Spatio-temporal patterns of coastal macrofaunal community influenced by sewage discharges

Abstract

Sewage discharges represent a very common source of anthropogenic impact in coastal areas, contributing to generate a high intake of organic matter with consequent reduction of oxygen in the water column and sediments. Among the coastal benthic communities, macrofaunal community is widely involved in the main ecological processes, therefore environmental alterations that threaten these communities, affect their stability over time, and consequently their resistance and resilience.

The main objective of this Ph.D. project was to evaluate both on a spatial and temporal scale, structural, functional, and trophic variations of the coastal macrofaunal community, influenced by the sewage discharge of urban and domestic wastewater in the Gulf of Trieste (North Adriatic Sea). The sampled macrofauna was investigated through new analytical approaches that can best highlight the community response with all its characteristics as well as its change towards a functioning evaluation of the target ecosystem. In general, the community was characterized by high biodiversity values in conjunction with the presence of stress-tolerant species which suggested that the communities are subject to a moderate level of stress. Through the functional traits analysis, the expression of traits related to semi-continuous reproduction, sediment transporters, and sub-surface depositories were increased by moving from the most distant stations to those closest to the main discharge pipeline, suggesting the presence of a continuous wastewater supply. On the contrary, in the more distant stations, a greater expression of the suspension feeder was observed, indicating the presence of lower inputs of organic matter. However, from the analysis of functional richness and β-diversity emerged that following improvement of the plant, which took place over the years studied, the community responded with an increase in the number of species, particularly in the stations near the sewage discharge loading. Furthermore, the bioturbation and bio-irrigation potential were not directly connected with the variation of allochthonous substance but rather with the granulometric characteristics of the area. On the contrary, macrofaunal secondary production also seems to have been influenced by the presence of sewage discharge. High values of the $P/\overline{B}$ ratio were calculated at the stations furthest from the sewage pipelines and a negative relationship was observed between biomass and trophic efficiency (TE) values. Therefore, it appears that sewage discharge induces an increase in TE at stations close to the pipelines. In our case, the sewage discharge has determined the presence of tolerant species with small size, low biomass, high reproduction, and growth rate, which seem to have allowed a greater transfer efficiency of energy at higher trophic levels.

Overall, community analyses both on a spatial and temporal scale have shown a good degree of resilience to environmental variations induced by the pronounced contribution of organic matter, as well as different patterns of organic matter use in terms of secondary production, productivity, and TE. These aspects (P, $P/\overline{B}$ and TE) were also treated for the first time in the context of macrozoobenthic communities subject to the influence of sewage and organic enrichment.

The variety of methods applied suggest how the use of integrated approaches that consider macrofaunal communities, analyzing them both from a structural and functional-trophic point of view, can

provide useful tools to better understand, monitor and evaluate the functioning of coastal ecosystems, including to multiple disturbing factors.

Riassunto

Gli scarichi fognari, rappresentano una sorgente di impatto antropico molto comune nelle aree costiere, contribuendo a generare un elevato apporto di sostanza organica con conseguenti possibili fenomeni di riduzione della normale concentrazione di ossigeno ambientale. Tra le comunità animali costiere, la macrofauna è ampiamente coinvolta nei principali processi ecologici, pertanto alterazioni ambientali che minacciano tali comunità, influiscono sulla loro stabilità nel tempo sia in termini di resistenza che resilienza.

L'obiettivo principale del presente dottorato è stato quello di valutare sia su scala spaziale che temporale le variazioni strutturali, funzionali e trofiche delle comunità macrofaunali influenzate dallo scarico di reflui urbani e domestici nel Golfo di Trieste (Alto Adriatico). La macrofauna campionata è stata indagata attraverso nuovi approcci analitici che contribuiscono ad evidenziare al meglio la risposta delle comunità, in tutte le sue caratteristiche, nonché a favorire la valutazione dei cambiamenti e del funzionamento dell'ecosistema in esame. In generale, le comunità sono risultate caratterizzate da elevati valori di biodiversità, osservati contestualmente alla presenza di specie stress tolleranti che hanno suggerito come tali comunità risultino soggette ad un moderato livello di stress. Attraverso l'analisi dell'espressione dei tratti funzionali come quelli relativi alla riproduzione semi-continua, al trasporto di sedimenti e ai deposivori sub-superficiali, sì è osservato un aumento della loro importanza, procedendo dalle stazioni più lontane a quelle più vicine alla condotta principale di scarico, suggerendo una correlazione tra l'espressione di questi tratti e la presenza di un apporto continuo di reflui. Contrariamente, nelle stazioni più lontane, è stata osservata una maggiore espressione del tratto relativo all'alimentazione sospensivora, indicando la presenza di inferiori apporti di sostanza organica. Inoltre, dall'analisi della ricchezza funzionale e dalla β-diversità è emerso come a seguito dell'aggiornamento dei processi depurativi, avvenuti nel corso degli anni studiati, le comunità abbiano risposto con un aumento del numero di specie, in particolare nelle stazioni vicine dello scarico. Tuttavia, i potenziali di bioturbazione e bioirrigazione non sono risultati direttamente collegati con la variazione di sostanza alloctona ma bensì, con le caratteristiche granulometriche dell'area. La produzione secondaria macrofaunale sembra, al contrario, essere stata influenzata dalla presenza dello scarico fognario. Sono stati osservati maggiori valori di produttività ($P/\overline{B}$) presso le stazioni più lontane dalla condotta di scarico ed è stata osservata una relazione negativa tra la biomassa e i valori di efficienza trofica (TE). Inoltre in prossimità, dello scarico delle acque reflue sono risultate favorite diverse specie considerate tolleranti, di piccola taglia, bassa biomassa ed elevati tassi di riproduzione e crescita, le quali sembrano aver consentito un maggiore trasferimento di energia ai livelli trofici superiori.

Nel complesso, le comunità analizzate sia su scala spaziale che temporale, hanno mostrato un buon grado di resilienza rispetto alle variazioni ambientali indotte dall'eccessivo apporto di sostanza organica, nonché diversi schemi di utilizzo della sostanza organica sia in termini di produzione secondaria, di produttività che in termini di TE. Tali aspetti (P, $P/\overline{B}$ e TE) risultano inoltre trattati per la prima volta nell'ambito di comunità macrozoobentoniche soggette all'influenza di scarichi fognari e arricchimento organico.

La varietà di metodi utilizzati suggerisce come l’impiego di approcci integrati che considerino le comunità macrofaunali analizzandole sia da un punto di vista strutturale che funzionale-trofico, possa fornire strumenti utili per meglio comprendere, monitorare e valutare il funzionamento degli ecosistemi costieri, anche soggetti a molteplici fattori di disturbo.

CONTENTS

1. Introduction

Marine biodiversity is a fundamental component of life in the oceans and on the earth (Simmonds, 2010). A large part of the world's population lives beside the sea and relies on the essential goods and services (such as food supply) that the marine ecosystems provide (Villnäs et al., 2018). In particular, coastal ecosystems are hotspots of environmental variability, biogeochemical transformations, and biological interactions, where dynamic exchanges of energy, mass, and nutrients arise between benthic and pelagic habitats through multiple routes and biodiversity is vast in benthic coastal areas (Gray, 1997). Marine coastal areas despite covering less than 10 % of the Earth's surface (Törnroos, 2014), are one of the most productive ecosystems in the world and frequently obtusely crowded and face plenty of anthropogenic pressures, including climate change, nutrient loading, and fishing (Griffiths et al., 2017). These human activities resulting in habitat degradation and loss of biodiversity (Lotze et al. 2006). Furthermore, in many cases, human activities have caused a homogenization of ecosystems due to the loss of food-chain complication, diversity within functional groups, species distribution range, biogenic habitats extinction and structure, and the reduction in the mean size of several organisms (Airoldi et al., 2008; Dulvy et al., 2008). Therefore, habitat degradation is evaluated as the most universal threat to diversity, structure, and functioning of marine coastal environments and to the goods and services they supply (Lotze et al., 2006; Hoegh-Guldberg et al., 2007; Airoldi et al., 2008; Halpern et al., 2008). Amidst these pressures, sewage discharge constitutes a considerable extent of waste input to the marine ecosystem. In addition to large amounts of suspended and particulate organic matter, wastewaters carry organic contaminants, fecal sterols, heavy metals, bacteria, and nutrients into the marine environments (de-la-Ossa-Carretero et al., 2016). Human disturbance in the world's oceans can be estimated directly from physico-chemical characteristics, or indirectly using the biota, that determines the ecological conditions of their habitats (Bigot et al., 2006). Hence, protecting the richness and diversity of organisms is extremely important, they are the ones who, by their activities, maintain ecosystem functioning and, successively, improve human health (Cardinale et al., 2012). Simultaneously, there is an urgent need to understand how these system functions and improve assessments and analysis tools that allow effective interpretation of these changes. In addition, studying the effects of anthropogenic pressure on marine ecosystems is essential to conduct appropriate economic and environmental management with adequate monitoring programs (Katsanevakis et al., 2011). In general, marine soft-sediment habitats, which are the main bottom components of the coastal zone, show some of the most functionally important ecosystems on Earth, being characterized by high biomass and diversity of biota that is basic to the intermediary of a wealth of goods and services (Queirós et al., 2013). The estimation of benthic conditions is one of the widely utilized and good applied indicators in marine ecosystem evaluation. In particular, macrofaunal invertebrates are of particular interest because : (I) most of them are sessile or have limited mobility and are thus directly depending on local environmental circumstances, (II) they show highlighted responses to environmental changes depending on their species-specific sensitivity/tolerance levels (Bigot et al., 2006), and (III) due to their highest biomass and species diversity compared with the other benthic communities (Snelgrove, 1998) could be considered a key biological component, which plays a crucial role in cycling nutrients, oxygenation of deeper sediment layers, sediment reworking, bio-irrigation, organic matter decomposition, and secondary production. In addition, the macrofaunal invertebrates represent the higher trophic level of the benthic food chain, performed a *top-down* control, since is a preferred pray for bento-demersal fishes (Balsamo et al., 2010; de-la-Ossa-Carretero et al., 2016).

1.1 FUNCTIONALITY AND BIODIVERSITY OF MARINE ENVIRONMENTS

Interactions between biodiversity and ecosystem functioning in marine systems and coastal areas have been given special attention for very long years. Understanding the effective relationship of structural aspects of faunal communities and their functional role can prepare clues to the assessment of environmental hazards, productivity, and services to humans owing to biodiversity variations compelled by climate change and anthropic impacts. Ecosystem processes complete the interactions between organisms and the environment and each other, and biodiversity reflects these interactions (Heip et al.,1998).

According to the fundamental structure of biological systems (communities or ecosystems), two aspects are the bases; the number of species and the number of individuals within each of these species (Hurlbert, 1971; May, 1975; Sugihara, 1980). The biodiversity described the numbers of entities, such as the species (but also genotypes or ecosystems), the evenness of their distribution, the differences in their functional traits, and their interactions (Simpson, 2002). Ecosystem functioning, which is the sum of physical, chemical, and biological processes that transform and translocate energy or materials in an ecosystem, generally describes the combined effects of individual functions, with the overall rate of functioning being governed by the interplay of abiotic (physical and chemical) and/or biotic factors. Of these biotic factors, the effect of biodiversity is widely cited as being influential and is referred to as the ‘Biodiversity and Ecosystem Function’ relationship (BEF). Furthermore, the BEF represents a significant component of ecosystem health and provided ecosystem services (Strong et al., 2015). BEF includes ecological processes that underlie the properties, goods, and services of an ecosystem that control changes in energy and matter over time and space, derived by biotic activities as well as by abiotic factors (i.e. physical and chemical), but can also be representative about the of ecosystem resilience and stability (Srivastava and Vellend, 2005; Hooper et al., 2005). The term resilience of an ecosystem can be generally depicted as a domain of stability, which describes ecosystem behavior as determined by its components (i.e. structure, functions, and environmental relationships). For instance, as a consequence of human impacts in coastal ecosystems, the structure (i.e. abundance, biodiversity, species composition) of benthic communities is constantly changing, hence reducing their stability (Turner et al., 1995).

As coastal ecosystems are relatively shallow, the light penetrates through the water column, reaching the bottom, and is also represented by a strong coupling between pelagic and benthic processes, from autotrophs to secondary consumers and further up the food web (Gazeau et al., 2004; Bremner, 2008). The zoobenthos influences decomposition rates and nutrient cycling in sediments through their active movements (i.e. bioturbation) and thus directly regulates fluxes of energies, representing an important role in ecosystem functions and services for humans. Additionally, traits such as size and longevity represent the stability of a community, as the presence of large, long-lived species is indicative of a mature climax community. Therefore, from a benthic ecosystem function perspective, dispersal, re-colonization, and connectivity are essential for maintaining the biodiversity and resilience of the ecosystem (Villnäs et al., 2018). Ultimately, the effects of disturbance will depend on its magnitude and the size of the systems' stability domain.

From a strictly ecological point of view, ecosystem function refers to roles that organisms have in an ecosystem. Based on the organism's role, they can be classified into functional groups involved in the building of the ecological system and its maintenance (Scheffer et al., 2001). Species interact with and respond to the physical and chemical habitats with different patterns depending on their abilities (Díaz and Cabido, 2001). Every single species can play a significant role in the various function of ecosystems and, because of either natural causes or human activities, any changes in their composition can have adverse effects on ecosystem processes (Gray et al., 2006).

1.2 DISTURBANCE TO ECOSYSTEM AND SOFT-SEDIMENT MACROFAUNAL COMMUNITIES

A disturbance may be defined as an event initiating species population change from mortality or removal and/or a change in the resources base of the community (Probert P.K., 1984), and relatively discrete biological, physical, or chemical events which alter or destroy available resources such as food and space. Therefore, disturbance influencing the structure and function of organism assemblages by directly or indirectly killing, displacing, or damaging components of the community. Further, disturbance events can occur over different spatial and temporal scales and at different frequencies (Ellis et al., 2000).

Soft-sediment habitats are often considered disturbance-dominated systems and recovery rates and patterns may provide important insights into the potential for broad-scale and abrupt changes in the structure and function of benthic communities. Faunal responses to disturbance are species-specific, with the potential for opportunistic population increases in response to the release of available food resources or decrease from predation or competition pressure (Norkko et al., 2010). Macrobenthic communities are frequently viewed as a mosaic of patches created by disturbance events (both from natural to anthropogenic origin) resulting in patches at different stages of recovery (Ellis et al., 2000). Soft-bottom communities can be affected by human activities such as fishing trawling, dredging, spoil and mining waste disposal, marine mining, organic and oil pollution (Probert P.K., 1984). However, natural changes in the environment could affect also macrofauna communities; e.g. seasonality and organic matter loads from rivers and/or surrounding lands (Occhipinti-Ambrogi et al., 2005).

Among the coastal areas, the effects of diffuse and synergistic forms of contamination are even more emphasized in enclosed basins, where the water circulation is restricted and, therefore, the dilution effect of contaminants is limited. Further, the coastal sediments act as a sink for contaminants and frequently contain higher concentrations of them compared to the water column (Trannum et al., 2004). Furthermore, benthic organisms living in or on sediments' surfaces may be exposed not only to contaminants in the overlying water or the pore water but also to contamination by direct contact with those substances adsorbed to sediment particles or by ingestion of those particles (Macken et al., 2008).

These organisms, especially macroinvertebrates have been long studied to evaluate the anthropic impact on marine ecosystems (Pearson and Rosenberg, 1978; Hyland et al., 2005). The resulting composition of species, replacements, eliminations, diversity, or abundance changes, can indicate the recent history of events affecting the area (Richardson et al., 2008). These macrofaunal responses can be quantitative, like abundance or biomass variations, and qualitative, in terms of species composition (presence/absence of sensitive and tolerant organisms). The tolerant taxa are those that can resist unfavorable abiotic (e.g. unpredictable weather conditions, natural high organic loads, and chemical contaminations), and biotic (e.g. parasites and pathogens) conditions, whereas sensible organisms are susceptible to stress factors responding with appreciable and specific changes (Markert, 2007). As reported in Pearson and Rosenberg's (1978) review, a major advantage of using macrobenthic communities as indicators of contamination principally by organic enrichment is their spatial and temporal stability. Their conceptual model **(Fig.1)** showing how species number (S), total abundance (A), and total biomass (B) change along a gradient of increasing organic input has provided much of the basis for current marine benthic indices known as “species/abundance/biomass (SAB) curve”.

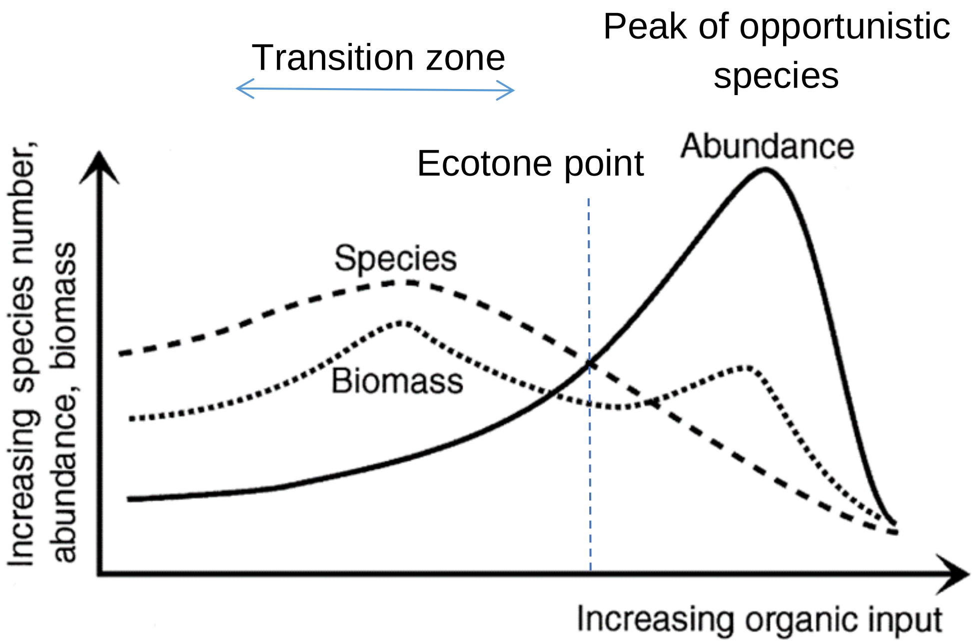

Figure 1: A generalized species abundance biomass (SAB) curve diagram adapted from Pearson and Rosenberg (1978). On the heavily contaminated side of the ecotone-point (right) the community is composed of a few pollution tolerant opportunistic species. On the less contaminated side of the ecotone-point (left) the community tends to approach that of an unpolluted site. The community at the ecotone point is a mixture of the two adjacent communities.

In the transition zone and ecotone point, the macrofaunal community undergoes a strong variation in terms of species composition, along a gradient of organic enrichment. Generally, at the transition zone, communities mostly consist of sensible species, that gradually decrease reaching the ecotone point, where pollution-tolerant opportunistic species dominant due to high organic matter load. Tolerant and less sensible species coexist at the ecotone point, which is a shift zone where biomass is temporarily low. Following the peak of opportunistic species, if organic enrichment increases, abundance, biomass, and species richness drop down into a grossly contaminated state that could end in an azoic environment (Pearson and Rosenberg, 1978) (**Fig.2**). Hence, a surplus of organic input leads to the substitution of sensitive and long-lived species (*K*-strategist) with tolerant and small body size ones (*r*-strategist).

