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VARIABLES AFFECTING THE PLANKTON NETWORK IN MEDITERRANEAN PORTS

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Abstract

Attention on port waters is increasing since these economically important areas are embedded in the coastal environment and their management needs to be considered in the monitoring programmes of coastal ecosystems. Specifically, planktonic taxa have mainly been studied in the marine environment as elements of food webs and proposed as early warning indicators of changing conditions. To implement the sustainable development (blue growth) of port areas, a general knowledge on the ongoing processes in their waters needs to be obtained, considering both abiotic and biotic variables.

We carried out seasonal samplings in three Mediterranean touristic ports and compared planktonic components (bacterioplankton, phytoplankton and zooplankton), physical and chemical water variables and analysed their relationships. Factor analysis revealed the effects of load of inland waters, seasonality, water turbulence and hydrocarbon pollution on the planktonic components and consumer dynamics in port sectors characterized by different depths and uses.

Keywords: plankton, zooplankton community, Mediterranean Sea, port, anthropogenic impact, hydrocarbon

1. Introduction

In recent decades international policies have converged toward a coherent management of coastal areas, integrating land and sea ecosystems in the coastal zone and their interactions (Newton and Icely, 2008) on one side, and environmental and economic issues on the other. The key concept ”sustainability” represents the main focus of many of the current policies, but a general failure is observed, primarily due to a lack of coordination among the different stakeholders (McGuire and Perivier, 2011) and the conflict between economic development and environmental quality. The European Community established a framework of actions and provided directives to define and improve the ecological water quality (Water Framework Directive, WFD 2000/60/EC; Marine Strategy Framework Directive, MSFD 2008/56/EC; Maritime Spatial Planning Directive, MSPD 2014/89/EU) with the aim of protecting the marine ecosystems and ensuring the delivery of their important ecological services.

In the restricted space of the Mediterranean coasts, the number of ports and marinas is constantly increasing, competing with bathing waters and protected areas (Piante and Ody, 2015). Port waters are not only directly linked to marine waters through the port entrance, but also to inland waters through inlets and discharge pipes. Therefore, the expansion of port areas could exponentially contribute to the decline of coastal water quality (Durrieu de Madron et al., 2011), potentially affecting the ecosystem services provided (e.g. fisheries, biodiversity, water regulation, cultural benefits). Despite their strategic role in coastal development, the main controls carried out in ports and adjacent areas are those related to chemical emissions and consequent pollution in water and sediments (Peris-Mora et al., 2005; Di Vaio and Varriale, 2018) as well as sanitary aspects linked to microbial pathogens (EEA, 2017), as basic controls to protect human health. Nevertheless, the most suitable Biological Quality Elements (BQEs: macroinvertebrates, angiosperms, macroalgae, phytoplankton and fish) as defined by WFD (2000/60/EU) for the environmental impact assessment in natural water bodies have been also suggested for ports, but not yet completely implemented (Hering et al., 2010; Ondiviela et al., 2013).

In ecological monitoring, the analysis of sediments and the associated benthic biota, as a biodiversity indicator, are currently the most used approaches to develop ecological quality indexes (Quintino et al., 2006; Teixeira et al., 2016). The stable and long-life characteristics of benthic communities, whose structure accumulates with time nutrients and contaminant loadings in the sediments, strikingly differ from the greater variability of the planktonic biota (Caroppo et al., 2013). Under the EU directive framework, water quality has been therefore analysed using indices based on benthos (Diaz et al, 2004) and standards have been developed for coastal waters. Only chlorophyll-*a* has been extensively used as an ecological quality indicator of the water column (Dimitriou et al., 2015).

From an ecological point of view, bacterioplankton, phytoplankton and zooplankton are the main planktonic components present in the water column in terms of abundance, biomass, diversity, trophic networks and ecosystem services provided (Beaugrand et al., 2010; Siokou-Frangou et al., 2010; Tweddle et al., 2018). Although planktonic taxa show a large spectrum of size, trophic and ecological roles, they have been rarely considered together as descriptors of the marine ecological quality (Caroppo et al., 2013). In the water column, physical-chemical and biotic parameters undergo higher variability at shorter temporal scales than benthic ones, with regard to seasonal changes and inflow/outflow from the connected marine and inland water bodies. The planktonic taxa can thus be perceived as a “moving interface”, rapidly reacting to the environmental variations and connected with the more stable benthic communities. Thanks to their biological properties, the planktonic communities have been proposed as early warning indicator of several types of impacts. More specifically, bacterioplankton abundance and activities have been acknowledged as sensitive sentinels of environmental changes (Munawar and Weisse, 1989; Caruso et al., 2016a; Caruso et al., 2016b), phytoplankton blooms have been related to eutrophication processes (Karydis, 2009) and several other pressures (MSFD criterion 4.1.), while zooplankton variation has been linked to regime shifts in ecosystem state (Pace et al., 2013), climatic changes (Roemmich and McGowan, 1995; Beaugrand, 2005; Sundstrom et al., 2017) and pollution (Uriarte et al., 2016). In this perspective, an all-inclusive control strategy is desirable in order to monitor the ecological status of water bodies (WFD, 2000/60/EC). Considering the continuity of coastal areas (i.e. marine protected areas, coastal lagoons, river mouths, bathing areas, tourist and commercial infrastructures, marinas and harbours), an in-depth knowledge of the planktonic components is an essential step to assess the mutual influence between linked water bodies, which are potential sources of abiotic and biotic variability (i.e. eutrophication, hydrocarbons, heavy metals, euryhaline species).

Based on these concepts, a pilot study using an integrated planktonic approach was needed to build a knowledge framework on trophic processes of the networks prevailing in port waters since morphology, hydrodynamics and human activities in harbours (heavily modified water bodies, WFD, 2000/60/EC) may specifically affect bacterio-, phyto-, and zooplankton as well as their relationships (Caroppo et al., 2013).

The present study was conducted under the framework of the ENPI-CDCMED project MAPMED (Management of Port areas in the Mediterranean Sea Basin), a multidisciplinary project aimed at improving the environmental sustainability in Mediterranean tourist ports (Zakhama-Sraieb et al., 2016; Chatzinikolaou et al., 2018; Massi et al., 2019; Vitali et al., 2019). In this study, the planktonic components of three Mediterranean tourist ports in different periods of the year, related to the tourist season, were analysed with the following specific aims: i) to assess zooplankton abundance and composition variability, ii) to define its relationships with the other planktonic components (bacterioplankton and phytoplankton) and with the water physical and chemical parameters, and iii) to identify the potential impacts of coastal pressures, port activities and linked infrastructures on the planktonic network. The different parameters (physical, chemical and biological) were analysed by means of a multivariate analysis in order to compare and interpret the ecological relationships in the water column of the three investigated ports.

2. Material and Methods

2.1. Brief description of the three ports

Among the three studied ports (Figure 1), Cagliari (Sardinia, Italy) and Heraklion (Crete, Greece) are both commercial and touristic harbours with a larger surface (2.07 km^2 and 0.87 km^2 respectively) than the artificial marina (0.04 km^2) of El Kantaoui (Sousse, Tunisia), where activities linked to tourism and fishing are operated. The three ports have an inner and more protected shallow area hosting small leisure and/or fishing boats; from there to the port entrance, higher depths and larger infrastructures (quays or docks) allow for activities involving bigger ships and cargoes in Cagliari and Heraklion. In El Kantaoui, the marina exclusively corresponds to the inner part of the two larger ports, which have similar structure and depth.

Within each port, three to five sampling stations were selected to achieve a good spatial coverage of the whole port area and represent discrete sectors dominated by specific port activities, potentially exposed to different impacts (Table 1, Figure 1). In detail, the Cagliari port hosts quays for the anchoring of small leisure boats (station C1), military navy, commercial and passenger ships (stations C2, C3, C4, respectively). The port entrance (station C5) faces south-west. The opening of Molentargius Lagoon loads brackish waters into the port main basin at station C1, while a canal running along the east side of the Santa Gilla Lagoon drains wastewater of urban runoff in the proximity of station C4 (Figure 1a and b). The marina of El Kantaoui is the second tourist port in Tunisia and hosts small and medium-size leisure boats (station E1). Only a small part of the marina is dedicated to other activities, as shipyard and fuel supplying (station E2). The port entrance (station E3) faces south-east (Figure 1c). The Heraklion port is constituted by the Old Venetian Harbour, hosting leisure and fishing boats (station H1), and the new Ferry Port, where passenger and cargo ships (stations H2, H4, respectively) are hosted along with a shipyard (station H5). Heraklion port entrance (station H7) faces east (Figure 1d). Discharges of sewage effluents from urban activities have been documented in the three port areas (Chatzinikolau et al., 2018; Massi et al., 2019; Vitali et al., 2019).

2.2. Sampling campaigns

Three sampling campaigns were performed in 2012 during winter (February), spring-early summer at the beginning of the tourist season (May), and late summer-autumn at the end of the tourist season (September). The samplings were performed during the day, from 9 am to 6 pm, to avoid the effects deriving from day-night plankton migrations. This sampling design does not account for the planktonic taxa life cycle variation (from few days, to months and years, depending on the taxon), but was conceived to capture the major environmental variations due to both climatic factors and human activities and the consequences they may have on the planktonic communities. In the Mediterranean region, winter and spring are normally rainy seasons, with lower temperatures and higher fresh water load, which accounts for a slightly lower salinity along the coasts (Mehta and Yang, 2008); summer is warm and dry, with major effects on water salinity in early autumn because of the increased evaporation. The tourist season starts in late spring and ends in early autumn, resulting in water scarcity, intense use of coastal facilities, high volume of marine traffic, as well as increased discharge/spill of wastewaters and pollutants (e.g. sewage effluents, lubricating oils, fuel oils and combustion products).

