Functional biodiversity of marine soft-sediment polychaetes from two Mediterranean coastal areas in relation to environmental stress

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ABSTRACT

Biological Traits Analysis (BTA) was used to identify functional features of infaunal polychaete assemblages associated with contamination in two Italian coastal areas: the harbour of Trieste (Adriatic Sea) and the Mar Piccolo of Taranto (Ionian Sea). The analysis was performed on 103 taxa, collected at four stations in each area. The two areas differed in species composition. The low diversity and the presence of stress-tolerant species in more polluted sites were not reflected in functional diversity, due to species contributing little to community functions or being functionally redundant. Sand and clay fractions were significant drivers of trait category expressions, however other environmental parameters (depth, total organic carbon and nitrogen, and Hg in sediments) influenced traits composition. *Motile* was the prevalent trait in environments with coarse sediments, and *tube-builder* were related to fine-grained ones. *Motile,* e*ndobenthic* and *burrower* were essential traits for living in contaminated sediments. *Epibenthic* and *sessile* polychaetes dominated at stations subjected to high organic loads. BTA offers an integrative approach to detect functional adaptations to contaminated sediments and multiple anthropogenic stressors.

1. Introduction

Benthic communities in coastal environments are under increasing stress due to anthropogenically-induced disturbances. One major source of concern is eutrophication, i.e. an increase in nutrient concentrations resulting in high primary production and sediment organic enrichment, principally due to domestic sewage wastes and also aquaculture farms (Villnäs et al., 2011; Franzo et al., 2016a). Furthermore, industries are usually established near highly populated areas such as coasts. Industrial areas are often characterized by the presence of large steel-works, oil refineries, shipbuilding and other industrial activities that are responsible for severe and persistent contamination in the form of heavy metals, asbestos, polycyclic aromatic hydrocarbons (PAHs), organic solvents, polychlorinated biphenyls (PCBs) and dioxins (Cardellicchio et al., 2016). Among coastal areas, the effects of diffuse and synergistic forms of pollution are even more emphasized in enclosed basins, where the water circulation is restricted and, therefore, the dilution effect of contaminants is limited (Trannum et al., 2004). Due to their capacity to retain pollutants from different sources, the sediments act as a sink in the aquatic environment reaching concentrations up to orders of magnitude higher than in the overlying water (Cardellicchio et al., 2007).

In soft-bottom sediments, benthic organisms, especially sessile or sedentary species that lack or have limited mobility, are exposed to high organic matter deposition and contaminants. These invertebrates respond to stress conditions both at the individual and community level through the variation in total numbers, selection of taxa and elimination of the sensitive ones (Solis-Weiss et al., 2004). Thus, changes in environmental conditions, induced by anthropogenic drivers, strongly affect the benthic community structure (i.e. species composition and biodiversity) and consequently the overall ecosystem functioning (Harley et al., 2006; Doney et al., 2012). Ecosystem functioning includes all processes that occur in a system as well as the chemical, physical and biological components involved (Naeem et al., 2002; Bremner, 2008). The biodiversity and ecosystem function (BEF) approach states that functioning of an ecosystem is not governed by the phylogeny of the biota, but by organisms' characteristics (i.e. traits) present in a system and their distribution (Naeem et al., 2002). The maintenance and regulation of functions in an ecosystem is thus essentially dependent on the ecological roles and traits of the taxa present. The BEF approach assumes that an increasing diversity, translated into an increasing number of expressed biological traits, could have
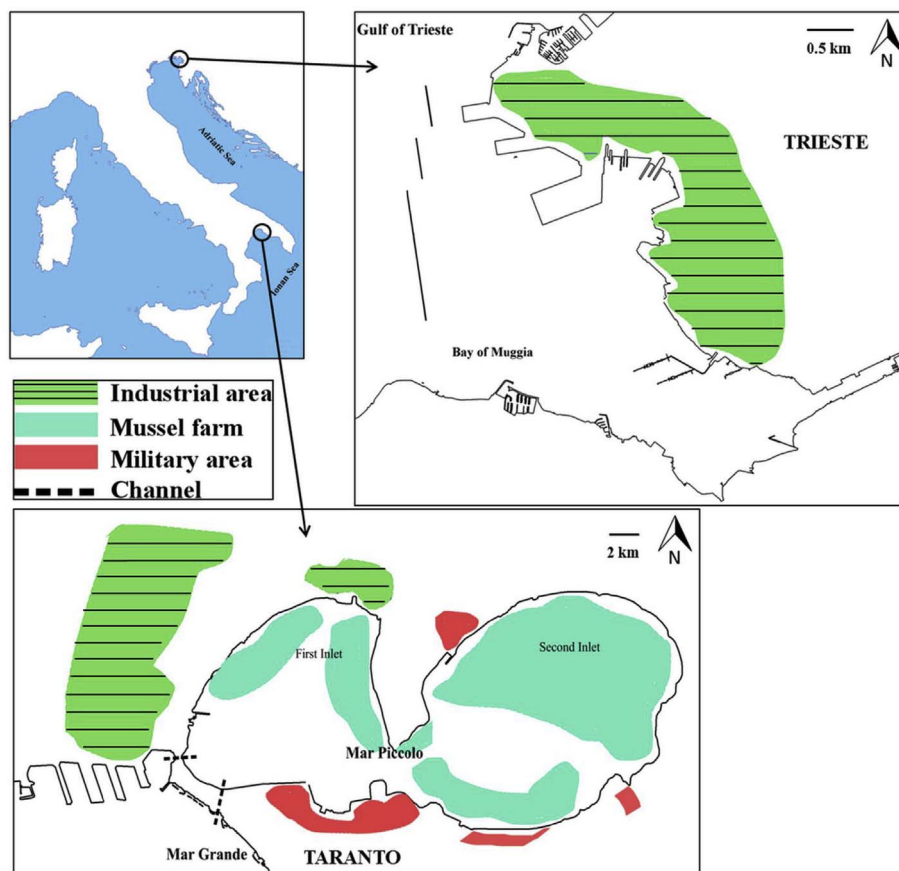
Fig. 1. Location of the sampling stations in the harbour of Trieste and Mar Piccolo of Taranto. The main anthropogenic pressures on their coasts are highlighted: industrial areas (*green-lined*), military areas (*red*), and mussel farms (*blue*). The channels connecting the Mar Piccolo with the Mar Grande are indicated by a dotted line. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

greater effects on ecosystem functioning, compared to less diverse assemblages that have a poorer functional expression (Chapin Iii et al., 2000; Hooper et al., 2005).

Macrofauna is a key biological component that drives important processes such as nutrient cycling, sediment reworking, bio-irrigation and organic matter decomposition (Widdicombe et al., 2004; Bremner et al., 2006; Olsgard et al., 2008). Individual species can contribute to different ecosystem functions, and hence shifts in species composition due to either natural or human-mediated changes may have fundamental implications for ecosystem functioning (Gray et al., 2006; Tillin et al., 2006; Schratzberger et al., 2007). This depends on the biological features of the species that may decrease or increase in response to human influence (Oug et al., 2012; Krumhansl et al., 2016).

