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# MACROZOOBENTHOS IN THE ADRIATIC SEA PORTS: SOFT-BOTTOM COMMUNITIES WITH AN OVERVIEW OF NON-INDIGENOUS SPECIES

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## A B S T R A C T

The present paper is a contribution to the first initiative of the Port Baseline Survey (PBS) for Non-indigenous species (NIS) in the Mediterranean Sea. It presents a report on the soft-bottom macrobenthos from the five Adriatic ports: Bari, Ancona (Italy), Koper (Slovenia), Pula, Rijeka (Croatia), with a focus on the presence and contribution of NIS to native assemblages. Out of 451 species identified, only four were common to all ports. A total of eight NIS were recorded, five in surveyed ports and three in the lagoon connected to the Port of Koper. The highest number of NIS was recorded in Bari, and the highest abundance in Ancona and Bari. Generally, the number, abundance and contribution of NIS seems too low to cause a substantial impact on native communities in surveyed ports. The suitability of methods adopted for PBS for soft-bottom NIS was discussed and suggestion for methodological improvement is provided.

Keywords: macrozoobenthos, soft-bottom community, ports, Non-indigenous species, Adriatic, Mediterranean

## 1. Introduction

Over the past century, marine environments were seriously threatened by a myriad of human activities. They are still subject to increasing anthropogenic pressure, but also to new threats driven by global changes. Since the turn of the century, an increasing public concern and awareness for the protection of marine life and sustainable functioning of marine ecosystems have launched a joint activity among policy makers and the scientific community. It resulted in the implementation of United Nations (UN) and/or European Commission (EC) strategies and instruments, aimed to: 1) preserve of marine biodiversity and habitats in a favourable conditions following the Convention on Biological Diversity and the Habitat Directive (CBD,1992; HD, 1992/43/EEC), 2) reach good ecological status of transitional and coastal waters in accordance with the EU Water Framework Directive (WFD, 2000/60/EC) and 3) ensure Good Environmental Status (GES) of marine ecosystems aimed at, *inter alia*, the reaching or maintenance of NIS introduced by human activities at levels that do not adversely alter the ecosystems according to the Marine Strategy Framework Directive (MSFD, 2008/56/EC). Important actions on the global and regional level address: the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) that requires ships to manage their ballast water to remove, render harmless, or avoid the uptake or discharge of aquatic organisms and pathogens within ballast water and sediments (IMO, 2004), 2) the identification of Pollution Hotspot Sites (PHS) in marine environments (EEA/UNEP/MAP, 2006) and 3) Pollution priority sites that require immediate actions to reach the desirable environmental conditions (Andričević et al., 2011).

Coastal waters are widely recognized as marine areas of high ecological and economic value, but also as highly threatened zone, exposed to multiple human activities and their negative impacts. The Adriatic Sea did not escaped this fate, particularly its northern part. It displays a wide range of features related to sensitive marine ecosystems: a semi-enclosed water body, shallow depth (<50 m), soft substrata, stratification of water column, high riverine input (e.g. Po River), and high productivity (Stachowitsch, 1991). Many kinds of biological and ecological threats negatively affect the Adriatic Sea ecosystem e.g., eutrophication, pollution, fragmentation of benthic habitats, invasion of NIS etc. (Stachowitsch, 1992; Simboura et al., 1995; Katsanevakis, 2011; Pećarević et al., 2013; Corriero et al., 2016) and substantially reduce its ability to respond to natural and anthropogenic disturbances. A long history of human use of the Adriatic Sea, together with global environmental changes (Conversi et al., 2010; Zenetos et al., 2010; Giani et al., 2012) have greatly altered the Adriatic marine environment and ecosystems (Coll al., 2009; Lotze et al., 2011), and ranked the basin as one of the most threatened regions of the Mediterranean Sea (Micheli et al., 2013). In the regional perspective it is highly positioned on the list of Priority issues related Mediterranean Sea (UNEP/MAP 2003, EEA/UNEP 2006), with twenty (15%) out of 131 hotspot sites in the Mediterranean - including all ports presented in this paper.

Recently, maritime transport and associated port activities are recognized as an increasing source of above-mentioned threats, with ballast water as one of main vectors for initial transport of NIS (Carlton, 1985; Grigorovich et al., 2003; Gollasch, 2007; Simkanin et al., 2009; Lawrence and Cordell, 2010). The quantity of ballast water released in the Adriatic ports of Italy, Slovenia and Croatia in 2003 was about 8 million tonnes, out of which about 80% was discharged in the Italian ports, while the remaining volume was shared between Slovenia's port of Koper and Croatian ports, with tendency to increase (Andričević et al., 2011). Six countries bordering the Adriatic Sea (Italy, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro and Albania) have taken a joint effort to increase awareness of environmental authorities to ballast water issues as a threat to the Adriatic Sea ecosystem. It was achieved through integrative activities on the project Ballast Water Management of the Adriatic Sea (BALMAS), which included several Port Baseline Studies (PBS) conducted within the project framework. This was the first target investigations of the matter on the regional scale and valuable support for the implementation of Ballast Water Management Convention (IMO, 2004), that entered into force in September 2017.

Biological invasions by NIS are one of the major threats to the integrity of native communities and they are considered as one of the drivers of global biodiversity changes (Mack et al., 2000; Bax et al., 2003). NIS - also termed as alien, exotic, invasive, foreign, non-native, immigrant, neobiota, naturalized or introduced species - are transported either intentionally or accidentally by human-mediated vectors outside of their native geographical range of habitat (Carlton, 1996). Successful settlement of NIS largely depends on the coincidence between arrival of the species to the new location and suitable conditions for establishment, including the absence of predators and the availability of resources (Hayden et al., 2009). Most NIS do not find optimal environmental conditions for reproduction, persistence, or survival, and are kept under control by unfavourable physical and chemical variables or by biotic interactions within the native community (Corriero et al, 2016), thus most of them do not pose a real threat to recipient ecosystems.

According to Williamson and Fitter (1996) successful invasions are relatively rare, 10% of NIS could become established in the recipient area but 1% of them will eventually become invasive. That ratio is not generally applicable and it differs among biogeographic areas and even sub-areas. In the Mediterranean, both the percentage of introduced NIS that become established (45%), and established NIS turning into invasive (18%) was much higher, and the ratio of the later ones is higer in the eastern (23%) then in the western (8%) Mediterranean basen (Zenetos et al., 2010; Çinar, 2013). Invasive Alien Species are a subset of established NIS which have spread, are spreading or have demonstrated their potential to spread elsewhere, and have an adverse effect on biological diversity, ecosystem functioning, socio-economic values (fishing, aquaculture, shipping, tourism etc.) and/or human health in invaded regions (Grosholz, 2002; Bax et al., 2003; Olenin et al., 2010). In the benthic subsystem, they present one of the most serious threats to integrity of the seafloor habitats and associated benthic communities. They can be drivers of shift in community composition (Carlton, 1989; Cohen and Carlton, 1998; Britton-Simmons 2004), as well as regression and/or displacement of native species (Neideman et al., 2003). The result could be in loss of keystone benthic species and type-specific communities (Gophen et al., 1995) and subsequent changes of structural and functional diversity of invaded benthic communities (Ruiz et al., 1999; Streftaris et al., 2005). In the worst-case scenario, the result could be a massive impact on the entire ecosystem functioning (Olenin et al., 2007) and global changes of biodiversity (Mack et al., 2000).

Furthermore, seaports are recipients of a myriad of organic and inorganic pollutants, directly or indirectly released into marine environment (Galanopoulou et al., 2005; Martínez-Lladó et al., 2007; Gibert et al., 2009; Kapsimalis et al., 2014). Mediterranean ports, populated and affected since historical times, undergo successive degradation of type-specific communities, intensified by growing urbanization, industrialization, tourism and maritime transport over the past century. Nowadays, ports are generally considered polluted marine environments where different sources of pollution interlace and interfere, and together with global changes result in complex ecological relations and processes, often disable the distinction between different environmental and/or natural impacts. A negative relationship between invader impacts and native species richness, among sites with different disturbance regimes, may be result of anthropogenic activities instead of genuine invader effect (Thiele et al., 2011). Furthermore, the abundance and impact of NIS often co-vary with environmental conditions and interactions with other NIS (Simberloff and Von Holle, 1999; Jesche et al., 2012), so in polluted and/or NIS susceptive areas such as seaports, increased caution is needed in identification of disturbance sources. A potential harmfulness and impact of NIS to native communities can be properly estimated only if sufficient information on the environmental pressures, native community structure, species composition and functional diversity in the invaded area are available.

Benthic macroinvertebrates are the most traditionally used biological indicators of ecosystem health in the marine environment, especially infaunal assemblages associated with soft-bottom habitats (Borja et al., 2003). Most of them are sessile, hemi-sessile or confined to restricted bottom areas and they spend complete life cycle or its greater part in direct contact with bottom sediments (Rhoads, 1974; Pearson and Rosenberg, 1978; Cortet et al., 1999; Gray and Elloitt, 2009). Thus, unlike plankton and nekton populations, the structure and spatial distribution of benthic communities can be directly linked with pollutant or disturbance exposure, especially in cases where a point source of pollution is present. However, seaports are recipients of non-point pollution sources, e.g. port service activities, organic pollution, transport related activities such as unintentional oil-spill and bulk cargo discharge, ballast water exchange, physical degradation caused by port construction, sometime combined with point source pollution. Obviously, pollution coming from diffuse and/or obscure sources is much more difficult to measure, identify and control than point sources. It could be the main reason of sparse comparative data related soft/bottom macrobenthos in the seaports (Zarkanellas, 1979; Simboura et al., 1995; Solis-Weiss et al., 2004; Martinez Lladó et al., 2007; Maiorano et al., 2011; Spagnolo et al., 2011; Riera et al., 2013, Award et al., 2014).

The goal of biological PBS is to provide inventories of marine life in and around commercial ports frequented by ships carrying ballast water in order to determine the presence, abundance and distribution of NIS that may have been either introduced by shipping, in ballast water or attached to hulls, as well as by other vectors (Award et al., 2014). Considering that biological and ecological information for port areas are generally scarce and seldom updated, Award et al. (2014), emphasized the importance of PBS as the source of biological data against which future changes in the structure and function of marine communities can be measured.