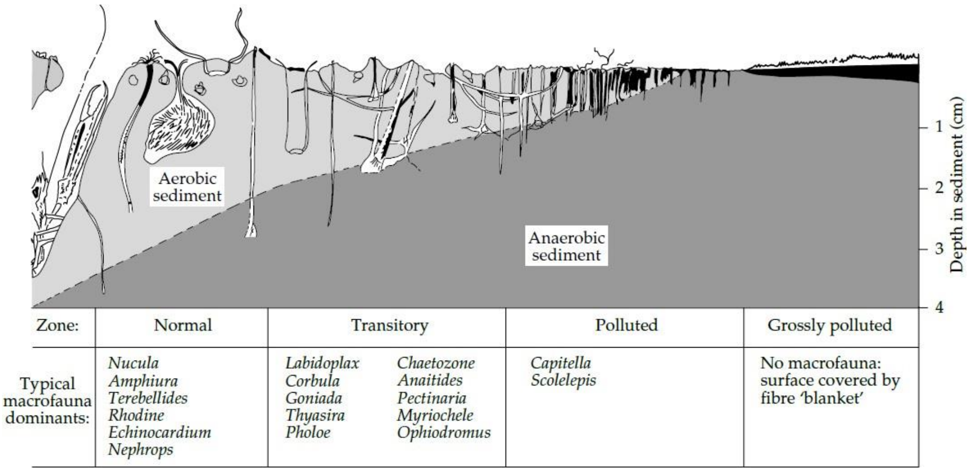

Figure 2: A schematic view of the effects of increasing organic enrichment on the fauna of soft sediments in northern Europe (from Pearson and Rosenberg, 1978). The gradient increases from left side to right.

The high levels of the organic matter whether from sewage, paper, and pulp-mill waste, or weathered oil may alter the organic content and biochemical composition of sediments in coastal areas and produce an organic enrichment, whose later degradation may lead to lower oxygen concentrations and hypoxia, with profound effects on ecosystem functioning. The lack of oxygen causes adverse effects on the benthic fauna, such as changes in density and species composition favoring opportunistic *r*-strategist polychaetes and the mortality of sensitive *K*-strategist species (de-la-Ossa-Carretero et al., 2016). Such effects may seriously interfere with the normal functioning of the ecosystem and may alter the topographical and biological make-up of the seafloor. Eutrophication-induced hypoxia alters the structure, diversity as well as trophic structure, and related food web of benthic communities (Islam and Tanaka, 2004). Besides, in coastal areas, benthic invertebrates that experience low oxygen concentrations may show different responses depending on their physiological abilities and survival strategies such as physiological changes (Forster et al., 1995).

1.3 TRAIT-BASED APPROACH

Trait-based approaches are widely applied in ecological and evolutionary researches. Historically, the term “trait” has naturally moved from the common language to a more scientific one in various disciplines (e.g. quantitative genetics, physiological ecology, functional ecology, population demography, evolutionary physiology, life-history evolution). A trait in its simplest form is described as a proxy for the functioning of the organism (Violle et al., 2007). Over the past three decades, the trait concept has evolved firstly in the terrestrial realm, from studies discussing its wider application for vegetation classifications (Grime, 1977) and habitat heterogeneity (Southwood, 1977) toward the introduction of the expression “functional traits” (Diaz and Cabido, 2001; Petchey and Gaston, 2002). A functional trait has been defined as a component of an organism’s phenotype that determines its effect on processes and its response to environmental factors (Reiss et al., 2009). Indeed, traits are often used as surrogates for ecosystem properties as they have been documented to affect multiple ecosystem functions, such as nutrient cycling, primary, and secondary production, and sediment erodibility (Bremner et al., 2006).

Trait approaches in marine benthic systems focused primarily on trophic functional groups. In general, the community was divided into groups of taxa (functional group) that share similar functional attributes or exploit common resource bases (guilds) using criteria like morphology of the feeding apparatus, feeding mode, nature, and origin of the food. The principal feeding habits identified in marine benthic communities are surface- and subsurface deposit feeders, suspension feeders, predators, omnivores, and grazers (Pearson and Rosenberg, 1978; Bonsdorff and Pearson, 1999; Jumars et al., 2015). This approach was implemented with the Biological Traits Analysis (BTA), introduced by Bremner et al., (2006). BTA is based on the habitat template theory, which states that species 'characteristics evolve in response to habitat constraints (Southwood, 1997). Biological traits are the morphological, physiological, phenological, or behavioral features of an organism that describe its performance (Violle et al., 2014). Importantly, biological traits are known to respond to environmental change (Lavorel et al., 2011) as well as anthropogenic disturbances (Villnäs et al., 2011, 2012; Mouillot et al., 2013). Biological traits analysis uses a series of life history, morphological, and behavioral characteristics of species present in assemblages to indicate aspects of their ecological functioning (Naeem et al., 1999). The approach of considering combinations of biological attributes, in terms of functional groups, has a long history in marine benthic ecology (e.g., Fauchald and Jumars, 1979; Bonsdorff and Pearson, 1999; Pearson, 2001) i.e., bioturbation, community stability, and juvenile dispersal potential. Further, the community structure is governed by habitat variability and the biological traits exhibited by organisms will provide information about how they behave and interact to stress (Lavorel et al., 1997), thereby indicating the state of the environment (Usseglio-Polatera et al., 2000). BTA combines structural data (species abundance or biomass) with information on functional features of each species (e.g. feeding habits, movement method, environmental position, and reproductive typology) thus it provides a link between species, environment, and ecosystem processes (Bermner et al., 2006). BTA uses multivariate ordination to describe patterns of biological trait composition over entire assemblages (i.e. the types of traits present in assemblages and the relative frequency with which they occur). Therefore, to give a comprehensive description of communities and ecosystems, Biological Traits Analysis (BTA) should be combined with taxonomic analysis (Diaz and Cabido, 2001). BTA is based on the assumption that phylogenetically unrelated organisms might have evolved similar biological adaptation, thus leading to functional similarity, combined with taxonomic dissimilarity (Usseglio-Polatera et al., 2000). The BTA was primarily developed in freshwater systems for describing characteristics of invertebrate communities in streams and as a tool for biomonitoring (Usseglio-Polatera et al., 2000; Lamouroux et al., 2004). Recently, marine studies have used this analytical approach to assess various natural effects on benthic functional structures: habitat heterogeneity (Dimitriadis et al., 2012; Sigala et al., 2012; Paganelli et al., 2012), organic enrichment from river flow (Linden van der, 2012; Zhang et al., 2015) and CO_2 vent-systems (Gambi et al., 2016). Functioning has also been assessed concerning the human-induced impact of climate changes (Neumann and Krönke, 2011; Weigel et al., 2016), organic enrichment related to aquaculture (Villnäs et al., 2011), fishing (de Juan et al., 2007), contaminated sediments (Oug et al., 2012), and sewage discharge (Gusmao et al., 2016; Krumhansl et al., 2016). In a previous study on the Adriatic Sea, Munari (2013) performed a BTA to explore and compare species and biological traits on landward and seaward coastal defense structures in the north-western Adriatic Sea (Italy). Moreover, a similar approach has been performed to improve the knowledge of benthic community functioning concerning transitional water ecosystems (Marchini et al., 2008) and the coastal environment (Paganelli et al., 2012). In the Gulf of Trieste (northern Adriatic Sea), BTA was used to explore polychaetes' responses to sediments contaminated by organic and metal pollutants (Nasi et al., 2018).

In the terrestrial realm, measuring traits (e.g. plant height, leaf area, and seed mass) is relatively easy to conduct for each plant in a community. Traits are thus often measured on a continuous scale and represent real-value traits. Below the water surface, this becomes more challenging, especially for marine animals living in the sediment or moving in the water column. Hence, traits used for studying function in benthic communities are principally discrete, i.e. of a categorical or discontinuous type. The trait value, although related to individual variability, is often not measured in a study per se but applied based on various previously published sources (Bremner et al., 2003). The tradition of collecting quantitative samples and knowledge of taxon characteristics, in general, is probably a reason for the larger amounts of studies focused on benthic functional structures, in particular on the macrofaunal community. This point highlights once again that macrofaunal organisms are useful in understanding the ecosystem functioning of coastal environments (Törnroos, 2014).

1.4 β-DIVERSITY

As previously stated, there is a growing body of evidence showing that biodiversity is important for generating and stabilizing ecosystem functions, and thus ensures the provisioning of numerous ecosystem services to society (Mori et al., 2018). Species diversity, now well known to community ecologists, was first discussed by Whittaker (1960, 1972) where he described the alpha, beta, and gamma diversity levels of natural communities. Alpha (α) diversity is the diversity on a relatively small scale, gamma (γ) diversity is that of the whole ecosystem (Legendre et al., 2005), and beta (β) diversity is conceptually the variation in species composition among sites within a geographical area of interest (Whittaker, 1960) **(Fig. 3)**. Under this definition, β-diversity has been suggested as a measure of ecosystem resilience across scales, where the difference between a local site's richness and the size of the regional species pool reflects the connectivity between biological assemblages and thus influences the recovery potential of ecological systems (de Juan et al., 2013).

The description of major patterns in α and β-diversity at multiple scales is important to understand how changes in community composition or species richness at local scales are connected to larger spatial and temporal scales (Barros et al., 2014), and understanding these processes is necessary for the effective management and conservation of coastal marine environments (Legendre et al., 2005; Casas-Güell et al., 2015). There are only a few studies on temporal changes in macrofaunal diversity over varying spatial scales that have been made (e.g., Zajac et al., 2013). Moreover, habitat homogenization and ecological fragmentation are amongst the main threats to biodiversity. β-diversity has been promoted as providing essential information for conservation management by highlighting the consequences of biodiversity loss to ecosystem resilience (de Juan et al., 2013). The interesting aspects of β-diversity are concerned not just with calculating an index but with going back and examining species lists to discover which species are changing along a gradient in temporal scale and then trying to understand the biology of how the species are changing and the life history characteristics that are associated with such changes. For instance, a coastal habitat consisting of small patches of shell debris and encrusting organisms surrounded by soft-sediments could be expected to have high within-habitat species turnover (β-diversity), even if sample (α) biodiversity were not high (Hewitt et al., 2005). Therefore, changes in β-diversity could thus be a potent indicator of threats on ecosystem functions and services and useful to infer environmental, spatial, and stochastic determinants of community assembly for numerous organism groups (Mori et al., 2018). Wilson and Shmida (1984) highlighted the importance of β-diversity in indicating the extent to which habitats are utilized by species. Previous works showed that spatial and temporal β-diversity in species can contribute towards simultaneously supporting different functions (Mori et al., 2018). Ellingsen and Gray (2002) suggest that species β-diversity is as important as local species richness in determining diversity at the regional scale. Nevertheless, β-diversity can be controlled by historical, evolutionary, and other biogeographical processes and also related to an organism's physiology (Barros et al., 2014).

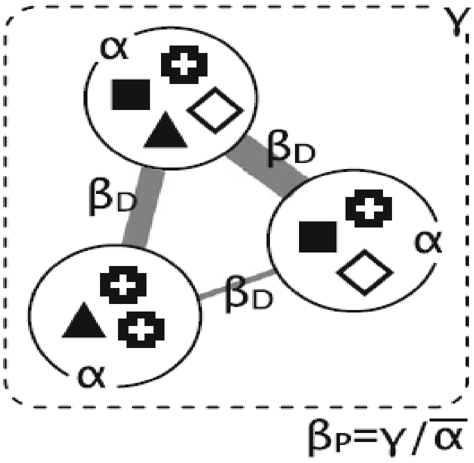

Figure 3: The schema of alpha, beta and gamma diversity from Jurasinsky et al. (2009). α – alpha diversity (number of species in the sample). γ – gamma diversity (number of species in the dataset, community or region). β_D – differentiation diversity, the similarity between pairs of samples (calculated e.g. by Jaccard similarity index); the wider the grey bar, the higher similarity (and lower dissimilarity). β_P –proportional diversity, the relationship between two scales of investigations considering species number on alpha and gamma level.

β-diversity can represent the difference in species composition between local and regional assemblages (de Juan et al., 2013) and is quite flexible because it can be based on any chosen ecologically meaningful dissimilarity measure (Koleff et al., 2003). Vellend, (2001) and Anderson et al., (2011) pointed out that studies of β-diversity might focus on community structures and directional change in community composition from one sampling unit to another along a gradient at spatial and temporal scales. Indeed, β-diversity is essential for identifying relevant scales of change and understanding ecosystem processes related to anthropogenic activities. Importantly, β-diversity may show non-linear responses to stress, as diffuse sources of anthropogenic disturbance can operate cumulatively, implying the importance of understanding thresholds of change in β-diversity (de Juan et al., 2013).

During the early phases of anthropogenic impacts in the coastal ecosystem, localized species losses and invader establishment might cause β-diversity to increase. Even when β-diversity decreases, compensatory changes in alpha-diversity can buffer gamma-diversity against declines in beta-diversity (Socolar et al., 2016).

1.5 BIOTURBATION OF MARINE SEDIMENTS

The term bioturbation is frequently used in scientific literature to describe how living organisms affect the substratum in (or on) which they live. In many cases, the original meaning from ichnology is strictly followed, and the term refers solely to the redistribution of particles and the formation of biogenic structures by burrowing animals. In other cases, bioturbation is used in the context of all physical disturbances caused by animals on the substratum, including particle (reworking) and water (ventilation) movements. Bioturbation is today a widely applied standard term in aquatic sciences, yet no consensus on its definition for fauna has been reached, and most studies use it implicitly for their purpose (Kristensen et al., 2012). Bioturbation also occurs over larger extents covered by the intermediate scale and of course is dependent on the size of the population of the key bioengineers. The key bioturbation processes are by individuals feeding at the surface and defecating deeper into the sediment, or by head-down feeders, which defecate at the surface, and also the infaunal predators move through the sediment in search of prey and thus are often active re-workers of sediment (Gray and Elliott, 2009).

It is the result of egestion, disturbance, and turnover of the bed, the increase in the ‘surface’ layer (being carried into the sediment), irrigation and increased oxygenation at depth, and the creation of habitats suitable for further colonization (Gray and Elliott, 2009). Indeed, the bioturbation activities of benthic macrofauna are known to profoundly affect essential ecosystem functions of benthic ecosystems, including biogeochemical cycling, carbon storage, and various chemical and microbial processes, as well as physical characteristics such as porosity and sediment surface topography (Bonaglia et al., 2013).

Macrofauna can enhance nutrient fluxes and more efficient decomposition of buried organic matter, because of amplifying physical exchanges and physiological factors. However, bioturbation effects are not uniform among nutrients in sediment layers; e.g. bioturbation by deep-burrowing polychaetes has been shown to strongly enhance sediment phosphorus retention while on the other hand increasing the fluxes of dissolved nitrogen to the water column (Bonaglia et al., 2013; Griffiths et al., 2017). By exposing deeper sediment layers, bioturbation promotes sediment aeration and stimulates aerobic microbial activity. Considering the differences in bioturbation capacities among macrobenthic species, changes in assemblage structure have clear implications for sediment-related processes mediated by the fauna (Mermillod-Blondin, 2011).

Indeed, bioturbation is such a denominator and acts as an ‘umbrella’ term that covers all faunal transport activities physically disturbing the substratum. It is separated into activities by animals that directly move and mix particles by the process of reworking and/or directly move water through burrows by the process of ventilation. Thus, faunal bioturbation in aquatic environments is defined as all transport processes carried out by animals that directly or indirectly affect sediment matrices. These processes include both particle reworking and burrow ventilation. Active particle reworking involves faunal displacement and biomixing of particles and includes burrowing, construction, and maintenance of galleries, as well as ingestion and defecation of particles. Depending on their mode of reworking, animals can be categorized as biodiffusors, upward conveyors, downward conveyors, and regenerators. Because of reworking, organic matter and microorganisms are displaced (bio mixed) within the sediment matrix (Kristensen et al., 2012)(**Fig. 4**).

A recent method to study macrofaunal bioturbation, taking into account the whole community in the exam is called: community bioturbation potential (BP_c). BP_c is a metric first described by Solan et al. (2004a), which combines abundance and biomass data with information about the life traits of individual species or taxonomic groups. This information describes modes of sediment reworking and mobility of taxa in a dataset, two traits known to regulate biological sediment mixing, a key component of bioturbation (Solan, 2004a and b). The BP_c is thus not a direct measure of the process of bioturbation. Rather, BP_c provides an estimate of the potential of a community to bioturbated sediment. Hence, where macrofaunal abundance and biomass data are available, BP_c provides a means to estimate the extent to which benthic communities are likely to affect important ecosystem properties that underpin ecosystem functioning. Another important feature is the sediments irrigation derived by organisms that influences the different biogeochemical processes at the seafloor. Bioirrigation is mainly caused by burrow-dwelling organisms that can ventilate the sediments, creating a fast water interchange between the overlying water and subsurface sediments. (Kristensen et al., 2012; Nasi et al., 2020). Accordingly, bioirrigation is predominantly related to body mass and feeding type (Christensen et al., 2000). Wrede et al. (2018) modified the BP_c index suggested by Solan et al. (2004a) into community irrigation potential (IP_c), as a new index, whereas in the bioturbation potential calculation-BPc, the mobility trait presumably underrates the contribution of sessile organisms with low mobility rate but high biorrigation efficiency.

The consequences of environmental and human-driven changes in biodiversity to BP_c and its relationship to ecosystem function have been explored considering: habitat structure and hypoxia (Villnäs et al., 2012), eutrophication (Dimitriou et al., 2017), chemical pollution (Mazik and Elliot, 2000), and at local (Teal et al., 2008) and regional scales (Solan et al., 2012). Despite the studies cited above, the macrofaunal bioturbation and the consequence to ecosystem function in the Mediterranean Sea are still lacking.

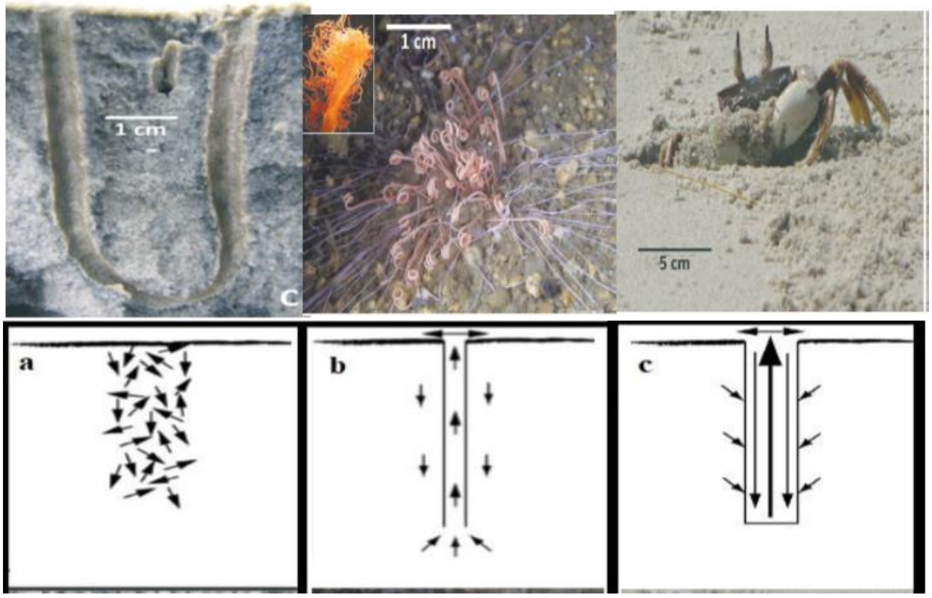

Figure 4: macrofauna reworking groups with diagram representation above (**a** = Biodiffuser; **b** = Conveyor; **c** = Regenerator) and examples below: the crustaceans *Scopimera* sp and *Ocypode* spp. (**a** and **c**, respectively) and the polychaete *Cirriformia grandis* (**b**) (Kristensen et al., 2012).

1.6 PRODUCTION, PRODUCTIVITY, AND TRANSFER EFFICIENCY

Marine coastal habitats offer a diversity of conditions such as soft and hard substrata, macrophytes, and a larger gradient of water depth and temperature than freshwater environments. They vary in their quality, particularly in the availability of food (quality and quantity) they supply to benthic organisms (Cusson and Bourget, 2005). The organic material produced within a community by plants is called primary production since green plants produce their organic matter directly from sunlight via photosynthesis. Animals have to obtain their energy by ingesting other animals or plants, and hence production by animals is called secondary production (Gray and Elliott, 2009). Secondary production, which is the incorporation estimate of organic matter or energy per unit of time and area, integrates the influence of numerous biotic variables and environmental conditions affecting individual growth and population mortality (Cusson and Bourget, 2005).