Studies on soft bottom macrozoobenthic species assemblages are fundamental in marine environmental monitoring and assessment of impacts deriving from human activities. The majority of ecological studies focus on the structural aspects of species assemblages, such as abundance, biomass or diversity, but rarely assess the functional adaptation to environmental variables. Species interact with and respond to their physical and chemical environment in various ways depending on their ability to do so. The use of species composition might be inadequate for investigating processes that sustain an ecological system (Diaz and Cabido, 2001), since the ecosystem processes are determined by the functional characteristics of the organisms involved, rather than by taxonomic identity (Grime, 1997). Biological Trait Analysis (BTA) is a useful analytical approach to better understand the relationships between organisms and ecosystem functioning (Solan et al., 2004; Bremner et al., 2006; Gagic et al., 2015). BTA combines structural data of a macrofaunal community (species abundance or biomass) with the information on functional features of species (Törnroos and Bonsdorff, 2012). BTA relies on a comprehensive set of functional traits (e.g. feeding type, body size and reproductive

technique), which can serve as indicators for ecosystem functioning. Hence, using this approach is possible to assess the functional diversity of the community. The functional diversity is the component of biodiversity that describes the variety of functions developed by organisms in an assemblage, community and ecosystem (Tilman et al., 1997). Functional diversity can be quantified by estimating the extent, dispersion and relative abundance of species functional traits and provides an informative complement to studies addressing the interrelationship between community structure, environmental heterogeneity and ecosystem function (Gusmao et al., 2016, and reference therein).

The biological trait concept was originally developed for and used in freshwater systems (e.g. Charvet et al., 2000; Usseglio-Polatera et al., 2000) and only recently several studies have applied this approach also to marine benthic assemblages to describe their ecological functioning (e.g. Törnroos and Bonsdroff, 2012; Darr et al., 2014; Gogina et al., 2014) as well as to investigate the effects of climate change (e.g. Neumann and Kröncke, 2011; Weigel et al., 2016). In contrast, the effects of chemical contamination on invertebrate biological features and consequently the changes in ecosystem functioning have been seldom considered (i.e. Oug et al., 2012; Krumhansl et al., 2016). Other studies focused on the functional traits of only one of the macrofaunal phyla (e.g. bivalves or polychaetes) (Montalto et al., 2015; Gambi et al., 2016; Otegui et al., 2016). Polychaetes typically contribute to a high percentage of the total macrofaunal community diversity and abundance. Moreover, they occupy a large part of the available niches in marine environment and dominate marine sediments in diverse forms and ways of life. In fact, the multi-faced response of polychaetes to environmental disturbances is probably a consequence of their high morpho-functional diversity (Giangrande, 1997; Jumars et al., 2015), which allows different species to occupy ecological niches along a gradient, from pristine to disturbed habitats (Giangrande et al., 2005).

In this study, Polychaeta was chosen as a target group to illustrate

the links between the environmental features and their functional response to environmental stress. BTA was used: i) to explore and characterize functional attributes of soft-sediment polychaete assemblages in two Mediterranean coastal basins subjected to different levels of contamination; ii) to study potential alterations of ecosystem functioning and the functional adaptation of macrofaunal invertebrates to this environmental stress. In particular, we focused on two functional aspects (diversity and identity) of polychaete assemblages, potentially modified by contaminants and other environmental variables (grain-size and anthropogenic organic enrichment). We aimed to answer the following questions: 1) Does environmental stress affect functional biodiversity? 2) Which trait categories are affected by different levels of contamination? 3) Which is the role of environmental factors in structuring assemblage and functional trait patterns?

2. Materials and methods

2.1. Study sites and sampling design

The study was performed in two Mediterranean coastal basins subjected to multiple impacts: the harbour of Trieste (Gulf of Trieste, northern Adriatic Sea), and the Mar Piccolo of Taranto (Ionian Sea) (Fig. 1 and Table 1). The Gulf of Trieste, located in the north-western Adriatic Sea, is a shallow embayment of about $600 \, km^2$ and with a coastline of about 100 km. Geographical, hydrological and sedimentological features are exhaustively described in Franzo et al. (2016b). Within the Gulf of Trieste, the Bay of Muggia is a shallow embayment (8-20 m) about 7 km long and 4 km wide and oriented NW– SE (Ghirardelli and Pignatti, 1968; www.porto.trieste.it). Sedimentation is controlled by low hydrodynamism and fluvial inputs: two streams enter the Bay, Rosandra and Ospo that may discharge large amounts of fine sediments containing chemical fertilizers (www.porto.trieste.it). The Bay of Muggia houses the port and industrial area of Trieste and its morphology makes it prone to the accumulation of contaminants, since it is sheltered from currents and characterized by an elongated shape and low depth (Cibic et al., 2017).

The Mar Piccolo of Taranto is an inner, semi-enclosed sea located in the northern area of Taranto (Fig. 1). It has a surface area of $20.72 \, km^2$ and is divided by a promontory in two smaller basins named First and Second Inlet. The area is characterized by shallow waters (maximum depth is about 13 m in the First Inlet) (Table 1). The First Inlet is directly connected with the Mar Grande through two channels: the 'Navigabile' and the 'Porta Napoli'. The fluxes of water through the two inlets are generally weak and depend on the difference in density between the two basins. Most of the water inputs derive from numerous small surface freshwater courses and 34 submarine freshwater springs, called "Citri", which influence salinity and temperature of both inlets (Caroppo et al., 2016). As more than 80% of the province of Taranto is used for farming, in particular for the cultivation of wheat, cereal crops and fodder, freshwater inputs contain chemicals drained from the surrounding agricultural soils in the basin (Caroppo et al., 2012). The

basin is generally characterized by low velocity currents (about $5–10 \, cm \, s^{-1}$) driven by the sea tides, with the maximum reached in the two connecting channels with the Mar Grande (up to $30–40 \, cm \, s^{-1}$) (Umgiesser et al., 2007). The two inlets are characterized by different levels of confinement, i.e. by a different degree of connection to the sea and of sea water renewal time (Canu et al., 2012). In particular, the Second Inlet, due to the low hydrodynamism and scarce water exchange with the nearby Mar Grande, represents the most confined part of the system (Cardellicchio et al., 2007). In the Mar Piccolo of Taranto, the long line mussel farms, although present in both inlets, are more widespread in the second one (i.e., ~66% of the whole Second Inlet's coverage against ~26% of the First one; Caroppo et al., 2012).

Two sampling campaigns, in both areas, were performed as part of a large-scale monitoring program: in February and April 2014 (in the Mar Piccolo); in May 2014 and March 2015 (in the port of Trieste). Sediment samples for grain-size, chemical analysis and macrofauna were taken at four stations in both areas. In the harbour of Trieste, the stations were located near the major industrial activities of the area: St. TS1 between the port's cargo piers; St. TS2 in an area dedicated to shipbuilding activities; St. TS3 close to the iron and steel plant and St. TS4 near the petroleum industry (Fig. 1). In the Mar Piccolo of Taranto, sampling was carried out at four stations selected as representatives of different environmental and anthropogenic impacts. In the First Inlet, St. TA1 represents the environmental features of the area in front of the "Navigabile" channel whereas St. TA2 of that nearby the military navy arsenal. In the Second Inlet, St. TA3 and St. TA4 were selected as representatives of the most enclosed part of the Mar Piccolo with intensive mussel aquaculture farming. The latter were chosen to follow an increasing distance from the main sources of industrial pollution (located in the First Inlet) (Fig. 1).

The major contaminants of concern are listed in Table 1, whereas data on contaminants are reported in Cibic et al. (2017) for Trieste sampling stations and Cibic et al. (2016) for Taranto ones. Based on the Environmental-quality status which has been development in Italy for the assessment of water quality status according to the European Water Framework Directive (Marin et al., 2008), the stations were classified from "Good" to "Warning" (Table 1).