2.3. Sampling procedures and laboratory analyses

2.3.1. Physical and chemical variables

The physical properties of surface water (temperature, salinity, oxygen and pH) were measured on board using a 3420 WTW multi-meter. Three replicate samples (5 L each) of surface seawater were collected at each station during each sampling campaign and used for chemical analyses.

For the chemical analyses of inorganic nutrients (NO_2, NO_3, NH_4, PO_4, SiO_2), particulate organic carbon (POC) and chlorophyll-*a*, the seawater samples were filtered immediately after collection through Whatman GF/F filters (47 mm). Filters were stored at -20°C and used for the determination of chlorophyll-*a* according to the fluorometric method of Yentsch and Menzel (1963) and Arar and Collins (1992). The filtered water samples were stored at -20°C and used for the determination of NO_2, NO_3, PO_4, SiO_2, following the techniques proposed by Strickland and Parsons (1972) and for NH_4, those by Ivančić and Degobbis (1984).

For hydrocarbons, samples of 1 L of unfiltered water were extracted with hexane spiked with surrogate standards of aliphatic hydrocarbons (AHs) and polycyclic aromatic hydrocarbons (PAHs). The extracts were concentrated, fractionated and the concentrations of AHs and PAHs were measured using gas chromatography-mass spectrometer (Agilent 6890 gas chromatograph interfaced with mass spectrometer).

Determination of heavy metals in water samples was performed by Inductively Coupled Plasma Spectrometry (ICP-OES, Perkin Elmer Optima DV 7000). Concentrations resulted below the detection limits of the analytical method in all samples for As, Cd, Cr, Ni, Pb, Sb, and V, and in more than 50% of the samples for Cu; therefore metals were not further included in the analyses (MAPMED Consortium, 2013).

2.3.2. Bacterioplankton

Water samples were collected at the surface (within 1m depth) using sterilized 15 L low-density polyethylene collapsible carboys (washed with 10% bleach and rinsed with sterile MilliQ water). Samples were immediately fixed with filtered formaldehyde (final concentration 1.8%) for 1 h at 4°C. One mL aliquots were filtered onto black polycarbonate membranes (0.2 µm pore size, 25 mm diameter), rinsed with ultra-pure water, air-dried, and transported to the laboratory at room temperature (Beardsley et al., 2008). The filters were stained with DAPI (10 µg mL^{-1}) for 5 minutes, then washed six times with ultra-pure water and six times with ethanol 80%. Filters were mounted onto microscope slides using UV-transparent fluorescence-free immersion oil. Cells (diameter < 20 µm) were counted via epifluorescence microscopy (Olympus BX51) equipped with a mercury burner power supply unit (OLYMPUS U-RFL-T) in five fields per filter on three replicate filters for each station and each sampling period.

2.3.3. Phytoplankton

Samples of 250 mL of water were collected at the surface (within 1 m depth) at each station and each sampling period, fixed with neutralized formalin (final concentration 1%) and stored in dark glass bottles. Subsamples of variable volumes were observed under an invertoscope (Zeiss IM35, ph. c., 40x) after sedimentation, following standard methods (Zingone et al., 2010).

Phytoplankton taxa were identified to the lowest possible taxonomic level and assigned to the classes of diatoms, dinoflagellates and coccolithophores; cryptophytes, chlorophytes, cyanobacteria and nanoflagellates, which could not be identified further, were included in the mixed group labelled as “other phytoplankton”.

2.3.4. Zooplankton

An Apstein net for zooplankton (200 µm mesh width, 40 cm mouth diameter, 1 m net length) was used and five vertical tows were performed (Zunini Sertorio, 1990; Camatti and Ferrari, 2010) at each station and during each sampling period in the three ports, avoiding the net touching the bottom. The volume of

filtered water was calculated as: V = mouth surface x station depth and was used to estimate the densities of zooplankton (ind m^{-3}). The samples were stored in 8% normalized formalin.
The individuals in the five replicates were sorted, counted and identified under a stereomicroscope using a Bogorov counting chamber for zooplankton (40 mL). The main taxa were identified to the lowest possible taxonomic level, except for the larval stages, which were identified only at higher taxonomic levels.

2.4. Statistical analysis

A data matrix with the physical-chemical parameters, total bacterial counts, total counts of phytoplankton and zooplankton, densities of the four phytoplankton groups (diatoms, dinoflagellates, coccolitophores, "other phytoplankton") and zooplankton taxa for each station and each sampling period in the three ports was constructed. The bacterioplankton, phytoplankton and zooplankton counts were log transformed to approach normal distribution. The same set of physical-chemical, bacteria and phytoplankton data collected in a station was used for the five zooplankton replicates collected in the same station.
For univariate analysis, 2-way-ANOVAs with port and sampling period as main effects and 1-way-ANOVAs by stations within each port were performed (Statistical Analysis System, SAS, package version 9.4). When ANOVA detected significant effects ($p<0.05$), the Scheffé multicomparison of means was performed. The Scheffé test was selected because it can compare groups with different number of observations (Scheffé, 1959).
The relationships among variables were evaluated with the multivariate technique of factor analysis (SAS, package version 9.4) to identify the environmental variables and groups of planktonic taxa accounting for the main variability of the data (Kim and Mueller, 1978; Milstein, 1993; Nourisson et al., 2018). In this analysis we included the physical-chemical variables, total bacterial counts, densities of the four phytoplankton groups and densities of the zooplankton taxa that presented at least 50 ind m^{-3} in the overall database. Among the several available techniques to extract factors, the Principal Component Analysis (PCA) calculated from the correlation matrix among variables was selected, which allows the factor analysis to be applied as exploratory tool, without requiring a normal distribution of all the variables included in the data matrix (Kim and Mueller, 1978). The method computes the linear combination of the original variables, which accounts for as much of the variation contained in the samples as possible, called first factor (Factor1). The second factor (Factor2) is the second linear function of the original variables, which accounts for most of the remaining variability, and so on. The factors are independent one from another, have no units and are standardized variables (normal distribution, mean=0, variance=1). The value of a factor in a given sample is the result of the sum of all the variables included in the factor calculation, each one multiplied by a coefficient. The coefficients of the linear functions defining the factors were used to interpret their meaning, considering the sign (+/-) and relative size of the coefficients as an indication of the weight of each variable.

3. Results

3.1. Description of the physical-chemical variables in the three ports

The average values of the measured variables by port and sampling period are presented in Table 2 and the results of the ANOVAs are reported in Table 3. Temperature and salinity were significantly lower and almost all the other variables were significantly higher in the Cagliari port than in El Kantaoui and Heraklion ports (Tables 2, 3). The only exception was the concentration of AHs in the El Kantaoui marina, which was not significantly different from the other two ports. No significant difference was found in pH among ports. As expected in the Mediterranean region, temperature increased from winter (February) to early summer (May) to late summer (September) and dissolved oxygen (DO) was significantly lower in September than in February and May. The variable pH was significantly higher in September than in February, with intermediate values in May. Both POC and chlorophyll-*a* were significantly higher in May than in February (Tables 2, 3), with intermediate values in September. On the overall dataset, polycyclic aromatic hydrocarbons (PAHs) were significantly higher in February than in the remaining months. Overall, the variability among ports was higher than the variability among sampling periods, since 12 of the 13 analysed physical-chemical variables presented significant differences among ports and only 6 among periods (2-way-ANOVAs, Table 3).

The variability among stations within each port was lower than the variability among ports and among periods, as indicated by the low number of variables with significant differences among stations when 1-way-ANOVAs were performed separately for each port (i.e. 7 in Cagliari, 1 in Heraklion, 0 in El Kantaoui, not shown). Only the data from Cagliari are shown (Table 3, Scheffé mean multicomparison by stations in Cagliari), where most of the among-station variability was recorded. In this port, nutrient concentrations were significantly higher and salinity significantly lower in the leisure boat area (C1) than in the other stations (Table 1S, Supplementary Material). The only exceptions were the intermediate values (not significantly different from those measured in the other stations) found at the port entrance (C5) for salinity and levels of nitrate, phosphate and silicate, as well as at the station hosting the military navy vessels (C2) for the concentration of nitrate. The concentration of total PAHs was significantly higher at the station hosting the military navy vessels (C2) than at the cargo ships station (C4), with intermediate values not significantly different from either in the remaining stations. The overall variance accounted by this model was only 18% of the total variance, as shown by the low coefficient of determination (r^2=0.18, Table 3). In the Heraklion port, the only difference found among stations was for silicate, with a coefficient of determination r^2=0.69. Silicate concentration in Heraklion was significantly higher in the cargo ship area (H4) than at the port entrance (H7), while values in the other stations were intermediate and not significantly different from either.

3.2. Planktonic biota in the three ports

The highest bacterial densities (around 10^7 cell mL^{-1}) were found in Cagliari port in May at all stations (Table 2, Table 1S, Supplementary Material) and in the three sampling periods at the station hosting leisure boats (C1). On the contrary, the lowest bacterial abundances (around 10^6 cell mL^{-1}) were detected in El Kantaoui port in February (all stations) and in Heraklion port in September (at the port entrance, H7). The total bacterial counts were significantly higher in Cagliari port than in the other two ports (ANOVA, Table 3). No significant differences were found between El Kantaoui and Heraklion ports.