The present paper is a contribution to the first initiative of the PBS for NIS in the Mediterranean Sea. It presents a report on the soft-bottom macrobenthos from the five Adriatic ports: Bari and Ancona (Italy), Koper (Slovenia), Pula and Rijeka (Croatia). The study provides structural and functional characterization of soft-bottom macrofauna, with a focus on the presence of NIS and their contribution to native assemblages.

Despite the low number of NIS identified, it could be a valuable basis for the future assessment for changes of soft-bottom macrobenthic assemblages, as well as in the identity, distribution and abundance of NIS in surveyed ports.

## 2. Material and methods

### *2.1.Study area, sampling and processing*

The PBS on the soft-bottom macrofauna were carried out in the five Adriatic Sea ports: Bari, Ancona (Italy), Koper (Slovenia), Pula and Rijeka (Croatia) in 2013-2015. Stations and sites description are presented in Table 1. Details related to geographical distribution and main characteristics of sampling ports (including reasons for selection of sampling sites), matching environmental data, comparative overview of NIS and hard-bottom macroinvertebrates from corresponding PBS are presented elsewhere (Kraus et al., this issue b; Kraus et al., this issue a; Spagnolo et al., this issue).

In each port, two seasonal surveys were performed as follows: in spring and late summer 2013 in Koper, spring and fall 2014 in Ancona and Bari, fall 2014 and spring 2015 in Pula and Rijeka. The sampling strategy followed the BALMAS PBS Protocol (Ninčević-Gladan et al., 2014), based on the CRIM Protocol (Hewitt and Martin, 2001). Depending on the seabed properties, sampling was performed at one or two horizontal transects, located perpendicular to the berth (hard bottom substrate) with the grid of hard-bottom sampling sites, arranged along three vertical transects at 15 m distance from each other (Spagnolo et al., this issue). An inner horizontal transect was located at a distance 0-1 m from the berth and an outer 50 m away. At Koper, Pula and Rijeka the edge belt of the seabed is covered by rocky bottom, thus soft-bottom macrofauna at these ports was sampled only along the outer transect. At each horizontal transect, core samples were taken at three sites located at a distance of 15 m from each other.

Sediment samples were collected in situ by SCUBA divers using tubular hand corers with a sharpened edge (Ø =18 cm, h= 30 cm, sampling area=0.025m$^2$) along the horizontal transects at three sites distant 15 m in all ports, except from Koper where samples were collected using a box corer (20x20x10 cm, sampling area 0.04 m$^2$). In all ports one sample were taken per site (minimum sampling area according PBS Protocol) (Ninčević-Gladan et al., 2014). Optionally, in the port of Ancona, three replicate samples were collected from the each site.

Each core sample was transferred to a net-bag (1 mm mesh). Immediately after diver's emergence, samples were rinsed, transferred into PVC jars, initially preserved in 4% formalin, and then transferred to 70% ethanol for determination. In the laboratory, organisms were separated from the sediment remains, sorted and counted up to higher taxa (phylum, order, class, family) level, and then identified to species level, whenever possible. Species determination was done using traditional identification keys (Fauvel, 1923; 1927; Tebble, 1966; Nordsieck, 1969; Parenzan, 1970; 1974; 1976.; Laubier and Ramos, 1974; Fauchald, 1977; Bianchi, 1981; San Martin, 2003; Viéitez et al. 2004). Names of identified taxa were checked after the World Register of Marine Species basis (WoRMS; http://www.marinespecies.orgFunctional identity of species was checked using AZTI software interface (AZTI; http://www.azti.es) following classification of the macrozoobenthos in five ecological groups: (E G I - the disturbance-sensitive species, EG II - the disturbance indifferent species, EG III - the disturbance tolerant species, EG IV - the second order opportunistic species and  EG V - the first-order opportunistic species (Borja et al, 2000; Muxika et al, 2005) according to their sensitivity to an increasing stress gradient (Grall and Glémarec, 1997; Borja et al., 2000). Non-indigenous species were identified according the most recent lists of NIS for the Mediterranean and Adriatic Sea (Steftaris and Zenetos, 2006; Zenetos et al., 2010, 2012; Katsanevakis et al., 2014;  Pećarević et al., 2013; Manini et al., 2016; Galil et al., 2017; Ulman et al., 2017; Zenetos et al., 2017).

### *2.2. Data analysis*

In order to harmonize data and use all possible information, available data from each port were pooled at the level of sampling sites, both in analyses related to whole ports and sampling seasons.

Furthermore, determination to the species level was not always possible, so one should notice the difference between macrofauna abundance (including all specimens, regardless of taxonomic level) and species abundance, related only to identified species. All results that describe species composition, i.e. species abundance (N), richness (S), Margalef's diversity index (d), Shannon-Wiener diversity index (H') and Pielou's evenness (J'), as well as those of similarity between samples, refer to pool of specimens identified up to the species level. In other words, data related to higher taxa are not included in the species composition analyses.

Statistical analyses were performed using PRIMER ecological software package PRIMER™ (Clarke, 1993; Clarke and Warwick, 2001; Clarke and Gorley, 2006). Univariate indices were calculated to describe species composition at a particular site. Multivariate analyses were applied to estimate and test similarity of species composition within and among surveyed ports. Non-metric Multidimensional Scaling (nMDS) was performed to enable visualization of group differences among compared samples. Subsequent multivariate analyses, i.e. sample discrimination and null hypothesis testing were provided using two complementary procedures: Analysis of Similarities (ANOSIM) and Similarity Percentages breakdown (SIMPER) a non-parametric test to routines.

## 3. Results

### *3.1. Overview of native soft-bottom communities*

A total of 8427 macroinvertebrate specimens were recorded; more than 97% specimens were identified to species level, and the rest to the family or, more rarely, to higher taxa levels. The most abundant were Polychaeta and Mollusca, comprising 52% and 40% of total macrofauna, respectively. Other invertebrate groups encompassed 8% of the macrobenthos: Crustacea (5%), Echinodemata (2%), Cnidaria, Sipuncula and Bryozoa <1% . In Bari, Ancona, Pula and Rijeka, the most abundant were Polychaeta (50-70%), followed by Mollusca (10-37%), and these two taxonomic groups accounted from 70% (Rijeka) to 79% (Ancona) of total macrofauna abundance. In the port of Koper Polychaeta and Mollusca accounted 90% of macrofauna, but Bivalvia numerically dominated (63%) over Polychaeta (27%). Four constitutive taxa (Gastropoda, Bivalvia, Polychaeta and Crustacea) were found in all ports and at all sampling stations, Echinodermata occurred at all but one station (RI1), while all other taxa, in all ports, were characterized by low abundance and frequency. Macrofauna densities, expressed as total number of specimens per unit area, amounted 137 (Rijeka), 176 (Ancona), 344 (Pula), 927 (Koper) and 1075 (Bari) specimens/0.1 $m^2$ area. The percentage share of the four most abundant taxa (pooled data of two seasonal surveyes) is presented in Figure 1**.**

Overall, 451 species were identified. The highest values of the species abundance (N), number of species (S) and species richness (d) were obtained in Bari; the highest values of Pielou's evenness (J') and Shannon-Wiener diversity (H') in Pula, while the lowest univariate indices were reported from Rijeka (Table 2). In all PBS, except the port of Rijeka, higher values of species abundances and species richness were recorded in spring compared to fall samples (Figure 2). Characterization of soft-bottom communities in surveyed ports is outlined in the supplementary material.

Comparative analysis of soft-bottom macrofauna revealed only four species common for all the five ports (the molluscs *Antalis inaequicostata* and *Bittium reticulatum* and the polychaetes *Melinna palmata* and *Owenia fusiformis*), while 76% of species were exclusive of one of five ports: 149 in Bari, 66 in Ancona, 58 in Pula, 59 in Koper and 3 in Rijeka. Most of them belonged to Polychaeta (72%), followed by Mollusca (23%), Crustacea (3,17%), Echinodermata (2%) and Bryozoa (<1%), while Anthozoa and Sipuncula were presented by single species with single specimens.

The low number of common species recorded within and among the five ports (Table 3) indicated the peculiarity of species composition in each port. An average similarity of qualitative and quantitative macrofauna composition within ports was rather low, except in Koper (Bari: 32-36%; Ancona: 16-29%; Koper: 22-61%; Pula: 16-23%; Rijeka: 3-31%), and between the ports was still more conspicuous (Table 4).

MDS plot enabled a visual distinction among four groups of samples - three fairly coherent related to the ports of Bari, Ancona and Koper and the fourth – mixed one, with no clear cut distinction between Pula and Rijeka ports (Figure 3). Subsequent ANOSIM test confirmed the indications obtained by MDS and provided a full discrimination among sampling stations. Summarizing, ANOSIM revealed no significant differences in the species composition among stations belonging to the same port except: AN1 *vs.* AN3, BA2 *vs.* BA3, PU1 *vs.* PU 2 and PU3, but also among all three sites in the Rijeka port. Significant differences (R=0.765, p=1%) were found among stations belonging to different ports, except AN1 *vs.* BA1, as well as all three stations from Pula port *vs.* RI1, and PU 2 and PU 3 *vs.* RI3. The last step, SIMPER analysis, provided an insight into highly contributing species explaining both, similarity in species composition within the same port and dissimilarity between different ports, as well as specific affinity between some stations belonged to different ports (Table 5). More than 50% difference between AN1 and AN3 was related to much higher abundances of five species (individual contribution in parentheses), i.e. *Heteromastus filiformis* (25%), *Sternaspis scuttata* (9%), *Mytilus galloprovincialis*, *Kurtiella bidentata* and *Aphelochaeta marioni* (each about 5%) at AN1 related to AN3. Similarly, about 50% of difference between BA2 and BA3 can be attributed to much higher abundances of seven species (*Pseudoleiocapitella fauveli*, 9%; *Leodice limosa* 6%; *Kirkegaardia* sp.1, 4%; *Cirrophorus furcatus*, 4%; *Lumbrineris geldiayi*, 3%; *Melinna palmata*, 2% and *Aonides oxycephala*, 2%) at BA2 related to BA3, as well as higher abundances of five species (*Capitella minima* 6%, *Loripes lucinalis* 5%, *Notomastus latericeus* 5%, *Heteromastus filiformis* 3% and *Mediomastus capensis* 3%), at BA3 related to BA2. The most contribution to differences between PU 1 *vs.* PU2 and PU3 can be attributed to higher abundance of *Heteromastus filiformis*, *Lumbrinereis latrelii*, *Lucinella divaricata* and *Tellina donacina*, as well as the high abundance of some species, e.g. *Peresiella clymenoides*, *Euclymene oerstedi*, *Notomastus aberans* and *Tellina donacina*, occurred only at station PU1, but it was also due to *Aponuphis bilineata*, fairly abundant at PU3 and absent from PU1. In terms of community structure, differences among sampling stations in the Rijeka port could be attributed to presence/absence of several species typical of a particular port. About 70% of difference in species composition between RI1 and RI2 resulted from the presence of polychaetes *Capitella capitata* and *Malacoceros fuliginosus* at RI1 and *M. palmata* at RI2, and its absence in the reverse case. The same percentage of difference between RI1 and RI3 was due to presence of *Lucinella divaricata*, *C. capitata* and