2.2. Polychaete sampling

Sediment samples for benthic fauna, and in particular polychaetes, were sampled using a van Veen grab ($0.01 \, m^2$ sampling surface; three replicates). Sediments were sieved on a 1.0 mm mesh to retain the fraction of macrozoobenthic organisms. The retained sediment and organisms were immediately fixed with a formaldehyde solution (4% v/v final concentration in seawater). After washing, organisms were separated from the sediment by tweezers, divided into the main taxonomical phyla, and preserved in ethanol 70°. Polychaetes were identified under a stereomicroscope at 8-100× final magnification and counted. For the taxonomic identification, the keys listed in Morri et al. (2004) were used.

Table 1

Coordinates, depths, contaminants values and Environmental-quality status (Eqs) at 4 stations located in the harbour of Trieste and in the Mar Piccolo of Taranto. PCBs = Polychlorobiphenyls; (Marin et al., 2008, Cibic et al., 2016, 2017).

Station	Latitude	Longitude	Depth m	Cu	Hg	Pb	Zn	total PCBs	Eqs
				$mg \, kg^{-1}$				$\mu g \, kg^{-1}$	
TS1	45° 38′ 01	13° 45′ 09	18.5	36.8	3.4	57.1	137.0	74.7	warning
TS2	45° 37′ 47	13° 46′ 11	15.0	112.0	4.4	388.0	770.0	907.0	warning
TS3	45° 37′ 04	13° 46′ 31	13.0	28.7	0.5	73.9	205.0	50.8	altering
TS4	45° 36′ 41	13° 47′ 04	10.5	16.6	0.1	4.0	57.0	1.0	good
TA1	40° 29′ 01	17° 14′ 46	11.2	180.3	1.3	80.0	231.0	164.9	warning
TA2	40° 28′ 46	17° 15′ 38	11.0	100.5	5.7	152.0	319.0	1067.6	warning
TA3	40° 28′ 57	17° 16′ 42	7.5	64.9	0.5	74.0	222.0	164.8	altering
TA4	40° 28′ 57	17° 17′ 41	7.0	55.2	0.1	51.0	189.0	39.0	altering

Table 2
Biological traits with their categories characterizing multiple dimensions of contaminated sediments interactions, including adults longevity, reproductive characteristics as well as adults environmental position, movement and feeding habits for the taxa studied. Codes of the categories are also presented.

Traits	Categories	Abbrev.	Examples of potential relationships
Adult longevity	≤1 yr 1-3 yrs	Al1 Al3	Adult longevity increases the contact time with the contaminated sediments;
	3-6 yrs 6-10 yrs	Al6 Al10	
Reproductive frequency	Semelparous Iteroparous	Sem Iter	Reproductive frequency indicates role in community development;
	Semi-continuous	Scon	
Mechanism development	Direct Epitokia	Dir Epit	Larval development is a proxy for recruitment success;
	Lecitotrophic Planktotrophic	Flec Fplan	
Larval environmental development	Benthic Pelagic	Lben Lpel	Larval development environment is important to avoid the contact of contaminated sediments, e.g. pelagic > benthic;
Adult environmental position	Endofauna Interface	Endo Inter	Spatial overlap of contaminated sediments could be dangerous for organisms;
	Epibiont	Epib	
Adult mobility	Sessile Semi-motile	Sess Smot	Motility may increase the possibility to avoid contaminated sediments, e.g. motile > sessile;
	Motile	Mot	
Adult movement method	Swimmer Crawler Tube-builder	Swim Craw Tub	Movement method may influence the survivorship in contaminated sediments, e.g. crawler > tube-builder;
	Burrower	Burw	
Adult feeding habit	Suspension feeder Surface deposit feeder	Susp Sdep	Feeding habit indicates role in trophic pathway;
	Subsurface deposit feeder Herbivore Predation Scavenger	Ssdep Herb Pred Scav	

2.3. Biological trait analysis

The effect of chemical contamination on polychaete assemblages was assessed using Biological Traits Analysis (BTA). The biological traits considered in this study (8 traits with 29 categories) were selected based on their ecological importance in characterizing multiple dimensions of contaminated sediments interactions and following previous studies that used this technique for similar purpose (Oug et al., 2012; Krumhansl et al., 2016) (Table 2).

The polychaete species were coded based on their affinity for the chosen traits (Table S1). For example, species were categorized by their feeding habits, as suspension feeder, surface- or subsurface deposit feeder, herbivore, predator or scavenger. For organisms that were strictly suspension feeders, a value of 3 was assigned for suspension feeding and 0 was assigned for all other habits. For organisms that were suspension or deposit feeders, values of 1–2 were assigned for each of these habits (depending on the degree of affinity for these traits) and 0 for the rest of the habits. This is known as a "fuzzy coding" procedure (Chevenet et al., 1994) that allows for species adherence to multiple

categories within each trait (0 = no affinity, 1 = low importance, 2 = moderate importance, 3 = dominant). Traits for each taxon were derived from literature sources (i.e. Giangrande, 1997; Rouse, 2000; Jumars et al., 2015) and databases (i.e. www.marlin.ac.uk/biotic; polytraits. lifewatchgreece.eu). Taxonomical resolution was kept at the species level whenever possible but adjusted to genus or family when the information on traits was available only on a higher taxonomic level. Overall, biological traits could not automatically be assumed to be response traits. However, a range of traits in our exploration of the relationship between the environment and the polychaete assemblages were included.

2.4. Sediment characteristics

Sediment grain-size, Total Organic Carbon (TOC) and Total Nitrogen (TN) contents were assessed at each station and area. Sediments were sampled using a KC Haps bottom corer (KC-Denmark, Silkeborg, Denmark) and a polycarbonate sample tube (13.3 cm Ø with a sample area of 127 cm^2). For logistic reasons, in February 2014 samples for these analyses were not collected from the Mar Piccolo of Taranto.

Sediment subsamples of about 10–15 g were taken for grain-size analysis. Small pebbles and shell fragments (> 2 mm) were separated from the sand and mud fractions by sieving at 2 mm. Each sample was disaggregated and the organic matter oxidized using 10% hydrogen peroxide (Carlo Erba, Rodano, Italy). The analyses were performed using a Malvern Mastersizer 2000 equipped with Hydro 2000s (Malvern Instruments Ltd. Malvern, UK). Data are expressed as percentages of sand, silt and clay. For TOC and TN analyses, sediments were freeze-dried and grounded using a mortar, then a pestle and the fraction > 250 μm was separated from the rest of the sample. Only the fraction < 250 μm was used for the analysis. Triplicate of subsamples (~8–12 mg) were weighed directly in a silver capsule (5 × 9 mm) on a microultrabalance Mattel Toledo model XP6 (accuracy 1 μm). Before TOC determination, subsamples were treated with increasing concentrations of HCl (0.1 N and 1 N) to remove carbonates (Nieuwenhuize et al., 1994). TOC and TN contents were measured using an elemental analyser CHNO-S Costech model ECS 4010 following the methods of Pella and Colombo (1973) and Sharp (1974). Capsule blanks were also analysed. The precision of the analysis was about 3%. The values are expressed as mg C g dry mass^{-1}.

