu/eurostat/statistics-explained/index.php/Maritime_transport_statistics_-_short_sea_shipping_of_goods)

Filella M, Belzile N, Chen Y-W (2002) Antimony in the environment: a review focused on natural waters: I. Occurrence. Earth-Science Reviews. 57: 125-176.

Fürsich F T (1981) Salinity-controlled benthic associations from the Upper Jurassic of Portugal. Lethaia. 14: 203–223.

Garilli V, Caruso T (2003) Records of *Cerithium scabridum* Philippi, 1848 (Caenogastropoda, Cerithiidae) from Northwestern Sicily. Bollettino Malacologico, Roma, 39 (9-12): 157-160.

Giangrande A, Fraschetti S (1993) Life cycle, growth and secondary production in a brackish-water population of the polychaete *Notomastus latericeus* (Capitellidae) in the Mediterranean Sea. Marine Ecology. 14: 313-327.

Gray J S (1989) Effects of environmental stress on species rich assemblages. Biological Journal of the Linnean Society. 37: 19-32.

Gray J S, Elliot M (2010). Ecology of marine sediments: From science to management. Oxford University Press.

Guerra-García J M, García-Gómez J C (2004) Soft bottom mollusc assemblages and pollution in a harbour with two opposing entrances. Estuar Coast Shelf Sci 60:273–283.

Gutperlet R, Capperucci R M, Bartholomä A, Kröncke I (2015) Benthic biodiversity changes in response to dredging activities during the construction of a deep-water port. Marine Biodiversity. 45:819–839.

Hilsenhoff W L (1988) Rapid field assessment of organic pollution with a family-level biotic index. Journal of North American Benthological Society. 7: 65-68.

Hrs-Brenko M (2006) The basket shell, *Corbula gibba* Olivi, 1792 (Bivalve Mollusks) as a species resistant to environmental disturbances: A review. Acta Adriatica. 47: 49-64.

Ivančić I, Degobbis D (1984) An optimal manual procedure for ammonia analysis in natural waters by the indophenol blue method. Wat. Res. 18: 1143-1147.

Killops S D, Al-Juboori M A H A (1990) Characterisation of the unresolved complex mixture (UCM) in the gas chromatograms of biodegraded petroleums. Organic geochemistry. 15(2): 147-160.

Kruskal W H, Wallis W A (1952) Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association. 47: 583–621.

Kruskal J B, Wish M (1978) Multidimensional scaling. Sage, Beverly Hills, CA.

Kundu S, Mondal N, Lyla P S, Ajmal Khan S (2010) Biodiversity and seasonal variation of macro-benthic infaunal community in the inshore waters of Parangipettai Coast. Environmental Monitoring and Assessment 163: 67-79.

Luoma S N, Davis J A (1983) Requirements for modelling trace metal partitioning in oxidized estuarine sediments. Marine Chemistry 12: 159-181.

Mandal S, Harkantra S N (2013) Changes in the soft-bottom macrobenthic diversity and community structure from the ports of Mumbai, India. Environmental Monitoring Assessment. 185: 653‐672.

Mandalakis M, Polymenakou P N, Tselepides A, Lampadariou N (2014) Distribution of aliphatic hydrocarbons, polycyclic aromatic hydrocarbons and organochlorinated pollutants in deep-sea sediments of the southern Cretan margin, eastern Mediterranean Sea: A baseline assessment. Chemosphere. 106: 28-35.

MAPMED Consortium. 2013. Site characterization reports: 1) Cagliari Port, Sardinia, Italy (134 p), 2) Heraklion Port, Crete, Greece (122 p), 3) El Kantaoui Port, Tunisia (133 p). Management of Port Areas in the Mediterranean Sea Basin.

MAPMED Consortium. 2015. Action Plan for the rational and sustainable management of water, sediments and linked ecosystems: a) Port of Heraklion - Greece (p. 168); b) Port of Cagliari - Italy (p. 275); c) Port of El Kantaoui - Tunisia (p. 184).