Considering the overall abundance of phytoplankton, Cagliari port had the significantly highest phytoplankton density at all (total, diatoms and "other phytoplankton"; Tables 2, 3), whereas no significant differences resulted for the other two ports. Heraklion was the port with the highest abundances of coccolithophores, which at El Kantaoui presented the lowest values with a significant difference between the two ports; intermediate densities were found in Cagliari (Tables 2, 3). The abundances of total phytoplankton showed a general increasing trend in the three ports from the lowest values in February (around 10 cell mL^{-1}) to the maxima recorded in May (El Kantaoui) and September (Cagliari and Heraklion), when phytoplankton reached densities over 10^3 cell mL^{-1} in Cagliari and El Kantaoui, and 10^2 cell mL^{-1} in Heraklion (Table 2). Overall, diatoms were the dominant class representing on average more than the 80% of the total phytoplankton in September in the three ports, in May in Cagliari and El Kantaoui, and in February exclusively in Cagliari at stations C1 and C2 (Table 2, Table 1S, Supplementary Material). Indeed, in February diatoms and dinoflagellates showed their lowest contribution, when coccolitophores dominated in Heraklion (all stations) and Cagliari (excluding C1 and C2) and "other phytoplankton" dominated in El Kantaoui (particularly cryptophytes) and Cagliari (particularly cryptophytes, freshwater chlorophytes and cyanobacteria). In El Kantaoui (May), Cagliari (May and September) and in Heraklion (September, to lesser extent) diatom blooms with different taxonomic contributions were responsible for the highest densities of total phytoplankton. Dinoflagellates prevailed in Heraklion in May, together with a lower contribution of "other phytoplankton" (cryptophytes and chlorophytes).

Regarding zooplankton, a total of 36,659 ind m^{-3} in Cagliari port, 5,791 ind m^{-3} in El Kantaoui and 63,228 ind m^{-3} in Heraklion port were counted. The zooplankton mean abundances ranged between 19 ind m^{-3} in El Kantaoui in February and 941 ind m^{-3} in Heraklion in the same month (Table 2) and had peaks in the single samples of 2,022 ind m^{-3} in Heraklion (February), 1,652 ind m^{-3} in El Kantaoui (September) and 1,175 ind m^{-3} in Cagliari (February). In Heraklion and Cagliari, the highest densities were mostly found in February at the stations hosting passenger and cargo ships (C3 and C4, H3 and H4) and the lowest at the stations hosting leisure boats (C1, H1) in the three sampling periods (Figure 2, Table 1S, Supplementary Material). A different pattern was identified in El Kantaoui marina, where the lowest densities were observed in February and the highest in September (Table 2, Figure 2). Overall, over 70 zooplankton taxa were identified, most of them occurring only once or rarely with few individuals (Table 1S, Supplementary Material). The most abundant zooplankters were copepods (55% of the total of all samples), followed by appendicularians (17%) and cladocerans (9%), all belonging to holoplankton (Figure 2a). Meroplanktonic organisms constituted 17% of the total zooplankton in all the analysed samples, with barnacle nauplii (Cirripedia) contributing with 6%, polychaetes with 4% (mainly spionid larvae) and gastropod larvae and Hydromedusae with 2% each.

Concerning holoplankton, Calanoida copepods represented the 45% of the total zooplankton, with the genus *Acartia* accounting for 31% of the total zooplankton and 60% of the copepods (Figure 2b). Other common calanoid genera identified in the three ports were *Isias* (11% of copepods), *Paracalanus*, *Parvocalanus, Clausocalanus* and *Calocalanus* (reported here as Calanoida spp., together with other less abundant and non-identified calanoids, 9% of copepods), *Centropages* (5%) as well as *Eucalanus*, *Pseudodiaptomus*, *Pontella*, *Diaixis*, and *Temora* (<1%) within the "others" zooplankton group. The genus *Acartia* was mostly present in February at Heraklion, where it formed swarms, declining in this port in May and September. *Acartia* was also observed in Cagliari, mainly in February and May at stations C4 and C5, and in El Kantaoui, mainly in September at the inner stations E1 and E2 (Figure 2b). Cyclopoid copepods were 6% of the total zooplankton, with the genus *Oithona* as the most represented (8% of copepods, 5% of total zooplankton, Figure 2b) and very low percentages of *Corycaeus*, *Oncoea*, *Farranula* and *Copilia* (3% on copepods, 2% on total), the last one exclusively in Heraklion. Harpacticoid copepods were recorded with 3% of the total (5% of copepods, Figure 2b), mostly *Diarthrodes* (El Kantaoui) and *Euterpina*.

The second holoplanktonic dominant taxon, Appendicularia, was mainly represented by the genus *Oikopleura*, mostly present in Cagliari in February, and in Heraklion and El Kantaoui in September (Figure 2a). Cladocerans were observed with all the three genera known for the Mediterranean: *Podon*, *Evadne* and *Penilia*. *Podon* was mostly present in Cagliari in February, with decreasing densities in May and September, whereas *Evadne* was the main genus in Heraklion in September. In El Kantaoui, all the three cladoceran genera occurred with low abundances during the three sampling periods, except for the numerically dominant cladoceran *Penilia* in September.

The results of the 2-way-ANOVAs, performed with port and sampling period as main effects on the abundances of total bacteria, total and main groups of phytoplankton, and total zooplankton (Table 3), showed that the variability of biota among ports was higher than the variability among sampling periods, since five of the seven variables presented significant differences among ports (highest abundances in Cagliari for the majority of the variables) and only two among periods (highest abundances of dinoflagellates in May and coccolitophores in February). The seasonal variation of single zooplankton taxa was very high and interspersed among ports and sampling periods, so that, when zooplankton was taken as a whole (Table 3), no significant differences were observed. Significant variation among stations within each port was not recorded by the 1-way-ANOVA for those seven variables (Table 3).

3.3. Linking abiotic condition and planktonic elements by factor analysis

The first four factors explain 57% of the whole data variability (Table 4). Each factor shows a different combination of variables with coefficients, which reflects different sources of variability in the studied data. Table 4 reports the coefficients of each variable and their relevance for the interpretation of the factor itself. The values of these factors calculated for each station and sampling period in the three investigated ports are reported in Figure 3.

The first factor (Factor1, Table 4) accounted for 24% of the overall data variability. It is a bipolar factor showing in its positive pole a strong positive correlation (high positive coefficients) among DO, concentrations of nutrients (NH_4, NO_2, NO_3, PO_4, SiO_2), POC, chlorophyll-*a*, total bacterial counts, as well as abundances of diatoms, "other phytoplankton" and zooplanktonic cladocerans and barnacles nauplii (Cirripedia), while a weaker correlation (mid positive coefficients) was found with the abundances of appendicularians and decapod larvae. These variables were negatively correlated with those in the negative pole, strongly (high negative coefficients) with salinity and weakly (mid negative coefficients) with the abundance of ichthyoplankton (Table 4). Factor1 differentiated Cagliari from the other two ports, with higher values for Cagliari as compared to El Kantaoui and Heraklion in all stations and sampling periods as result of lower salinity, higher nutrient levels and higher plankton abundances (Figure 3a). In time and at station scale, the Cagliari port presented higher values of Factor1 in May at all stations and in the three sampling periods at the stations hosting leisure boats (C1) and military navy vessels (C2) as well as at the port entrance (C5). In the ports of Heraklion and El Kantaoui, Factor1 values were rather similar among different stations in the same sampling period, showing an increasing trend with time.

The second factor (Factor2, Table 4) accounted for a further 15% of the remaining data variability. It reflected the abundances of most zooplankton taxa (holoplankton and meroplankton), more specifically, higher coefficients were found for copepods (harpacticoids, cyclopoids, calanoids and monstrilloids), hydromedusae, chaetognats, appendicularians, molluscs (both gastropods and bivalves) and polychaetes, while lower coefficient values were found for barnacle nauplii. Among the investigated ports, the variability range of Factor2 was wider and the differences through time were larger for El Kantaoui (Figure 3b), with lower values in February and higher in September, mainly at the port entrance (E3). On the contrary, the variability range was rather similar and narrow in Cagliari and Heraklion, with higher differences between ports than sampling periods. In the two largest ports, the Factor2 generally increased from the leisure boat area towards the port entrance (in Heraklion up to H4, not reaching H5 and H7) and at each station from February to May and September (Figure 3b). Overall, Factor2 differentiated zooplanktonic communities between El Kantaoui and the other two ports, but also among different stations within each port and through time, as the second main source of variability in the analysed data.

The third factor (Factor3, Table 4) accounted for a further 11% of the remaining data variability. It can be described as a bipolar factor showing negative correlations between concentrations of nitrate and silicate, abundances of coccolitophores and cyclopoid copepods on the positive pole, and temperature, abundances of dinoflagellates, diatoms and plathelminth larvae on the negative pole. A similar trend was recognized in Cagliari and Heraklion (Figure 3c), with higher values of Factor3 in February (as result of the maxima of coccolithophores and the minima of diatoms and dinoflagellates) and lower values in May and September (as result of the maxima of dinoflagellates in May and the maxima of diatoms in May and September). On the contrary, a different trend was found in El Kantaoui marina, with the lowest values of Factor3 in May, corresponding to a synchronous variation of the planktonic taxa included in the factor at the negative pole at the three sampling stations. In all ports and stations, Factor3 differentiated samples collected in winter (February) from samples collected during the warmer months (May and September, Figure 3c). Moreover, in February the Factor3 values discriminate between the two large ports (Cagliari and Heraklion with higher values of Factor3) and the marina of El Kantaoui (lower values of Factor3).