2.5. Data analysis

On trait profiles, univariate and multivariate analysis were performed to examine patterns and relationships with environmental factors. Trait category richness was calculated as the total number of categories expressed per sample. Functional diversity was measured as the diversity in distribution and range of expression of functional traits, with the latter reflecting characteristics of organisms that influence ecosystem processes (Petchey and Gaston, 2002). To assess the functional diversity and functional identity of polychaete assemblages in each sample, the library FD in the R program ver. 3.1.3 was used (Laliberté et al., 2014; www.R-project.org/). The Functional Dispersion (FDis) metric, developed by Laliberté and Legendre (2010), was chosen as a diversity index, as it has analytical advantages in comparison to other indices. The metric for FDis is unaffected by species richness. This feature allows it to be used with more traits than species present, to be computed from any distance or similarity measure, and to be capable of taking species abundance into account (Laliberté and Legendre, 2010). FDis describes the abundance-weighted mean distance of individual species to their group centroid (all species community) in multivariate trait space. Larger FDis values imply a more functionally spread community in the multivariate trait space, and hence a higher functional diversity (Laliberté and Legendre, 2010). Differences in number of taxa and FDis among stations and sampling periods were tested by

Mann–Whitney U test (STATISTICA 7 software).

To analyse changes in trait composition and expression, the functional identity as community level weighted means (CWM) of trait category expression was calculated. CWM is a widely used index that may reflect the trait strategies given by the species pool and environmental conditions of a site (Muscarella and Uriarte, 2016). For the calculation of CWM in our study, a community was defined as the species assemblage in each replicate sample. CWM values represent the expression of a trait by species in a given community, weighted by the abundance of species expressing that specific trait. CWM was calculated in the FD library for each sampling station and period. CWM values of trait expressions were tested by Spearman correlation in order to verify if they were complementary. Variables with Spearman values $r_s > 0.95$ were considered complementary and deleted from the dataset prior to subsequent analyses. In addition, in order to extend the functional information on polychaete assemblages to taxa composition, the species abundance matrix was included in the statistical analysis. The diversity was evaluated as the total number of taxa at a specific station/sampling.

Multivariate analyses of polychaete composition and trait expressions were performed on the taxa abundance matrix applying a non-metric multidimensional scaling analysis (nMDS) ordination model. Further, to assess differences in species composition and traits (CWM values) between areas and sampling periods, we used a PERMANOVA test, where 'area' and 'period' were fixed factors. Unrestricted permutations of row data and 9999 permutations were applied. To detect which taxa were mainly responsible for changing polychaetes composition, SIMPER (SIMilarityPERcentage) analysis was employed and factor (area) was assigned. A cut-off at 50% was applied.

Environmental variables were tested by Spearman correlation in order to verify if they were complementary. Variables with Spearman values $r_s > 0.95$ were considered complementary and deleted from the dataset prior to subsequent analyses. A BIO-ENV analysis was used as a first exploratory approach, to assess which environmental variables correlate best with the pattern of species composition and CWM values at both areas, using the average value of each abiotic variable. Data were normalized before entering the analysis and the BIO-ENV routine was calculated using the Spearman's coefficient. The main environmental parameters correlated with species composition and trait modalities expressions were used to perform Distance-based Linear Models (DistLM). The latter was carried out to asses which variables explained differences ($p < 0.05$) in species and traits composition. The environmental parameters were normalized and selection based on R^2 was applied.

For each multivariate analysis, nMDS, PERMANOVA, SIMPER, BIO-ENV, and DistLM, the two matrices (i.e. species composition and CWM values) were square root transformed and Bray-Curtis similarity was used. These analyses were performed using PRIMER 7 (PRIMER-E Ltd. Plymouth,UK) (Clarke et al., 2014).

Redundancy analysis (RDA) was used as constrained ordination analysis, to look for relationship between species composition/CWM values and sediment variables in each location independently. The two matrices were square root transformed. RDA ordination, using the Vegan package in the R program ver. 3.1.3, was performed (Oksanen et al., 2007; www.R-project.org/).

3. Results

3.1. Structural and functional biodiversity of polychaete assemblages

A total of 103 taxa of polychaetes were found at the Taranto and Trieste stations. The number of taxa varied from a minimum of 7 (at St. TA3) to a maximum of 45 (at St. TS4) in April and May 2014, respectively. No significant spatial differences in number of taxa were observed between Taranto and Trieste stations, whereas significant temporal variations were detected in May 2014 and March 2015 ($z = 2.0$; $p < 0.05$) in the harbour of Trieste (Fig. 2a). For trait category

richness, no variations were observed between areas or sampling seasons (average: 24.8 ± 1.0; range: 24-27) (Fig. 2a).

FDis values measured in Trieste varied from a minimum of 4.1 (March 2015) to a maximum of 4.7 (May 2014) whereas in Taranto they ranged from 4.0 (April 2014) to 5.3 (February 2014). Overall, no significant variations of functional dispersion on temporal and spatial scale were observed (Fig. 2b).

The nMDS analysis based on polychaete taxa composition highlighted a higher variability of assemblages between areas rather than sampling periods (Fig. 3a). St. TA1, in both sampling periods, was positioned closer to Trieste stations. Overall, the spatial dissimilarity between Trieste and Taranto was corroborated by the PERMANOVA main test that highlighted a significant difference between the two areas ($t = 4.3$; $p < 0.01$). In contrast to polychaete taxonomic composition, the uniformity of the functional traits between areas and sampling periods was confirmed by nMDS and PERMANOVA analysis. In fact, the latter showed no significant differences between CWM values for either of the factors 'area' and 'season'. In the nMDS ordination the maximum distance (Bray-Curtis maximum dissimilarity) was observed between St. TA4 and St. TA3 in February 2014 (Fig. 3b). The SIMPER test performed on polychaete assemblages showed that dissimilarity between the two areas (85.2%, average dissimilarity) was mainly due to the species *Pseudoleiocapitella fauveli* (Contribution % = 5.1) at Taranto sites (particularly at St.TA1) and *Hilbigneris gracilis* (Contribution % = 4.9) at Trieste sites (average value: 30.4 ± 29.4 ind. m^{-2}). Also the species *Notomastus aberans* was dominant in Trieste sites compared to Taranto ones (Contribution % = 3.2). (Table S2).

Among the CWM values of trait expressions tested by Spearman correlation, none were highly correlated ($r_s \geq 0.95$) and therefore all were included in the subsequent analyses. Since no statistical differences were observed in functional traits composition for both areas and sampling periods, trait modalities which were most expressed in the full dataset were highlighted. Thus, average values of CWM for each sampling period were plotted to visualize a functional meta-composition (Fig. 4). The polychaete assemblage for both areas showed higher proportions of five out of all the considered trait categories, for which highly expressed CWM values were obtained: *iteroparous, larval pelagic development, endofauna, motile* and *burrower*. On the contrary, less represented categories of traits (CWM values < 1) were: *6-10 years of adult longevity, semelparous, semi-continuous, larval benthic development, sessile, swimmer, tube-builder, herbivore* and *scavenger*. However, variations in categories belonging to 'adult mobility', 'adult movement method' and 'adult feeding habit' traits were observed among sites. In particular, this variation was more evident in Taranto sites (Fig. 4 e, f, g and h) rather than Trieste ones (Fig. 4 a, b, c and d). The CWM values of *motile* as trait category, decreased from TA2 and TA3 to TA4 (CWM: 2.4, 2.3 and 1.6, respectively) (Fig. 4 f, g and h). Conversely, a slight increase of *sessile* modality was noticed at St. TA4. At the latter station, highest CWM values of *sessile* and *tube-builder* were obtained (CWM: 0.9 and 1.0, respectively).