Production may be defined as the increase in biomass (organic matter) by organisms whether that organic matter is accumulated as growth or as reproduction which may, in the case of many benthic organisms, be liberated as eggs, sperm, and larvae. Production estimation is fundamental for determining the material available to support higher predators (Gray and Elliott, 2009). Besides, marine invertebrates represent an important link in matter cycling and energy flow from primary producers to top consumers in the marine food chain (Brey, 1990). Benthic macrofaunal production is an important pathway in the energy flow through ecosystems and has assumed a fundamental role in the quantification of ecosystem dynamics (Tumbiolo and Downing, 1994; Bolam et al., 2010). Analyzing the different components that contribute to benthic production (e.g., biomass, life span, and body size) and how this production is affected by external factors is very important for understanding ecosystem dynamics and its function under environmental and anthropogenic stress (Dolbeth et al., 2012). Further, anthropogenic pressures significantly impact the species diversity and composition, consequently, could lead to changes in trophic interactions within communities (Nordström and Bonsdorff, 2017). Hence, benthic macrofaunal production, as a useful integrative indicator of the trophic capacity of marine ecosystems, has been estimated by several proposed and improved empirical methods. They have been developed to examine the link between macrobenthic production, population features (e.g., biomass and lifespan), biotic and environmental variables (e.g., depth, temperature, and habitat type), and to estimate production from environmental data without the requirement of intense sampling programs (Robertson, 1979; Brey, 1990; Edgar, 1990; Sprung, 1993; Tumbiolo and Downing, 1994; Brey, 2001). On the one hand, an assessment of the annual production of a population or a community is a time-consuming endeavor that involves the collection of a long series of data on the numbers and weights of individuals. On the other hand, analysing the production of these organisms allows energy flow estimates within ecosystems and represents the formation of community biomass by growth through time (Benke et al., 2010; Dolbeth et al., 2012). Therefore, to avoid this laborious task of obtaining a series of growth and/or survival estimates on populations, it is tempting to simply calculate production (P) from much easier obtainable values of biomass (B) by application production/biomass ($P/\overline{B}$) ratio values from lecturers (Bolam et al., 2010). The global patterns of benthic production and $P/\overline{B}$-ratios are mainly affected by life-history characteristics such as population biomass and density, body mass, recruitment, age, life span, taxonomy, trophic status, etc. (Waters, 1977, Rigler and Downing, 1984). For example, Robertson (1979) proposed the use of a life span of macrobenthic invertebrates. A review of available data for marine macrozoobenthos found the expected negative relationship between $P/\overline{B}$ and life span. However, the usefulness of life span as a predictor of $P/\overline{B}$ is limited, because estimates of life span depend, among other factors, on the accuracy of age determinations and the applicability of a literature-derived longevity estimate (mostly from a different population) for the population studied. As another method, it has been proposed that the relationship between $P/\overline{B}$ values and body weight can be assessed using maximal weight (Tumbiolo and Downing, 1994), weight on reaching maturity (Banse and Mosher, 1980), or mean weight of all individuals within the population (Brey 1990, 2001). However, there are some limitations to apply this method such as (1) estimates of maximal body weight suffer from the same possible bias as those of life span; (2) the exact time of reaching maturity may be somewhat arbitrary, and (3) estimates of weights or ages obtained from the published literature may not be fully representative for the population studied.

In this thesis, Brey's (2012) model (Modified Version 01-2012) was used to estimate $P/\overline{B}$ ratios of composite taxonomical groups. The following to the mean-individual-weight method proposed by Brey (2001) for 2 reasons: (1) the Brey model is now used more frequently than other models so the results can be better comparable, and (2) it performed better than any other empirical model for the prediction of $P/\overline{B}$ or P values. By collecting data of >1000 populations of a wide range of invertebrate species, Brey (1990, 2001) empirically established relationships between $P/\overline{B}$ and mean individual weight, W (a logarithmic relationship), and between $P/\overline{B}$ and annual instantaneous rate of mortality, Z (a linear relationship: $P/\overline{B} = Z$). Though the Brey (1990, 2001) model is based on (mostly) annual estimates of the population parameters W and B, it has frequently been used to estimate annual P from a single assessment of these 2 parameters. The relationship between $P/\overline{B}$ and W (Brey, 1990) offers a real shortcut for the prediction of $P/\overline{B}$. Species or populations that are characterized by relatively small individuals with relatively low individual weights show relatively high values of Z and $P/\overline{B}$ (Brey and Clarke, 1993). Besides, knowledge of food-web structure is critical to the management of anthropogenic disturbances and the conservation of species due to the highly complex interactions among species (Duffy, 2002). Moreover, benthic invertebrates represent a link in the energy transfer from primary producers to higher trophic levels (TLs) which are the number of energy levels from the primary producers to the top consumers (Nilsen et al., 2006; Libralato and Solidoro, 2010). Thus the transfer of efficiency (TE) study provides an understanding of energy flows along benthic trophic pathways, which links to the trophic level and shows the fraction of energy transferred from one trophic level to another (Stergiou and Karpouzi, 2002; Andersen et al., 2008). Higher TE means that a greater proportion of production at low trophic levels is converted to production at higher trophic levels (Eddy et al., 2020). TE is a vital factor in shaping marine ecosystems because even small changes in TE can accumulate at the trophic levels and cause severe differences in movement in the upper TL until influence the highest predator abundance (Moore et al., 2018). Moreover, research on macroinvertebrates has traditionally focused on taxonomy and species diversity, and little effort has been made to assess the ecological significance of benthic invertebrates as producers and/or consumers. Although the assessment of macrofauna production and productivity have been reported considering: estuarine habitats (e.g., Ponti et al., 2007; Kon et al., 2010), continental shelf (Bolam et al., 2010), in a heterogenic subarctic with effects of the recent invasion by the predatory red king crab (Fuhrmann et al., 2015), in a high latitude ecosystem (Nilsen et al., 2006), for a typical shelf slope-basin region (Lin et al., 2015), effects of fishing disturbance (Reiss et al., 2009), despite the above studies, patterns of macrobenthic production and productivity have been less clearly attention with differences in habitats and contaminate.

1.7 STUDY AREA

The Gulf of Trieste is situated in the northernmost part of the Adriatic Sea (Central Mediterranean Sea) and is an epicontinental, semi-enclosed shallow marine basin (Biolchi et al., 2015) located between Italy, Slovenia, and Croatia with an average depth of 17 m (a maximum of about 25m) and a volume of 9.5 km3 (Olivotti et al., 1986) **(Fig. 5)**.

The Gulf endures annual oscillations of temperature altering from 5°C to >24°C at the surface and from 6 °C to >20°C at the bottom. The salinity of the water in the gulf is typically marine, ranging between 33 and 38.5‰ (Ogorelec et al., 1991). The Eastern Adriatic Current (EAC), a current flowing northwards along the Istrian coast and advecting warmer and saltier waters coming from the Ionian Sea (Poulain and Cushman-Roisin, 2001), also influences the Gulf of Trieste and causes a general cyclonic circulation. The Gulf is usually described as a three-layer structure: the circulation in the surface layer (3-5 m deep) is mainly wind-driven, while the lower layer (from 10 m bottom) and the intermediate transition layer follow a general cyclonic circulation (Stravisi, 1983). However, due to its shallow depth, the general circulation of the Gulf and the vertical structure may be rapidly modified in response to intense local atmospheric forcing (winds) and river plumes (Malačič and Petelin, 2001; Querin et al., 2007).

The Gulf is influenced by large and variable freshwater inputs (Fonda-Umani et al., 1992; Russo and Artegiani, 1996); from W to E, by the Tagliamento River, the Isono River which is the main freshwater input, and the Tiavo River from the Norwest part of the basin, whereas the Riana and Dragonja Rivers from the southern one (Conversi et al., 2009).

Due to the vertical mixing of the surface freshwater with seawater across the halocline, this plume induces an inflow of water masses near the bottom of the Gulf (Malačič and Petelin, 2001). It is generally classified as a region of freshwater influence (Simpson et al., 1993) due to intense riverine discharges and undergoes a marked seasonal variability. The hydrodynamism of the Gulf is driven by wind regimes (Bora and Sirocco), characterized by strong, abrupt wind events, by the interaction with the general circulation of the Adriatic Sea (Cossarini et al., 2002; Cossarini and Solidoro, 2007), tides, buoyancy effects of the Isonzo River plume and exchanges of water masses with the Adriatic Sea (Falcieri et al., 2016). When a strong Bora wind blows from E-NE in association with river flood events, the fluvial plume cannot expand symmetrically and is diverted and stretched NE–SW with sediments carried away south-westwards along the coast. Conversely, when winds from the SE and SW (locally called the Libeccio and Scirocco) are dominant, the riverine suspended load is trapped in the Gulf of Panzano where fine particle settling is completed over several days (Covelli et al., 2001).

The concurrence of tidal mixing (Malačič et al., 2000), the alternation between winter mixing and summer stratification of the water column, the variable wind patterns, the occurrence of severe wind storms, and the variable freshwater contributions determine a very large inter-annual, seasonal and even short-term variability in the hydrological and trophic conditions of the basin (Lipizer et al., 2012). In the basin the main sedimentary processes are the transport and deposition of terrigenous sediments by the main rivers flowing into the Gulf, which provision is continuous and variable depending on the season, and the erosion and deposition locally due to the marine waves and currents (2-10 cm/s), both characterized by moderate action. Furthermore, also precipitation and sedimentation by marine bio-organisms influence the seabed granulometric and sedimentary distribution. Grain-size variability in the sediments is strictly connected to the sedimentary load from the Isonzo River in the north, seabed morphology, and meteomarine conditions. The Isonzo River produces a microtidal, low-energy, and fine-grained deltaic system. It is affected by long periods of low-medium discharges and short peaks of intense riverine flow, associated with high suspended sediment load, following heavy rainfall (Covelli et al., 2004). The sediment accumulation rates are approximately 1mm yr^{-1} in the central part of the gulf and increase to about 2.5 mm yr^{-1} towards the mouth of the Isonzo River, located in Panzano Bay (Ogorelec et al., 1991). Surface sediments in this area are mostly silty clays and clayey silts (Zuschin and Piller, 1994).

The Gulf of Trieste is affected by many sources of organic and inorganic pollutants, coming from agricultural and industrial activities in the hinterland, as well as from tourist and maricultural activities along its coasts (Notar et al., 2001; Covelli et al., 2006). The Gulf's outlandish hydrologic and geomorphologic statuses make it amenable to the accumulation of contaminants, as regards its lengthened, sheltered embayment with reduced hydrodynamic (Solis-Weiss et al., 2004).

The Servola sewage disposal plant is the most important sewage plant in the Gulf. It is a mixed-type collecting and treating both meteoric and wastewaters. Until the early 1980s, the Servola sewage discharge, in place since 1938, and served a maximum of 100 000 inhabitants. Though equipped for biological treatment, it drained directly into the Gulf after simple 'trash screening' (F.V.G., 1985). The latter was replaced in 1992 by two adjacent submarine ducts (6.5 and 7.5 km). These wastewaters are released through the two pipelines ending into the sea at a depth between 20 and 23 m with several diffusers with a total diffusional zone length of 1.5 km, (1 km the longest and 0.5 km the shortest duct respectively). Since 1992, the plant was characterized by the initial three treatment phases, serving up to 270,000 inhabitants of Trieste, with a maximum flow of 6000 L sec^{-1}(Solis-Weiss et al., 2007). In recent years the plant serving up to 190.000 equivalent inhabitants for about 35 million m^3 yr^{-1} of wastewaters. In addition, definitively since June 2018, the plant added new treatments, that including pumping station, primary sedimentation, UV disinfection, and biofilters for pre-denitrification, nitrification/oxidation for post-denitrification, and phosphorous removal (www.acegasapsamga.it).

One of the main areas subject to strong eutrophication was the Adriatic Sea (Degobbis et al., 2000). In particular, in the past, the Gulf of Trieste has long been a strongly eutrophicated area, characterized by the highest incidence of mucilage and anoxia events which have led to catastrophic mortality events of benthic communities (Stachowitsch, 1991). Additionally, the macrofaunal community dynamics concerning the analysis of the effects of sewage outfalls are less known in the Adriatic Sea, especially in the Gulf of Trieste (e.g. Auriemma et al, 2016; Cibic et al., 2008; Solis-Weiss et al., 2007). Therefore, the studies included in this thesis are the first ones presenting data on macrofaunal community in terms of functional diversity and occurrences of functional identity, β-diversity bioturbation attributes, secondary production, productivity, and transfer efficiency of macrofaunal communities influenced by sewage-derived materials.

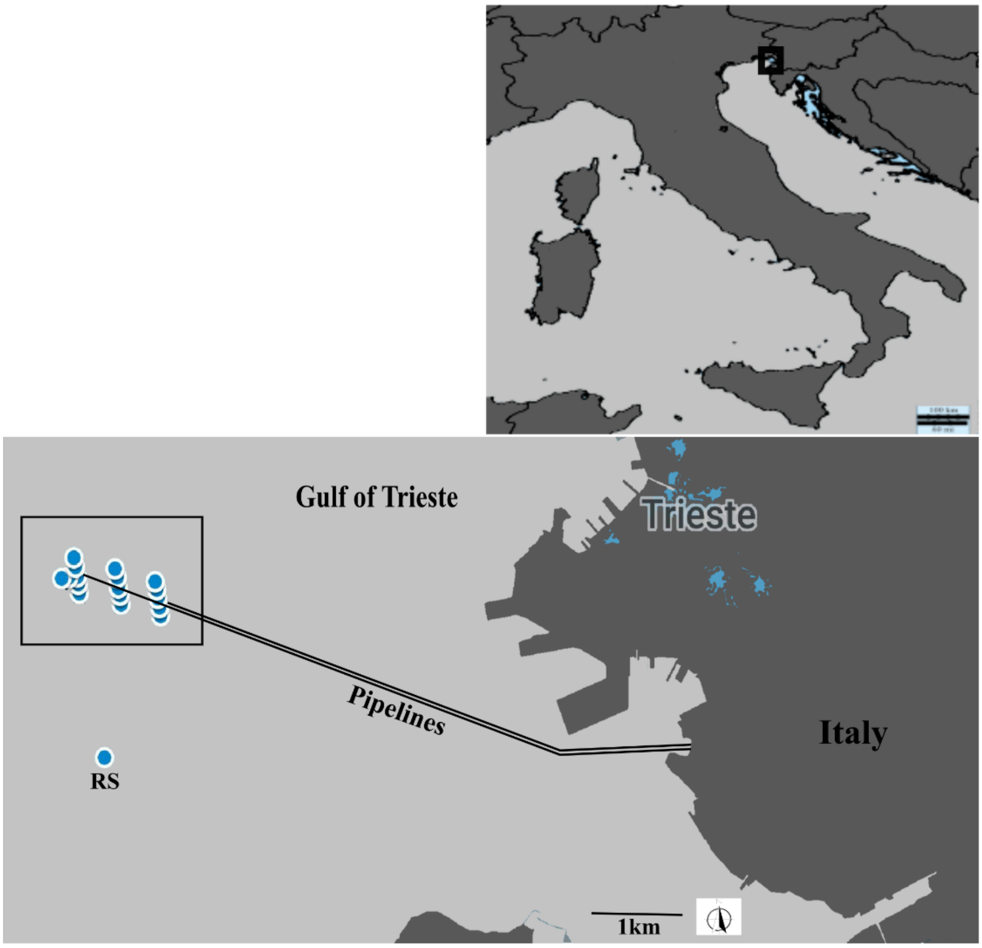

Figure 5: Location of the Servola pipelines and study area in the Gulf of Trieste.

1.8 AIM OF THE THESIS

The main scientific question asked in this thesis was to investigate the response of the coastal macrofaunal community influenced by sewage-derived materials in the Gulf of Trieste. To achieve that, any structural (abundance, biomass, production, productivity, biodiversity, and species composition) and functional changes (functional diversity, identities, entities, and bioturbation potential) were considered. In addition, information on structural and functional features have implemented with new analytical approaches that give more insights into the effects of sewage effluent on macrofaunal communities, also from the point of view of ecosystemic functioning. This doctoral thesis is focused on how the macrofaunal community responds to organic enrichment along the gradient of sewage discharges but considering different features of the community investigates, both from spatial to temporal scales.

More precisely, in the four sections, this thesis assessed:

- **Paper I**: Spatial and temporal variability of macrofaunal functional diversity and trait occurrences achieved by the Biological Traits Analyses (BTA). In particular, the formulated questions were: i) Are the structural and functional patterns driven by specific sediment physical-chemical variables and contaminants? ii) Are there any differences in functional diversity and identity considering the structural abundance and biomass information? iii) Is it possible to include BTA of macrofaunal communities in coastal monitoring programs?

- **Paper II**: Spatial and temporal changes of macrofaunal community in terms of functional richness and beta-diversity, influenced by sewage effluents following also an improvement of treatments. In particular, I sought to answer the following questions: i) Is functional diversity influenced by sewage effluent variations along a distance gradient? ii) Is functional diversity buffered by functional redundancy, such that the loss of functional diversity with changes in sewage deposition than taxonomic diversity? iii) Does biodiversity (α and β) vary following the variation of treatments?

- **Paper III:** Spatial variability of bioturbation attributes (i.e. sediment reworking and biorrigation) influenced by high organic matters from sewage. In addition, to underline differences in macrofaunal bioturbation features, influenced by allochthonous organic matters, I compared the community of the Gulf of Trieste with one influenced by riverine-derived materials. More specifically, the questions addressed were: i) Does the structure of the benthic macrofauna community affect bioturbation processes in different sedimentary environments? ii) Do macrofaunal bioturbation attributes show spatial variability associated with different OM inputs of terrigenous/freshwater allochthonous and sewage-derived materials? iii) Are the bioturbation attribute patterns driven by specific sediment physicochemical parameters?

- **Paper IV**: Spatial analysis on the effects of sewage discharges on biomass, production, productivity ($P/\bar{B}$), and transfer efficiency of macrofaunal community. Precisely, i) What are the spatial differences in biomass, production, ($P/\bar{B}$), and transfer efficiency along a gradient of sewage effluents discharge? ii) Is there any relationship between the spatial variability of biological factors with the environmental variables? iii) Does TE reflect the variations in benthic communities' compositions and structure due to sewage effluents?

References

Airoldi, L., Balata, D., Beck, M.W., 2008. The Gray Zone: Relationships between habitat loss and marine diversity and their applications in conservation. Journal of Experimental Marine Biology and Ecology 366, 8–15.

Ambrogi, O. A., Savini, S., Forni, G., 2005. Macrobenthos community structural changes off Cesenatico coast (Emilia Romagna, Northern Adriatic), a six-year monitoring programme. Science of the Total Environment, 353, 317– 328.

Andersen, K. H., Beyer, J. E., Lundberg P., 2008. Trophic and individual efficiencies of size-structured communities. Proceedings of the Royal Society B 276:109–114.

Anderson, M.J., Crist, T.O., Chase, J.M., Vellend, M., Inouye, B.D., Freestone, A.L., 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol. Lett., 14, 19– 28.

Auriemma, R., Nasi, F., Del Negro, P., 2016. The macrozoobenthic fauna of the bottoms nearby the underwater sewage duct of Trieste (Northern Adriatic Sea). Biologia Marina Mediterranea,23(1), 90.

Balsamo, M., Albertelli, G., Ceccherelli, V.U., Coccioni, R., Colangelo, M.A., CuriniGalletti, M., Danovaro, R., D'Addabbo, R., De Leonardis, C., Fabiano, M., Frontalini, F., Gallo, M., Gambi, C., Guidi, L., Moreno, M., Pusceddu, A., Sandulli, R., Semprucci, F., Todaro, M.A., Tongiorgi, P., 2010. Meiofauna of the Adriatic Sea: present knowledge and future perspectives. Chem. Ecol. 26, 45–63. https://doi.org/10.1080/ 02757541003705492.

Banse, K., Mosher, S., 1980. Adult body mass and annual production/biomass relationships of field populations. Ecol Monogr 50:355–379.

Barros, F., Blanchet, H., Hammerstrom, K., Sauriau, P. G., Oliver, J., 2014. A framework for investigating general patterns of benthic β-diversity along estuaries. Estuarine, Coastal and Shelf Science, 149, 223–231. https://doi.org/10.1016/j.ecss.2014.08.025.

Benke, A. C., 2010. Secondary production as part of bioenergetic theory—contributions from freshwater benthic science. River Research Applications. ; 26:36–44. doi: 10.1002/rra.1290.