The fourth factor (Factor4, Table 4) accounted for a further 7% of the remaining data variability. It is a bipolar factor showing negative correlation between the abundances of siphonophores, echinoderms larvae and ichthyoplankton in the positive pole, and concentrations of AHs and PAHs as well as abundances of amphipods in the negative pole. The shallow El Kantaoui marina and the deep Heraklion port presented opposite patterns, while the intermediate-depth Cagliari port was similar to El Kantaoui in February and to Heraklion in May and September (Figure 3d). In El Kantaoui, the coefficients of Factor4 were lower as compared to the other ports at all stations and in all sampling periods (Figure 3d), with the exception of the samples collected in winter in Cagliari at the stations hosting the military navy vessels (C2), passenger (C3) and cargo ships (C4) (Figure 3d). The generally lower Factor4 values in El Kantaoui were the result of higher levels of AHs and amphipods and lower (or zero) levels of the three positive coefficient variables (i.e. abundances of siphonophores, echinoderms larvae and ichthyoplankton). An opposite situation was found in Heraklion, where the coefficients of Factor4 were higher than in the other two ports in almost all the sampling stations and periods, as related to higher abundances of ichthyoplankton, echinoderm larvae and siphonophores and lower levels of AHs, PAHs and amphipods (Figure 3d). In Cagliari port, the coefficients of Factor4 presented lower values in February (more similar to those measured in El Kantaoui) than in the other sampling periods, when higher levels of hydrocarbons and amphipods were measured in the port waters. On the contrary, higher Factor4 values (more similar to those observed in Heraklion) were found at all the stations of the Cagliari port in May, when concentrations of PAHs were lower and echinoderm larvae were more abundant (Table 2).

4. Discussion

4.1. Differences in freshwater inputs and nutrient loading

At the space and time scales of the present study, the main variability occurred among ports as it was identified by Factor1. Overall, statistical analyses cogently differentiated the port of Cagliari from the port of Heraklion and the marina of El Kantaoui (Table 3 and Figure 3a). The specificity of the Cagliari port can be attributed to the input of inland brackish waters into the port area, an hydrological feature that does not occur in the other two harbours. The Natural Protected Area of Molentargius is a system of coastal lagoons, ponds and saltworks protected by EU (http://eunis.eea.europa.eu/sites/ITB040022) for the conservation of wild animals, plants and natural habitats and included in the RAMSAR Convention. Within this system, the San Bartolomeo canal (Figure 1b) receives water from the brackish lagoon "Stagno del Molentargius" and collects treated wastewaters from the surrounding Municipalities, discharging brackish waters into the leisure boat area (C1) of the Cagliari port (Figure 1a, b). A plume of this water likely extends to cover the area of the port entrance (C5) providing a distinct water profile within the port. The effects were mainly visible in February in the area hosting leisure boats (C1, minimum salinity 28‰) and at the port entrance (C5, minimum salinity 30‰). This discharge affected not only salinity but also nitrate, phosphate and silicate (at the negative pole of Factor1, Table 4) with high levels of the three nutrients at stations C1 and C5, and high level of nitrate at station C2 (1-way-ANOVA, Table 3). In the proximity of the quays for the anchoring of cargo ships (C4), the Santa Gilla canal drains wastewaters of urban runoff into the port area (Figure 1b). Despite this freshwater inlet, the station C4 seemed to be marginally influenced, probably due to the low flow rate and/or good quality of the discharged waters. Finally, the sector of the Cagliari port hosting passenger ships (C3), more distantly located from the two discharges of inland waters, was the least affected area of the port (highest salinity and lowest nutrient levels during all the studied periods).

The brackish water input of the Molentargius lagoon not only drains into the Cagliari port high levels of inorganic nutrients and organic matter (Massi et al., 2019), but also plankton. Freshwater chlorophytes, cyanobacteria (namely "other phytoplankton", Table 3) and the freshwater pennate diatom *Tabellaria fenestrata*, typical of eutrophic waters, were exclusively found in Cagliari among the studied ports, particularly at stations C1 and the nearby C2. The high trophic level of waters in the Cagliari port was also likely responsible for the development of diatom blooms and the proliferation of bacteria, which affected station C1 (and also C2 for diatoms) in the three studied periods, and influenced all the stations in May, probably due to persistent effects of nutrient rich brackish waters from the Molentargius lagoon after the winter-spring rainy period. More specifically, these high trophic conditions promoted the May-September blooms of typical coastal fast-growing and individually small diatoms, as *Skeletonema pseudocostatum* and *Thalassiosira pseudonana*. Indeed, *Skeletonema* species are well known coastal and estuarine blooming diatoms in Mediterranean waters (Moncheva et al., 2001; Kooistra et al., 2008; Abboud-Abi Saab et al., 2008), while *T. pseudonana*, typical of coastal and brackish environments, is favoured by high temperatures (Hegseth and Sakshaug, 1983).

Concerning zooplankton, three variables of the positive pole of Factor1 highlighted the association of the eurihaline and neritic cladoceran *Podon* (Factor1, Table 4) with crustacean larvae and *Oikopleura dioica*. The first two groups are known in disturbed and shallow areas subjected to river discharges (Christou et al., 1995) and the latter depends on chlorophyll and temperature for its development (Harris et al., 2005). Specifically, the genus *Podon* is described as a raptorial feeder on big particles, such as microzooplankton and large phytoplankton (Jagger et al., 1988) that are abundant in the water column in eutrophicated areas with high nutrient loading. Accordingly, this taxon was present in Cagliari with the highest densities during all the sampling periods.

As compared to the Cagliari port, more saline waters with lower levels of nutrients, bacteria, phytoplankton and lower abundance of zooplankton with positive coefficients in Factor1 prevailed in Heraklion and El Kantaoui ports in all the studied periods. A trend is observed in these ports with a mild increase of Factor1 from winter to summer (Factor1, Figure 3a), which may be attributed to an increased nutrient loading during the tourist warm season together with increases of some plankton organisms. In El Kantaoui, the diatom blooming in May was found exclusively for *S. pseudocostatum.* As a matter of fact, *Skeletonema* blooms have been observed in the port waters of Malta (DeBono, 2001/2002; Nuccio, unpublished data) and in other southern Mediterranean ports (Abdel-Halim and Khairy, 2007; Heneash et al., 2014). In September, a contribution to the aforementioned increasing trend was given by the large occurrence in El Kantaoui port of the filter-feeder *Penilia avirostris*, described as more typical of warm season and waters

(Margaritora, 2010) , and by the high abundances in Heraklion of Cirripedia and *Oikopleura* (Figure 2a), the latter probably dependent on food availability as one of the most critical limiting factors (Tomita et al., 2003).
The further variable contributing to the different patterns observed between Cagliari port, on one side, and El Kantaoui and Heraklion, on the other side, is the higher density of ichthyoplankton that occurred in these last two ports. Ichthyoplankton seemed more connected with open sea processes and was indeed mainly observed at the outer stations of El Kantaoui and Heraklion ports. It seemed instead that fish did not find suitable conditions and released few quantities of eggs and larvae in the Cagliari port waters.

4.2. Consumer dynamics

The comparison of zooplankton densities among different studies is complex, due to its wide fluctuations in space and time and the high number of variables involved (e.g. salinity, depth, currents, nutrients in the water column and sampling mesh size). In the Mediterranean open sea, Siokou-Frangou et al. (2010) described a decrease in zooplankton abundances as independent of the seasonality, that seems, instead, to be caused by the large water circulation from the Strait of Gibraltar to the eastern Mediterranean Sea Basin. The abundances of meso-zooplankton (captured using 200 μm mesh nets in the first 50 m water layer in October-November) ranged from 551 ind m^{-3} in the Strait of Sicily to 108 ind m^{-3} in the Cretan Sea, while values lower than 100 ind m^{-3} were documented in a station along the Cretan coast (Mazzocchi et al., 1997). On the other hand, zooplankton abundance changed depending on the vicinity to the coast (Calbet et al., 2001). More specifically, annual values were reported in the range of 1-2 x 10^3 ind m^{-3} in the gulf of Naples (Italy) (Ribera d'Alcalà et al., 2004), and peaks of 2 x 10^4 ind m^{-3} in Elefsis bay (Greece) with a density in February-April doubled compared to January (Siokou-Frangou et.al., 1995).
Local dynamics may strongly influence the local abundances of zooplankton: if domestic, industrial and river discharges favour zooplankton abundance (Siokou-Frangou and Papathanassiou, 1991), a reduction of zooplankton density in the inner area of a gulf may be attributed to the high turbidity and low water circulation, as observed by Christou et al. (1995) in Maliakos gulf (Greece) and by Belmonte et al. (2018) in the Gulf of Vlorë (Albania). Therefore, it is expected that the local conditions existing in ports areas, with a high concentration of nutrients, low hydrodynamics and high levels of particle suspension, could enhance or reduce zooplankton densities. The total number of individuals counted in the three investigated ports highlighted abundances in line with those observed in literature along the Mediterranean coasts and do not fit with the data obtained by Siokou-Frangou et al. (2010) across the east-west open sea gradient.
Besides the seasonality of the different zooplanktonic species, local processes as upwelling, plumes, tidal oscillations, bottom processes, wind stress and turbulence, are considered the main drivers structuring zooplanktonic communities and their biological interactions in harbours and bays (Dawson and Pieper, 1993). Parallel considerations may be made for the present study in which Factor2 shows a not homogeneous distribution of the relevant taxa and their variability among stations within each port and with season (Factor2, Table 4, Figure3b).
Predators like hydromedusae and chaetognaths that have restricted ranges of tolerance to pollution and variation of environmental parameters, were present in the outer port stations, while other more opportunistic or tolerant taxa like polychaetes, bivalves and appendicularians seemed to take advantage of the port environment mainly occupying the intermediate area of the port, but not the inner one (Table 1S Supplementary Material). A similar pattern was described for the Gulf of Thermaikos (Greece) (Siokou-Frangou and Papathanassiou, 1991).
In both large ports of this study (Cagliari and Heraklion), the wide connection between the port area and the open sea through their wide port entrance (400 m in Cagliari and 300 m in Heraklion), the relatively deep bathymetry (up to 13.5 m in Cagliari and 19.5 m in Heraklion) and the wide area between docks and breakwaters (C3-C5 and H3-H5, Figure 1) allow a rather free water penetration from the open sea. This condition results in a higher abundance of the majority of zooplankton taxa (Factor2, Table 4) at the port entrance (C5 and H7) as compared to the respective shallower and more protected areas (C1, C2 and H1, Figure 3b), where open sea water penetration is more difficult. Furthermore, in C1 and C2 a disturbance element for zooplankton grazing could have been the presence of cyanobacteria (included in "other phytoplankton" and found exclusively in Cagliari), for their eventual toxin production and poor