*M. fuliginosus* at RI1 and their absence at RI3, while 50% of difference between RI2 and RI3 resulted from presence of three molluscs (*L. divaricata, Loripes lucinalis, Hydrobia acuta*) and one polychaete (*M. palmata*) recorded at RI2 and absent from RI3.

### *3.2. Contribution of NIS to native communities*

In the PBS of the five Adriatic Sea ports, a total number of 5 species and 86 specimens were identified as NIS, both accounting for 1,1% of the total species richness and abundance. Three species belonged to Polychaeta (*Hydroides elegans, Notomastus aberans* and *Pseudopolydora paucibranchiata*) and two to Bivalvia (*Anadara transversa, Ruditapes philippinarum*). Five NIS were reported from Bari, and only one from Ancona, Koper, Pula and Rijeka ports (Table 6). Additionally, three NIS were found in Škocjanski zatok Nature Reserve, a lagoon connected with port of Koper (data not included in faunistic overview): the bivalve *Arcuatula senhousia*, the polychaete *Ficopomatus enigmaticus* and the cirriped *Amphibalanus eburneus*. The last one was represented by single specimen, accidentally imported from nearby rocky substrate.
The contribution of NIS in total species abundance amounted for 0.05% (Koper); 0.19% (Pula); 0.49% (Rijeka); 1.05% (Bari) and 2.44% (Ancona), while their participation in total number of species was 0.79% (Pula); 0.83% (Koper); 0.89% (Bari); 2.33% (Rijeka) and 2.34% (Ancona).
*Arcuatula senhousia* was observed in high numbers (11-235 per site) at seven out of eight sites in the Škocjanski zatok lagoon, where 716 specimens were found in winter and 11 specimen in summer 2014. The low frequency of NIS, i.e. their presence at only one site in Koper, Pula and Rijeka, does not provide a suitable dataset for multivariate analysis on the similarity/dissimilarity of NIS among surveyed ports.

## 4. Discussion

In terms of taxonomic composition, dominance and frequency observed at higher taxa level, the soft-bottom macrofauna from the five Adriatic ports was rather similar and fully comparable. In terms of macrofauna densities, there were differences between less abundant assemblages (176-344 ind./0.1m$^2$) from Rijeka, Ancona and Pula and highly abundant assemblages from Koper (927 ind./0.1m$^2$) and Bari (1075 ind./0.1m$^2$). In the shallow and closed or semi-closed coastal systems with limited water circulation, poor flushing and weak tidal exchange, the abundance of soft-bottom macrofauna is variable and usually governed by local environmental factors (Sakellariadou, 2015). Areas characterized by such hydrographic conditions are generally susceptible to eutrophication, siltation, organic overloading and oxygen crises and often are associated with more or less degraded soft-bottom benthic communities (Zarkanellas, 1979; Stachowitsch, 1991; Diaz and Rosenberg, 1995; Diaz, 2001; Levin et al., 2009; Tillin and Tyler-Walter, 2013). Apart from unfavourable physical background, ports are exposed to long-term anthropogenic pressure enhanced by urban, industrial and shipping development. They are generally indicated as pollution hotspots or areas of stagnation (Sakellariadou, 2015), with a variety of organic and inorganic pollutants, releasing intentionally or unintentionally into marine environment, compromising its environmental quality and additionally affecting soft-bottom communities. In both, physically disturbed and/or polluted areas, macrofauna abundances can increase, decrease or stay unchanged, depending on the relative abundances of opportunistic, sensitive and tolerant species (Clark and Warwick, 1990). It means that assemblages from impacted habitats can reach very high or very low densities, depending on the functional community structure driven and/or supported by local environmental conditions. Those differences in macrofauna abundance cannot be simply attributed to pollution and/or to other environmental stressor.

Numerous studies have demonstrated that soft-bottom macrofauna respond in a predictable manner to a variety of natural and anthropogenic disturbances (Pearson and Rosenberg, 1978; Dauer, 1993; Tapp et al., 1993; Warwick, 1993) and that stress response is often corresponding to Pearson-Rosenberg model (PRm) of faunal succession. That model, was originally developed to describe predictable changes of the benthic assemblages along the spatial and/or temporal gradient of organic overloading (Pearson and Rosenberg, 1978). It was repeatedly tested against different sources of pollution and appear to be universally applicable for most physical, chemical, or biological disturbances in sublittoral, soft-bottom habitats (Rhoads and Germano, 1986; Rosenberg et al., 2004; Magni et al., 2009). Nowadays PRm is widely accepted and applied, either directly, i.e. throughout ecological studies of benthic communities or indirectly - as the baseline concept used in development of different descriptors and indices (e.g., AMBI, M-AMBI) implemented into EU legislative frameworks for assessment of the ecological status in marine ecosystems (WFD/2000/60/EC; Muxika et al., 2005; MSFD/2008/56/EC). The concept is based on the relationship between the life strategy of soft-bottom invertebrates, i.e., affiliation of each species to one of five functional categories associated with sensitivity to pollution/disturbance, and the percentage contribution of these five functional groups to overall community structure (Grall and Glémarec, 1997; Borja, et al., 2000). Soft-bottom macrozoobenthos inhabiting port areas is highly conditioned by environmental factors such as water renewal, hydrodynamics and land surface and subsurface runoff water inflows and characterised by low mobility, adaptation to high levels of organic matter and resilience and capacity to recover following disturbance (Tillin and Tyler-Walters, 2013). Considering given determinants and PRm presumptions, we expected that macrobenthic assemblages from surveyed ports could be impoverished, rather uniform (highly dominated by one or a few species physiologically well adapted to stress conditions) and characterized by low species richness, diversity and evenness. Checking the most comprehensive database for functional identity of infaunal species (AZTI; http://ambi.azti.ess) we found that benthic assemblages in the five Adriatic ports were dominated and substantially composed by tolerant and opportunistic species, as expected. Surprisingly, the species richness, diversity and evenness expressed by univariate indices, indicated rich, diverse and evenly distributed species composition, despite evident modifications of the assemblage structure towards the r-selection model (MacArthur and Wilson, 1967), characteristic for unstable and/or polluted environments. Hence, univariate descriptors of benthic communities indicated the presence of modified, but quite diverse and presumably well-established soft-bottom communities in all surveyed ports, except of Rijeka. It might reflect the successful adaptation of many pollution-tolerant species to long-term pollution and unstable environmental conditions in the ports of Ancona, Bari, Koper and Pula. Spatial heterogeneity of macrozoobenthos in combination with significantly lower values of all univariate indices, suggested that Port of Rijeka is the most impacted port in the context of this study. That is the largest and the eldest cargo port on the eastern coast of the Adriatic Sea, with crude oil terminal and two shipyards in the close vicinity. It is distinguished from the other four ports by extremely poor soft-bottom assemblages in terms of abundance, species richness and species diversity. Although it might be considered as an obvious example of severe pollution caused by shipping activities, such assumption should be taken with caution, since it is the only port situated in the zone of transitional waters (Rječina River estuary). Transitional waters are naturally stressed environments, that discourage the settlement of many organisms and generally characterize with low diversity. Hence, the low diversity and abundance of macrofauna in the Port of Rijeka cannot be simply attributed to pollution and/or to other environmental stressor. It could be attributed to antrophogenic or natural stress or their synergistic effect. To clarify that issue, further invesigation is required. It should be based on comparison of recent and historical data on the target benthic community, as well as its relationship with environmental factors and antrophogenic pressures. No matter what, we support the concern and decision of Environmental Protection Agency (EPA), which recently has included the Port of Rijeka on the list of the six Adriatic ports, classified as priority sites requiring immediate actions to reach the desirable environmental conditions (Andričević et al., 2011).

The results of multivariate analyses figured out significant differences in macrofauna communities structure between all the ports, except Pula *vs.* Rijeka.  Despite differences in univariate aspects of macrobenthic assemblages between these two ports, there were partial overlapping in their multivariate aspect. It could be the result of sharing some "influential species" and/or differences derived from the spatial heterogeneity (Rijeka) or homogeneity of macrofauna (Pula). Dominance of opportunistic and tolerant species everywhere implied pollution as important driver of benthic assemblages structure, but distinction in dominant species identity and very low number of common species suggested that local ecological set-up prevailed in shaping and moderating a specific species composition over pollution effects in each particular port. These results match the findings related to the hard-bottom macrofauna from the same sampling stations in a way that particular macrofaunal assemblages were established in each port (Spagnolo et al., this issue), but differ from them in terms of numbers and similarity of non-indigenous species (NIS). The relationship between soft-bottom communities and available data on the environmental factors (including anthropogenic pollution) in particular ports will be the subject of future publications.