Bigot, L., Conand, C., Amouroux, J. M., Frouin, P., Bruggemann, H., Grémare, A., 2006. Effects of industrial outfalls on tropical macrobenthic sediment communities in Reunion Island (Southwest Indian Ocean). Marine Pollution Bulletin, 52(8), 865–880. https://doi.org/10.1016/j.marpolbul.2005.11.021.

Biolchi, S., Furlani, S., Covelli, S., Busetti, M., Cucchi, F., 2015. Morphoneotectonics and lithology of the eastern sector of the Gulf of Trieste (NE Italy). Journal of Maps, 11.

Bolam, S.G., Barrio-Froján, C.R.S., Eggleton, J.D., 2010. Macrofaunal production along the UK continental shelf. Journal of Sea Research 64, 166–179.

Bonaglia, S., Bartoli, M., Gunnarsson, J. S., Rahm, L., Raymond, C., Svensson, O., Yekta, S. S., Brüchert, V., 2013. Effect of reoxygenation and Marenzelleria spp. bioturbation on Baltic Sea sediment metabolism. Marine Ecology Progress Series, 482, 43–55. https://doi.org/10.3354/meps10232.

Bonsdorff, E., Pearson, T. H., 1999. Variation in the sublittoral macrozoobenthos of the Baltic Sea along environmental gradients: A functional-group approach. Austral Ecology, 24(4), 312-326.

Bremner, J., Frid, C. L. J., Rogers, S. I., 2003. Assessing Marine Ecosystem Health: The long term effects of fishing on functional biodiversity in North Sea benthos. Aquatic Ecosystem Health and Management, 6(2), 131–137.

Bremner, J., Rogers, S. I., Frid, C. L. J., 2006. Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). Ecological Indicators, 6(3), 609–622. https://doi.org/10.1016/j.ecolind.2005.08.026.

Bremner, J., 2008. Species' traits and ecological functioning in marine conservation and management. J. Exp. Mar. Biol. Ecol. 366, 37-47.

Brey, T., 1990. Estimating productivity of macroinvertebrates from biomass and mean individual weight. Meeresforschung 32, 329–343.

Brey, T., Clarke, A., 1993. Population dynamics of marine benthic invertebrates in Antarctic and subantarctic environments: are there unique adaptations? Antarct Sci 5: 253–266.

Brey, T., 2001. Population dynamics in marine benthic invertebrates. A virtual handbook. Alfred Wegener Institute for polar and marine research, Germany. Version 01.2. Available at http://www.thomas-brey.de/science/virtualhandbook/navlog/index.html.

Brey, T., 2012. A multi-parameter artificial neural network model to estimate macrobenthic invertebrate productivity and production. Limnol. Oceanogr. Methods 10, 581–589. http://dx.doi.org/10.4319/lom.2012.10.581.

Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A., Mace, G. M, Tilman, D. Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B., Castelli, A., Lardicci, C., Tagliapietra, D., 2004. Soft bottom macrobenthos. Mediterranean marine benthos: a manual of methods for its sampling and study. Biologia Marina Mediterranea, 11(Suppl 1), 99-131.

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., Narwani, A., Mace, G. M., Tilman, D., Wardle, D. A., Kinzig, A. P., Daily, G. C., Loreau, M., Grace, J. B., Larigauderie, A., Srivastava, D. S., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67. doi:10.1038/nature11148

Casas-Güell, E., Teixidó, N., Garrabou, J., Cebrian, E., 2015. Structure and biodiversity of coralligenous assemblages over broad spatial and temporal scales. Mar. Biol. 162, 901–912. http://dx.doi.org/10.1007/s00227-015-2635-7

Christensen, B., Vedel, A., Kristensen, E., 2000. Carbon and nitrogen fluxes in sediment inhabited by suspension feeding (*Nereis diversicolor*) and non-suspension feeding (*N. virens*) polychaetes. Mar. Ecol. Prog. Ser. 192, 203–217.

Cibic, T., Acquavita, A., Aleffi, F., Bettoso, N., Blasutto, O., De Vittor, C., Falconi, C., Falomo, J., Faresi, L., Predonzani, S., Tamberlich, F., Fonda Umani, S., 2008. Integrated approach to sediment pollution: A case study in the Gulf of Trieste. Marine Pollution Bulletin, 56, 1650–1667.

Conversi, A. Peluso, T, and Fonda-Umani, S., 2009. Gulf of Trieste: A changing ecosystem. Journal of geophysical research, 114, C03S90, doi:10.1029/2008JC004763.

Cossarini, G., Solidoro, C., Crise, A., 2002. A model for the trophic food web of the Gulf of Trieste, paper presented at 1st Biennial Meeting, Int. Environ. Modell. and Software Soc., Lugano, Switzerland.

Cossarini, G., C. Solidoro., 2007. Global sensitivity analysis of a trophodynamic model of the Gulf of Trieste, Ecol. Modell., 212, 16– 27, doi:10.1016/j. ecolmodel.2007.10.009.

Covelli, S., Faganeli, J., Horvat, M., Brambati, A., 2001. Appl. Geochem., 16, 541−558.

Covelli, S., Piani, R., Faganeli, J., Brambati, A., 2004. Circulation and suspended matter distribution in a microtidal deltaic system: The Isonzo river mouth (northern Adriatic Sea). Journal of Coastal Research, 41, 130–140.

Covelli, S., Fontolan, G., Faganeli, J., and Ogrinc, N., 2006. Anthropogenic markers in the Holocene stratigraphic sequence of the Gulf of Trieste (northern Adriatic Sea). Mar. Geol., 230, 29–51, https://doi.org/10.1016/j.margeo.03.013, 2006.

Cusson, M., Bourget, E., 2005. Global patterns of macroinvertebrate production in marine benthic habitats. Marine Ecology Progress Series, 297, 1–14. https://doi.org/10.3354/meps297001.

De Juan, S., Thrush, S.F., Demestre, M., 2007. Functional changes as indicators of trawling disturbance on a benthic community located in a fishing ground (NW Mediterranean Sea). Marine Ecology Progress Series ,334, 117–129.

De Juan, S., Thrush, S. F., Hewitt, J. E., 2013. Counting on β-Diversity to Safeguard the Resilience of Estuaries. PLoS ONE, 8(6), 1–11. https://doi.org/10.1371/journal.pone.0065575.

Degobbis, D., Precali, R., Ivancic, I., Smodlaka, N., Fuks, D., Kveder, S., 2000. Long-term changes in the northern Adriatic ecosystem related to anthropogenic eutrophication. International Journal of Environment and Pollution, 13(1/2/3/4/5/6), 495.

De-la-Ossa-Carretero, J. A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lizaso, J. L., 2016. Monitoring the effects of wastewater treatment strategies. Environmental Monitoring and Assessment, 188(2), 1–11. https://doi.org/10.1007/s10661-016-5092-y.

Díaz, R., Rosenberg, R., Díaz, R., Corral, R., Diaz-Garcia, R., Rosenberg, R. D., 1995. Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and Morine Biology, 33, 245–303.

Díaz, S., Cabido, M., 2001. Vive la différence: Plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution, 16(11), 646–655.

Dimitriadis, C., Evagelopoulos, A., Koutsoubas, D., 2012. Functional diversity and redundancy of soft bottom communities in brackish waters areas: local vs regional effects. Journal of Experimental Marine Biology and Ecology, 426, 53−59.

Dimitriou, P. D., Papageorgiou, N., Karakassis, I., 2017. Response of benthic macrofauna to eutrophication in a mesocosm experiment: Ecosystem resilience prevents hypoxic conditions. Frontiers in Marine Science, 4, 391.

Dolbeth, M., Cusson, M., Sousa, R., Pardal M.A., 2012. Secondary production as a tool for better 14 understanding of aquatic ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 69, 1230–1253.

Duffy, J.E., 2002. Biodiversity and ecosystem function: the consumer connection. Oikos, 99, 201–219.

Dulvy, N.K., Rogers, S.I., Jennings, S., Stelzenmüller, V., Dye, S.R., Skjoldal, H.R., 2008. Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. Journal of Applied Ecology 45, 1029– 1039.

Eddy, T. D., Bernhardt, J. R., Blanchard, J. L., Cheung, W. W. L., Colléter, M., du Pontavice, H., Fulton, E. A., Gascuel, D., Kearney, K. A., Petrik, C. M., Roy, T., Rykaczewski, R. R., Selden, R., Stock, C. A., Wabnitz, C. C. C., & Watson, R. A., 2020. Energy Flow Through Marine Ecosystems: Confronting Transfer Efficiency. Trends in Ecology and Evolution, 36(1), 76–86. https://doi.org/10.1016/j.tree.2020.09.006

Edgar, G.J., 1990. The use of the size structure of benthic macrofaunal communities to estimate faunal biomass and secondary production. Journal of Experimental Marine Biology and Ecology 137, 195–214.

Ellingsen, K. E., Gray, J. S., 2002. Spatial patterns of benthic diversity: is there a latitudinal gradient along the Norwegian continental shelf? Journal of Animal Ecology 71:373–389.

Ellis, J. I., Norkko, A., Thrush, S. F., 2000. Broad-scale disturbance of intertidal and shallow sublittoral soft-sediment habitats; Effects on the benthic macrofauna. Journal of Aquatic Ecosystem Stress and Recovery, 7(1), 57–74. https://doi.org/10.1023/A:1009923530894.

F.V.G., 1985. Piano di risanamento del 'bacino idrografico' delle acque marittime costiere—Rapporto conclusivo. Regione Autonoma Friuli-Venezia Giulia (Direzione Regionale dei Lavori Pubblici), Trieste, p. 173.

Falcieri, F. M., Kantha, L., Benetazzo, A., Bergamasco, A., Bonaldo, D., Barbariol, F., Malačič, V., Sclavo, M., Carniel, S., 2016. Turbulence observations in the Gulf of Trieste under moderate wind forcing and different water column stratification, Ocean Sci., 12, 433–449, https://doi.org/10.5194/os-12-433-2016.

Fauchald, K., Jumars, P. A., 1979. The diet of worms: a study of polychaete feeding guilds. Oceanography and Marine Biology: An Annual Review 17:193–284.

Flint, R. W., Kalke, R. D., 2005. Reinventing the wheel in ecology research? Science 307:1875.

Fonda-Umani, S., Franco, P., Ghirardelli, E., Malej, A., 1992. Outline of oceanography and the plankton of the Adriatic Sea, in Marine Eutrophication and Population Dynamics, edited by G. Colombo et al., pp. 347–365, Olsen and Olsen, Fredensborg, Denmark.

Forster, S., Graf, G., Kitlar, J., Powilleit, M., 1995. Effects of bioturbation in oxic and hypoxic conditions: A microcosm experiment with a North Sea sediment community. Marine Ecology Progress Series, 116(1–3), 153–162. https://doi.org/10.3354/meps116153.

Fuhrmann, M. M., Pedersen, T., Ramasco, V., Nilssen., E. M., 2015. Macrobenthic biomass and production in a heterogenic subarctic fjord after invasion by the red king crab. J. Sea Res. 106: 1–13. Doi: 10.1016/j.seares.2015.09.003

Gambi, M. C., Musco, L., Giangrande, A., Badalamenti, F., Micheli, F., Kroeker, K. J., 2016. Distribution and functional traits of polychaetes in a CO_2 vent system, winners and losers among closely related species. Marine Ecology Progress Series, 550, 121–134.

Gazeau, F., Smith, S. V., Gentili, B., Frankignoulle, M., Gattuso, J. P., 2004. The European coastal zone: characterization and first assessment of ecosystem metabolism. Estuar. Coast. Shelf Sci. 60, 673-694.

Gray, J. S., 1997. Marine biodiversity: Patterns, threats and conservation needs. Biodiv. Conser. 6: 153–175.

Gray, J. S., Wu, R. S. S., Or, Y. Y., 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Marine ecology progress series, 238, 249−279.

Gray, J.S., Dayton, P., Thrush, S., Kaiser, M.J., 2006. On effects of trawling, benthos and sampling design. Marine Pollution Bulletin, 52 (8), 840–843.

Gray, J.S., Elliott, M., 2009. Ecology of Marine Sediments. New York, NY: Oxford University Press Inc. 225 pages.

Griffiths, J. R., Kadin, M., Nascimento, F. J. A., Tamelander, T., Törnroos, A., Bonaglia, S., Bonsdorff, E., Brüchert, V., Gårdmark, A., Järnström, M., Kotta, J., Lindegren, M., Nordström, M. C., Norkko, A., Olsson, J., Weigel, B., Žydelis, R., Blenckner, T., Niiranen, S., Winder, M., 2017. The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. In Global Change Biology. 23(6), 2179–2196. https://doi.org/10.1111/gcb.13642.

Grime, J. P., 1997. Biodiversity and ecosystem function, the debate deepens. Science, 277(5330), 1260−1261.

Gusmao, J. B., Brauko, K. M., Eriksson, B. K., Lana, P. C., 2016. Functional diversity of macrobenthic assemblages decreases in response to sewage discharges. Ecological Indicators, 66, 65−75.

Halpern, B. S., et al. 2008. A global map of human impact onmarine ecosystems. Science 319:948–952.

Heip, R., Warwick, L., d'Ozouville European Marine and Polar Science (EmaPS)., 1998. A European Science Plan on Marine Biodiversity ESF (European Science Foundation) Marine Board Report, Strasbourg 48 pp.

Hewitt, J. E., Thrush, S. F., Halliday, J., Duffy, C., 2005. The importance of small-scale habitat structure for maintaining beta diversity. Ecology, 86(6), 1619–1626. https://doi.org/10.1890/04-1099.

Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Greenfield, P., Gomez, E., Harvell, C.D., Sale, P.F., Edwards, A.J., Caldeira, K., Knowlton, N., Eakin, C.M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R.H., Dubi, A., Hatziolos, M.E., 2007. Coral reefs under rapid climate change and ocean acidification. Science 318, 1737–1742.

Hooper, D.U., Chapin, F.S., III, Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs, 75: 3-35. https://doi.org/10.1890/04-0922

Horvat, M., Covelli, S., Faganeli, J., Logar, M., Mandic, V., Rajar, R., Sirca, A., Zagar, D., 1999. Mercury in contaminated coastal environments; a case study: The Gulf of Trieste. Sci Total Environ 237/238:43–56.

Hyland, J., Balthis, L., Karakassis, I., Magni, P., Petrov, A., Shine, J., Warwick, R., 2005. Organic carbon content of sediments as an indicator of stress in the marine benthos. Marine Ecology Progress Series, 295, 91−103.

Islam, M. S., Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. Marine Pollution Bulletin, 48(7–8), 624–649. https://doi.org/10.1016/j.marpolbul.2003.12.004.

Jumars, P. A., Dorgan, K. M., Lindsay, S. M., 2015. Diet of worms emended: an update of polychaete feeding guilds. Annual review of marine science, 7, 497−520.

Jurasinsky, G., Retzer, V., Beierkuhnlein, C., 2009. Inventory, differentiation, and proportional diversity: a consistent terminology for quantifying species diversity. Oecologia 159:15–26.

Katsanevakis, S., Zenetos, A., Mačić, V., Beqiraj, S., Poursanidis, D., Kashta. L., 2011. Invading the Adriatic: spatial patterns of marine alien species across the Ionian–Adriatic boundary. AB 13,107−118.

Koleff, P., Gaston, K.J., Lennon, J.J., 2003. Measuring beta diversity for presence-absence data. J. Anim. Ecol., 72, 367–382.

Kon, K., Kurokura, H., Tongnunui, P., 2010. Effects of the physical structure of mangrove vegetation on a benthic faunal community. Journal of Experimental Marine Biology and Ecology 383:171−180 DOI 10.1016/j.jembe.2009.11.015.

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C. O., Banta, G. T., 2012. What is bioturbation? the need for a precise definition for fauna in aquatic sciences. Marine Ecology Progress Series, 446, 285–302. https://doi.org/10.3354/meps09506.

Krumhansl, K., Jamieson, R., Krkosek, W., 2016. Using species traits to assess human impacts on near shore benthic ecosystems in the Canadian Arctic. Ecological Indicators, 60, 495-502.

Laliberté, E., Legendre, P., Shipley, B., Laliberté, M. E., 2014. Package 'FD'. Measuring functional diversity from multiple traits, and other tools for functional ecology. R package ver.1.0–12.

Lamouroux, N., Dolédec, S., Gayraud, S., 2004. Biological traits of stream macroinvertebrate communities: effects of microhabitat, reach, and basin filters. Journal of the North American Benthological Society, 23(3), 449−466.

Larigauderie, A., Srivastava, D., Naeem, S., 2012. Biodiversity loss and its impact on humanity. Nature 486:59−67.

Lavorel S., McIntyre S., Landsberg J., Forbes D., 1997. Plant functional classifications: from general groups to specific groups based on response to disturbance. Trends in Ecology and Evolution 12:474–478.

Lavorel, S., Grigulis, K., Lamarque, P., Colace, M. P., Garden, D., Girel, J., Pellet, G., Douzet, R., 2011. Using plant func-tional traits to understand the landscape distribution of multiple ecosystem services. Journal of Ecology 99:135–147.

Legendre, P., Borcard, D., Peres-Neto, P.R., 2005. Analysing Beta diversity: Partitioning the spatial variation of community composition data. Ecological Monographs, 75: 435-450. https://doi.org/10.1890/05-0549.

Libralato, S., Solidoro, C., 2010. Comparing methods for building trophic spectra of ecological data. ICES Journal of Marine Science, 67(3), 426–434. https://doi.org/10.1093/icesjms/fsp249.

Lin, H., Wang, J., Liu, K., He, X., Lin, J., Huang, Y., Zhang, S., Mou, J., Zheng, C., Wang, Y., 2016. Benthic macrofaunal production for a typical shelf-slope-basin region in the western Arctic Ocean. Continental Shelf Research, 113, 30–37. https://doi.org/10.1016/j.csr.2015.12.001

Linden van der, P., Patrício, J., Marchini, A., Cid, N., Neto, J.M., Marques, J.C., 2012. A biological trait approach to assess the functional composition of subtidal benthic communities in an estuarine ecosystem. Ecol. Indic. 20, 121–133.

Lipizer, M., De Vittor, C., Falconi, C., Comici, C., Tamberlich, F., & Giani, M., 2012. Effects of intense physical and biological forcing factors on CNP pools in coastal waters (Gulf of Trieste, Northern Adriatic Sea). Estuarine, Coastal and Shelf Science, 115, 40–50. https://doi.org/10.1016/j.ecss.2012.03.024

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., Jackson, J. B. C., 2006. Depletion, degradation and recovery potential of estuaries and coastal seas. Science312:1806–1809.

Macken, A., Giltrap, M., Foley, B., McGovern, E., McHugh, B., Davoren, M., 2008. A model compound study: the ecotoxicological evaluation of five organic contaminants employing a battery of marine bioassays. Environmental pollution, 153(3), 627−637.

Malačič, V., Viezzoli, D., Cushman-Roisin, B., 2000. Tidal dynamics in the northern Adriatic Sea, J. Geophys. Res., 105(C11), 26,265 – 26,280.

Malačič, V. and Petelin, B., 2001. Gulf of Trieste, in Physical Oceanography of the Adriatic Sea, Past, present and Future, edited by: Cushman-Roisin, B., Gacic, M., Poulain, P. M., and Artegiani, A., 167–177, Kluwer Academic Press, Dordrecht.

Marchini, A., Munari, C., Mistri, M., 2008. Functions and ecological status of eight Italian lagoons examined using biological traits analysis (BTA). Marine Pollution Bulletin, 56(6), 1076−1085.

Markert, B., 2007. Definitions and principles for bioindication and biomonitoring of trace metals in the environment. Journal of Trace Elements in Medicine and Biology, 21(SUPPL. 1), 77–82.

May, R.M., 1975. Patterns of species abundance and diversity. M.L. Cody, J.M. Diamond (Eds.), Ecology and evolution of communities, Harvard University Press, Cambridge, 81-120.

Mazik, K., Elliott, M., 2000. The effects of chemical pollution on the bioturbation potential of estuarine intertidal mudflats. Helgoland Marine Research, 54(2), 99.

Mermillod-Blondin, F., 2011. The functional significance of bioturbation and biodeposition on biogeochemical processes at the water−sediment interface in freshwater and marine ecosystems. J N Am Benthol Soc 30: 770−778.