McPherson C, Chapman P M, Bruyn A M H, Cooper L (2008) The importance of benthos in weight of evidence sediment assessments — A case study. Science of the Total Environment. 394: 252–264.

Melbye A G, Brakstad O G, Hokstad J N, Gregersen I K, Hansen B H, Booth A M, Rowland S J, Tollefsen K E (2009) Chemical and toxicological characterization of an unresolved complex mixture-rich biodegraded crude oil. Environmental Toxicology and Chemistry. 28: 1815–1824.

Moreira L B, Castro I B, Hortellani M A, Sasaki S T, Taniguchi S, Petti M A V, Fillmann G, Sarkis J E S, Bícego M C, Costa-Lotufo L V, Abessa D M S (2017) Effects of harbor activities on sediment quality in a semi-arid region in Brazil. Ecotoxicology and Environmental Safety. 135: 137–151.

Mori C, Orsini A, Migon C (1999) Impact of arsenic and antimony contamination on benthic invertebrates in a minor Corsican river. Hydrobiologia. 392: 73–80.

Morrisey D J, Underwood A J, Howitt L (1996) Effects of copper on the faunas of marine soft-sediments: An experimental field study. Marine Biology. 125: 199-213.

Munari C, Rossi R, Mistri M (2005) Temporal trends in macrobenthos community structure and redundancy in a shallow coastal lagoon (Valli di Comacchio, Northern Adriatic Sea). Hydrobiologia. 550: 95–104.

Olsgard F, Somerfield P J, Carr M R (1998) Relationships between taxonomic resolution, macrobenthic community patterns and disturbance. Marine Ecology Progress Series. 172: 25-36.

Ondiviela B, Juanes J A, Gomez A G, Samano M L, Revilla J A (2012) Methodological procedure for water quality management in port areas at the EU level. Ecological Indicators. 13: 117–128.

Ondiviela B, Gómez A G, Puente A, Juanes J A (2013) A pragmatic approach to define the ecological potential of water bodies heavily modified by the presence of ports. Environmental Science and Policy. 33: 320 - 331.

Pearson T H, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Ann. Rev. 16: 229–311.

Pearson S, Windupranata W, Pranowo S W, Putri A, Ma Y, Vila-Concejo A, Fernández E, Méndez G, Banks J, Knights A M, Firth L B, Breen B B, Jarvis R, Aguirre J D, Chen S, Smith A D H, Steinberg P, Chatzinikolaou E, Arvanitidis C (2016) Conflicts in some of the World harbours: what needs to happen next? Maritime Studies. 15:10.

Pekey H, Karakaş D, Ayberk S, Tolun L, Bakoğlu M (2004) Ecological risk assessment using trace elements from surface sediments of Izmit Bay (Northeastern Marmara Sea) Turkey. Marine pollution bulletin. 48: 946-953.

Raman A V (1995) Pollution effects in Visakhapatnam harbour, India; an overview of 23 years of investigations and monitoring. Helgoländer Meeresunters. 49: 633 - 645.

Raman A V, Ganapati P N. (1983) Pollution effects on ecobiology of benthic polychaetes in Visakhapatnam Harbour (Bay of Bengal). Marine Pollution Bulletin. 14: 46-52.

Reddy C M, Eglinton T I, Hounshell A, White H K, Xu L, Gaines R B, Frysinger G S (2002) The West Falmouth oil spill after thirty years: The persistence of petroleum hydrocarbons in marsh sediments. Environmental Science and Technology. 36: 4754–4760.

Reish D J, Martin J M, Piltz P M, Word J Q (1976) The effect of heavy metals on laboratory populations of two polychaetes with comparisons to the water quality conditions and standards in Southern California marine waters. Water Research. 10(4): 299-302.