manageability by zooplankton (Hogfors et al., 2014, and references therein). In contrast, the shallow and narrow port entrance (up to 4 m depth and 60 m width) in the smaller El Kantaoui port is likely to reduce water penetration inside the marina. This low hydrodynamism may not only be attributed to the profile of the port, but also to its geographical position, which is more distant from the main Mediterranean currents compared to the other two ports (Siokou-Frangou et al., 2010), as well as to the calm weather conditions typical of this coastal area (Ben Haj, 2004). At El Kantaoui in February, the relatively higher winter water mixing and the zooplankton seasonality would explain the similar low zooplankton abundance in the port entrance (E3) and intermediate area (E2), and the even lower abundance in the leisure boat station (E1) (Figure 3b); this spatial difference suggests that the open sea water should have penetrated into the port up to station E2. From May to September, the low hydrodynamism seems to have favoured a local zooplankton development inside the port (positive value of Factor2 at station E1, Figure 3b). Indeed, very still water was documented during the whole sampling campaign in September, which could account for the much higher zooplankton abundance found at the port entrance (E3) than at the inner stations (peak of Factor2, Figure 3b). It is worth noting that at station E3 the open sea taxa (e.g. Hydromedusae, Appendicularia and Gastropoda larvae) were observed in higher abundances compared to the densities in the other stations, while opportunistic taxa (e.g. Calanoida and Cirripedia) were more abundant in the inner station E1 (Table 1S, Supplementary Material).

Among copepod genera, harpacticoids, cyclopoids and calanoids had a considerable weight in the consumer dynamics as indicated by Factor2 (Table 4). Among cyclopoids, the most abundant taxon was *Oithona*, which is representative of opportunistic zooplankters, belongs to the coastal neritic or shallower waters (Williams and Muxagata, 2006) and it is known to feed on a wide range of particles, such as organic matter and phytoplankton of small particle size (Lampitt and Gamble, 1982; Turner, 2004). The calanoid *Acartia* was the most conspicuous copepod at the three ports (Figure 2b). It is a well-known swarming genus (Ueda et al, 1983; Santu et al 2016) that may take advantage of coastal areas with suitable conditions like sheltered bays rich in nutrients, but also port areas (Siokou-Frangou et al., 1995; Belmonte et al., 2018, Vidjak et al., 2018). It was shown that much of *Acartia* seasonality depends on temperature, salinity and hydrology and eventually oxygen or chlorophyll-*a* (proxy of phytoplankton) (Siokou-Frangou et al., 1998; Kang, 2011). The *Acartia* abundances observed in this study (Figure 2b) apparently followed the trends typical of naturally enclosed coastal areas with the booster effect of nutrient abundance that favoured phytoplankton blooms. An inverse relationship between the relative quantities of phytoplankton and zooplankton have been observed by many authors and were explained with zooplankton grazing, animal exclusion in phytoplankton patches, or the different reproduction rates of vegetal and animal populations (Cattani and Corni, 1992). In this study, the sampling periodicity was not enough frequent to define a seasonal relationship between phyto- and zooplankton. Nevertheless, in El Kantaoui high abundance of calanoids (*Acartia*) were observed in May and September along with low abundances of diatoms, most likely ascribable to the action of copepods grazing (Ryther and Sanders, 1980) favoured by the sheltered waters of the inner stations E1 and E2 (Table 1S, Supplementary Material).

4.3. Seasonality and water turbulence

Seasonal differences among ports were highlighted by Factor3 (Table 4, Figure 3c) with rather opposite trends between Cagliari and Heraklion on one side, and El Kantaoui, on the other, reasonably related to changes in water temperature and seasonal dominance of specific phytoplankton groups (diatoms, dinoflagellates, coccolitophores). Winter conditions of major wind stress, water mixing and turbulence, likely contributed to particle re-suspension from the bottom and favoured higher concentrations of nitrate and silicate in the water column (Table 2). This phenomenon was more evident in the two larger ports of Cagliari and Heraklion (Figure 3c), situated in windier coastal areas, as compared to the marina of El Kantaoui that is characterized by a low water turbulence (Siokou-Frangou et al., 2010) and very infrequent gales (Maurice and Lockyear, 1983; MAPMED Consortium, 2013). Under these environmental conditions, seasonal changes in phytoplankton composition and cyclopoids density have an important role in accounting the variance in Factor3 (Tables 3, 4).

Coccolitophores resulted generally more abundant in winter, most noticeably in the two larger ports and at the stations most exposed to seawater fluxes, likely shaped by currents and greatly reduced during the warmer periods (Table 2). This taxon was demonstrated to constitute a large part of the nano-phytoplankton fraction in the Mediterranean Sea, recorded mainly in autumn and winter in south-eastern waters (Siokou-Frangou et al., 2010). Moreover, their presence in ports was linked to seawater flux by Massi et al. (2019). Compared to coccolithophores, in Cagliari and Heraklion diatoms and dinoflagellates had an opposite trend, increasing their abundances from February to May and September (Table 2). Moreover, the observed negative correlation of diatoms with silicate concentration at opposite poles of Factor3 (Table 4) may depend on the feeding of diatoms consuming the nutrient. In El Kantaoui a different pattern of Factor3 was evident for the absence of coccolithophores in winter, except for a very scarce density at the port entrance, as well as the high diatom bloom and the increment of dinoflagellates in May. The above-cited blooming diatoms are typical of eutrophic coastal and estuarine waters for their opportunistic features of exploiting nutrients and organic matter in enriched shallow waters over a wide range of temperatures (Carstensen et al., 2015).

Concerning the zooplanktonic components at the Factor3 positive pole, almost all the cyclopoids belonged to the genus *Oithona*, which are typical of neritic areas and enclosed systems. They have been demonstrated to be negatively influenced by water temperature (Wang et al., 2017), confirming the present data on Cagliari and Heraklion ports. On the other hand in El Kantaoui an opposite trend was observed, characterised by increasing concentrations of *Oithona* and the other cyclopoids from February and May to September as previously reported by Calbet et al. (2001). As for the other planktonic components, in rich and favourable conditions the environmental variables affecting life cycles of specific zooplanktonic groups shift to a different hierarchy driven by local dynamics.

Factor3 highlighted the importance of the variation of abundances of Platyhelminthes, a group that showed similar pattern of diatoms and dinoflagellates and was mostly found in calm and warmer conditions. The taxon was mainly represented by Müller larvae and was collected mostly in May at El Kantaoui. This result may depend on the species local life cycle and feeding preferences (Rawlinson, 2014). As a matter of fact, a high concentration of Müller larvae was as well found during a further sampling in June 2015 in the same marina (Rossano, unpublished data).

4.4 Impacts of anthropogenic activities

The last factor explaining the variability in the analysed biotic and abiotic parameters is related to anthropogenic activities as the main sources of PAHs and AHs contaminating port waters (Factor4, Table 4). Pyrogenic emission sources associated to the incomplete combustion of fuels and biomasses (e.g. fuel combustion in engines) are the main origin of PAHs in the three studied ports (Vitali et al., 2019) in line with several other Mediterranean harbours (Merhaby et al., 2015; Schintu et al., 2015). In addition, accidental oil spills and leakages of refined oil products (e.g. diesel, lubricating oils) are anthropogenic sources of petrogenic PAHs and AHs entering the port waters, even if AHs may also derive from natural origins, such as biomass of marine microorganisms (i.e. phytoplankton, algae and bacteria) and transfer of terrestrial plant detritus from the land into the sea (Head et al., 2006; Mandalakis et al., 2014; Chatzinikolaou et al., 2018; Vitali et al., 2019).

The concentrations of PAHs in surface waters of the three ports (24 - 336 ng L^{-1}, Table 1S, Supplementary material) were within the ranges previously recorded in Mediterranean open sea (10 - 30 ng L^{-1} in North Aegean Sea, Abdulla and Linden, 2008) and coastal waters (20 - 40,000 ng L^{-1} in Turkish coasts, Abdulla and Linden, 2008) and the levels were well below the concentrations leading to 50% mortality (300,000 - 2,500,000 ng L^{-1}, Kennish, 1998) or producing chronic effects on most marine organisms (50,000 - 150,000 ng L^{-1}, ANZECC, 1999). Therefore, the concentration of PAHs does not seem high enough to have a strong impact on the zooplankton, as it was also established by Chatzinikolaou et al. (2018) for benthic macrofauna from the same sites.