Moore, J. K., Fu, W., Primeau, F., Britten, G. L., Lindsay, K., Long, M., Doney, S. C., Mahowald, N., Hoffman, F., Randerson, J. T., 2018. Sustained climate warming drives declining marine biological productivity. Science, 359 (6380), 1139–1143. https://doi.org/10.1126/science.aao6379.

Mori, A. S., Isbell, F., Seidl, R., 2018. β-Diversity, Community Assembly, and Ecosystem Functioning. Trends in Ecology and Evolution, 33(7), 549–564. https://doi.org/10.1016/j.tree.2018.04.012.

Mouillot, D., Graham, N. A., Villéger, S., Mason, N. W., Bellwood, D. R., 2013. A functional approach reveals community responses to disturbances. Trends in ecology & evolution, 28(3), 167-177.

Munari, C., 2013. Benthic community and biological trait composition in respect to artificial coastal defence structures: a study case in the northern Adriatic Sea. Marine environmental research, 90, 47−54.

Naeem, S., Chapin, F.S., Costanza, R., Ehrlich, P.R., Golley, F.B., Hooper, D.U., Lawton, J.H., O'Neill, R.V., Mooney, H.A., Sala, O.E., Symstad, A.J., Tilman, D., 1999. Biodiversity and Ecosystem Functioning: Maintaining Natural Life Support Processes Issues in Ecology, vol. 4. Ecological Society of America, Washington, pp. 11.

Nasi, F, Nordström, M., Bonsdrorff, E., Auriemma, R., Cibic, T., del Negro, P., 2018. Functional biodiversity of marine soft-sediment polychaetes from two Mediterranean coastal areas in relation to environmental stress. Marine environmental research. 137, 121–132. https://doi.org/10.1016/j.marenvres.2018.03.002.

Nasi, F., Ferrante, L., Alvisi, F., Bonsdorff, E., Auriemma, R., Cibic, T., 2020. Macrofaunal bioturbation attributes in relation to riverine influence: What can we learn from the Po River lagoonal system (Adriatic Sea)? Estuarine, Coastal and Shelf Science, 232, 106405.

Neumann, H., Kröncke, I., 2011. The effect of temperature variability on ecological functioning of epifauna in the German Bight. Marine Ecology, 32(1), 49-57.

Nilsen, M., Pedersen, T., Nilssen, E. M., 2006. Macrobenthic biomass, productivity (P/B̄) and production in a high-latitude ecosystem, North Norway. Marine Ecology Progress Series, 321(September), 67–77. https://doi.org/10.3354/meps321067

Nordström, M. C., Bonsdorff, E., 2017. Organic enrichment simplifies marine benthic food web structure. Limnology and Oceanography, 62(5), 2179–2188. https://doi.org/10.1002/lno.10558.

Norkko, J., Norkko, A., Thrush, S. F., Valanko, S., Suurkuukka, H., 2010. Conditional responses to increasing scales of disturbance, and potential implications for threshold dynamics in soft-sediment communities. Marine Ecology Progress Series, 413, 253–266. https://doi.org/10.3354/meps08745.

Notar, M., Leskovšek, H., and Faganeli, J., 2001. Composition, Distribution and Sources of Polycyclic Aromatic Hydrocarbons in Sediments of the Gulf of Trieste, Northern Adriatic Sea, Mar. Pollut. Bull., 42, 36–44.

Ogorelec, B., Misic, M., Faganelli, J., 1991. Marine geology of the Gulf of Trieste (northern Adriatic): sedimentological aspects. Marine Geology 99, 79−92.

Olivotti, R., Faganelli, J., Malej, A., 1986. Impact of ‘organic’ pollutants on coastal waters, Gulf of Trieste, Water Sci. Technol., 18, 57– 68.

Oug, E., Fleddum, A., Rygg, B., Olsgard, F., 2012. Biological traits analyses in the study of pollution gradients and ecological functioning of marine soft bottom species assemblages in a fjord ecosystem. Journal of Experimental Marine Biology and Ecology, 432–433, 94–105.

Paganelli, D., Marchini, A., Occhipinti-Ambrogi, A., 2012. Functional structure of marine benthic assemblages using Biological Traits Analysis (BTA), a study along the Emilia-Romagna coastline (Italy, North-West Adriatic Sea). Estuarine, Coastal and Shelf Science,96, 245−256.

Pearson, T. H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review, 16(January), 229–311.

Pearson, T. H., 2001. Functional group ecology in soft-sediment marine benthos: the role of bioturbation. Oceanography and Marine Biology: An Annual Review 39:233–267.

Petchey, O. L., Gaston, K. J., 2002. Functional diversity (FD), species richness and community composition. Ecology Letters, 5(3), 402−411.

Ponti, M., Colangelo, M. A., Ceccherelli, V. U., 2007. Composition, biomass and secondary production of the macrobenthic invertebrate assemblages in a coastal lagoon exploited for extensive aquaculture: valle Smarlacca (northern Adriatic Sea). Estuarine Coastal and Shelf Science 75:7989 DOI 10.1016/j.ecss.2007.01.021.

Poulain, PM., Kourafalou V. H., Cushman-Roisin, B., 2001. Northern Adriatic Sea. Physical Oceanography of the Adriatic Sea, 143-165.

Probert, P.K., 1984. Disturbance, sediment stability, and trophic structure of soft-bottom communities. Journal of Marine Research, 42, 893–921.

Queirós, A. M., Birchenough, S. N. R., Bremner, J., Godbold, J. A., Parker, R. E., Romero-Ramirez, A., Reiss, H., Solan, M., Somerfield, P. J., Van Colen, C., Van Hoey, G., & Widdicombe, S., 2013. A bioturbation classification of European marine infaunal invertebrates. Ecology and Evolution, 3(11), 3958–3985. https://doi.org/10.1002/ece3.769.

Querin, S., Crise, A., Deponte, D., Solidoro, C., 2007. Numerical study of the role of wind forcing and freshwater buoyancy input on the circulation in a shallow embayment (Gulf of Trieste, northern Adriatic Sea). Journal of Geophysical Research, 111, 1–19.

Reiss, H., Greenstreet, S. P. R., Sieben, K., Ehrich, S., Piet, G. J., Quirijns, F., Robinson, L., Wolff, W. J., Kroncke, I., 2009. Effects of fishing disturbance on benthic communities and secondary production within an intensively fished area. Marine Ecology Progress Series 394, 201–213.

Reiss, J., Bridle, J. R., Montoya, J. M., Woodward, G., 2009. Emerging horizons in biodiversity and ecosystem functioning research. Trends in ecology and evolution, 24(9), 505−514.

Richardson, B. J., Cibic, T., Acquavita, A., Aleffi, F., Bettoso, N., Blasutto, O., Vittor, C. De, Falconi, C., Falomo, J., 2008. Integrated approach to sediment pollution: A case study in the Gulf of Trieste. Marine Pollution Bulletin, 56(June 2004), 1650–1657.

Rigler, FH., Downing, J. A., 1984. The calculation of secondary production. In: Downing JA, Rigler FH (eds) A manual on methods for the assessment of secondary productivity in freshwaters. Blackwell Scientific Publications, Oxford, p 19–86.

Robertson, A.I., 1979. The relationship between annual production ratio and lifespans for marine macrobenthos. Oecologia 38, 193–202.

Rosenfeld, J. S., 2002. Functional redundancy in ecology and conservation. Oikos, 98(1), 156–162. Yachi, S., Loreau, M., 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. Proceedings of the National Academy of Sciences, 96(4), 1463–1468.

Russo, A., Artegiani, A., 1996. Adriatic Sea hydrography, Sci. Mar., 60, 3 – 43.

Hurlbert, S.H., 1971. The non-concept of species diversity: a critique and alternative parameters Ecology, 52, pp. 577−586.

Scheffer, M., Carpenter, S., Foley, J. A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature, 413(6856), 591.

Sigala, K., Reizopoulou, S., Basset, A., Nicolaidou, A., 2012. Functional diversity in three Mediterranean transitional water ecosystems. Estuarine, Coastal and Shelf Science, 110, 202−209.

Simmonds, K. R., 2010. The protection and preservation of the marine environment. Recueil Des Cours, Collected Courses, Volume 218 (2007), 74–107. https://doi.org/10.1163/ej.9780792313243.009-166.5.

Simpson, R. D., 2002. Definitions of biodiversity and measures of its value. Resources for the Future, Washington D. C., Discussion Paper, 02-62, 17.

Snelgrove, P. V. R., 1998. The biodiversity of macrofaunal organisms in marine sediments. Biodiversity and Conservation, 7(9), 1123–1132.

Socolar, J. B., Gilroy, J. J., Kunin, W. E., Edwards, D. P., 2016. How Should Beta-Diversity Inform Biodiversity Conservation? Trends in Ecology and Evolution, 31(1), 67–80. https://doi.org/10.1016/j.tree.2015.11.005.

Solan, M., Cardinale, B. J., Downing, A. L., Engelhardt, K. A., Ruesink, J. L., Srivastava, D. S., 2004a. Extinction and ecosystem function in the marine benthos. Science, 306(5699), 1177-1180.

Solan, M., Wigham, B. D., Hudson, I. R., Kennedy, R., Coulon, C. H., Norling, K., Rosenberg, R., 2004b. In situ quantification of bioturbation using time-lapse fluorescent sediment profile imaging (f-SPI), luminophore tracers and model simulation. Marine Ecology Progress Series, 271, 1−12.

Solan, M., Scott, F., Dulvy, N. K., Godbold, J. A., Parker, R., 2012. Incorporating extinction risk and realistic biodiversity futures: implementation of trait-based extinction scenarios. Marine biodiversity and ecosystem functioning: frameworks, methodologies, and integration. Oxford University Press, Oxford, UK, 127−148.

Solis-Weiss, V., Aleffi, F., Bettoso, N., Rossin, P., Orel, G., Fonda-Umani, S., 2004. Effects of industrial and urban pollution on the benthic macrofauna in the Bay of Muggia (industrial port of Trieste, Italy). Science of the total environment,328(1-3), 247−263.

Solis-Weiss, V., Aleffi, I.F., Bettoso, N., Rossini, P., Orel, G., 2007. The benthic macrofauna at the outfalls of the underwater sewage discharges in the Gulf of Trieste (Northern Adriatic Sea, Italy). Annales Series Historia Naturalis, 17 (1), 1−16.

Simpson, J. H., Bos, W. G., Schirmer, F., Souza, A. J., Rippeth, T. P., Jones, S. A., Hydes, D., 1993. Periodic stratification in the Rhine ROFI in the North Sea, Oceanol. Acta., 16, 23−32

Southwood T. R. E., 1977. Habitat, the templet for ecological strategies – presidential address to the British Ecological Society, Journal of Animal Ecology 46:337–365.

Sprung, M., 1993. Estimating secondary production from body weight and mass—a field test in a non-boreal intertidal habitat. Marine Ecology Progress Series 100, 103–109.

Srivastava, D. S., Vellend, M., 2005. Biodiversity-ecosystem function research: is it relevant to conservation? Annual Review of Ecology Evolution and Systematics, 36, 267−294.

Stachowitsch, M., 1991. Anoxia in the Northern Adriatic Sea: rapid death, slow recovery. Geological Society, London, Special Publications, 58(1), 119–129.

Stergiou, K. I., Karpouzi, V. S., 2002. Feeding habits and trophic levels of Mediterranean fish. Reviews in Fish Biology and Fisheries, 11: 217–254.

Stravisi, F., 1983. The vertical structure annual cycle of the mass field parameters in the Gulf of Trieste. Boll Oceanol Teor Applic 1:239–250.

Strong, J. A., Andonegi, E., Bizsel, K. C., Danovaro, R., Elliott, M., Franco, A., Garces, E., Little, S., Mazik, K., Moncheva, S., Papadopoulou, N., Patrício, J., Queirós, A. M., Smith, C., Stefanova, K., Solaun, O., 2015. Marine biodiversity and ecosystem function relationships: The potential for practical monitoring applications. Estuarine, Coastal and Shelf Science, 161, 46–64. https://doi.org/10.1016/j.ecss.2015.04.008.

Sugihara, G., 1980. Minimal community structure: an explanation of species abundance patterns. American Naturalist, 116, 770−787.

Teal, L. R., Bulling, M. T., Parker, E. R., Solan, M., 2008. Global patterns of bioturbation intensity and mixed depth of marine soft sediments. Aquatic Biology, 2(3), 207−218.

Törnroos, A., 2014. Interpreting marine benthic ecosystem functioning in coastal waters: validating the biological trait concept. Åbo Akademi University.

Trannum, H. C., Olsgard, F., Skei, J. M., Indrehus, J., Øverås, S., Eriksen, J., 2004. Effects of copper, cadmium and contaminated harbour sediments on recolonisation of soft-bottom communities. Journal of Experimental Marine Biology and Ecology,310(1), 87-114.

Tumbiolo, M.L., Downing, J.A., 1994. An empirical model for the prediction of secondary production in marine benthic invertebrate populations. Marine Ecology Progress Series 114, 165–174.

Turner, S.J., Thrush, S.F., Pridmore, R.D., Hewitt, J.E., Cummings, V.J., Maskery, M., 1995. Are soft-sediment stable? An example from a windy harbour. Marine Ecology Progress Series, 120, 219–230.

Usseglio-Polatera P., Bournaud M., Richoux P., Tachet H., 2000. Biological and ecological traits of benthic freshwater macroinvertebrates: relationships and definition of groups with similar traits. Freshwater Biology 43:175–205.

Vellend, M., 2001. Do commonly used indices of b-diversity measure species turnover? J. Veg. Sci., 12, 545–552.

Villnäs, A., Perus, J., Bonsdorff, E., 2011. Structural and functional shifts in zoobenthos induced by organic enrichment - Implications for community recovery potential. Journal of Sea Research, 65(1), 8–18.

Villnäs, A., Norkko, J., Lukkari, K., Hewitt, J., Norkko, A., 2012. Consequences of increasing hypoxic disturbance on benthic communities and ecosystem functioning. PloS one, 7(10), e44920.

Villnäs, A., Hewitt, J., Snickars, M., Westerbom, M., Norkko, A., 2018. Template for using biological trait groupings when exploring large-scale variation in seafloor multifunctionality: Ecological Applications, 28(1), 78–94. https://doi.org/10.1002/eap.1630.

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier, E., 2007. Let the concept of trait be functional! Oikos, 116(5), 882-892.

Violle, C., Reich, P. B., Pacala, S. W., Enquist, B. J., Kattge, J., 2014. The emergence and promise of functional biogeography. Proceedings of the National Academy of Sciences USA111:13690–13696.

Waters, T.F., 1977. Secondary production in Inland waters. Advances in Ecological Research 10, 91–164.

Weigel, B., Blenckner, T., Bonsdorff, E., 2016. Maintained functional diversity in benthic communities in spite of diverging functional identities. Oikos,125(10), 1421-1433.

Whittaker, R.H., 1960. Vegetation of the Siskiyou mountains, Oregon and California. Ecol. Monogr., 30, 279–338.

Whittaker, R. H., 1972. Evolution and measurement of species diversity. Taxon, 21, 213–251.

Wilson, M., and Shmida, A., 1984. Measuring beta diversity with presence–absence data. Journal of Ecology 72:1055– 1064.

Wrede, A., Beermann, J., Dannheim, J., Gutow, L., Brey, T., 2018. Organism functional traits and ecosystem supporting services. A novel approach to predict bioirrigation. Ecol. Indicat. 91, 737–743.

Zajac, R. N., Vozarik, J. M., Gibbons, B. R., 2013. Spatial and Temporal Patterns in Macrofaunal Diversity Components Relative to Sea Floor Landscape Structure. PLOS ONE 8(6): e65823. https://doi.org/10.1371/journal.pone.0065823

Zhang, Q., Warwick, R. M., McNeill, C. L., Widdicombe, C. E., Sheehan, A., Widdicombe, S., 2015. An unusually large phytoplankton spring bloom drives rapid changes in benthic diversity and ecosystem function. Progress in Oceanography, 137, 533−545.

Zuschin, M., Piller, W. E., 1994. Sedimentology and facies zonation along a transect through the Gulf of Trieste, Northern Adriatic Sea, Beitr. Palaeontol., 18, 75–114.www.acegasapsamga.it

2. Biological Traits Analyses

This chapter is adapted as Paper I from: Seyed Ehsan Vesal, Federica Nasi, Jessica Pazzaglia, Larissa Ferrante, Rocco Auriemma, Federica Relitti, Matteo Bazzaro, Paola Del Negro. The effect of sewage discharges on soft-bottom macrofauna thoroughly traits-based approach. Original manuscript accepted in Marine Pollution Bulletin.

2.1 Introduction

A large part of the world's population lives beside the sea and relies on the essential goods (e.g. fishing and aquaculture) and services (e.g. wastes assimilation) that the marine ecosystems provide (Costanza et al., 1997; Villnäs et al., 2018). However, the recent expansion of human activities in the marine domain has resulted in habitat alterations and biodiversity degradation (Lotze et al., 2018). Indeed, during the last decades, there was a large increase of studies focused on marine communities inhabiting coastal areas. Studying the effects of anthropogenic pressure on marine ecosystems is essential to conduct appropriate economic and environmental management with adequate monitoring programs (Katsanevakis et al., 2011).

Due to their ability to adapt their composition and structure in response to a different source of disturbance, soft macrofaunal communities are fundamental in assessments of impacts from human activities (Pearson and Rosenberg, 1978; Oug et al., 2012). In this direction, most studies focused on the structural aspect of macrofaunal species 'assemblages, such as abundance, biomass, and diversity. A wide variety of valid biotic indices relating to species composition and biodiversity of macrobenthic communities have been proposed, e.g., AMBI (AZTI's Marine Biotic Index; Borja et al., 2000), M-AMBI (multivariate AMBI; Bald et al., 2005) and BENTIX (Simboura and Zenetos, 2002), as an attempt to address the objectives of many European Directives (e.g., Marine Strategy Framework Directive-MSFD, EU Biodiversity Strategy and Water Framework Directive-WFD; D'Alessandro et al., 2020). These indices focusing on structural aspects of macrofaunal communities and relying on the taxonomic identification of species, rarely assessed the functional adaptation of macrofaunal invertebrates to stressed environments. In fact, species interact with and respond to the physical and chemical habitats with different patterns depending on their abilities (Díaz and Cabido, 2001). Every single species can play a significant role in the various function of ecosystems and, because of either natural causes or human activities, any changes in their composition can have adverse effects on ecosystem processes (Gray et al., 2006). Thus, the sole application of species composition may not be enough to explore the processes that sustain ecological systems (Díaz and Cabido, 2001). Following this issue, the new approach of the Biological Traits Analysis (BTA; Bremner et al., 2003) was recently developed, combining structural data of a macrofaunal community (species abundance or biomass) with the information on functional traits of every single species (Törnroos and Bonsdorff, 2012). Traits have been defined as a component of an organism's phenotype (e.g. body size, feeding structure, and life span) and represent the proxy of an individual's performance (Violle et al., 2007). Functional features of traits determine different responses to environmental factors and effects on ecosystem processes (Reiss et al., 2009). Therefore, BTA is nowadays considered a useful analytical method to better understand the relationships between organisms and ecosystem functioning (Solan et al., 2004; Bremner et al., 2006; Gagic et al., 2015). Besides, using this approach is possible to implement two functional aspects. Functional diversity represents the variety of functions settled by organisms in a community, comprising a component of biodiversity (Nasi et al., 2018). The other aspect is the functional identity, which indicates the role of a single species in the ecosystem, or rather, if a single trait is strongly linked to an ecosystem function, high occurrences of these traits may best predict the functioning (Gagic et al., 2015).