3.2. Relationships between species or traits, and environmental variables

The measured environmental factors are presented in Table 3. Since, according to Spearman correlation, Pb was highly correlated with Zn and PCBs (Polychlorobiphenyls) ($r_s = > 0.95$), this parameter was removed from the dataset for the subsequent analysis.

According to the BIO-ENV analysis that related the polychaetes assemblages with the considered environmental variables in both study areas, species distribution was best correlated with depth, sand and clay fractions, TOC and TN contents ($r_s = 0.47$). A similar output of the BIO-ENV analysis was obtained considering the trait categories expression. In fact, the functional distribution was best correlated with depth, sand and clay fractions, TN content and Hg concentration ($r_s = 0.63$).

The significant variables obtained from both BIO-ENV analyses (i.e. depth; sand and clay fractions; TOC and TN contents; Hg) were used to
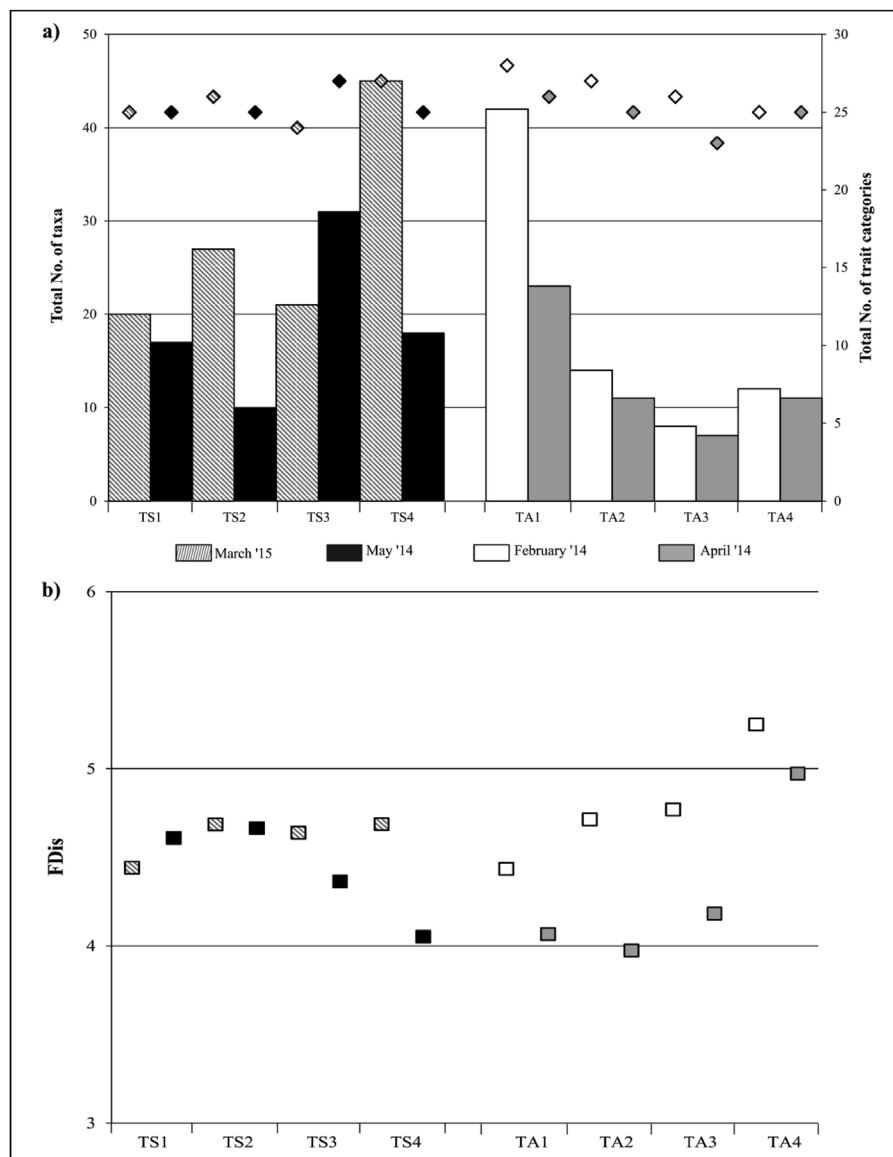
Fig. 2. Total number of taxa (histogram) and trait categories (diamond) (a) and functional dispersion (FDis) (b), for each sampling station and period.

perform a Distance-based Linear Model (DistLM) considering all Trieste and Taranto sites. The six variables were used to build the best model, applied to both species and functional composition ($R^2 = 0.87$ and 0.84, respectively). According to the model output, TN content and depth were the significant drivers for polychaete assemblages (Pseudo-F = 3.24 and 2.44; p < 0.01 and p < 0.05, respectively), whereas clay and sand fractions were the significant variables for trait expressions (Pseudo-F = 2.67 and 2.17; and p < 0.01 p < 0.05, respectively).

The results obtained from the linear model (DistLM) were further confirmed by the constrained ordination Redundancy analysis (RDA) that was carried out on the same environmental parameters. The variance of the most important polychaete taxa, selected by SIMPER analysis (cut off 50% of Cumulative contribution %) on the basis of the factor "area", could be principally related to differences in depth, sand and clay fractions and TN content (Fig. 5a). The first axis (RDA1: var.exp. = 43%) represented an increasing gradient of TN content (RDA loading = 0.89) and decreasing depth (RDA loading = -0.77). The second axis (RDA2: var.exp. = 15%) highlighted an increase of TOC content and sand fraction toward stations located at the bottom side of the plot (RDA loading = -0.52 and -0.51, respectively). The

polychaetes *Lanice conchilega* (RDA loading = 0.66) and *Nereis lamellosa* (RDA loading = 0.63) were plotted on the first axis at high values of TN (right-hand side of the plot). The species *Eunice vittata*, was positioned on the second axis (bottom of the plot) at increasing values of sand fraction and TOC content (RDA loading = -0.75).