Among the investigated harbours, the Cagliari port exhibited the highest concentrations of PAHs in surface water, according to the levels of PAH contamination in sediments (Vitali et al., 2019). Nevertheless, sediment levels of PAHs in the three studied ports spanned within a wider range (25 - 49,000 ng g^{-1}) as compared to concentrations in the water column (Vitali et al., 2019). Hydrocarbons, and particularly PAHs, tend to associate with particulate matter due to their low water solubility, sinking to the bottom and accumulating in sediments over time (Readman et al., 2002; Zakaria et al., 2002). This explains why their concentrations in the studied sites were considerably higher in sediments than in the overlying water column, as frequently observed in literature (Abdulla and Linden, 2008). On the other hand, hydrocarbon degrading bacteria were found to be abundant in surface waters at the three studied ports, where they seem to be involved in the fate of hydrocarbons in the water column (Bullita et al., 2014; Bullita, 2016). Indeed, decreasing concentrations from winter to the warm periods was evident in Cagliari both for AHs and PAHs and in El Kantaoui for PAHs, which may be at least partially ascribed to the low degradation rate at winter temperature (Head, 2006; Bullita, 2016).

In shallow waters, besides the direct inputs of hydrocarbons by anthropogenic activities, sediment re-suspension occurs through different processes (e.g. navigating vessels, turbulence due to storms, wind and winter water mixing, tides) and may cause hydrocarbon re-mobilization into the water column (Roberts et al., 2012). In the investigated ports, this phenomenon seems to occur in the small marina of El Kantaoui, characterized by a low bathymetry in the whole port area (below 4 m), as well as at the shallow stations of the two bigger ports of Cagliari (C2, 4.5 m) and Heraklion (H1, 3.7 m), where low Factor4 values and high hydrocarbon levels were mostly found (Figure 3d). More specifically, benthos samples collected at station C2 in Cagliari revealed a black fatty substance that glued the sediments making the sieving process very difficult (Chatzinikolau et al., 2018; Rossano C., personal field observations). Indeed, the levels of PAHs in sediments of station C2 (49,000 ng g^{-1}) resulted one order of magnitude higher than the concentrations found in the other sectors of the Cagliari port and the highest among the three investigated harbours (Vitali et al., 2019); consistently, the highest concentration of PAHs in surface water were found in the present study at station C2 (336 ng L^{-1} in February). In Cagliari, burning of coal and biomass are the main source of PAHs entering the port water by atmospheric deposition and street run-offs emitted from the adjacent city (Vitali et al., 2019), a pollution usually characterised by recalcitrant compounds with a long-term persistence in marine environments (Yunker et al., 2002; Duran et al, 2016). Therefore, the local high contaminations of PAHs at the station C2 could be reasonably attributed to the presence in the past of a water drainage channel, which collected city run-offs to this shallow area of the Cagliari port channel (RAS-ARDIS local Authorities, personal communication, 2015).

In line with the close interconnection between water column and benthos, Factor4 was significantly correlated with water depth (Pearsons r^2= 0.34; r^2= 0.44 excluding the winter Cagliari samples), a parameter that is directly linked to the specific anthropogenic activities operated in each port sector (Table 1). Therefore, water depth seems to be an important descriptor of the on-going processes in ports. An exception to this trend was the negative Factor4 values found in February in the Cagliari port not only at the shallow water station C2, but also in the deep water stations (Figure 3d), which may be reasonably explained by an increased water turbulence and consequent sediment re-suspension under the windy conditions during the winter sampling.

Concerning fauna at the shallow water stations, benthic amphipods (the only biotic component at the negative pole of the Factor4) were found in the water column likely because of their increased mobility, or due to the particle re-suspension and consequent hydrocarbon mobilisation. On the opposite, the positive pole organisms (siphonophores, echinoderms, ichthyoplankton) prevailed in Cagliari and Heraklion ports and were more abundant in the deep stations (Figure 3d, high values in Heraklion in H3-H5 and in Cagliari in C3 and C4), likely related to local variation in community composition and more favourable conditions in waters with less sediment re-suspension.

Conclusions

Regarding the aims of the study, the description of the planktonic biota in three Mediterranean ports (aims i and ii) revealed an acceptable ecological water status, with generally abundant and complex communities. This result is in line with that obtained on the benthic fauna by Chatzinikolaou et al. (2018) in the same ports and stations. The relationships of the planktonic communities with water abiotic components (aim ii) appeared different in the three ports, with a high influence of brackish eutrophic water discharge in the port of Cagliari, which caused high abundances of bacterioplankton and spring-summer blooms of phytoplankton. Seasonality was also clear, depending more on meteorological factors (temperatures, winds and storms) than on human activities (tourist season). It is worth adding that, if the continental shelf is considered as an area with high productivity, where plankton communities are strongly dependent on the nutrient recycling and upwelling as reported by many authors (e.g.: Dawson and Pieper, 1993; Siokou-Frangou et al 2010; Miloslavić et al 2012), the studied port areas seem to guarantee similarly rich environments, favouring the growth of zooplanktonic communities with a specific periodicity (aim iii). The different port activities apparently did not affect planktonic networks, which were more linked to station depth and hydrodynamics (turbulence and connection to the coastal waters, depending on the distance from the port entrance). Hydrocarbon pollution did not exceed acceptable levels and seems to be controlled by degradation activities of bacterioplankton (Head et al., 2006). Compared to the benthic community (Chatzinikolaou et al., 2018), the planktonic community is more dependent on freshwater inputs, seasonality and water turbulence. For these reasons the analysis of interactions among planktonic components and environmental variables may contribute to clarify the ecosystem dynamics and suggest eventual critical points to take into account in view of a sustainable management of port areas. It provides information on the functioning of the coastal ecosystem (inland, port and marine waters) and may represent an early warning indication of change or loss in ecosystem health status. It is therefore suggested to include the planktonic community with its three components, bacterioplankton, phytoplankton and zooplankton, in the monitoring programs of port areas to control the anthropogenic impacts on coastal waters.

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Table 1. Location of the sampling stations in the three Mediterranean ports (Cagliari, Sardinia, Italy; El Kantaoui, Sousse, Tunisia; Heraklion, Crete, Greece) and main uses of the sampling stations within each port (Chatzinikolaou et al., 2018; Massi et al., 2019; Vitali et al, 2019).

Station	Latitude	Longitude	Water Depth (m)	Station use
Cagliari				
C1	39°12'11.40"N	9° 7'24.12"E	7.8	Leisure - small boats
C2	39°12'20.46"N	9° 7'15.06"E	4.5	Intermediate (military navy vessels)
C3	39°12'27.12"N	9° 6'46.44"E	8.3	Passenger ships
C4	39°12'25.98"N	9° 6'18.60"E	13.5	Cargo ships
C5	39°11'52.94"N	9° 6'41.10"E	11.4	Port entrance (oriented to south)
El Kantaoui				
E1	35°53'38.64"N	10°35'53.16"E	2.5	Leisure - small boats
E2	35°53'34.44"N	10°35'58.92"E	4.0	Intermediate (fuel station)
E3	35°53'34.65"N	10°36'4.44"E	3.2	Port entrance (oriented to south-east)
Heraklion				
H1	35°20'36.32"N	25° 8'9.93"E	3.7	Leisure - small boats
H3	35°20'44.70"N	25° 8'40.87"E	19.5	Passenger ships
H4	35°20'42.70"N	25° 8'52.28"E	10.5	Cargo ships
H5	35°20'48.72"N	25° 9'7.94"E	19.0	Shipyard
H7	35°20'50.82"N	25° 9'17.88"E	7.0	Port entrance (oriented to east)

Table 2. Mean values (of replicates and stations) of physical, chemical and biological variables recorded in each sampling period in the three Mediterranean ports