Many marine studies applied BTA to benthic assemblages concerning several environmental variables (e.g. Paganelli et al., 2012; Weigel et al., 2016; Nasi et al., 2020) and human-induced impacts (e.g., Gusmao et al., 2016; Krumhansl et al., 2016, D'Alessandro et al., 2020). The growing of human pressure on marine environments is enhancing the need for ecosystem understanding and reliable indicators of environmental health (Bremner, 2008). Since traits represent the link between life-history and habitat, the use of the multiple traits approach could support the marine management and policies to curbing human impacts on coastal environments (Beauchard et al., 2017). However, the BTA in marine management is still challenging because the development of marine indicators based on functional approaches needs more studies and should be based on complete sets of magnitude traits (Beauchard et al., 2017). Further, few studies have focused on the relationship between functional diversity and principal biotic indices (e.g., AMBI and BENTIX), to evaluate functional attributes of macrofaunal communities as an indicator in marine coastal management (Gusmao et al., 2016; Krumhansl et al., 2016; D'Alessandro et al., 2020). In particular, Gusmao et al., (2016) integrated and compared the functional information based on macrofaunal abundance (commonly used in BTA studies) with functional diversity carried out with biomass data. The use of biomass matrix in BTA approach to underline the effect of human pressure on coastal environments remains seldom investigated.

In this study, we focus on the macrofaunal community inhabiting a coastal area influenced by sewage discharges. Several studies described the structural responses of macrofaunal invertebrates subjected to high organic loads and contaminants from wastewaters (e.g. Pearson and Rosenberg, 1978; Solis –Weiss et al., 2007; Suoza et al., 2013), and others assessed the validity of different biotic indices, for the correct monitoring of this type of impact (e.g. Pinto et al., 2009; Ferrera et al., 2011). However, to the best of our knowledge, the influence of sewage discharges on the functional diversity of macrofaunal communities and trait composition in the sublittoral area of the Mediterranean Sea has not been investigated yet.

In this study, BTA was used: i) to explore and characterize the functional attributes of macrofaunal invertebrates nearby an area subject to wastewater loads; ii) to assess which matrices of structural data (i.e. species abundance and biomass) best explain the difference in functional diversity and identity of the community investigated iii) to assess the potential application of functional analyses to developing management practice for wastewater treatment systems. In particular, we aimed to answer the following questions: 1) Are the structural and functional patterns driven by specific sediment physical-chemical variables and contaminants? 2) Are there any differences in functional diversity and identity considering the structural abundance and biomass information? 3) Is it possible to include BTA of macrofaunal communities in coastal monitoring programs?

2.2 Material and methods

2.2.1 Study site

The Gulf of Trieste is located at the northernmost part of the Adriatic Sea. It is a shallow embayment of about 600 km^2 and a coastline of 100 km (Brambati and Catani, 1988). The Gulf is connected to the rest of the Adriatic Sea by a sill (~22 m depth) located in the southern part of the basin (Ogorelec et al., 1991); 10% of its area is <10 m and the maximum depth is about 25 m. The Gulf endures annual oscillations of temperature altering from 5°C to >24°C at the surface and from 6 °C to >20°C at the bottom. Marine sedimentation is strongly influenced by local river plumes, particularly supplied by the Isonzo River (Covelli and Fontolan, 1997). The annual average sedimentation rate is about 1 mm y^{-1} in the middle of the Gulf and 2.5 mm y^{-1} in front of River Isonzo (Covelli et al., 1999). The Gulf is also affected by the Eastern Adriatic Current (EAC), flowing along the Istrian coast to the northward and advecting warmer and saltier waters coming from the Ionian Sea (Poulain and Cushman-Roisin, 2001), which leads to general cyclonic circulation. However, the Gulf general pattern of currents may be quickly modified in response to intense local atmospheric forcing (winds) and river plume (Querin et al., 2007; Malaćić and Petelin, 2009). In particular, the basin is highly influenced by the Bora, a north-easterly wind characterized by a strong intensity that can mix the entire water column also favoured by the shallow depth of the basin (Querin et al., 2007).

The city of Trieste discharges its main urban sewage in the centre of the Gulf by the Servola disposal plant, which serves up to 270,000 inhabitants, with a maximum flow of 6,000 L sec^{-1} (Solis-Weiss et al. 2007). It is a mixed type plant, collecting and treating both meteoric and wastewaters of about 50 million m^3 per year (Novelli, 1996). Since 1992, the disposal plant depurates wastewaters by chemical-physical treatment and finally releases them into the sea through two adjacent pipelines (6.5 and 7.5 km). The last part of the pipes is characterized by the presence of 600 turrets for a total diffusional zone length of 1.5 km (1 km the longest and 0.5 km the shortest duct respectively). These conducts are located in the north-eastern part of the Gulf of Trieste at a depth between 20 and 23 m (45°38'36.30" N; 13°40'51.70"E).

2.2.2 Sampling design

Sediments were sampled during November 2016 and April 2018. To assess the influence of municipal wastewater discharge on macrofaunal community nearby the sewage outfalls and to best cover the entire diffusion zone, sediments were collected at 18 stations, gathered in three transects (“distal”, “medial” and “proximal”). In each transect, the stations were placed at increasing distance from the duct (at 5, 100, and 200 meters) (Fig.1). Besides, a reference station was positioned 2 km far from the distal end of the pipeline in the opposite direction to the average annual current (SSE 170 °).

Sediment samples for analyses of physical and chemical parameters (grain-size, total Organic Carbon-Corg, Total Nitrogen-TN contents, and redox potential-Eh) were collected by a Box Corer (Haps Frame-Supported

Bottom Corer, KC-Denmark) using PVC liners with a diameter of 13.5 cm and a height of 30 cm. For the macrofaunal community, sediments were retrieved with a van Veen grab (0.1 m^2) in three replicates of each station.

2.2.3 Redox potential, sediment grain-size, organic matter content and contaminants

Measures of redox potential (Eh) were carried out to assess the physical-chemical state of marine sediments, providing indications of oxidation-reduction processes that determine sediment oxic conditions (Colman and Holland 2000). The Eh was estimated from undisturbed superficial sediment (0-1 cm), obtained immediately after collecting core liners. The analysis was performed with Metrohm 704 voltimet, after the platinum electrode standardization (CRISON 5265) in Light's solution (Clesceri et al., 1996).

For grain-size analysis, sediments were sieved at 2 mm and pre-treated with 10% hydrogen peroxide before being analysed with a BECKMAN COULTER LS 13 320 Laser Diffraction Particle Size Analyzer. Data are expressed as percentages of sand, silt, and clay following the Udden-Wentworth grain-size classification (Wentworth, 1922). For chemical analyses, sediments were freeze-dried, homogenised and ground to fine powder. The contents of total organic carbon (Corg) and total nitrogen (TN) were measured using an elemental analyser CHNO-S Costech model ECS 4010, as described in Franzo et al., (2019). Corg and TN values were expressed as mg g^{-1}. Organic carbon to nitrogen molar ratio (C:N) was calculated and used as a proxy to infer the organic matter origin (Rumolo et al., 2011).

The concentration of mercury was carried out by atomic absorption spectrophotometry by cold vapour (Analyst 100, PerkinElmer, USA). The concentrations of 16 USEPA priority pollutant Polycyclic aromatic hydrocarbons (PAHs) were also measured according to Cassin et al., (2018) were detected. For this study, we used the sum of the analysed PAHs.

2.2.4 Macrofaunal samples processing and benthic indices

The sediments for the macrofauna community were sieved on a 1.0 mm mesh to retain the fraction of macrofaunal organisms. The retained sediment and organisms were immediately fixed in ethanol 80°. Macrofaunal invertebrates were identified under a stereomicroscope (Zeiss Discovery V.12, 8-110× final magnification) and counted. For the taxonomic identification, the keys listed in Morri et al., (2004) were used. The abundance number is expressed as individual per m^2.

Macrofaunal biomass was performed for each sampling station and period. To reduce the bias due to different developmental stages or size classes, the average biomass of species was achieved by weighting all individuals belonging to the same taxon and sampling station. Wet weight measurements, were obtained after 30 seconds in absorbent paper. Where biomass was not available for measurements, we applied the conversion factor proposed by Ricciardi and Bourget (1998) and Brey et al., (2001).

Biogenic Remain (BR) represents the fraction retained together with macrofaunal organisms after sieving and is mainly composed by fragments of death molluscs' shells, echinoderm skeletal parts, and polychaetes carbonate pipes. The volume of BR was measured after the removal of invertebrates alive at the time of sampling and is expressed as mL.

BENTIX index was estimated according to Simbura and Zanetos (2002). Besides, abundance (M-AMBI) and biomass (M-bAMBI) based indices were calculated using AMBI 5.0 software (Borja et al., 2000). For both indices, invertebrates are assigned a score for I to V, based on their tolerance, following AMBI library (Borja and Muxika, 2005). M-AMBI index is based on the following metrics: AMBI, Shannon diversity, and taxa richness (Mistri et al., 2018).

2.2.5 Biological traits analysis

The effect of sewage discharge on the benthic community was assessed using Biological Traits Analysis (BTA). BTA was applied on 276 species, considering 11 biological traits with 48 categories. The selected set of traits described important morphological, behavioural, and life history characteristics of marine benthic invertebrates and similarly were used in previous studies (Gusmao et al., 2016; Krumhansl et al., 2016) (Table 1). The taxa were coded based on their affinity for the chosen traits using the 'fuzzy coding' procedure. Through 'fuzzy coding', taxa can exhibit trait categories to different degrees taking into account the interspecific variations in trait expressions (Bremner et al., 2006). Traits for each species were derived from literature sources (Trainito and Doneddu, 2005; Hayward and Ryland, 2017) and databases (i.e. www.marlin.ac.uk/biotic; www.polytraits.lifewatchgreece.eu). In particular, for information on life histories, we followed the authors Giangrande (1997) and Rouse (2000). Polychaetes 'feeding habit' modes were obtained from Jumar et al., (2015). We obtained information from the literature for 'adult activity' traits (Queirós et al., 2016; Kristensen et al., 2012). Lastly, information on 'tolerance' traits were attributed following the five ecological groups from AMBI index (Borja and Muxika, 2005). The taxonomic resolution was kept at species level whenever possible but adjusted to genus or family when the information on traits was available only at a higher taxonomic level.

Functional Dispersion (FDis; Laliberté and Legendre, 2010) was calculated based on the fuzzy coding traits matrix and species abundance. FDis describes the abundance-weighted mean distance of individual species to their group centroid (all species community) in a multivariate functional space constructed by a principal coordinate analysis (PCoA) based on the Euclidian dissimilarity matrix of species traits (Villéger et al., 2008; Scheiner et al., 2017). FDis was calculated from the abundance matrix (FDis-abu) and biomass (FDis-biom). The functional identity was calculated as community level weighted means (CWM) of trait category expression. CWM is a widely used index that may reflect the traits strategies given by the species pool and environmental conditions of a site (Muscarella and Uriarte, 2016). CWM values represent the occurrence of a trait by species in each community values were weighted by the abundance(CWM-abu) and biomass (CWM-biom).

2.2.6 Data analysis

Firstly, all data were tested for normality and collinearity following Shapiro Wilk's and Spearman's rank correlation coefficient, respectively. Environmental parameters, biotic indices, and macrofaunal functional features (functional diversity indices and CWM values based on abundance and biomass data) were compared among sampling area and periods using univariate (Mann-Whitney U test and Kruskal-Wallis H) and multivariate analyses (one-way PERMANOVA with an unrestricted permutation of raw data and 9999 permutations). For each test, the following fixed factors were applied: i) 'transect'; ii) 'distance from the duct'. Abiotic parameters and macrofaunal community values (i.e., structural and functional) of the reference site were not included in the previous analyses.

The relationships between each functional and diversity index were tested with linear regressions (R^2). We assumed that biotic indices, that describe the ecological status of macrofaunal community, must be considered as predictor variables. To avoid mistakes due to covariation among variables, the linear regressions were previously tested without 'AMBI index' traits. Further, Spearman' rank correlation was performed to investigate the relationships between functional diversity and biotic indexes, and environmental parameters.

To test the significant relations between the different matrices, the RELATE routine was applied as follows i) abundance *vs* biomass; ii) CWM-abu *vs* CWM-biom.

Similarly, Principal Coordinates Analysis (PCoA) was performed on traits occurrences to highlight differences in functional modalities (CWM-abu and -biom, separately) among sampling stations and periods. Pearson correlations with PCO axes were also tested.

Also, environmental parameters were used to perform the Distance-Linear Modelling (DistLM) to assess the variables that explain the differences ($p<0.05$) in species abundance, biomass, and trait occurrences (CWM-abu and -biom). Before the analysis, the abiotic parameters were normalized and the option 'All specified' and R^2 were used as the selection procedure and criterion, respectively.

In this study, we applied the RLQ analysis (Dolédec et al., 1996) to look for relationships between trait-categories occurrences (abundance- and biomass-based, separately) and sediment variables. As a first step, we carried out the analysis separately on each of the following three tables: environmental variables (R), abundance (L), and traits (Q). For the abundance table, we applied Correspondence Analysis (CA) whereas, for the environmental tables, we applied Principal Component Analysis (PCA). Regarding fuzzy-coded trait data, a fuzzy correspondence analysis (FCA) was conducted. Afterward, we carried out RLQ analysis, considering simultaneously the three components R, L, and Q. This analysis estimates the correlation between functional traits and environmental components through the computation of a crossed array (cross-covariance matrix weighted by abundances). The fourth-corner methods were applied to measure, one at a time, the

associations between the species traits and the environmental variables. We combined two permutation models (one for the samples and the other one for the species; Dray et al., (2014), carrying out 49,999 permutations and applying the adjustment of p-values for multiple testing (False Discovery Rate [FDR] method; Benjamini and Hochberg, 1995). The correlations between traits and environmental variables were considered significant if the largest p-values of the permutation models (i.e., samples and species permutation models) were lower than α. Before the analyses, the Hellinger transformation was applied to abundance and biomass data.
Univariate and multivariate analyses were carried out using STATISTICA 7 and PRIMER 7 (PRIMER-E Ltd. Plymouth, UK), respectively. For the multivariate analyses, the two matrices (i.e., species composition and CWM values) were square root, and the Bray- Curtis similarity was applied. Lastly, functional analyses, RLQ-fourth corner tests, and correlations were computed using the software program R, version 3.5.2, R packages: 'FD', 'ade4', and 'corrplot', respectively (R Development Core Team, 2018).

2.3 Results

2.3.1 Environmental variables

In the study area, sediments were mainly composed by silt (average value: 54.7±6.8 %) in both periods. Besides, higher percentages of silt were observed in 2016, compared to 2018, (U test: z=4.1; p<0.01). In the first sampling period, sand fraction was completely absent only at -2D, whereas the maximum was measured at -1M (24.8%). Conversely, higher sand contents were noticed in 2018 (U test: z=-3.1 p<0.01). Sand percentages varied between 5.7% at 2M and 24.8% at -1M. In both sampling periods, sand fraction significantly increased at stations nearby the duct (H= 7.7; p<0.01). Clay percentages varied from a minimum of 25.3 % at -1M and 38.9% at -1D in 2016; whereas ranged between 22.4% at 0D and 42.5% at RS, in 2016 and 2018, respectively (Table 2). Overall, high volumes of BR were detected at stations located nearby the duct compared to the farther ones.

In both sampling periods, higher values of Corg and TN were observed in the diffusion area compared to RS. In particular, Corg values at stations gathered in the 'distal' transect were significantly higher than stations in the 'medial' one (H test *post-hoc* comparison z=2.8; p<0.05). In April 2018 greater Corg and TN contents were noticed at 0D (60.5 and 3.8 mg g^{-1}), whereas the lowest value was observed at RS (10.6 and 1.2 mg g^{-1}, respectively). A similar pattern was noticed for C:N ratio. In both sampling periods, a higher ratio was observed in the diffusion area compared to RS. In particular, greater C:N values were detected at stations located nearby the duct (11.1 and 18.6 at 0D, in 2016 and 2018, respectively). As supported by H test, significant differences in C:N ratio were observed at stations placed at 5 m from the duct compared to ones located at 200 m (*post-hoc* comparison z=2.6; p<0.05). Regarding Eh values, significant differences were detected among transects (H=11.6; p<0.01; 'distal' *vs* 'medial' z=2.7; p<0.01). In particular, very low Eh values were noticed at the station nearby the main outfall (-255 and -290 mV at 0D) if compared to RS (95

and 54 mV) in 2016 and 2018, respectively. The highest amount of BR was estimated in 2016 than in 2018 (U test z=4.8; $p<0.01$). In both sampling periods, mercury concentrations significantly varied among stations located at increasing distance from the duct (H=7.7; $p<0.01$). In particular, Hg significantly increased at stations located at 200 meters from the pipe compared to the nearest ones (200m *vs* 100m z=2.7; $p<0.02$).
At stations 2FD (Hg: 0.77 mg Kg^{-1}) and 1FD (0.74 mg Kg^{-1}) the highest Hg contents were detected in 2016 and 2018, respectively. On the contrary, in both sampling periods, significant differences in PAHs concentrations were noticed among transects (H=12.0; $p<0.01$). In particular, higher PAHs contents were observed at stations gathered in the distal transect compared to the 'medial' one ('distal' *vs* 'medial' z=2.7; $p<0.01$), except for the maximum PAHs value observed at 0P (660 mg Kg^{-1}) in 2018.

2.3.2 Macrofaunal abundance and biomass

Total abundance ranged from 326.6±90.7 ind. m^{-2} at RS to 2546.7±2281.0 ind. m^{-2} at 0D in 2016; whereas a minimum of 360.0±157.2 at 0P and a maximum of 3436.6 ± 464.9 at -1D were observed in 2018. In 2016, the total values of biomass varied between 18.1±10.2 (-2P) to 546.6 ±876.3 g m^{-2} (-2M). Conversely, lower values were measured in 2018, with a minimum at RS (18.7±13.1 g m^{-2}) and a maximum at -1M (2094.6±1741.1 g m^{-2}). The PERMANOVA main-test performed on macrofaunal community abundance highlighted the difference between years, transects, and distance from the pipeline (Pseudo-F=5.18; 1.96; 2.64; $p<0.001$). In particular, by PERMANOVA pair-wise tests, differences in species composition were observed between stations gathered in 'distal' and 'medial' transects (t=1.69; $p<0.001$) and among stations located at 5 m vs 100 m (t=1.53; $p<0.05$) and 5 m vs 200 m (t=2.06; $p<0.01$). These differences were, principally related to the high dominance of polychaete *Capitella capitata* at stations gathered in distal transect and at 5 m from the duct. In particular, a great number of *C. capitata* was observed at 0D in 2016 (2116.6±2317.5 ind. m^{-2}). Moreover, comparing the macrofaunal species abundance and biomass matrices, they were significantly related (RELATE test: rs=0.62; $p<0.01$). Similarly, PERMANOVA main-test carried out on biomass matrix highlighted significant differences between sampling periods (Pseudo-F=2.25; $p<0.05$), as well as among the stations located at a different distance from the duct (Pseudo-F=2.55; $p<0.001$). On the contrary, no significant differences were noticed among transects. Besides, by pairwise comparisons, significant differences were highlighted among stations located nearby the duct to the farther ones (5 m *vs* 100 m: t=1.39; $p<0.05$ and 5 m vs 200 m: t=2.11; $p<0.001$). The dissimilarity among station position is linked to the highest biomass at stations located far from the duct, principally due to the presence of the bivalve *Atrina fragilis.* The highest weight of *A. fragilis* was measured at -1M in 2018 (1809.6±1566.3 g m^{-2}). The distance-based Linear Model (DistLM) performed on macrofaunal abundance highlighted sand, silt, and clay fractions, Corg, C:N, Eh, BR and Hg as important drivers in species distribution among stations and periods. Similarly, the same drivers were obtained with DistLM calculated on biomass, except for Corg, TN, Eh, and PAHs. The outputs of DistLM analyses are summarized in Table S1.

2.3.3 Biotic and functional indices

The results of biotic indices are listed in Table 3. The calculation of BENTIX showed overall ‘moderate’ ecological quality in many stations of the diffusion zone. For both sampling periods, ‘poor’ conditions were detected at RS. In particular, in 2018 stations located in the ‘distal’ transect were characterized by lower values of BENTIX index conferring ‘poor’ EcoQS. Overall high values of M-AMBI and M-bAMBI indices were calculated in the whole sampling area for both sampling periods. Many stations were classified as ‘good’ and ‘high’ EcoQS. However, the lowest values of M-AMBI were measured at 0D in 2016 (0.22) and 2018 (0.39), and conferred thus a ‘poor’ and ‘moderate’ EcoQS, respectively. Moreover, as corroborated by H test, significantly lower values of M-AMBI were noticed at stations gathered in the ‘distal’ transect compared to stations in ‘medial’ and ‘proximal’ one (‘distal’ *vs* ‘medial’ $z=3.15$ $p<0.01$; ‘distal’ *vs* ‘proximal’ $z=2.62$ $p<0.01$). Further, M-bAMBI highlighted ‘poor’ condition at 0D in 2016 (0.36), but also ‘moderate’ EcoQS in 2016 at 2M (0.51) and RS; and in 2018 at RS (0.49).