Shipping is generally identified as one of the main vectors responsible for introduction of NIS, either by transfer of fouling organisms attached to submerged part of the vessels hull or organisms transported by water and/or sediment contained in ballast tanks (Carlton, 1985; Ruiz et al., 1999; Leppäkoski et al. 2002; Simkanin et al., 2009; David and Gollasch, 2015). Hence, facing with a double effect of shipping activities and environmental disturbance, ports can be considered coastal areas especially susceptible and vulnerable to introduction and/or establishment of NIS.  The BWM Convention (IMO, 2004) is a key tool to address the issue. It states that ships in international traffic must manage their ballast water to specific standards, ensuring that no, or minimum, harmful organisms are transferred to the next port of call. To achieve that goal, the BMW Convention "encouraged signatory countries to undertake scientific and technical research and monitoring including observation, measurement, sampling, evaluation and analysis of the effectiveness and adverse impacts of any technology or methodology as well as any adverse impacts caused by such organisms and pathogens that have been identified to have been transferred through ships' ballast water" (IMO, 2004). Although PBS are not a specific requirement under the BWM Convention, their implementation is recommended by IMO, as an important tool for the shipping and ballast water risk assessment.

The fouling organisms, living attached to a variety of natural and artificial hard substrata can be more likely transferred and introduced into ports by shipping activities than infaunal species (Ferrario et al., 2017; Marić et al., 2017). Thus, the presence of *Amphibalanus eburneus*, *Ficopomatus enigmaticus*, *Arcuatula senhousia* and *Hydroides elegans* in soft-bottom macrofauna samples from Škocjanski zatok lagoon and respectively the Port of Bari, can be considered accidental. The bivalve *Anadara transversa* found in the soft-bottoms of Koper is typically associated with soft-bottom sediments, but occasionally attach to rocks and shells and it was reported as a member of the hard-bottom assemblage in the Ancona. *A. transversa* and above mentioned fouling species were present both on hard- and soft-bottom of investigated area, but only *H. elegans* was found in both types of bottom in the same port. Geographical distribution and status of above-mentioned species are already described in the parallel study from the hard-bottom assemblages (Spagnolo et al., this issue), so iteration of these data in this paper is needless. Here, it should be noted that three species common for hard- and soft-bottoms (*Bugulina fulva*, *Chaetozone corona* and *Hydroides dianthus*) and identified as NIS in the fouling assemblages from the same study area (Spagnolo et al., this issue) are omitted from the present NIS listing, after thorough checking of the most recent literature. There is no evidence of human-mediated introduction of bryozoan *B. fulva* in the Mediterranean, so it is likely Atlanto-Mediterranean species and the Adriatic Sea could be a part of its natural range. The polychaete *C. corona* has a non-confirmed NIS status, and it is possible cryptogenic species for the Mediterranean (Çinar and Ergen, 2007; Zenetos et al., 2010; Le Garrec et al., 2017). *H. dianthus* has been recently re-assessed as a species complex consisting of two cryptic species, one of which is likely native to the Mediterranean Sea (Sun et al., 2017). In this paper a brief information on the geographical distribution of three NIS, reported only from the soft-bottom of surveyed ports is provided. *Pseudopolydora paucibranchiata* is a tube-building spionid polychaete. It was described from Japan and its native range extends from Hong Kong to the southern Kuril Islands (NEMESIS; https://invasions.si.edu/nemesis/databases.html). Later it was reported from the Pacific Ocean, (Radashewski, 1993) and the Northeast Atlantic (Ramberg and Schram, 1983). In the Mediterranean, it was firstly reported from Turkey (Levantine sea) and Greece (Aegean Sea), where supposed to be transported with ballast waters as a possible vector of invasion (Dagly and Çinar, 2008). In The Bay of Izmir, together with two other spionids, it comprised about 95% of zoobenthos, replacing previously known opportunistic species, but seems that could invade only areas where perturbations due to high organic loads or the fresh water influx occur (Dagly and Çinar, 2008). In the Adriatic Sea, *P. paucibranchiata* was so far known from the Port of Pula and North Adriatic offshore waters (Mikac, 2015). The most recent finding from Bari was the first record of that NIS in Italian waters and pointed out westward expansion of *P. paucibranchiata* which can be related to shipping activities. In present study four specimens were reported from Bari.

*Notomastus aberans* arrived in the Mediterranean from the Indian Ocean and Red Sea, probably as Lessepsian migrant *via* the Suez Canal (Zenetos et al., 2010). It is successfully established all over the Mediterranean, including eastern and western coast of the northern, central and southern basins of the Adriatic Sea. *Ruditapes philippinarum* is an infaunal bivalve originates from the western Pacific, having natural populations distributed from the Philippines up to the southern Kuril Islands (Scarlato 1981). In 1972, it was introduced into France for commercial hatchery, and its expansion in the Mediterranean is attributed to intentional and/or unintentional spreading from the aquaculture. *R. philippinarum* was introduced in the northern Adriatic (Venice Lagoon) to supplement the local fishery of the autochthonous *R. decussatus* (Cesari and Pellizzato 1985, Breber 2002). In a relatively short period it colonized the entire Venice Lagoon (Breber 2002), expanding also into other nearby locations (Zentilin; 1990; Carrieri et al. 1992). The first record from the eastern (Croatian) Adriatic coast was recently reported from Zelena laguna, the west Istrian coast (Nerlović et al., 2016). In present study one specimen was found in Bari.

The number of NIS recorded in an area is the basic indicator addresses anthropogenic pressure regarding NIS introduction (Olenin et al., 2010). In the investigated area, both the number and abundance of NIS were generally low. The highest number of NIS was recorded in Bari, and the highest abundance in Ancona and Bari. In present study, the polychaete *N. aberans* was the most common and the most abundant invader, recorded in all ports, except Koper. From total of 53 specimens, 39 were found in Ancona. *A. transversa* were found in Bari and Koper, and other three NIS only in Bari. Apart from *N. aberans*, NIS represented with ten or more specimens were *A. transversa* and *H. elegans*. Rough approximation of ratios for NIS to native species, as a measure of change in species composition, may be 1:40 in majority of the European marine waters, 1:20 at open coasts, and 1:5 in estuaries and lagoons (Olenin et al., 2010). In that sense, the ratio for NIS to native species in Bari and Rijeka (1:42) are comparable with measure reported for majority of European marine waters. Here, it should be noted that the Port of Rijeka belongs to transitional waters, and from that aspect, the ratio for NIS to native species is considerably lower from the benchmark that was set up for estuaries (Olenin et al., 2010). The three other ports (Pula 1:126; Koper 1:120; Bari 1:111) and entire investigated area (1:122) attain even better score, in favour of native species. The participation of NIS in total number of species was low in general (Pula 0.79%, Koper 0.83%, Ancona 0.89%, Rijeka 2.33 %, Bari 2.34%) and in comparison with hard bottom assemblages from the same ports (Bari 6.8%, Ancona 7.5%, Koper 3,6; Pula 2.5%), except Rijeka (2.9%) (Spagnolo et al, this issue) where close contribution of NIS was found for hard- and soft-bottom habitats. The general pattern can be applied to the number (29 *vs* 5), and contribution (4% *vs* 1%) on the hard- and soft-bottoms of the five Adriatic ports.

A low number of NIS - half of which belong to fouling organisms, call into question either the suitability of soft-bottom macrofauna as a tool for monitoring of NIS or the PBS method adopted for soft-bottom surveys. Since the most of lists related the Mediterranean NIS involved many species associated with soft-bottoms (Zenetos et al., 2010, 2012, 2017; Pećarević et al., 2013; Katsanevakis et al., 2014; Galil et al., 2017), it is more likely that sampling methodology adopted for PBS and NIS monitoring was not suitable choice. Like a worldwide our survey was designed around CRIMP Protocol (Hewitt and Martin 2001), with adaptations and compromises depending on the local circumstances. One of the more significant adaptations is the use of non-diving based sampling methods and another one is a reduction in sampling intensity within particular sites or habitats (Award et al., 2014. In present study, the later adaptation measure was applied. The number of samples collected in each port met the minimum requirements of PBS Protocol, due to personnel constraints in all partner institutions, except Ancona. It was additionally reduced in Koper, Pula and Rijeka - where the occurrence of hard-bottom prevented sampling along the inner horizontal transect. Hence, the low number of NIS could be related to minimum sampling efforts allowed by PBS Protocol, but it is not likely. The number of NIS detected in Bari is five times higher, despite a triple number of samples collected in Ancona. Experience gained from the present study suggests that reasonable increase in sampling intensity, i.e. more replicates, more sites, use of unsieved material etc., could not increase efficiency of NIS detection. Experience from previous environmental studies imply that an increase in sampling intensity could not be feasible, due to time-consuming laboratory work on separation, sorting and counting of macrobenthos. Global taxonomy crises and insufficient number of taxonomic experts for soft-bottom benthic invertebrates is an additional argument against increase sampling intensity. A solution could be find in selection of alternative sampling device for PBS and NIS monitoring in soft-bottom habitats.

Sampling strategy based on SCUBA diving method, recommended for both hard- and soft bottoms, is accompanied with decreasing feasibility and increasing both, physical risk and monitoring costs. Still, SCUBA diving is the only suitable method for sampling of hard-bottom macrofauna. Due to a higher number of NIS and the fact that hard-bottom macrofauna is more likely transported by shipping compared to soft-bottom invertebrates, sampling design of the BALMAS PBS Protocol for hard-bottom communities might be efficient and feasible for monitoring of NIS, despite all limitations. The better resolution of NIS should be tested by increasing the number of sampling sites on the hard-bottom. On the other side, sampling protocol addressing the soft-bottom macrobenthos seems to be unsuitable and infeasible for PBS and NIS survey in the seaports. Due to different species composition and high diversity of macroinvertebrates, soft-bottom communities in the seaports should be monitored, as well. Samples collected by hand-operated corers contain much less volume of the sediment compared to Van Veen grab, i.e. the standard sampling device in the most of the Mediterranean countries. A probability for detection of NIS using hand-operating corers (sampling area of 0,025 $m^2$) or small box-corer (sampling area < 0.1 $m^2$), is lower than using standard Van Veen grab. Moreover, the qualitative results obtained by hand-operated corers are poorly comparable with data resulted from most of regional benthic research. In Mediterranean countries, a huge quantity of data from soft-bottom habitats was provided from grab samples.