Variable	Unit	Cagliari			El Kantaoui			Heraklion		
		Feb	May	Sep	Feb	May	Sep	Feb	May	Sep
ENVIRONMENT										
Temperature	°C	10.3	21.0	22.3	11.7	23.7	26.5	14.6	21.0	25.3
Salinity	‰	33.2	33.2	34.2	37.0	36.9	36.1	38.2	38.3	37.3
DO	mg L^{-1}	9.3	11.5	7.5	8.7	6.3	4.5	7.8	7.0	6.7
pH		8.1	8.3	8.4	8.2	8.2	8.2	8.2	8.2	8.4
Ammonia	µM	10.9	14.9	12.6	0.3	0.2	2.3	0.5	0.5	0.5
Nitrite	µM	1.65	3.00	1.62	0.00	0.03	0.17	0.05	0.01	0.04
Nitrate	µM	26.7	18.5	12.9	0.1	0.7	0.6	7.7	9.8	6.9
Phosphate	µM	1.40	3.78	3.08	0.07	0.05	0.22	0.14	0.04	0.02
Silicate	µM	11.52	9.93	6.29	1.39	0.39	1.81	6.26	3.74	4.44
POC	µg L^{-1}	1,270	2,781	1,768	1,013	906	1,202	1,202	1,068	1,094
Chlorophyll-*a*	µg L^{-1}	2.80	10.50	5.18	0.35	0.95	0.34	0.39	0.50	0.99
AHs	ng L^{-1}	5,257	3,888	2,720	3,133	3,867	3,196	2,340	1,969	3,031
PAHs	ng L^{-1}	137	68	43	103	42	37	46	60	67
BACTERIA & PHYTOPLANKTON										
Total bacterial counts $x10^6$	cells mL^{-1}	7.6	8.9	7.0	1.9	4.2	6.3	4.7	3.8	2.7
Total phytoplankton	cells mL^{-1}	221.00	3,813.00	4,364.00	29.00	1,421.00	141.00	24.00	65.00	100.00
Diatoms	cells mL^{-1}	171.00	3,280.00	3,754.00	10.10	1,338.00	115.00	1.40	11.90	73.30
Dinoflagellates	cells mL^{-1}	3.40	16.00	14.00	1.20	38.00	2.90	4.10	27.00	13.00
Coccolitophores	cells mL^{-1}	6.70	0.40	1.80	0.06	0.20	0.00	14.00	3.40	1.40
Other phytoplankton	cells mL^{-1}	40.00	516.00	594.00	17.00	45.00	23.00	3.80	23.00	12.00
ZOOPLANKTON										
Total zooplankton	ind m^{-3}	540.0	475.0	128.0	19.0	140.0	910.0	941.0	112.0	322.0
Holoplankton										
Cnidaria Scyphomedusae	ind m^{-3}	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cnidaria Siphonophora	ind m^{-3}	0.1	0.0	0.0	0.0	0.0	0.0	2.7	0.2	0.0
Ostracoda	ind/m^3	0.3	0.7	0.0	0.1	0.0	1.0	0.1	0.7	0.1
Amphipoda	ind m^{-3}	3.5	0.1	0.1	0.0	2.2	1.3	0.1	0.6	0.2
Cladocera	ind m^{-3}	126.0	85.0	18.0	0.3	1.8	36.0	0.1	0.0	10.0
Calanoida copepods	ind m^{-3}	91.0	184.0	18.0	3.3	11.0	289.0	862.0	65.0	106.0
Cyclopoida copepods	ind m^{-3}	44.0	13.0	3.2	3.0	1.4	139.0	24.0	3.6	19.0
Harpacticoida copepods	ind m^{-3}	6.0	8.4	2.9	1.0	3.9	116.0	2.6	2.7	3.0
Monstrilloida copepods	ind m^{-3}	0.2	0.4	0.1	0.0	0.9	2.9	0.1	0.1	0.4
Siphonostomatoida copepods	ind m^{-3}	0.0	0.6	2.2	0.0	0.2	0.2	0.0	0.0	0.1
Chaetognata	ind m^{-3}	0.1	0.0	0.0	0.0	0.0	29.0	2.4	0.2	0.1
Appendicularia	ind m^{-3}	229.0	50.0	29.0	0.3	14.0	85.0	2.2	1.3	127.0
Meroplankton										
Proportion of meroplankton	%	5.6	27.3	42.2	51.1	74.1	22.4	4.4	32.8	17.0
Cnidaria Hydromedusae	ind m^{-3}	5.4	3.1	4.8	0.3	0.3	45.0	0.8	3.1	3.1
Ascidiacea	ind m^{-3}	0.4	10.9	0.6	0.3	3.9	3.7	0.4	2.1	3.3
Platyhelminta Müller larvae	ind m^{-3}	0.0	0.3	0.9	0.1	70.0	0.7	0.0	0.2	0.0
Polychaeta larvae	ind m^{-3}	8.8	8.2	4.4	3.6	15.0	77.0	20.0	11.0	6.0
Gasteropoda larvae	ind m^{-3}	3.0	7.9	8.0	0.0	2.7	27.0	6.2	11.2	1.3
Bivalvia larvae	ind m^{-3}	2.6	2.2	0.8	0.1	2.7	11.7	0.9	0.7	2.6
Echinodermata larvae	ind m^{-3}	0.0	0.7	0.5	0.0	0.0	0.0	0.2	0.9	1.1
Cirripedia nauplia	ind m^{-3}	5.8	82.0	32.0	0.5	8.4	37.0	0.6	2.5	29.0
Decapoda larvae	ind m^{-3}	3.1	13.0	1.5	0.2	0.3	0.9	0.8	0.2	2.8
Ichthyoplankton	ind m^{-3}	1.1	1.3	0.5	4.6	0.4	1.2	11.3	4.8	5.6

DO: dissolved oxygen; POC: particulate organic carbon; AHs: aliphatic hydrocarbons; PAHs: polycyclic aromatic hydrocarbons.

Table 3. Results of 2-way-ANOVAs of physical, chemical and biological variables by port and sampling period (Cagliari: C, El Kantaoui: E, Heraklion: H) and 1-way-ANOVA by stations in Cagliari port.

Variable	2-way-ANOVA by port and sampling period		Scheffé mean multicomparison by port main effect			Scheffé mean multicomparison by sampling period main effect			1-way-ANOVA	Scheffé mean multicomparison by stations in Cagliari					
	Significance	r^2	C	E	H	Feb	May	Sep	Significance	r^2	C1	C2	C3	C4	C5
number of observations			15	9	14	12	13	13			3	3	3	3	3
ENVIRONMENT															
Temperature	***	0.95	b	a	a	c	b	a	ns						
Salinity	***	0.59	b	a	a				**	0.76	b	A	a	a	ab
DO	***	0.68	a	b	b	a	a	b	ns						
pH	**	0.37				b	ab	a	ns						
Ammonia (NH_4)	*	0.26	a	b	b				**	0.80	a	B	b	b	b
Nitrite (NO_2)	**	0.37	a	b	b				***	0.83	a	B	b	b	b
Nitrate (NO_3)	**	0.35	a	b	ab				**	0.73	a	ab	b	b	ab
Phosphate (PO_4)	*	0.28	a	b	b				**	0.77	a	B	b	b	ab
Silicate (SiO_2)	*	0.32	a	b	ab				**	0.81	a	B	b	b	ab
POC	***	0.52	a	b	b	b	a	ab	ns						
Chlorophyll-*a*	***	0.54	a	b	b	b	a	ab	ns						
AHs	*	0.26	a	ab	b				ns						
PAHs	***	0.32	a	b	b	a	b	b	**	0.18	ab	A	ab	b	ab
BACTERIO-, PHYTO- & ZOO-PLANKTON															
Total bacterial counts	***	0.57	a	b	b				ns						
Total phytoplankton	***	0.43	a	b	b				ns						
Diatoms	***	0.43	a	b	b				ns						
Dinoflagellates	***	0.46				b	a	b	ns						
Coccolitophores	***	0.51	ab	b	a	a	b	b	ns						
Other phytoplankton	**	0.35	a	b	b	.			ns						
Total zooplankton	ns								ns						

r^2 = coefficient of determination of the ANOVA model. In each of the three multi-comparisons, different letters indicate significant differences at p<0.05. Letter 'a' represents a higher value than letter 'b' that represents a higher value than letter 'c'. Ns and empty spaces: not significant (*p*>0.05). DO: dissolved oxygen; POC: particulate organic carbon; AHs: aliphatic hydrocarbons; PAHs: polycyclic aromatic hydrocarbons.

Table 4. Results of factor analysis of the physical-chemical data and log-transformed densities of planktonic organisms in three Mediterranean ports. Factor coefficients in bold were used for interpretation. n=190 observations.

Variable	**Coefficients**			
	Factor1	**Factor2**	**Factor3**	**Factor4**
ENVIRONMENT				
Temperature	-0.01	0.34	**-0.77**	0.12
Salinity	**-0.87**	0.06	-0.21	0.10
DO	**0.58**	-0.18	0.26	0.25
Ammonia	**0.76**	-0.24	0.25	0.02
Nitrite	**0.86**	-0.20	0.21	0.09
Nitrate	**0.65**	-0.11	**0.49**	0.02
Phosphate	**0.79**	-0.22	0.13	0.12
Silicate	**0.68**	-0.14	**0.50**	0.12
POC	**0.76**	0.04	-0.17	0.38
Chlorophyll-a	**0.85**	-0.02	-0.15	0.31
AHs	0.40	-0.11	0.21	**-0.44**
PAHs	0.16	-0.19	0.36	**-0.42**
BACTERIA & PHYTOPLANKTON				
Total bacterial counts	**0.70**	0.20	0.04	0.01
Diatoms	**0.77**	0.01	**-0.53**	-0.11
Dinoflagellates	-0.19	0.06	**-0.73**	0.27
Coccolitophores	-0.38	-0.09	**0.48**	0.23
Other phytoplankton	**0.79**	-0.11	-0.36	-0.01
ZOOPLANKTON				
Holoplankton				
Cnidaria Scyphomedusae	0.04	0.18	0.32	-0.28
Cnidaria Siphonophora	-0.35	-0.01	0.41	**0.49**
Ostracoda	0.04	0.18	0.03	0.08
Amphipoda	0.03	0.05	-0.04	**-0.47**
Cladocera	**0.65**	0.40	0.20	-0.21
Calanoid copepods	-0.13	**0.67**	0.31	0.38
Cyclopoid copepods	-0.03	**0.76**	**0.47**	-0.05
Harpacticoid copepods	0.07	**0.85**	0.00	-0.13
Monstrilloida copepods	-0.05	**0.59**	-0.09	-0.09
Siphonostomatoida copepods	0.43	-0.17	-0.30	0.15
Chaetognata	-0.24	**0.65**	0.17	-0.04
Appendicularia	**0.46**	**0.58**	0.09	-0.26
Meroplankton				
Cnidaria Hydromedusae	0.09	**0.76**	0.09	-0.16
Ascidiacea	0.36	0.35	-0.37	0.18
Platyhelminta Müller larvae	-0.05	-0.09	**-0.56**	-0.29
Polychaeta larvae	-0.14	**0.71**	0.08	0.04
Gasteropoda larvae	0.09	**0.58**	-0.03	0.33
Bivalvia larvae	0.02	**0.74**	-0.04	-0.19
Echinodermata larvae	0.00	0.03	-0.12	**0.43**
Cirripedia nauplia	**0.58**	**0.46**	-0.35	0.03
Decapoda larvae	**0.46**	0.41	0.00	0.28
Ichthyoplankton	**-0.44**	0.07	0.33	**0.52**
Variance Explained (%)	24	15	11	7
Interpretation	Freshwater input, nutrient loading	Consumer dynamics	Seasonality, water turbulence	Anthropogenic activities