The number of species varied from a minimum of 24 (at 0D) to a maximum of 93 (at 1M) in 2016 and 2018, respectively. In particular, higher values of species richness were noticed at stations gathered in the ‘medial’ transect compared to the ‘distal’ one ($z=3.54$; $p<0.01$). Overall, as confirmed by the U test, a higher number of species was observed in 2018 compared to 2016 ($z=-1.99$; $p<0.05$). The FDis-abu followed the trends observed for the species richness in both sampling periods. The lowest values of FDis-abu were recorded at stations located nearby the main outfall (0D: 2.18 in 2016 and 3.86 in 2018), whereas the highest values were noticed at -2M (6.91) and -1M (7.58) in 2016 and 2018, respectively (Fig. 2). As species richness, FDis-abu showed significantly lower values at stations gathered in the ‘distal’ transects compared to the ‘medial’ one ($z= 2.77$; $p<0.01$). FDis-biom varied from 0.56 (at 2P) to 6.98 (at 0M). Moreover, significant different values were observed between station positions ($H=7.00$; $p<0.05$) (Fig. 2).

Regarding the relation between functional and biotic indices, M-AMBI and M-bAMBI showed a relation with FDis-abu (i.e., increased with environmental healthy). In particular, FDis-abu was significantly related to M-AMBI and the number of species with a higher value of R^2 (Fig. 3). On the contrary, for FDis-biom no relations with biotic indices and number of species were noticed. Lastly, the number of species and biotic indices (i.e. M-AMBI and M-bAMBI) were significantly correlated with sand percentage and Eh, but were negatively related with silt and Hg. FDis-abu highlighted a high positive correlation with Eh and a negative one with biogenic remains (BR) (Fig. 4).

2.3.4 Functional traits occurrences

The PERMANOVA tests carried out on CWM values based on species abundance and biomass, highlighted significant differences among stations located at 5, 100, and 200 meters from the pipeline (Pseudo-F=2.82 and 2.47 $p<0.05$, respectively). Besides, for both matrices, significant variations in functional traits occurrences were noticed between stations nearby the duct and farther ones (PERMANOVA pairwise tests: $t= 2.09$; $p<0.01$

and 2.00; p<0.05, respectively). On the contrary, CWM-abu significantly differed from sampling periods (t=3.27; p<0.01). The latter result confirmed the different traits occurrences described by CWM-abu and -biom, among sampling stations and periods, since no match was tested by RELATE analysis.

The DistLM performed on CWM-abu highlighted sand and silt fractions, Corg, C:N, Eh, BR, and Hg act as important drivers in species distribution among stations and periods. On the contrary, none of the selected abiotic variables were the drivers for CWM-biom values. The outputs of DistLM analyses are summarized in Table S2.

The Principal Coordinates Analyses (PCoA) performed on CWM species abundance showed clear separation in assemblage functional trait composition from the station nearby the duct (0D in 2016 and 2018) (Fig. 5a). This separation was associated mainly with the first axes (PCoA1, Table S3) for modalities belonging to the main group of traits as 'life histories', 'adult activity', and 'response to anthropogenic pressure'. In particular, at this station high occurrence of *conveyors, surface- subsurface-deposit feeders,* and *Group V* species were noticed. Besides, the PCoA 2 plotted a group of stations positioned at 100, 200, and RS (2018) characterized by higher occurrences of *sessile, epibionte, no movement*, and *no bioturbation modalities*. On the contrary, no clear pattern of distance gradients was observed at PCO performed on CWM-biom values. However, the great functional variation among stations was associated both for PCoA1 and PCoA2, with the main group of traits as 'adult size and shape', 'life history', 'Adult activity'. Lastly, a group of stations was plotted separately from the rest, resulting in a higher correlation with the negative axes PCoA1. These stations were characterized by high occurrences of modalities as *shell protection, adult longevity 6-10 yrs, suspension feeder, superficial modifier*, and *interface*. (Fig. 5b and Table S3).

The RLQ and fourth root analyses were carried out on traits occurrences, abundance and, biomass. However, since no significant relationships among traits occurrences biomass-based and environmental parameters were observed, the results were not reported. The RLQ analysis, performed on species abundances accounted for 78.9 % of the total variance (Fig. 6a and Table 4) and the sampling stations were plotted based on the distance from the pipeline. Station nearby the main outfall was plotted along RLQ1 axis (left- the hand of the plot), conversely, the farthest stations were positioned at the negative part of RLQ1 axis. Fig. 6 underlines the significant relationships between the RLQ environmental axes and individual traits (b), and between the RLQ trait axes and individual environmental variables (c). After the application of the FDR adjustment method significant high correlations were recorded (p<0.001). Significant positive correlations were also recorded between *semi-continues* and AxcR1 (on the right of the diagram). Negative correlations were found for the same axis vs *iteroparous* (on the left of the diagram). Regarding the second axis (AxcR2), positive correlations were recorded for the *dorso-ventally compressed* (on the top of the diagram), whereas negative correlations were recorded for the *30-80mm adult size* and *vermiform* traits (on the bottom of the diagram). Concerning environmental variables, positive relations were recorded between AxcQ1 and TN, Corg and C:N, whereas negative correlations with AxcQ1 and Hg and Clay. The second AxcQ2 was positively related with Eh and sand and negatively with silt and BR.

2.4 Discussion

In this study, we explored the soft-sediment invertebrates assemblages influenced by sewage-derived materials. We covered the entire diffusion area with an accurate sampling design, that considered not only the sediments in front of the main outfall but also the entire diffusion zone of the pipelines. Important information of macrofaunal community features were obtained as well, considering the stations placed at gradually increasing distances from the contamination sources. Doing that, we were able to highlight the structural and functional attributes of macrofaunal invertebrates in an area subject to wastewater discharges, determining, in particular, which structural data matrices (i.e., species abundance and biomass) can best explain the difference in functional diversity and identity of the community investigated. Furthermore, we tested the applicability of functional approaches in an area deeply influenced by anthropogenic pressures for the potential integration of BTA in monitoring activities.

2.4.1 Abiotic features

The wastewaters discharged into coastal environments contain considerable loads of organic matter. Moreover, an increase in the concentration of the nutrients and suspended particles are expected near the sewage-affected areas (Waldron et al., 2001). In this study, we did not find an evident pattern of Corg and TN contents depending on the distance from the pipeline, or rather, a decrease in Corg content was not observed at stations about 200 meters from the duct. Waldorn et al., (2001) suggested that the combined effect of wind and sea current actions might modify organic matter deposition throughout the sampling area. Cozzi et al., (2008) also demonstrated that meteorological characteristics and winds could have affected the material sedimentation nearby the outfall. The authors illustrated that the direction and intensity of all currents played an important role in the horizontal flow of wastewaters. Nevertheless, in agreement with Filgueiras et al., (2007) we found higher values of Corg and TN in the diffusion area in comparison with RS. Furthermore, we evidenced allochthones input of organic matter by sewage-derived materials, nearby the main diffusion zone (higher values of C:N ratio). In both periods, the very low values of redox potential (Eh) in the diffusion area compared to reference suggested hypoxic conditions at sediments. In fact, under excessive nutrient load, the sedimentation of organic matter may exceed the rate of its degradation (Taylor et al., 1998), and microbial decomposition severely depletes dissolved oxygen, lacking sufficient oxygen to support most organisms (Arend et al., 2011). Also, the higher values of sand at stations located adjacent to the duct could be linked to the presence of the sewage pipeline as also confirmed by Melis et al., (2019). Moreover, the same distribution pattern of the sandy fraction was observed for biogenic remains (BR). High values of BR were measured at stations close to the pipeline in both sampling periods, especially that one located at the main outfall. The progressive increase of sand fraction and BR could be ascribable to the characteristics of the disposal plant. The sewage system is a mixed type, collecting not only wastewater but also meteoric ones. Therefore, in addition to shell fragments which are normally present in marine sediments (Díaz et al., 1995), a high amount of vegetal debris coarse sediments (i.e., gravel) is commonly observed at the stations close to the duct. In this study, we also analysed the concentrations of contaminants such as Hg and PAHs, as potentially bring by sewage sludge. Hg values significantly increased at stations located at 200 meters from the duct, that are characterized by high percentages of clay. Indeed, the concentrations of heavy metals generally increase as particle size decreases (Yao et al., 2015). However, it is well documented that the high values of Hg in the Gulf of Trieste are generally linked with the Isonzo River, which is known to be the main source of particulate Hg due to long-term cinnabar extraction activity at the Idrija mining district (Slovenia) (Covelli et al., 2007). On the contrary, higher PAHs contents were measured at stations gathered in the 'distal' transect compared to 'medial' one that could potentially be related to sewage outfall. Bolam et al., (2011) illustrated that the highest mean values of PAHs were detected close to the disposal site, which follows the same pattern found in our study.

2.4.2 Macrofaunal community structure and functional characteristics

The macrofauna community investigated is typical of coastal environments influenced by high and long-term deposition of sewage materials (Solis –Weiss et al., 2007; Suoza et al., 2013). This community followed the model of Pearson and Rosenberg (1978) in both sampling periods. We observed significant differences in community structure among stations and transects. At stations nearest the pipe, we noticed high macrofaunal densities and low biomasses, whereas the opposite was observed at the farthest stations. Also, a great variation in taxa composition following the distance gradient was detected. This difference is mainly ascribable to the dominance of the polychaete *Capitella capitata*. The presence of wastewater discharge is mirrored by the dominance of this opportunistic *r*-strategist species. Our results showed a high dominance of *C. Capitata* at stations gathered in a 'distal' transect, in particular at 0D in both periods. In fact, *C. capitata*, which is a mainly stress-tolerant species, is commonly observed, often in high abundance, in areas influenced by the large amount and long-lasting deposition of organic matter and oxygen depletion (Pearson and Rosenberg, 1978; Grémare et al., 1989).

In our study, that species abundances and biomass are not affected by Hg and PAHs but are rather affected by the amount and feature sewage-derived materials origin. For macrofaunal density, the grain-size distribution and organic matter together with Eh and BR were the main drivers of species distribution. Sediments composition is a key element in structuring macrofauna community and the distribution of dominant species, also when related to organic enrichment (Rhoads and Boyer, 1982; Hermand, 2008). Species such as *Atrina fraglis* and *Maldane glebifex* were typically observed in the Gulf of Trieste muddy-sediments at these depths (Nasi et al., 2017). Conversely, typical sandy fraction species were observed at stations close to the duct, in particular the polychaete *Owenia fusiformis* (Pinedo et al., 2000). The species distribution pattern resulted also influenced by Eh levels. The hypoxic conditions measured mostly at 0D likely caused an increased presence of opportunistic and stress-tolerant species as observed in our study case. Thus, our results agree with those Solis –Weiss et al., (2007) and Grey et al., (2002) pointing out that sewage discharges could influence the oxygen concentration within sediments, thus promoting the stress-tolerant macrofaunal species. To confirm this, in addition to *C. Capitata* other polychaetes with opportunistic behaviour were observed at 0D (e.g. *Heteromastus filiformis* and *Lumbrineris latreilli*).

The influence of contamination on the reduction of species richness has been largely documented (e.g., Johnston et al., 2015; Mutlu et al., 2010). In conditions of moderate organic enrichment, an increase in species numbers can occur (Solis-Weiss et al., 2007), while in conditions of high organic load a decrease in species diversity is expected (Simonini et al., 2004; Auriemma et al., 2016). In fact, in our study, the highest number of species was found at the 'medial' transect, whereas 0D showed the lowest number of taxa. Besides, the condition of the mixed grain size with a high amount of sand in 'medial' transect, resulted in additional ecological niches, increasing the possibility of a major number of species establishment Ergen et al., (2007). This was also confirmed by the biotic indices, which support the occurrence of moderate organic stress on macrofaunal community. 'Good' ecological status was conferred to stations in 'medial' transect, whereas lower values of AMBI were measured at 0D.

In our study, the FDis values were higher if compared to heavily contaminated sites (Nasi et al., 2018), except for the lowest values recorded in front of the main outfall. The major amount of sewage-origin materials seems to affect functional diversity, as corroborated by the significant correlation between BR and Eh. Conversely, in 'medial' transect FDis was comparable to coastal areas not influenced by anthropogenic pressures (Weigel et al., 2016). FDis-abu, in particular, showed spatial patterns with higher values in the non-contaminated condition and was significantly related with species numbers and M- and Mb-AMBI (see Fig. 3). The higher values of functional diversity occurred together with higher values of species richness, meaning that several species strictly perform different functions and therefore the community might be more susceptible to changes in ecosystem functions caused by species loss (Gladstone-Gallagher et al., 2019).

Regarding the functional identity, patterns in the distribution of modalities among sampling positions and transects were highlighted by CWM-abu values (see Fig.5a and 6a). As observed by Oug et al., (2012) and Krumhansl et al., (2016), the functional attributes were affected by organic effluents, showing distance-related gradients from the most contaminated stations. PCO and RLQ-fourth corner analyses have plotted high *conveyors*, *surface-subsurface-deposit feeders,* and *Group V* modalities nearby the stations directly influenced by sewage discharges. In particular, the traits as *30-80 mm maximum size, vermiform,* and *semi continues* were related with Eh, BR and organic matter. The high expression of *Vrm, M/L,* and *Ssdep* modalities were likely due to the presence of polychaetes *C. capitata, Naineris Laevigata,* and *Marphysa sanguinea*. These results agree with those of Gaston et al., (1998) and Oug et al., (2012), which found a preponderance of *Sddf* in sediments subject to anthropogenic stress, and low proportion of other trophic groups (e.g., predators, suspension feeders). Gaston et al., (1998) suggested that subsurface deposit feeders, especially *C. capitata*, which is recognized as a general indicator of disturbance would be the trophic group most likely to develop pollution tolerance because they may regularly encounter allochthones organic matter released from sediments.

Furthermore, we observed a reduction of ‘mobility’ and body size in stations more influenced by sewage-derived materials. The high expression of *semi-motile* trait category was observed at 0D, whereas an increase in the abundance of *sessile* organisms was noticed toward the stations placed at 100 and, in particular, at 200 meters from the duct (see Fig 5a). At the same stations, the high dominance of *Dorso-ventral compressed, shell-protection,* and *suspension feeder* modalities related to high contents of sands were observed. The latter traits belong principally to bivalve invertebrates (e.g., *Musculus subpictus; Atrina Fragilis)*. The presence of suspension feeder, in particular bivalves, suggests that sediments might be affected by the low disposal of organic materials (Trusch et al., 2004). The dominance of these traits, performed by bivalves, make them fundamental players in benthic-pelagic coupling. They can enhance sediment primary productivity, capturing large quantities of suspended organic matter and sinking phytoplankton, and then incorporating them into sediments through pseudo-faces, stimulating the microbial loop (Jones et al., 2011; Törnroos and Bonsdorff, 2012). Conversely, nearby the main outfall *conveyor* species, such as *C. Capitata*, classified as head-down conveyor-belt feeders, can promote benthic cycling (Kristensen et al., 2012). In general, *conveyors* move sediment particles through their gut by ingestion and secretion, transferring particles from deeper to superficial layers and vice-versa enhancing the organic matter remineralization (Belley and Snelgrove, 2016). This activity may contribute to modify sediment properties and promote microbial population resulting in accelerated degradation of organic matter (Kinoshita et al., 2008; Wild and Huettel, 2005).

Regarding the ‘life history’ modalities, the different reproductive frequencies represent survival strategy in presence of a periodic disturbance, being therefore identified as typical of unstable environments (Paganelli et al., 2012). In this study, opportunistic species showed *semi-continues* traits, especially *C. capitate* which tends to spread larvae (pelagic development) if food is supplied, rather than retained larvae (non-pelagic development) if the food is no longer available (Grémare et al., 1989). This confirms the high plasticity of these species to environmental constraints. Lastly, the *iteroparous* trait category was the dominant reproductive modality for the benthic invertebrates at stations located at 200 meters from the duct and reference site. This kind of reproduction frequency generally belongs to K-strategists that breed several times during a lifetime. These species have delayed reproduction, slow growth, and longer life span with a less pronounced numerical fluctuation than semelparous, such as *semi-continues* invertebrates (Giangrande, 1997). The presence of *iteroparous* invertebrates confers more stability to the community throughout the time due to the low variation in species turnover (Törnroos and Bonsdorff, 2012).

2.4.3 Differences between abundance and biomass-based analysis

In this study, the structural and functional features of macrofaunal community followed the classical model of Pearson and Rosenberg (1978), which predicts the responses of abundance, biomass, and species richness for different levels of organic contamination (Pearson and Rosenberg, 1978; Rosenberg, 2001). However, the BTA results, derived from both abundance- and biomass-based functional analyses, show a clear inconsistency in functional trait composition among the stations in both study periods, as corroborated also by RELATE analyses. Besides, a relationship between functional diversity abundance-based and the M-AMBI and M-bAMBI was found, but no relation was highlighted with FDis-biom. The latter result was confirmed also by Gusmao et al., (2016). The authors underlined that results could differ depending on the use of abundance or biomass as predictive measures. Our results evidenced that BTA (CWM and RLQ-fourth corner) abundance-based underlined differences in functional attributes along the distance gradient. This result is related to the numerical dominance of small-sized and thin stress-tolerant species at enriched conditions, which do not display an adequate part of the biomass. The opportunistic polychaete *C. capitata* was dominant at stations gathered in distal transect and at 5 m from the duct and represented the major drivers of abundance-based results in both sampling periods. The biomass-based followed the weight of bivalves, which seem to benefit from a moderate level of sewage inputs. However, in our case, the main driver of the biomass-based results is represented by the high weights of the bigger Mediterranean bivalve, *Atrina fragilis.*

The analysis performed with CWM values (abundance- and biomass-based) evidence a strict relation with the matrices applied and the presence of high numerical values that drove the traits occurrences. On the other hand, the RLQ-fourth corner (abundance-based) showed clear trait-environment relationships along the distance gradient from the main outfall. Peres-Neto et al., (2017) indicated that the fourth-corner approach outperforms the CWM-based one in terms of statistics' sampling accuracy and statistical power. Since permutations were done for species, traits, and environmental variables separately, this analysis better highlighted the variation of traits along environmental gradients.

On a general basis, we infer that abundance-based analysis in functional assessments is more appropriate even in presence of species numerically dominant or with higher weights that are expected to survive in a moderate environmental disturbance. Biomass-based analysis confirmed to be an unpredictable surrogate for abundance, especially in response to anthropogenic organic enrichment.

2.4.4 Functional attributes in monitoring programs

Macrofaunal is traditionally used in monitoring programs, in particular, to assess the influence of sewage discharges in the surrounding environments. Moreover, this aspect is extensively integrated into the monitoring programs due to the long-term variations of the sludges, caused by the implementation and modification of treatments in the sewage plants (Calabretta et al., 2008). Since the ecosystem processes are influenced by the functional characteristics of the organisms, rather than by taxonomic identity (Grime, 1997), the use of structural features of macrofaunal communities alone might give misleading information regarding the function performed by macrofaunal communities that sustain an ecological system (Díaz and Cabido, 2001). Therefore, the monitoring program, in the framework of ecosystem-based management needs to balance the expected outcomes due to various anthropogenic activities and requires metrics, indices, and systematic methods able to assess the variation of ecosystem functioning due to anthropogenic contaminations (Mangano et al., 2017).