Recently, there are substantial quantity of corresponding data provided from WFD survey for many Mediterranean ports. These data could be used as a benchmark (substitution for PBS) addressed to soft-bottom macrobenthic communities in implementation of BWMC. Moreover, EU member states are starting to carry out PBS for NIS in the framework of MSFD. In need of feasible and cost-effective solutions, these data might be used as suitable basis for testing efficiency/feasibility of monitoring focuses on the soft-bottom macrofauna and NIS in the seaports. If prove to be efficient, PBS and/or NIS observing system could be derived from WFD data, increasing cost-effectiveness of PBS and/or NIS monitoring in the seaports. To test this assumption further research is needed. In the interim, the results of number of NIS abundance and diversity seems too low to cause a substantial negative impact on native communities in surveyed ports.

Despite the low number of NIS identified, we suppose this contribution will be a valuable basis for the future assessment for changes of soft-bottom macrobenthic assemblages, as well as in the identity, distribution and abundance of NIS in surveyed ports.

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Table(s)

Table 1.

| PORT | STATION | COORDINATES | | PROFILE | | | BOTTOM TYPE |
|---|---|---|---|---|---|---|---|
| | | Latitude | Longitude | Distance (m) | No. sites and (replicates) | Depth (m) | |
| **Bari** | BA1 | 41° 08.536' | 16° 52.064' | 0<br>50 | 3<br>3 | 14 | fine phytal detritus |
| | BA2 | 41° 08.061' | 16° 52.134' | 0<br>50 | 3<br>3 | 7 | fine phytal detritus |
| | BA3 | 41° 08.473' | 16° 51.143' | 0<br>50 | 3<br>3 | 9 | fine phytal detritus |
| **Ancona** | AN1 | 43°37'31.45'' | 13°29'48.42'' | 1<br>50 | 3 (3)<br>3 (3) | 9 | silty-sand |
| | AN2 | 43°37'17.71'' | 13°29'50.06'' | 1<br>50 | 3 (3)<br>3 (3) | 10 | silty-sand |
| | AN3 | 43°40'04.89'' | 13°24'21.70'' | 1<br>50 | 3 (3)<br>3 (3) | 13 | silty-sand |
| **Koper** | KO1 | 45°33.390' | 13°43.837' | 50 | 3 (no) | 6 | silty-sand |
| | KO2 | 45°32.822' | 13°42.508' | 50 | 3 (no) | 8 | silty-sand |
| | KO3 | 45°34.275' | 13°43.931' | 50 | 3 (no) | 8 | silty-sand |
| | KO4 | 45°35.988' | 13°42.393' | 50 | 3 (no) | 8 | silty-sand |
| **Pula** | PU1 | 44°53.509' | 13°48.129' | 50 | 3 (no) | 10 | silty-sand |
| | PU2 | 44°51.991' | 13°49.697' | 50 | 3 (no) | 9 | detritic sand |
| | PU3 | 44°52.563' | 13°47.579' | 50 | 3 (no) | 26 | sandy-silt |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Rijeka** | RI1 | 45°19.707' | 14°25.836' | 50 | 3 (no) | 22 | sandy-silt |
| | RI2 | 45°19.340' | 14°26.328' | 50 | 3 (no) | 27 | sandy-silt |
| | RI3 | 45°18.851' | 14°28.147' | 50 | 3 (no) | 35 | sandy-silt |

Table 2.

| SITES | S | N | d | J’ | H’ |
|---|---|---|---|---|---|
| BA1 | 112 | 736 | 16.82 | 0.78 | 3.70 |
| BA2 | 136 | 1601 | 18.30 | 0.79 | 3.87 |
| BA3 | 104 | 962 | 14.99 | 0.75 | 3.50 |
| **Bari** | **214** | **3229** | **26.29** | **0.77** | **4.13** |
| AN1 | 86 | 1119 | 12.11 | 0.68 | 3.03 |
| AN2 | 56 | 334 | 9.47 | 0.69 | 2.79 |
| AN3 | 26 | 147 | 5.01 | 0.76 | 2.47 |
| **Ancona** | **112** | **1598** | **15.05** | **0.69** | **3.25** |
| KO1 | 34 | 260 | 5.94 | 0.72 | 2.55 |
| KO2 | 82 | 624 | 12.59 | 0.76 | 3.33 |
| KO3 | 69 | 645 | 10.51 | 0.76 | 3.22 |
| KO4 | 74 | 954 | 10.64 | 0.79 | 2.96 |
| **Koper** | **121** | **2223** | **15.57** | **0.70** | **3.34** |
| PU1 | 67 | 204 | 12.41 | 0.86 | 3.63 |
| PU2 | 63 | 177 | 11.98 | 0.90 | 3.72 |
| PU3 | 47 | 135 | 9.38 | 0.84 | 3.22 |
| **Pula** | **127** | **516** | **20.17** | **0.88** | **4.26** |
| RI1 | 13 | 121 | 2.50 | 0.57 | 1.47 |
| RI2 | 19 | 61 | 4.38 | 0.78 | 2.29 |
| RI3 | 19 | 23 | 5.74 | 0.98 | 2.87 |
| **Rijeka** | **43** | **205** | **7.89** | **0.68** | **2.54** |

Table 3.

| | Bari | Ancona | Koper | Pula | Rijeka |
|---|---|---|---|---|---|
| **Bari** | 149 | | | | |
| **Ancona** | 7 | 66 | | | |
| **Koper** | 5 | 8 | 56 | | |
| **Pula** | 7 | 5 | 8 | 58 | |
| **Rijeka** | 4 | 3 | 2 | 2 | 8 |

**Table(s)**

Table 4.

| | BA1 | BA2 | BA3 | AN1 | AN2 | AN3 | KO1 | KO2 | KO3 | KO4 | PU1 | PU2 | PU3 | RI1 | RI2 | RI3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **BA1** | | | | | | | | | | | | | | | | |
| **BA2** | 31.8 | | | | | | | | | | | | | | | |
| **BA3** | 35.9 | 34.7 | | | | | | | | | | | | | | |
| **AN1** | 10.6 | 8.5 | 13.6 | | | | | | | | | | | | | |
| **AN2** | 10.5 | 5.7 | 7.3 | 29.4 | | | | | | | | | | | | |
| **AN3** | 18.1 | 7.6 | 8.7 | 16.1 | 20.5 | | | | | | | | | | | |
| **KO1** | 8.4 | 4.7 | 7.0 | 19.1 | 40.9 | 17.2 | | | | | | | | | | |
| **KO2** | 8.7 | 11.2 | 17.2 | 17.6 | 10.0 | 9.9 | 30.5 | | | | | | | | | |
| **KO3** | 8.4 | 9.1 | 9.7 | 10.2 | 10.6 | 9.1 | 36.9 | 55.9 | | | | | | | | |
| **KO4** | 5.8 | 10.4 | 20.8 | 17.4 | 5.8 | 5.3 | 22.4 | 61.1 | 53.5 | | | | | | | |
| **PU1** | 8.5 | 7.4 | 6.5 | 5.1 | 6.7 | 8.5 | 8.2 | 12.1 | 10.1 | 7.3 | | | | | | |
| **PU2** | 6.8 | 5.5 | 7.0 | 2.9 | 4.7 | 1.9 | 3.2 | 11.0 | 6.8 | 8.0 | 22.6 | | | | | |
| **PU3** | 4.1 | 3.8 | 4.7 | 1.8 | 1.7 | 2.8 | 5.1 | 4.2 | 4.9 | 3.7 | 15.9 | 22.4 | | | | |
| **RI1** | 5.4 | 1.6 | 2.4 | 1.1 | 3.1 | 2.2 | 1.0 | 1.9 | 1.8 | 1.1 | 14.8 | 3.4 | 4.7 | | | |
| **RI2** | 5.3 | 3.0 | 5.1 | 1.2 | 1.5 | 1.0 | 0.6 | 3.5 | 2.8 | 2.0 | 21.1 | 9.2 | 9.2 | 30.8 | | |
| **RI3** | 1.8 | 1.4 | 2.2 | 1.4 | 1.7 | 2.4 | 2.8 | 2.8 | 1.5 | 1.6 | 8-8 | 10.0 | 11.4 | 2.8 | 9.5 | |

Table(s)

Table 5.

| SPECIES / PORT | ECOLOGICAL GROUP | BA | AN | KO | PU | RI |
|---|---|---|---|---|---|---|
| *Sternaspis scutata* | EG III | | 18.6 | 3.0 | | |
| *Heteromastus filiformis* | EG IV | 4.7 | 17.3 | 10.9 | | |
| *Mytilus galloprovincialis* | EG III | | 12.5 | | | |
| *Notomastus aberans* | EG III | | 7.1 | | | |
| *Corbula gibba* | EG IV | 7.3 | 4.3 | | | |
| *Nephthys hystricis* | EG II | | 4.3 | | | |
| *Nassarius nitidus* | EG II | | 4.0 | | | |
| *Tritia pygmaea* | EG II | | 3.8 | | | |
| *Lumbrinereis gracilis* | EG II | | 3.4 | 18.7 | | |
| *Pseudoleiocapitella fauveli* | EG V | 9.0 | | | 1.7 | |
| *Capitella minima* | EG V | 8.3 | | | | |
| *Aonides oxycephala* | EG III | 7.2 | | | 6.0 | |
| *Mediomastus capensis* | EG IV | 5.5 | | | | |
| *Loripes orbiculatus* | EG I | 3.3 | | | | |
| *Leodice limosa* | n.a. | 2.9 | | | | |
| *Melinna palmata* | EG III | 2.8 | | 3.4 | 2.1 | |
| *Nucula nitidosa* | EG I | 2.8 | | | | 16.8 |
| *Kirkegaardia* sp.*A* | n.a. | 2.6 | | | | |
| *Chaetozone gibber* | EG III | 2.4 | | | | |
| *Lumbrineris geldiayi* | EG II | 2.3 | | | | |
| *Protodorvillea kefersteini* | EG V | 2.2 | | | | |
| *Kurtiella bidentata* | EG III | 2.1 | | | 1.9 | |
| *Parvicardium exiguum* | EG I | 2.1 | | | | |
| *Schistomeringos rudolphi* | EG IV | 2.1 | | | | |
| *Cirrophorus furcatus* | EG II | 1.8 | | | | |
| *Aricidea fragilis* | EG I | 1.4 | | | | |
| *Sigambra tentaculata* | EG IV | 1.4 | | | 1.9 | |
| *Neanthes caudata* | EG IV | 1.4 | | | | |
| *Lumbrineris latreilli* | EG II | | | 2.7 | 11.2 | |
| *Lysidice unicornis* | n.a. | | | | 8.4 | |
| *Aponuphis bilineata* | EG I | | | | 6.3 | |