In the RDA performed on CWM data, two principal components were identified which together explained 55% of the total variance (RDA1 = 34%; RDA2 = 21%) (Fig. 5b). The clay and sand fractions were the predominant elements of the first factor (RDA loading = 0.73 and -0.62, respectively), whereas the major contributors of the second one were TN content and depth (RDA loading = 0.87 and 0.68, respectively). The modalities (RDA1 loading > ± 0.50) *direct development, iteroparous, endofauna, burrower* and *subsurface deposit feeder* were divided on the first axis at high values of sand fraction, TOC and Hg (left-hand side of the plot). *Sessile, tube-builder* and *suspension feeder* were positioned toward high values of clay fraction (right-hand side of the plot). *Semi-motile* category was separated from the other traits and positioned on the second axis (top of the plot) at increase values of depth (RDA loading = 0.52).
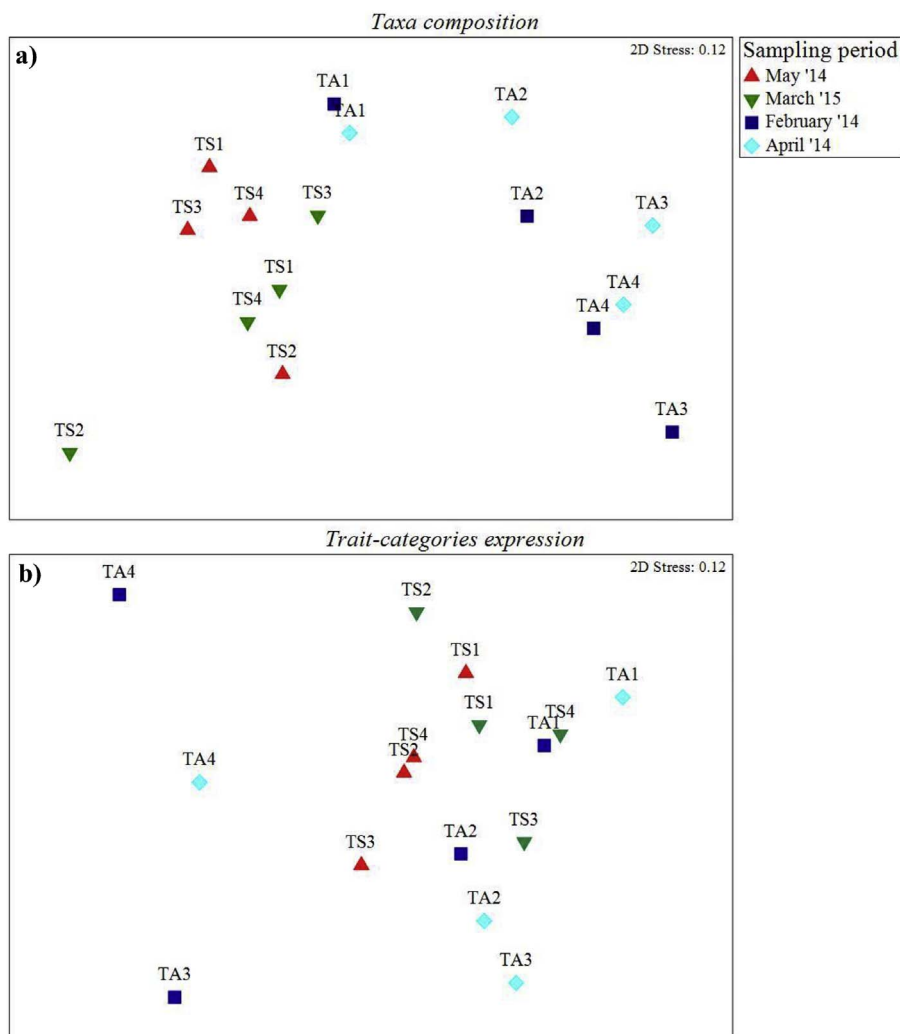
Fig. 3. Nonmetric multidimensional scaling (nMDS) ordination plot of taxa composition (a) and trait category expressions (b) (stress value 0.12 for both plots). (1.5 column image).

4. Discussion

We explored functional attributes of soft-sediment invertebrate assemblages in two Mediterranean coastal basins subject to different levels of contamination, assessing in particular potential structuring effects of contaminants and environmental variables on polychaete functional diversity and identity. We found that habitat and sediment descriptors, such as fractions of sand or clay, and depth together with TN and TOC content, were related to the taxonomic and functional structure of polychaete assemblages. We did not find, however, any strong structuring effects of the considered contaminants *per se*, suggesting that these two study areas are similarly affected (both coastal basins have a long history of anthropogenic environmental stress) and/or display functional redundancy to the degree that contamination effects do not manifest.

The influence of contamination on the reduction of species richness has been largely documented worldwide (Johnston et al., 2015 and references therein). However, in our study, the lowest values of polychaete richness were observed in the Second Inlet of the Mar Piccolo of Taranto (St. TA3 and St.TA4), that is less contaminated compared to those in the First one. The low number of polychaete taxa could be ascribable to the confinement gradient present in the basin. The nMDS analysis carried out on species composition highlighted a different assemblage in Taranto and Trieste (see Fig. 3 a), corroborated also by the PERMANOVA main-test. In fact, St. TA1, characterized by more marine features, was placed close to Trieste stations in both sampling periods

whereas the position of the other Taranto sites (particularly St. TA3 and St. TA4) seemed to depend upon the confinement gradient present in the Second Inlet. The geomorphological features of the Mar Piccolo of Taranto appear to play a pivotal role on polychaetes diversity and distribution within this area. This semi-enclosed basin has peculiarities of coastal transitional environments, with decreasing salinity, limited depth, and low water renewal rates, going from the 'Navigabile' channel towards the Second Inlet (Canu et al., 2012). Polychaetes respond to this confinement gradient with low taxonomical diversity and a gradual reduction of marine species, which are replaced by taxa adapted to brackish conditions. As reported by Franzo et al. (2016a), during both sampling campaigns, marine species such as *Notomastus aberans* and *Hilbigneris gracilis*, were observed at St. TA1 whereas in the Second Inlet, species typical of paralic environments were found (i.e. *Nereis lamellosa* and *Lanice conchilega*) (Fig. 5a). The species selection and distribution in coastal areas are directly related to depth (Gray and Elliott, 2009, and reference therein). The sampling stations in Trieste were deeper than those in Taranto. The species that inhabit sediments in the harbour of Trieste such as *Labioleanira yhleni*, *Maldane glebifex* and *Aricidea (Strelzovia) claudiae* are tipically observed in muddy-sediments of the Gulf of Trieste at these depths (Nasi et al., 2017). Overall, from the variables considered in this study, it was not possible to ascribe the decline of taxonomic richness to contamination, not even at the stations closest to the main anthropogenic sources of contamination. However, variation in species richness was observed between sampling periods. The lower values observed at the Trieste sites
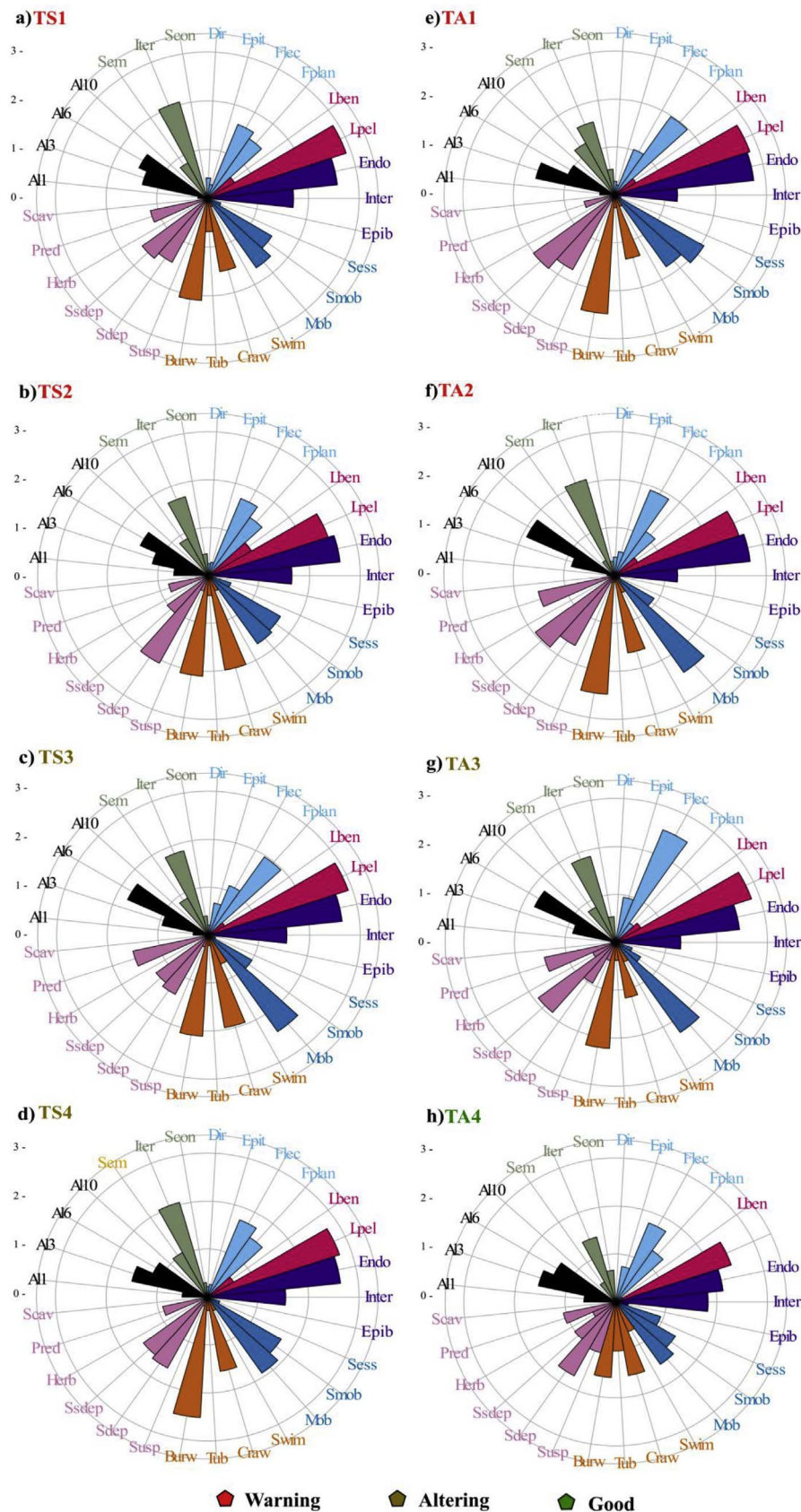
Fig. 4. Community-weighted means of trait category expression (CWM), average values for sampling seasons in Trieste sites (a. b, c d) and Taranto ones (e, f, g and h). Colour codes and individual bars represent trait affiliation and trait category expression, respectively (for labels see Table 2). Environmental-quality status of stations is indicated with different colours. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3