DO: dissolved oxygen; POC: particulate organic carbon; AHs: aliphatic hydrocarbons; PAHs: polycyclic aromatic hydrocarbons

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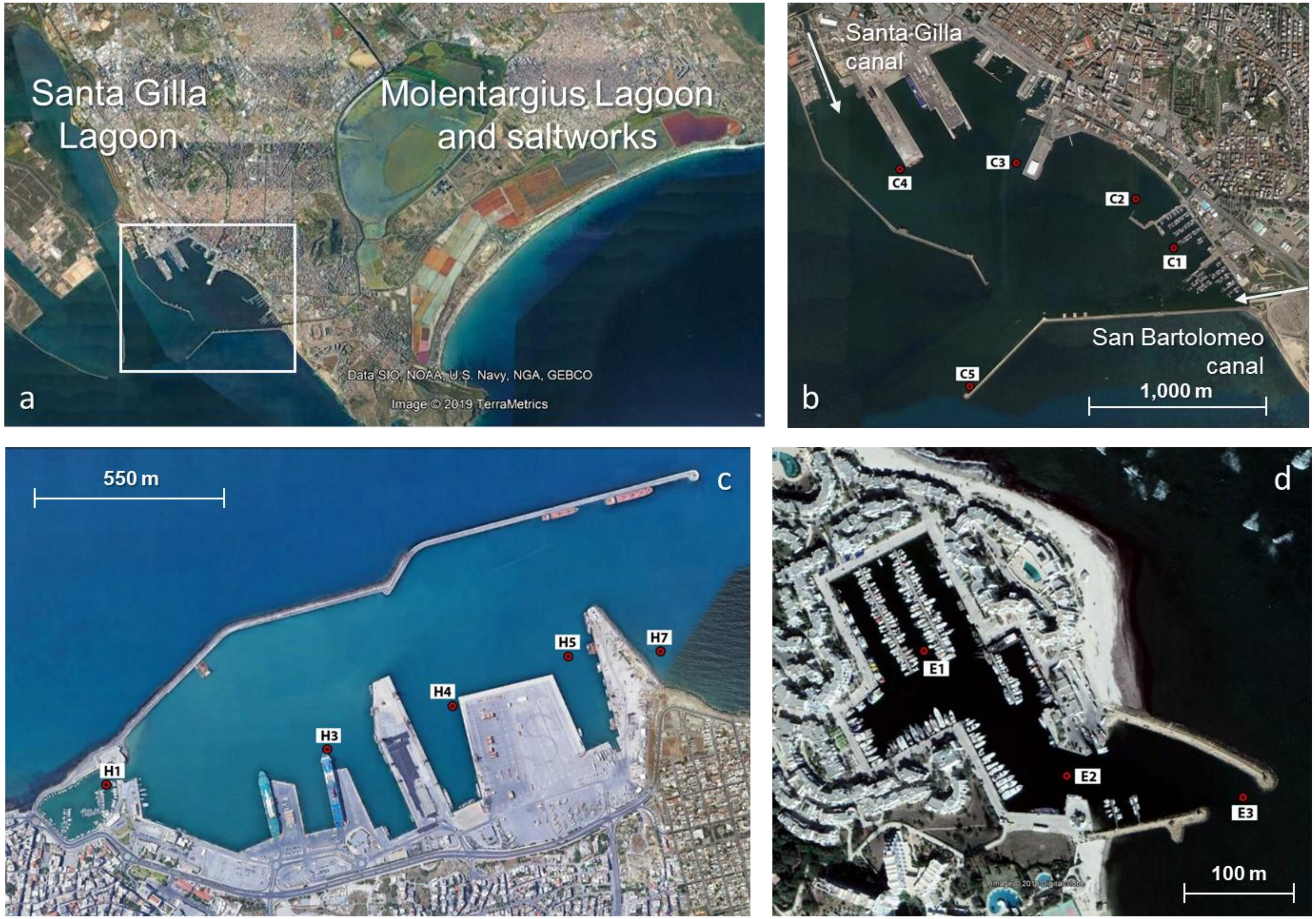
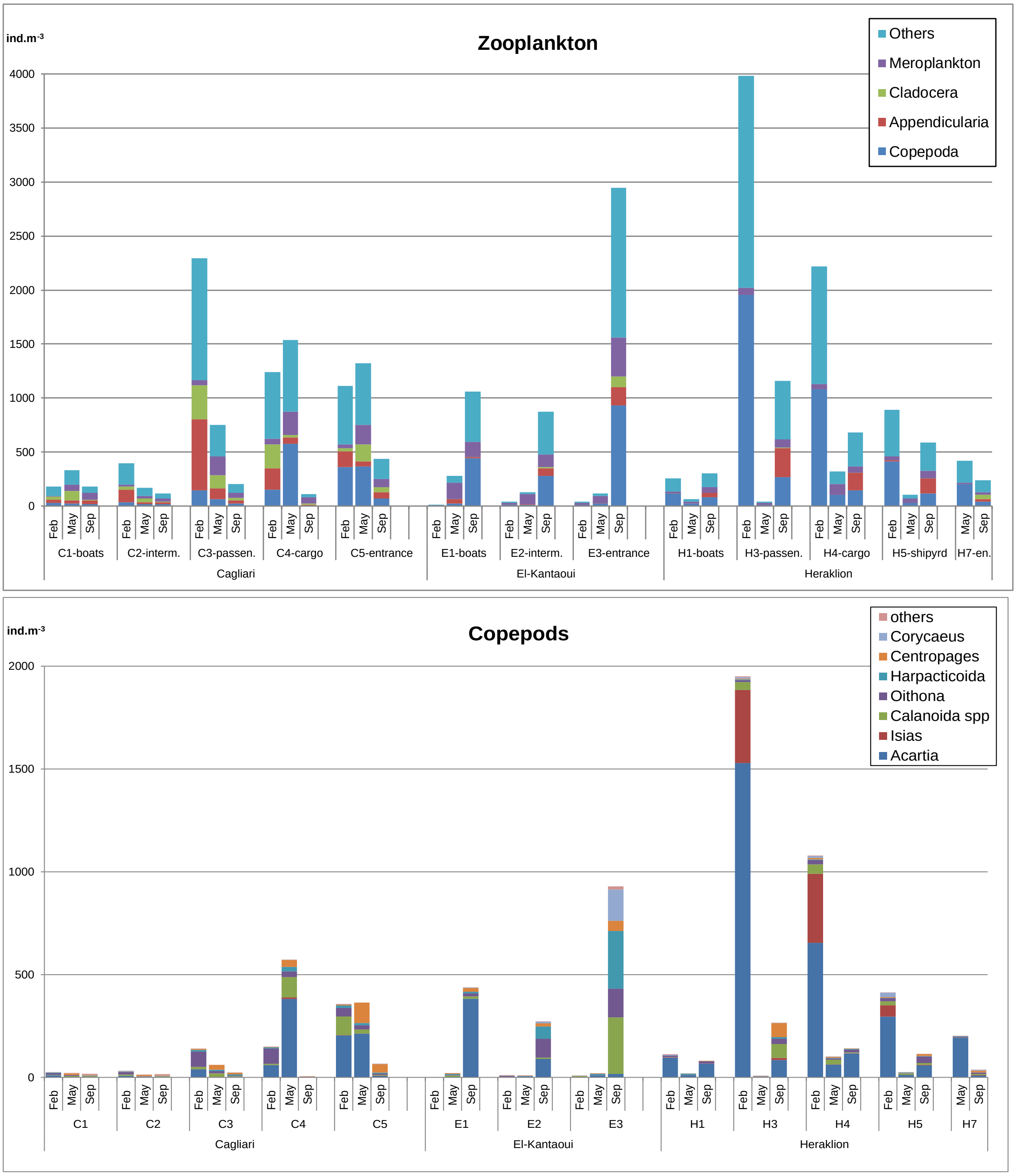


Figure 1. Maps of the three Mediterranean ports and sampling stations. **a)** Systems of lagoons and canals surrounding the Port of Cagliari (in the box); **b)** Port of Cagliari (Italia, Sardinia) and inlets of freshwaters (arrows); **c)** Port of Heraklion (Crete, Heraklion); **d)** Port of El Kantaoui (Sousse, Tunisia) (Google Earth © 2017, v. 7.1.8.3036).



a)

b)

Figure 2. Densities of the main taxonomic groups of **a)** zooplankton and **b)** copepods in different stations and sampling periods. Explanations of the labels and station uses are in Table 1.

Figure 3. Variation of each factor through space (ports, stations) and time (seasons); the most relevant variables for each factor, based on the coefficients in **Table 4**, are reported on the left. **a)** Factor 1; **b)** Factor 2; **c)** Factor 3; **d)** Factor 4. Arrows indicate the presence of both positive and negative coefficients (Factors 1,3,4) or only positive (Factor 2). Explanations of the labels and station uses are in Table 1.