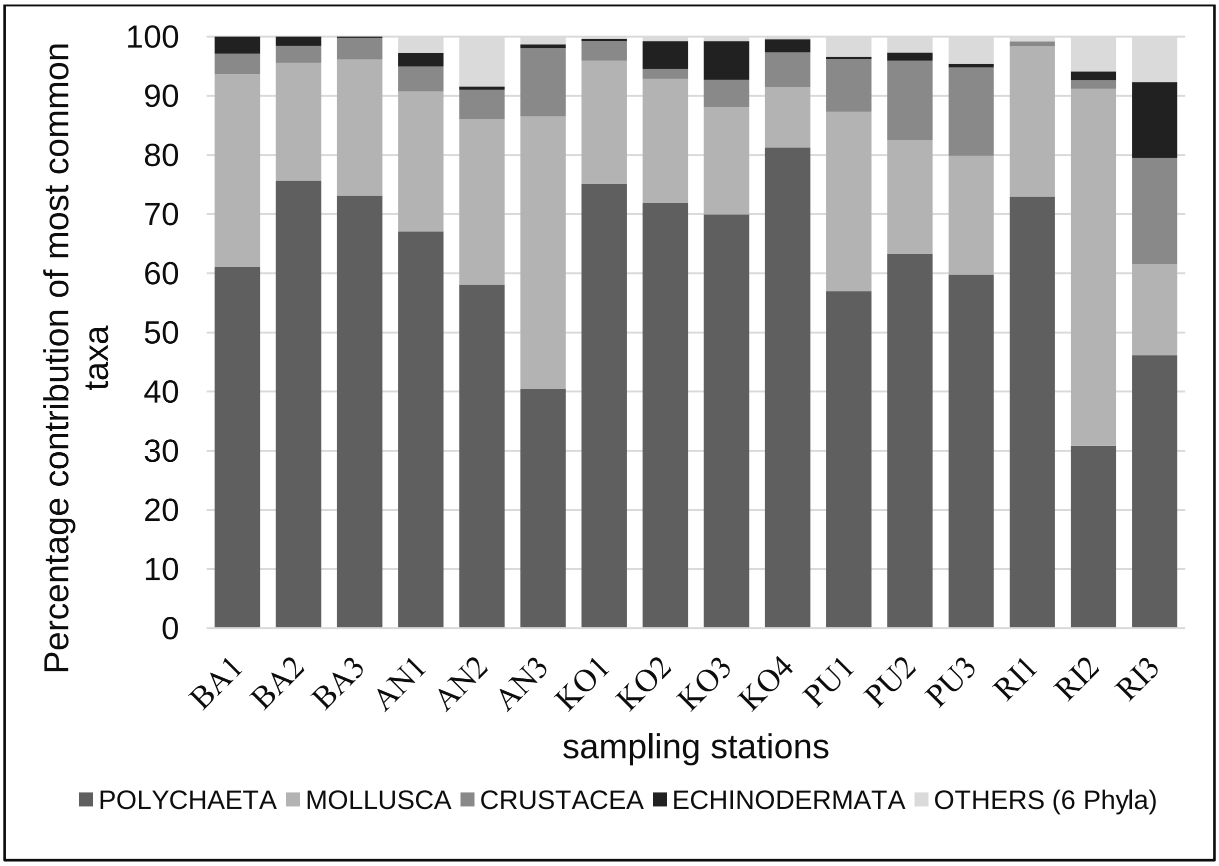
| | | | | | | |
|---|---|---|---|---|---|---|
| *Glycera unicornis* | EG II | | | | 5.8 | |
| *Euspira nitida* | EG II | | | | 3.9 | |
| *Lumbrinereis lusitanica* | EG II | | | | 3.8 | |
| *Papillicardium papillosum* | EG I | | | | 3.8 | |
| *Lucinella divaricata* | EG I | | | | 2.9 | 56.1 |
| *Lumbrinereis coccinea* | EG II | | | | 2.9 | |
| *Aponuphis grubii* | EG I | | | | 2.9 | |
| *Nereis rava* | EG III | | | | 2.1 | |
| *Tellina donacina* | EG I | | | | 1.9 | |
| *Tritia incrassata* | EG II | | | | | 11.3 |
| *Loripes orbiculatus* | EG I | | | | | 7.7 |
| *Laonice cirrata* | EG III | | | 13.0 | | |
| *Terebellides stroemii* | EG II | | | 51.7 | | |
| *Tellina distorta* | EG I | | | 6.4 | | |
| *Ampelisca* sp. | EG I | | | 2.9 | | |
| *Euclimene* sp. | n.a. | | | 2.5 | | |

**Table(s)**

Table 6.

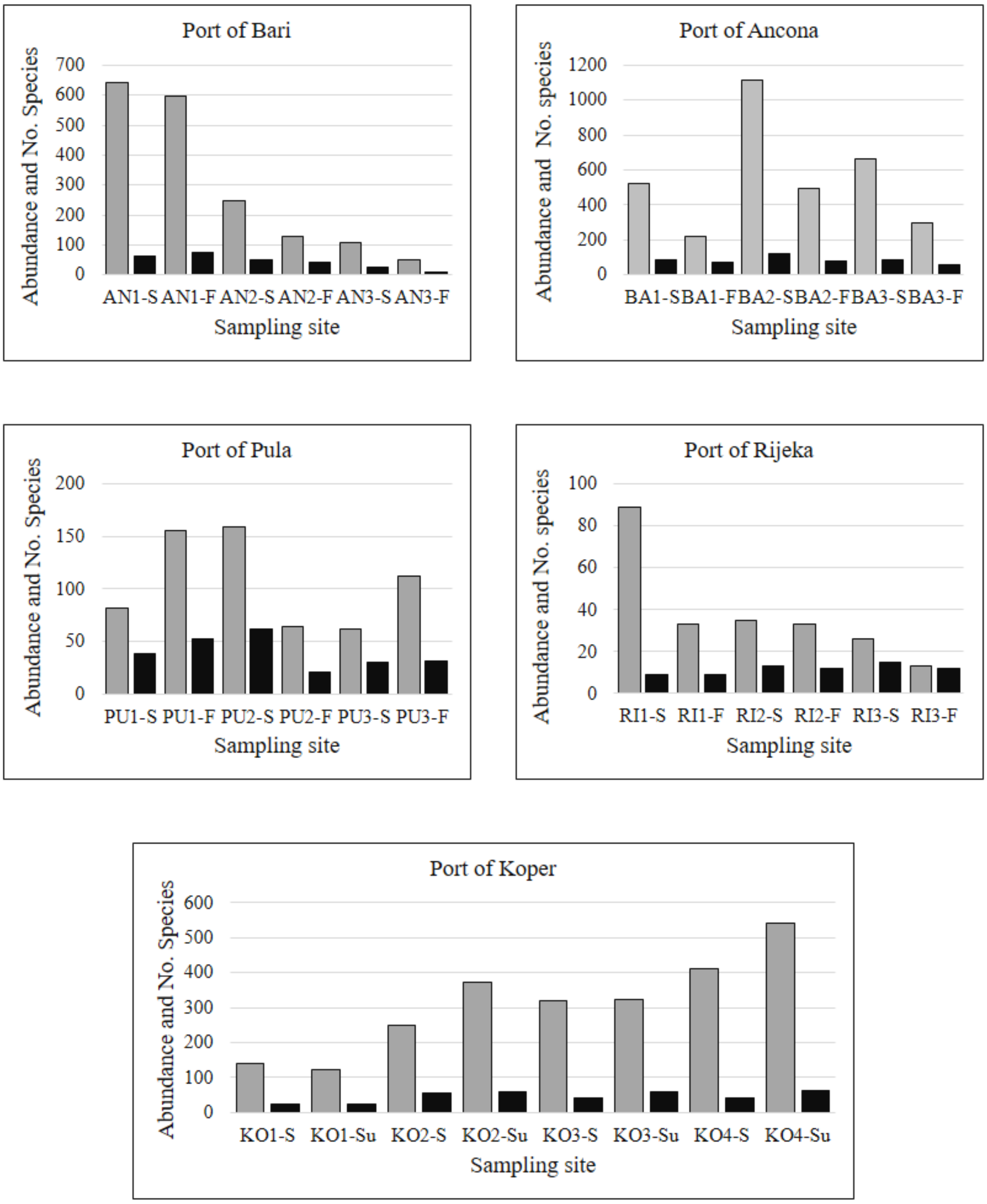
| PORT | BA | | AN | | KO | | PU | | RI | |
|---|---|---|---|---|---|---|---|---|---|---|
| **NIS / SEASON** | **spring** | **fall** | **spring** | **fall** | **spring** | **summer** | **spring** | **fall** | **spring** | **fall** |
| *Anadara transversa* | 6 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Pseudopolydora paucibranchiata* | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Notomastus aberans* | 1 | 1 | 27 | 12 | 0 | 0 | 0 | 11 | 1 | 0 |
| *Hydroides elegans* | 4 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ruditapes philippinarum* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TOTAL | 15 | 19 | 27 | 12 | 1 | 0 | 0 | 11 | 1 | 0 |

Figure 1.