Grain-size, chemical data values measured at the sampling sites. TOC = Total Organic Carbon; TN = Total Nitrogen.

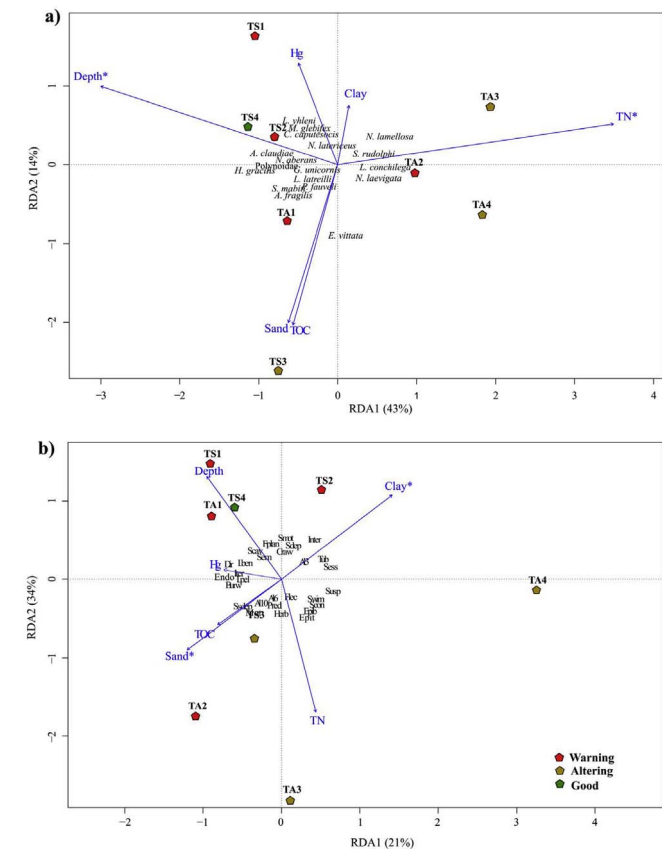| Station	Sand	SD	Silt	SD	Clay	SD	TOC	SD	TN	SD
	%						mg kg^{-1}			
TS1	6.24	5.14	60.24	6.25	33.52	1.11	29.45	0.40	1.82	0.05
TS2	9.40	4.38	58.13	1.51	32.47	2.87	36.10	0.95	1.64	0.03
TS3	17.96	0.37	54.67	2.79	27.38	3.17	44.35	1.40	1.70	0.03
TS4	10.16	2.15	62.39	0.34	27.45	2.49	20.30	0.65	1.52	0.04
TA1	14.35	_	58.77	_	26.88	_	34.47	0.15	2.53	0.03
TA2	13.20	_	62.23	_	24.58	_	38.06	1.00	2.69	0.04
TA3	14.33	_	59.67	_	25.99	_	32.24	0.36	4.01	0.04
TA4	2.92	_	60.33	_	36.75	_	22.34	0.26	2.77	0.05

Fig. 5. Redundancy analysis (RDA) of the most important polychaete taxa (from SIMPER analysis) (a) and traits-categories expression (b) with environmental factors in Trieste and Taranto. Environmental-quality statuses were indicated with different colours. Values with asterisk explained the best variability (DisTLM analysis) in species and traits composition. See Table S2 and Table 2 for complete species name and trait labels, respectively. Star TN = Total Nitrogen; PCBs = Polychlorobiphenyls. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in March 2015, compared to May 2014 were likely due to reproduction and larval recruitment rates more frequent during late spring time (Giangrande, 1997).

Stress-tolerant species were observed in both areas and were more abundant at stations with environmental "Warning" values (*sensu* Marin et al., 2008), i.e. *Lumbrineris latreilli*, *Naineris laevigata*, *Notomastus latericeus*, and *Schistomeringos rudolphi*. To some extent, the benthic ecosystem is able to adapt to anthropogenic pressure by trying to minimize its impact through the development of stress resistant communities that occupy new ecological niches (Cibic et al., 2012). In fact, our findings are in accordance with several studies on structural changes of macrofaunal communities in relation to human impacts (e.g. Solis-Weiss

et al., 2004; Lu and Wu, 2007; Mutlu et al., 2010).

Integrative approaches that consider the sets of environmental features in relation to structural and functional macrofaunal patterns provide useful tools for understanding, monitoring and assessing ecosystem functioning in highly impacted areas. The usefulness of the BTA depends on the selected traits and metrics (in our case abundance-based) to quantify the impact of contaminants on macrofaunal functional proprieties. The a priori selection of traits and categories is fundamental when performing biological traits analysis, because certain types of categories are more relevant in some circumstance than others (Bremner, 2008). In this study, the included traits and categories were based on (i) the potential relation with sediments subject to different level of contamination; (ii) expert judgement and personal knowledge on the investigated benthic community; and (iii) the availability of information on the traits for the collected benthic species. Differences in functional features were also depending on the index used: functional dispersions did not highlight any clear variation related to human pressures in this study; on the contrary, variations in functional adaptation in response to sediments contaminated to a different degree were obtained using functional identity. Our results revealed that polychaete functional structure can be successfully used as a complement to traditional taxonomic information to describe the anthropogenic impacts through biological traits analysis.