REMPEC - Regional Marine Pollution Emergency Response Centre for the Mediterranean Sea (2008) Study of Maritime Traffic Flows in the Mediterranean Sea. Final Report standards in Southern California marine waters. Water Research. 10: 299–302.

Rivero M S, Elías R, Vallarino E A (2005) First survey of macroinfauna in the Mar del Plata Harbor (Argentina), and the use of polychaetes as pollution indicators. Revista de Biología Marina y Oceanografía. 40(2): 101 – 108.

Rosenberg R, Blomqvist M, Nilsson H C, Cederwall H, Dimming A (2004) Marine quality assessment by use of benthic species-abundance distributions: a proposed new protocol within the European Union Water Framework Directive. Marine Pollution Bulletin. 49: 728–739.

Rowland S, Donkin P, Smith E, Wraige E (2001) Aromatic hydrocarbon ‘‘humps’’ in the marine environment: Unrecognized toxins? Environmental Science and Technology. 35: 2640–2644.

Rowland S J, Volkman J K (1982) Biogenic and pollutant aliphatic hydrocarbons in *Mytilus edulis* from the North Sea. Marine Environmental Research. 7: 117-130.

Russo G F, Fraschetti S, Terlizzi A (2002) Population ecology and production of *Bittium latreillii* (Gastropoda, Cerithidae) in a *Posidonia oceanica* seagrass bed. Italian Journal of Zoology. 69(3): 215-222.

Sanger D M, Holland A F, Hernandez D L (2004) Evaluation of the Impacts of Dock Structures and Land Use on Tidal Creek Ecosystems in South Carolina Estuarine Environments. Environmental Management. 33(3): 385-400.

Scarlett A, Canty M N, Smith E L, Rowland S J, Galloway T S (2007) Can Amphipod Behavior Help to Predict Chronic Toxicity of Sediments? Human and Ecological Risk Assessment: An International Journal. 13(3): 506-518.

Siegel S, Castellan N J (1988) Nonparametric statistics for the behavioral sciences (2nd ed.) McGraw-Hill. New York.

Smith J L, Burns K (1978) Hydrocarbons in Westernport Bay mussels. Final Report, Hydrocarbons in Victorian Ecosystems, Task T01-702. Westernport Regional and Marine Chemistry Unit, Ministry for Conservation, Australia.

Solomon F (2008) Impacts of copper on aquatic ecosystems and human health. Environment and Communities. 25-28.

Somerfield P J, Clarke K R (1995) Taxonomic levels in marine community studies, revisited. Marine Ecology Progress Series. 127: 113–119.

Spies R B, Hardin D D, Toal P T (1988) Organic enrichment or toxicity? A comparison of the effects of kelp and crude oil in sediments on the colonization and growth of benthic infauna. Journal of Experimental Marine Biology and Ecology. 124: 261–282.

Strickland J D H, Parsons T R (1972) A Practical Handbook of Seawater Analysis. Fish.Res.Bd. Canada.

Tsutsumi H (1987) Population dynamics of *Capitella capitata* (Polychaeta; Capitellidae) in an organically polluted cove. Marine Ecology Progress Series. 36: 139-149.

UNEP (United Nations Environment Programme) (2006) Illegal Oil Discharge in European Seas. Environment Alert Bulletin, No 7. Athens: UNEP.

Villna S, Norkko A (2011) Benthic diversity gradients and shifting baselines: implications for assessing environmental status. Ecological Applications. 21: 2172–2186.

Yentsch C S, Menzel D W (1963) A method for the determination of phytoplankton chlorophyll and pheophytin by fluorescence. Deep Sea Research. 10: 221-231.

Zavatarelli M, Mellor G L (1994) A numerical study of the Mediterranean Sea circulation. Journal of Physical Oceanography. 25: 1384-1414.

Weis J S, Weis P (1994) Effects of contaminants from chromated copper arsenate-treated lumber on benthos. Archives of Environmental Contamination and Toxicology. 26: 103-109.