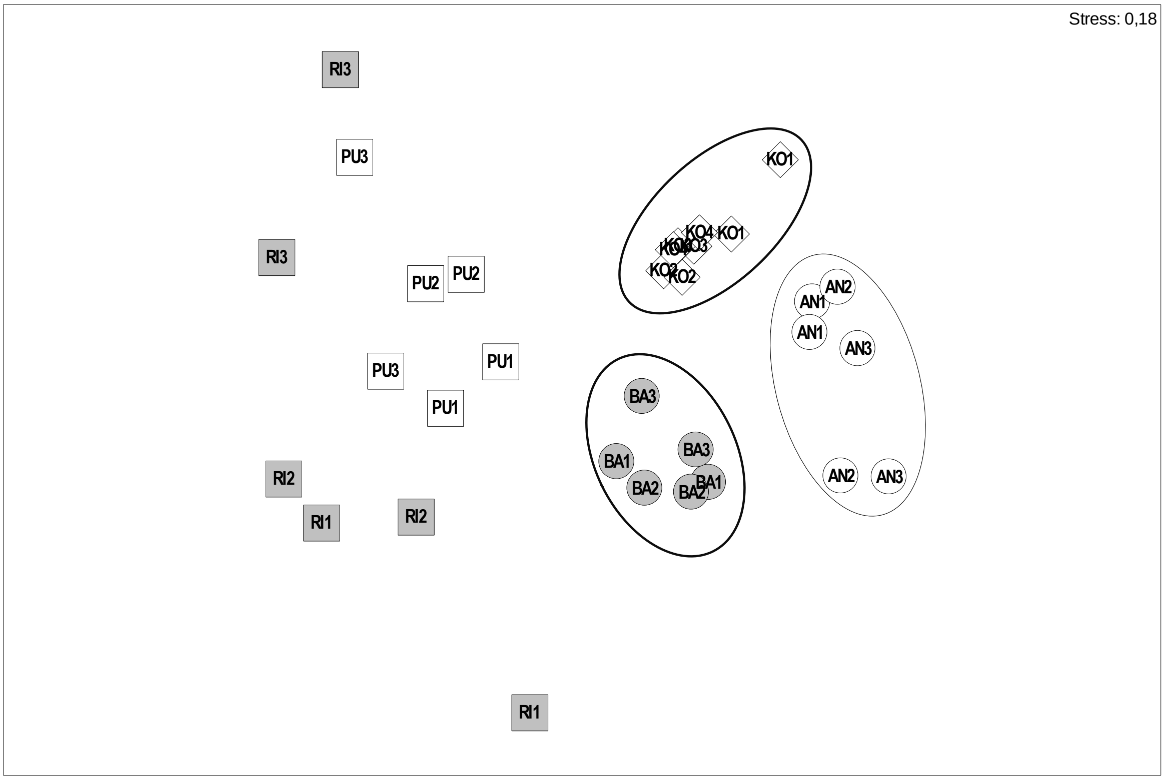
Percentage contribution of most common taxa
100
90
80
70
60
50
40
30
20
10
0
BA1
BA2
BA3
AN1
AN2
AN3
KO1
KO2
KO3
KO4
PU1
PU2
PU3
RI1
RI2
RI3
sampling stations
POLYCHAETA
MOLLUSCA
CRUSTACEA
ECHINODERMATA
OTHERS (6 Phyla)

Figure2.



Port of Bari
Abundance and No. Species
700
600
500
400
300
200
100
0
AN1-S AN1-F AN2-S AN2-F AN3-S AN3-F
Sampling site
Port of Ancona
Abundance and No. species
1200
1000
800
600
400
200
0
BA1-S BA1-F BA2-S BA2-F BA3-S BA3-F
Sampling site
Port of Pula
Abundance and No. Species
200
150
100
50
0
PU1-S PU1-F PU2-S PU2-F PU3-S PU3-F
Sampling site
Port of Rijeka
Abundance and No. species
100
80
60
40
20
0
RI1-S RI1-F RI2-S RI2-F RI3-S RI3-F
Sampling site
Port of Koper
Abundance and No. Species
600
500
400
300
200
100
0
KO1-S KO1-Su KO2-S KO2-Su KO3-S KO3-Su KO4-S KO4-Su
Sampling site

Figure 3.



Stress: 0,18
RI3
PU3
RI3
PU2
PU2
PU3
PU1
PU1
RI2
RI1
RI2
RI1
KO1
KO4
KO1
KO3
KO2
KO2
AN2
AN1
AN1
AN3
AN2
AN3
BA3
BA3
BA1
BA2
BA2
BA1

**SUPPLEMENTARY MATERIAL**

**Port of Bari** - Total abundance of infaunal taxa was 3306 specimens, 2301 in spring and 1005 in fall. All samples were numerically dominated by Polychaeta (72%), followed by Mollusca (24%). Out of 214 species identified during both surveys and along both profiles, 89 were noted in both sampling seasons, 90 only in spring and 35 only in fall samples. Species richness was higher in spring (179 species) than in fall (125 species). Forty-three species, corresponding to 20% of total species number, were recorded at all sampling stations, while 116 species (50%) were observed from only one station (BA1: 36; BA2: 50; and BA3: 30). In terms of dominance, 186 species were poorly represented (<1%), 24 species counted between 1 and 5%, and four polychaete species – *Pseudoleiocapitella fauveli* (8%), *Capitella minima* (6%), *Flexopecten glaber* (6%) and *Leodice limosa* (5%) contributed more than 5% in total infauna abundance. The first two belong to the group of the 1$^{st}$ order opportunistic species (EG V), *F. glaber* is a sensitive species (EG I), while affiliation of *L. limosa* is unknown.

**Port of Ancona** - A total of 1776 specimens were found, 1002 in spring and 774 in fall. In all samples Polychaeta (63%), Mollusca (27%) and Crustacea (5%) numerically dominated, while the rest of 16 taxa that occurred in this port accounted to 4% of total macrofauna abundance. Out of 112 species identified during both surveys and along both profiles, 41 species were noted in both sampling seasons, 35 only in spring and 36 only in fall samples. Sixteen species were found at all three sites, while 75 species were recorded only at one site – 48 species at AN1, 21 at AN2 and 6 at AN3, respectively. In terms of dominance, 93 species (83%) were poorly represented (<1%), 18 species contributed to entire species abundance between 1 and 5% and three species more than 5% - bivalve *Mytilus galloprovincialis* (7%) and polychaetes *Sternaspis scutata* (12%) and *Heteromastus filiformis* (24%). The first two belong to the guild of tolerant species (EG III), and the third one is a 2$^{nd}$ order opportunistic species.

**Port of Koper** - 2572 specimens were counted, 1156 in spring and 1416 in summer. All samples were numerically dominated by Mollusca (76%), followed by Polychaeta (16%), Crustacea (4%) and Echinodermata (4%), while seven remaining taxa accounted 0.04% of total macrofauna abundance. Out of 121 species recorded during both surveys along the outer profile, 61 were noted in both sampling seasons, 17 only in spring and 43 only in fall samples. Species richness was higher in fall (105 species) than in spring (79 species). Twenty-two species (18%) were present at all sampling stations, while the number of species recorded only at a single station was 1 (KO1), 23 (KO2), 12 (KO3) and 17 (KO4). In terms of dominance, 105 species were poorly represented (<1%), 12 species contributed between 1 and

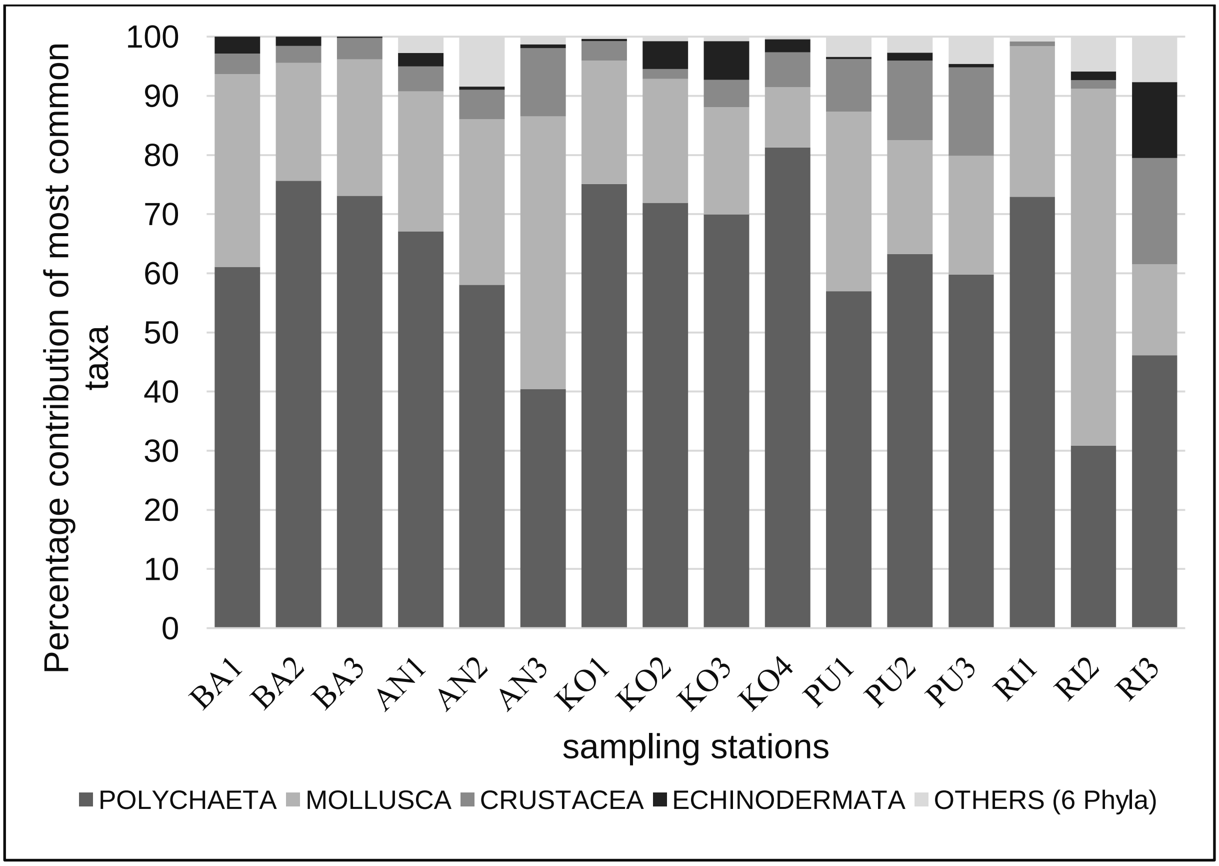
5% and only 4 species contributed more than 5% in the total infauna abundance: *Lumbrinereis gracilis* (17%), *Terebellides stroemi* (11%), *Heteromastus filiformis,* (10%) and *Laonice cirrata* (8%). Regarding sensitivity to pollution, the first two species are indifferent (EG II), *T. stroemi* is tolerant (EG III), and *H. filiformis* is a 2$^{nd}$ order opportunistic species.