The functional diversity did not highlight any pattern with species richness. Contamination and environmental features did not seem to affect the functional diversity of the two areas (Fig. 2). The perceived stability of these functional characteristics could be linked to functional redundancy in the polychaete assemblage, since higher species richness was not associated with higher FDis (Cadotte et al., 2011). Theory also suggests that, if there is evidence of functional redundancy in the macrofaunal community structure, the community may be less susceptible to changes in the ecosystem functioning caused by a species' loss (Gamfeldt et al., 2008). Indeed, the redundancy hypothesis states that if several species perform the same function and thus express similar trait values, in case of one being eliminated, others remain to provide or potentially even increase functioning (Yachi and Loreau, 1999). Recent studies are in accordance with our results, which suggest that multiple anthropogenic stressors might cause declines in biodiversity but not affect the functional diversity, if they reduce species that contribute little to function or that are functionally redundant in these communities (McMahon et al., 2012).

In the present study, sand and clay fractions were principal drivers of the traits of the polychaetes (DistLM analysis) in both areas. In addition, other environmental parameters such as depth, TOC and TN contents and Hg (by BIO-ENV analysis) could have influenced the difference in traits expression among sampling stations.

According to Oug et al. (2012); Piló et al. (2016); Krumhansl et al. (2016), to assess anthropogenically-induced modifications on the environment, BTA applied to polychaetes may be more informative than that applied to benthic invertebrates. However, it is difficult to determine whether the observed faunal pattern is structured solely by the environmental variables included in the analysis, or if including other environmental variables in the analysis would change this pattern (Clarke, 1993). Furthermore, many anthropogenic stressors (e.g. chemical contaminants, sewage discharge and mussel farming) could be added to the natural environmental variables (e.g. depth, seawater circulation and the renewal time of seawater), all these factors and stressors act simultaneously and their synergistic effect is not easy to predict (Sigala et al., 2012). In fact, in the present study the functional adaptations were more pronounced at the different level of contamination in the Mar Piccolo of Taranto than in the Gulf of Trieste. This may be due to diverse geomorphological characteristics of the areas: the Mar Piccolo is a semi-enclosed basin, with decreasing salinity, limited depth, and low water renewal rates, going from the First towards the Second Inlet, and with a severe but localized contamination (Alabiso et al., 2006). In contrast, the sediments in the harbour of

Trieste are slightly less contaminated (Cibic et al., 2017) likely due to the fact that the basin is a more open system compared to the Mar Piccolo, with higher sea water renewal and dilution and dispersion rates of contaminants. Further, in these two areas the macrofaunal invertebrates might have adapted to long-term and persistent contamination and therefore none of the considered anthropogenic stressors (allochthonous organic matter and contaminants) were the main drivers of the trait modalities distribution. In fact, the trait features were rather related with natural environmental factors such as the grain-size distribution (DistLM analysis).

In accordance with Oug et al. (2012), *motile* is a prevalent trait in environments with mostly coarse sediments. This may be an adaptation strategy to higher hydrodinamism which leads to coarser sediments. These conditions were present at sampling stations in the First Inlet of the Mar Piccolo and St. TS3. In contrast, we found that *sessile* and *tube-builder* traits were related to fine-grained sediments (Fig. 5b). However, at the highly contaminated stations, *motile* together with *endofauna* and *burrower* were the most expressed functional adaptations, represented by the species *L. latreilli* and *Eunice vittata* (Fig. 5c, f and g). The distribution of contaminants in sediments is often patchy (Stockdale et al., 2009). The heterogeneity of contaminants concentration within the sediments varies the exposure of mobile benthic organisms to contaminants. Thus, the mobile organisms likely have a higher probability to survive because they are able to avoid hotspots of contaminants through their active movements (Ward et al., 2013). Their movement ability makes them good bioturbators, through which they cause indirect contamination dispersion from the surface sediment to the deeper layers (De Vittor et al., 2016). A high expression of *tube-builder* and *sessile* traits was observed in the Second Inlet of the Mar Piccolo of Taranto (Fig. 5h) where the high percentage of clay particularly at St. TA4 is due to the low hydrodinamism of the basin (Canu et al., 2012). In addition to the natural environmental features of the basin, the high biodeposition (feces and pseudofeces) continuously produced by the mussel farms that are widely spread and long-lasting over the Second Inlet of the Mar Piccolo of Taranto have led to organic enrichment in sediments (Karuza et al., 2016). The high TN content indeed indicated a situation of substantial organic enrichment likely ascribable to mussels. This enrichment condition favoured *sessile* and *suspension feeder* polychaetes as *Pista lornensis* and *Lanice conchilega*. Usually, deposit feeders (particularly surface deposit ones) prevail in low hydrodynamic conditions where small-grained sediment particles are found, that retain more organic matter, therefore representing a higher food source availability. In fact, following organic enrichment these invertebrates have shown an opportunistic behaviour (Rossi, 2003). However, mussel farm depositions likely stimulated the growth of suspension feeders. This functional adaptation was previously observed by Zhang et al. (2015), who reported that suspension feeders respond slightly more slowly to the increase of organic load compared to surface deposit feeders. This feeding typology was indeed highly expressed in the Mar Piccolo of Taranto where sediments are enriched with long-lasting and continuous bio-depositions. In addition, the presence of suspension feeders suggest that major organic loads from the water column could change ecosystem functioning and processes, principally the prokaryotic secondary production (Cibic et al., 2016). In fact, suspension feeder are fundamental players in the benthic-pelagic coupling, capturing large quantities of suspended organic matter and phytoplankton, and then incorporating them into the sediments through pseudo-faces, stimulating the microbial loop (Newell, 2004; Törnroos and Bonsdorff, 2012).

Overall, in the present study, the *surface deposit feeder* modality was found in all the sampling sites. The well spread presence of this feeding modality could be principally linked to the grain-size distribution pattern. Sediments at the sampling stations were mostly characterized by sandy-mud or mud and many studies reported the close link of this feeding modality with the grain-size distribution has been already reported (Donald and Larry, 1982). Regarding the *subsurface feeder*

modality, our result is consistent with that of Oug et al. (2012). They observed high numbers of surface deposit feeders in sites contaminated mainly by heavy metals. We observed subsurface deposit feeders such as *N. latericeus*, *L. latreilli*, and *Pseudoleiocapitella fauveli* in both areas at sites with 'Warning' environment quality status. Gaston et al. (1998) suggested that subsurface deposit feeders are the trophic group most likely to endure contamination because they regularly encounter toxic metals and synthetic organic pollutants present in the sediments.

5. Conclusions

In the present study, BTA was used for the first time to identify shifts in functional features of infaunal polychaete assemblages associated with different levels of contamination. The synergistic effect of anthropogenic stressors and the environmental features of the two areas considered in this study did not seem to affect the polychaete functional diversity due to the presence of functional redundancy. 'Mobility', 'environmental position', 'movement methods', and 'feeding habits' were the traits more affected by the environmental stressors. The naturally occurring difference in grain-size was the main driver of functional patterns in sampling sites, even though environmental variables of anthropogenic origin (allochthonous organic matter and contaminants) were relevant for trait expressions, too. Further, in Trieste and Taranto sites the macrofaunal invertebrates might have adapted to the long-term and continuous anthropogenically-induced contamination. Integrative approaches that consider sets of environmental features in relation to structural and functional macrofaunal patterns provide useful tools for understanding, monitoring and assessing ecosystem functioning in highly impacted areas.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2018.03.002.

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