**Port of Pula** - The total abundance of infaunal taxa was 634 specimens, 303 of which were recorded in spring and 331 in fall. All samples were numerically dominated by Polychaeta (60%) and Mollusca (24%) which comprised 84% of total macrofauna abundance. Out of 127 species recorded during both surveys and along outer profile, 38 species were observed in both sampling seasons, 54 only in spring and 33 only in fall samples.  Species richness was higher in spring (91 species) than in fall (71 species). In terms of frequency, seven species were found at all the sites: the gastropod *Euspira nitida*, the bivalve *Parvicardium papillosum* and the polychaets *Glycera unicornis*, *Lumbrinereis coccinea*, *L. latreilli, L. lusitanica* and *Notomastus latericeus*, while 81 species were present at only one site (33 at PU1, 30 at PU2 and 21 at PU3). In terms of dominance, 101 species were poorly represented (<1%), 22 species contributed to the entire species abundance between 1 and 5% and two species more than 5% (polychaetes *Aponuphis bilineata* 7.7% and *L. latrelii* 6. 4%). In terms of sensitivity to pollution, *P. papillosum* belongs to EG I group (sensitive), *Lumbrinereis* spp. to EG II (indifferent to pollution), and *N. latericeus* to EG III (tolerant to pollution).

**Port of Rijeka** - 229 specimens were counted, 150 in spring and 79 in fall. All sediment samples were numerically dominated by Mollusca (39%) and Polychaeta (34%) which comprised 73% of total macrofauna abundance.  Out of 43 species recorded during both surveys and along outer profile, 7 species were recorded in both sampling seasons, 20 only in spring and 15 only in fall samples.  Species richness was slightly higher in spring (27 species) than in fall (22 species). Only two species, the gastropod *Tritia incrassata* and the bivalve *Nucula nitidosa*, were recorded at all sampling stations, and 36 species were found only at single station (7 at RI1, 13 at RI2 and 16 at RI3. In terms of dominance, 30 species were poorly represented (<1%), 10 species contributed to entire species abundance between 1 and 5% and 3 species more than 5%: *Malacocerus fuliginosus* (9%), *Lucinella divaricata* (23%) and *Capitella capitata* (32%). Regarding sensitivity to pollution, *C. capitata* and *M. fulignosus* are 1$^{st}$ order opportunistic species, while *L. divaricata* is attributed as a sensitive one.

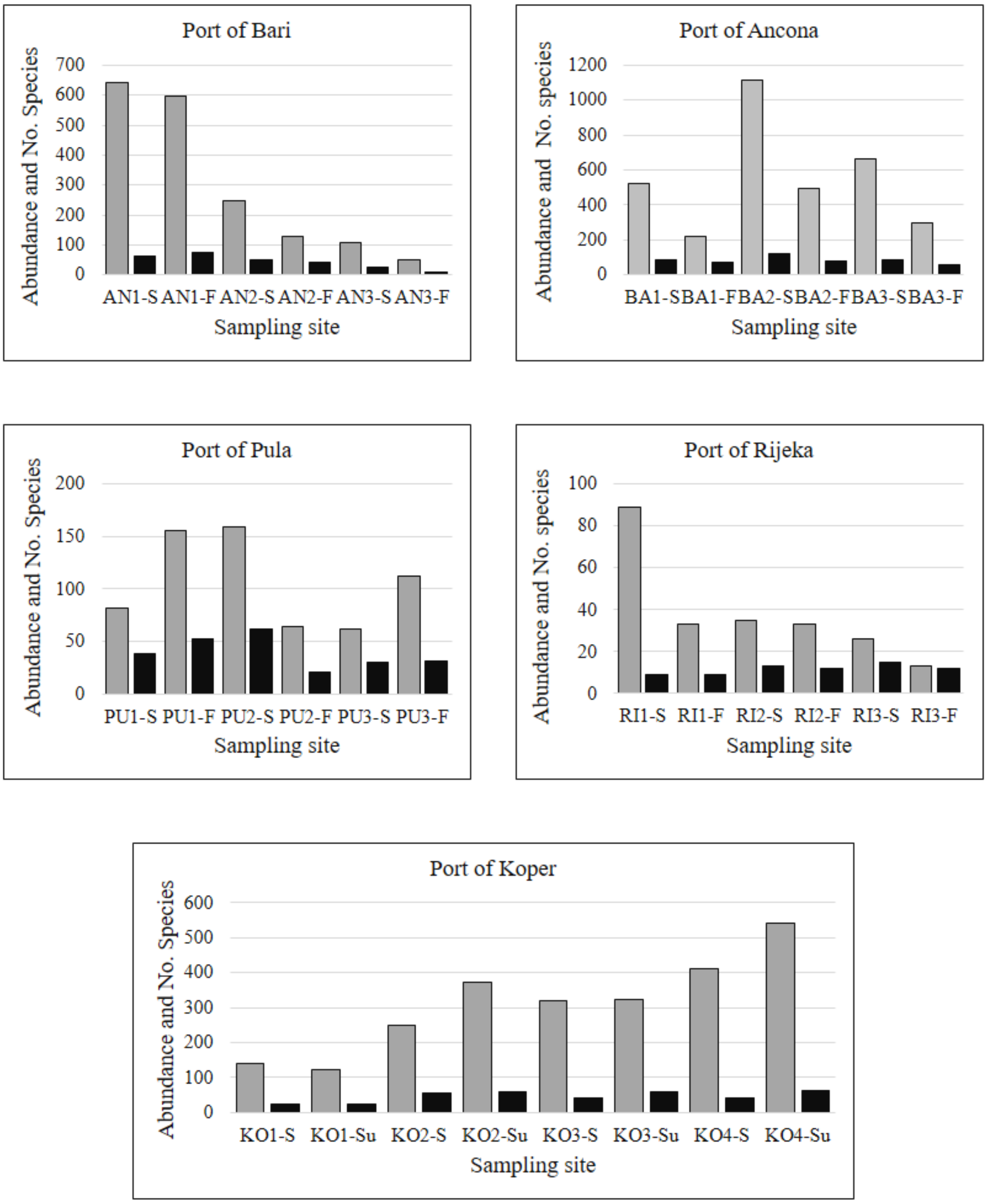
**Figure(s)**

Figure 1.



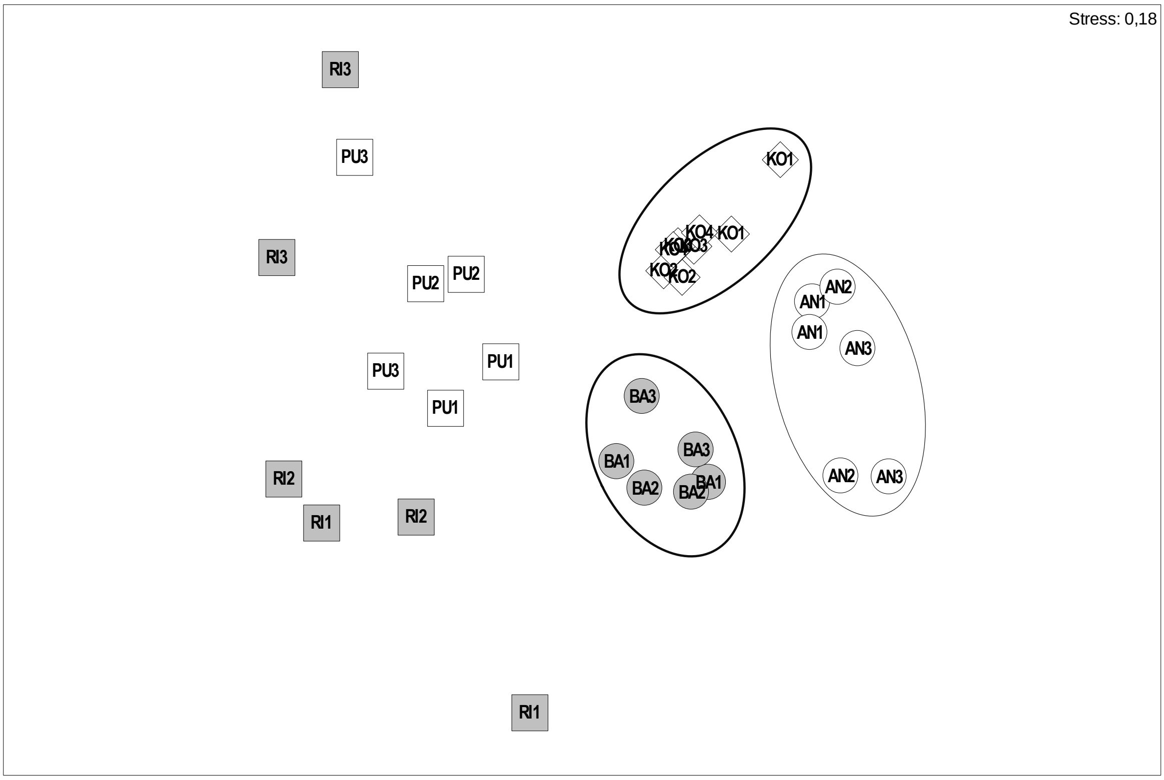
Figure(s)

Figure2.



**Figure(s)**

Figure 3.



Legend

Click here to download Supplementary Data: Travizi et al. Legend.docx

Characterization of soft-bottom communities in surveyed ports