In our study, functional diversity resulted significantly related to the benthic indices (i.e. AMBI and Mb-AMBI). Moreover, FDis-abu evidenced significant relationships with the abiotic variables strictly linked to sewage contaminations (i.e., Eh and Br). Our results support the validity of FDis index in the study of environmental health; as also confirmed by other studies (i.e., D'Alessandro et al., 2020; Gusmao et al., 2016). Overall, the interpretation of the BTA and the functional diversity indices depend on the selected traits. The *a priori* selection of traits and categories is fundamental when performing BTA because certain types of categories are more relevant in some circumstances than others (Bremner, 2008). However, in this study, for the calculation of macrofaunal functional features, we used a huge set of traits to cover the different functions performed by macrofaunal invertebrates. Along with traits relevant for sediment-related processes, as 'adult activities' (e.g., bioturbation and mobility) and 'feeding habits', we integrated the analyses with information regarding the 'longevity' and 'reproductive frequencies'. Life-cycle traits are related to the reproductive strategy of a species and its habit (Paganelli et al., 2012). This can give information on the development over time of communities influenced by human impact

Even though we found a relationship between biotic indices and functional diversity, the traits related to the AMBI index did not evidence a pattern in distance gradient from the main outfall. The *Group V* resulted highly expressed at the 'distal' station because of the high density of *C. Capitata*, since this species belongs to *Group V* in the AMBI index. Notwithstanding these results and the type of traits (they do not represent a function exploited by the invertebrates), we should not consider inserting into this kind of trait in the functional identities assignment.

Lastly, these sets of traits, together with a sampling design that considered the effect of different levels of sewage contamination evidenced their possible application in monitoring programs. The sampling of a distance gradient from the main source of pollution, considering also the entire diffusion area, would represent a robust way to test the macrofaunal functional features and their consistency of the described patterns in response to sewage contamination.

2.5 Conclusion

This study described spatial and temporal structural attributes and functional features of macrofaunal community influenced by sewage discharge. We observed significant differences in community structure among station positions and transects. High densities and low biomasses were observed nearby the pipeline whereas the opposite was observed at the farther stations. The abundance-based metric better evidenced variation patterns than those based on biomass. Functional abundance-based analyses highlighted that *vermiform*, *semi-continues*, *conveyors*, and *subsurface-deposit feeders* dominated stations subject to continuous sewage discharge. On the contrary*, shell-protection,* and *suspension feeder* modalities at stations far from the main outfall suggest that sediments might be affected by the low disposal of organic materials. Overall, the functional and structural analyses of macrofaunal community indicated that surrounding environments are affected by moderate organic stress in both sampling periods. Moreover, the similar pattern observed between FDis (abundance-based) and biotic indices displayed that functional diversity is related to benthic environmental health. Hence, we suggest applying abundance-based BTA (FDis and functional identity) as a reliable approach to detect the effect of sewage discharge on functional trait composition. Moreover, the use of approaches able to give an accurate characterization of the association between traits and their sensitivity to environmental stressors could be useful to predict uncertain events. Thus, we infer that BTA could hold a high potential for application in real-world assessments and monitoring environmental quality.

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References

Arend, K.K., Beletsky, D., Depinto, J.V., Ludsin, S.A., Roberts, J.J., Rucinski, D.K., Scavia, D., Schwab, D.J., Hook, T.O., 2011. Seasonal and interannual effects of hypoxia on fish habitat quality in central Lake Erie. Freshwater Biology, *56*, 366-383. https://doi.org/10.1111/j.1365-2427.2010.02504.x

Auriemma, R., Nasi, F., Del Negro, P., 2016. The macrozoobenthic fauna of the bottoms nearby the underwater sewage duct of Trieste (Northern Adriatic Sea). Biologia Marina Mediterranea, 23(1), 90.

Bald, J., Borja, A., Muxika, I., Franco, J., Valencia, V., 2005. Assessing reference conditions and physico-chemical status according to the European Water Framework Directive: A case-study from the Basque Country (Northern Spain). Marine Pollution Bulletin, 50, 1508–1522.

Beauchard, O., Veríssimo, H., Queirós, A.M., Herman, P.M.J., 2017. The use of multiple biological traits in marine community ecology and its potential in ecological indicator development. Ecological Indicators, 76, 81-96. https://doi.org/10.1016/j.ecolind.2017.01.011

Belley, R & Snelgrove, P., 2016. Relative contributions of biodiversity and environment to benthic ecosystem functioning. Frontiers in Marine Science, 3, 242. https://doi.org/10.3389/fmars.2016.00242

Benjamini, Y & Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society, 57(1), 289-300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bolam, S.G., Barry, J., Bolam, T., Mason, C., Rumney, H.S., Thain, J.E., Law. R.J., 2011. Impacts of maintenance dredged material disposal on macrobenthic structure and secondary productivity. Marine Pollution Bulletin, 62(10), 2230-2245. https://doi.org/10.1016/j.marpolbul.2011.04.012

Borja, A., Franco, J., & Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine Pollution Bulletin, 40(12), 1100-1114. http://ambi.azti.es.

Borja, A. & Muxika, I., 2005. Guidelines for the use of AMBI (AZTI's Marine Biotic Index) in the assessment of the benthic ecological quality. Marine Pollution Bulletin, 50(7), 787–789.

Brambati, A. & Catani, G., 1988. Le coste e i fondali del Golfo di Trieste dall'Isonzo a Punta Sottile: Aspetti geologici, geomorfologici, sedimentologici e geotecnici. Hydrores Trieste, 6, 13–28.

Brey, T., 2001. Population Dynamics in Benthic Invertebrates. A Virtual Handbook. Version 01.2. Institute for Polar and Marine Research, Germany. http://www.awi-bremerhaven.de/Benthic/Ecosystem/FoodWeb/Handbook/main.html Alfred Wegener.

Bremner, J., Rogers, S., Frid, C., 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. Marine Ecology Progress, Series, 254, 11–25.

Bremner, J., Rogers, S. I., Frid, C. L. J., 2006. Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). Ecological Indicators, 6(3), 609-622.

Bremner. J., 2008. Species' traits and ecological functioning in marine conservation and management. Journal of Experimental Marine Biology and Ecology, 366(1–2), 37-47. https://doi.org/10.1016/j.jembe.2008.07.007

Calabretta, C. J., & Oviatt, C. A., 2008. The response of benthic macrofauna to anthropogenic stress in Narragansett Bay, Rhode Island: a review of human stressors and assessment of community conditions. Marine Pollution Bulletin, 56(10), 1680-1695.

Cassin, D., Dominik, J., Botter, M., 2018. PAH and PCB contamination in the sediments of the Venice Lagoon (Italy) before the installation of the MOSE flood defence works. Environmental Science and Pollution Research, 25, 24951–24964. https://doi.org/10.1007/s11356-018-2524-y.

Clesceri, L.S., Eaton, A.D., Greenberg, A.E., Franson, M.A.H., American Public Health, A., American Water Works, A., Water Environment, F., 1996. Standard Methods for the Examination of Water and Wastewater: 19th Edition Supplement. American Public Health Association, Washington, DC.

Colman, AS. and Holland, HD., 2000. The global diagenetic flux of phosphorus from marine sediments to the oceans: redox sensitivity and the control of atmospheric oxygen levels. Marine Authigenesis: from Global to Microbial (C. R. Glenn, L. Prévôt-Lucas, J. Lucas, eds). Society of Economic Paleontologists and Mineralogists, Spec. Publ., 66, 53–75.

Costanza, R., Darge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., Oneill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., van den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature, 387, 253–260.

Covelli, S. & Fontolan, G., 1997. Application of a normalization procedure in determining regional geochemical baselines. Environmental Geology, 30, 34–45.

Covelli, S., Faganeli, J., Horvat, M., Brambati, A., 1999. Porewater Distribution and Benthic Flux Measurements of Mercury and Methylmercury in the Gulf of Trieste (Northern Adriatic Sea). Estuarine, Coastal and Shelf Science, 48(4), 415-428.

Covelli, S., Piani, R., Acquavita, A., Predonzani, S., Faganeli, J., 2007. Transport and dispersion of particulate Hg associated with a river plume in coastal Northern Adriatic environments. Marine Pollution Bulletin, 55(10–12), 436-450.

Cozzi, S., Reisenhofer, E., Di Monte, L., Cantoni, C., Adami, G., 2008. Effect of environmental forcing on the fate of nutrients, dissolved organic matter and heavy metals released by a coastal wastewater pipeline. Chemistry and Ecology, 24, 87-107.

D'Alessandro, M., Porporato, E. M., Esposito, V., Giacobbe, S., Deidun, A., Nasi, F., Ferrante, L., Auriemma, R., Berto, D., Renzi, M., Scotti, G., Consoli, P., Del Negro, P., Andaloro, F., Romeo, T., 2020. Common patterns of functional and biotic indices in response to multiple stressors in marine harbours ecosystems. Environmental Pollution, 259, 113959.

Díaz, R., Rosenberg, R., Díaz, R., Corral, R., Díaz-Garcia, R., Rosenberg, R. D., 1995. Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and Marine Biology, 33, 245–303.

Díaz, S. & Cabido, M., 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology & Evolution, 16, 646– 655.

Dolédec, S., Chessel, D., Ter Braak, C.J.F., Champely, S., 1996. Matching species traits to environmental variables: a new three-table ordination method. Environmental and Ecological Statistics, 3, 143–166.

Dray, S., Choler, P., Dolédec, S., Peres-Neto, P. R., Thuiller, W., Pavoine, S., 2014. Combining the fourth-corner and the RLQ methods for assessing trait responses to environmental variation. Ecology, 95(1), 14-21.

Ergen, Z., Çinar, M. E., Dagli, E., Kurt, G., 2007. "Seasonal dynamics of soft-bottom polychaetes in Izimir Bay (Aegean Sea, eastern Meditterranean)," in Scientific Advances in Polychaete Research, eds R. Sarda, G. San Martin, E. Lopez, D. Martin, D. George (Barcelona), 197–207.

Ferrera, I., Gasol, J. M., Sebastián, M., Hojerová, E., Koblížek, M., 2011. Comparison of Growth Rates of Aerobic An oxygenic Phototrophic Bacteria and Other Bacterioplankton Groups in Coastal Mediterranean Waters. Applied and environmental microbiology, 77, 7451–7458.

Filgueiras, V.L., Campos, L.S., Lavrado, H.P., Frensel, R., Pollery, R.C.G., 2007. Vertical distribution of macrobenthic infauna from the shallow sublittoral zone of Admiralty Bay, King George Island, Antarctica. Polar Biology 30, 1439–1447.

Franzo, A., Celussi, M., Bazzaro, M., Relitti, F., Del Negro, P., 2019. Microbial processing of sedimentary organic matter at a shallow LTER site in the northern Adriatic Sea: an 8-year case study. Nature Conservation, 34, 397.

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., Slade, E. M., Steffan-Dewenter, I., Emmerson, M., Potts, S. G., Tscharntke, T., Weisser W., Bommarco, R., 2015. Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. Proc. R. Soc. B 282: 2620.

Gaston, G. R., Rakocinski, C. F., Brown, S. S., Cleveland, C. M., 1998. Trophic function in estuaries: response of macrobenthos to natural and contaminant gradients. Marine and Freshwater Research, 49(8), 833-846.

Giangrande, A., 1997. Polychaete reproductive patterns, life cycles and life histories: an overview. Oceanography and marine Biology. An annual review, 35, 323-386.

Gladstone-Gallagher, R. V., Pilditch, C. A., Stephenson, F., Thrush, S. F., 2019. Linking traits across ecological scales determines functional resilience. Trends in ecology & evolution, 34(12), 1080-1091.

Gray, J. S., Wu, R. S. S., Or, Y. Y., 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Marine ecology progress series, 238, 249-279.

Gray, J.S., Dayton, P., Thrush, S., Kaiser, M.J., 2006. On effects of trawling, benthos and sampling design. Marine Pollution Bulletin, 52 (8), 840–843.

Grémare, A., Marsh, A., Tenore, K., 1989. Secondary production and reproduction of *Capitella capitata* type I (Annelida. Polychaeta) during a population cycle. Marine Ecology Progress Series, 51, 99–105.

Grime, J.P., 1997. Biodiversity and ecosystem function, the debate deepens. Science, 277(5330), 1260–1261.

Gusmao, J. B., Brauko, K. M., Eriksson, B. K., Lana, P. C., 2016. Functional diversity of macrobenthic assemblages decreases in response to sewage discharges. Ecological Indicators, 66, 65-75.

Hayward, PJ., & Ryland, JS., 2017. Handbook of the marine fauna of North-West Europe. Oxford university press. ISBN, 978-0-19-954944-3 (hbk.).

Hermand, R., Salen-Picard, C., Alliot, E., Degiovanni, C., 2008. Macrofaunal density, biomass and composition of estuarine sediments and their relationship to the river plume of the Rhone River (NW Mediterranean). Estuar. Coastal and Shelf Science 79, 1–10.

Johnston, E.L., Mayer-Pinto, M., Crowe, T.P., 2015. Review: chemical contaminant effects on marine ecosystem functioning. Journal of Applied Ecology, 52 (1), 140–149.

Jumars PA, Dorgan KM, Lindsay SM., 2015. Diet of worms emended: an update of polychaete feeding guilds. Annual Review of Marine Science, *7*:497–520. doi:10.1146/annurev-marine-010814-020007.

Jones, H. F., Pilditch, C. A., Bruesewitz, D. A., Lohrer, A. M., 2011. Sedimentary environment influences the effect of an infaunal suspension feeding bivalve on estuarine ecosystem function. Plos one, 6(10), e27065.

Katsanevakis, S., Zenetos, A., Mačić, V., Beqiraj, S., Poursanidis, D., Kashta. L., 2011. Invading the Adriatic: spatial patterns of marine alien species across the Ionian–Adriatic boundary. *AB 13*,107-118.

Kinoshita, K., Tamaki, S., Yoshioka, M., Srithonguthai, S., Kunihiro, T., Hama, D., Tsutsumi, H., 2008. Bioremediation of organically enriched sediment deposited below fish farms with artificially mass-cultured colonies of a deposit-feeding polychaete Capitella sp. I. Fisheries Science, 74(1), 77–87.

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C. O., Banta, G. T., 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Marine Ecology Progress Series,446, 285–302.

Krumhansl, K., Jamieson, R., Krkosek, W., 2016. Using species traits to assess human impacts on near shore benthic ecosystems in the Canadian Arctic. Ecological Indicators, 60, 495-502.

Laliberté, E., & Legendre, P., 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology, 91, 299–305.

Lotze, H. K., Haley Guest, H., O'Leary, J., Tuda, A., Wallace, D., 2018. Public perceptions of marine threats and protection from around the world. Ocean & Coastal Management, 152, 14-22.

Malačič V., & Petelin, B., 2009. Climatic circulation in the Gulf of Trieste (northern Adriatic). Journal of geophysical research, 114, 1– 15.

Mangano, M. C., Bottari, T., Caridi, F., Porporato, E. M. D., Rinelli, P., Spanò, N., 2017. The effectiveness of fish feeding behaviour in mirroring trawling-induced patterns. Marine environmental research, 131, 195-204.

Marchini, A., Munari, C., Mistri, M., 2008. Functions and ecological status of eight Italian lagoons examined using biological traits analysis (BTA). Marine Pollution Bulletin, 56, 1076–1085. https://www.marlin.ac.uk/biotic.

Melis, R., Celio, M., Bouchet, V. M., Varagona, G., Bazzaro, M., Crosera, M., Pugliese, N., 2019. Seasonal response of benthic foraminifera to anthropogenic pressure in two stations of the Gulf of Trieste (northern Adriatic Sea, Italy): the marine protected area of Miramare versus the Servola water sewage outfall. Mediterranean Marine Science, 20(1), 120-141.

Mistri, M., Borja, A., Aleffi, I. F., Lardicci, C., Tagliapietra, D., Munari, C., 2018. Assessing the ecological status of Italian lagoons using a biomass-based index. Marine Pollution Bulletin, 126, 600-605.

Morri, C., Bellan-Santini, D., Giaccone, G., Bianchi, C., 2004. Principles of bionomy: definition of assemblages and use of taxonomic descriptors (macrobenthos). Mediterranean marine biology, 11, 573–600.

Muscarella, R. & Uriarte, M., 2016. Do community-weighted mean functional traits reflect optimal strategies? Proceedings of the Royal Society B: Biological Sciences, 283, 1827, 20152434.

Mutlu, E., Çinar, M.E., Ergev, M.B., 2010. Distribution of soft-bottom polychaetes of the Levantine coast of Turkey, eastern Mediterranean Sea. Journal of Marine Systems, 79 (1), 23–35.

Nasi, F., Auriemma, R., Bonsdorff, E., Cibic, T., Aleffi, I. F., Bettoso, N., Del Negro, P., 2017. Biodiversity, feeding habits and reproductive strategies of benthic macrofauna in a protected area of the northern Adriatic Sea: a three-year study. Mediterranean Marine Science,18(2), 292-309.

Nasi, F., Nordström, M.C., Bonsdorff, E., Auriemma, R., Cibic, T., Del Negro. P., 2018. Functional biodiversity of marine soft-sediment polychaetes from two Mediterranean coastal areas in relation to environmental stress. Marine Environmental Research, 137, 121-132.

Nasi, F., Ferrante, L., Alvisi, F., Bonsdorff, E., Auriemma, R., Cibic, T., 2020. Macrofaunal bioturbation attributes in relation to riverine influence: What can we learn from the Po River lagoonal system (Adriatic Sea)? Estuarine, Coastal and Shelf Science, 232, 106405.

Novelli, G., 1996. Gli scarichi a mare nell`alto Adriatico. Rassegna tecnica del Friuli Venezia Giulia, 3, 11-19.

Ogorelec, B., Misic, M., Faganelli, J., 1991. Marine geology of the Gulf of Trieste (northern Adriatic): sedimentological aspects. Marine Geology, 99, 79-92.

Oug, E., Fleddum, A., Rygg, B., Olsgard, F., 2012. Biological traits analyses in the study of pollution gradients and ecological functioning of marine soft bottom species assemblages in a fjord ecosystem. Journal of Experimental Marine Biology and Ecology, 432–433, 94-105.

Paganelli, D., Marchini, A., Occhipinti-AmBRogi, A., 2012. Functional structure of marine benthic assemblages using Biological Traits Analysis (BTA): A study along the Emilia-Romagna coastline (Italy, North-West Adriatic Sea). Estuarine, Coastal and Shelf Science, 96, 245-256.

Pearson, T. H., & Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology: An Annual Review, 16, 229-311.

Peres-Neto, P. R., Dray, S., ter Braak, C. J., 2017. Linking trait variation to the environment: critical issues with community-weighted mean correlation resolved by the fourth-corner approach. Ecography, 40(7), 806-816.

Pinedo, S., Sardá, R., Rey, C., Bhaud, M., 2000. Effect of sediment particle size on recruitment of *Owenia fusiformis* in the Bay of Blanes (NW Mediterranean Sea): an experimental approach to explain field distribution. Marine Ecology Progress Series, 203, 205-213.

Pinto, R., Patrício, J., Baeta, A., Fath, B. D., Neto, J.M., Marques, J. C., 2009. Review and 832 evaluation of estuarine biotic indices to assess benthic condition. Ecological indicators, 9(1), 1-25.
http://www.polytraits. lifewatchgreece.eu.

Poulain PM., Kourafalou V.H., Cushman-Roisin B., 2001. Northern Adriatic Sea. Physical Oceanography of the Adriatic Sea, 143-165.

Queirós, A. M., Huebert, K. B., Keyl, F., Fernandes, J. A., Stolte, W., Maar, M., Kay, S., 2016. Solutions for ecosystem-level protection of ocean systems under climate change. Global Change Biology, 22, 3927–3936.

Querin, S., Crise, A., Deponte, D., Solidoro, C., 2007. Numerical Study of the Role of Wind Forcing and Freshwater Buoyancy Input on the Circulation in a Shallow Embayment (Gulf of Trieste, northern Adriatic Sea). JGR C, 111, 1–19.

R Core Team., 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org

Reiss, J., Bridle, J. R. Montoya, J. M., Woodward, G., 2009. Emerging horizons in biodiversity and ecosystem functioning research. Trends in Ecology and Evolution, 24, 505– 514.

Rhoads, D. C., & Boyer, L. F., 1982. The effects of marine benthos on physical properties of sediments. In Animal-sediment relations (pp. 3-52). Springer, Boston, MA.

Ricciardi, A., & Bourget, E., 1998. Weight-to-weight conversion factors for marine benthic macroinvertebrates. Marine Ecology Progress Series, 163:245-251.

Rosenberg, R., 2001. Marine benthic faunal successional stages and related sedimentary activity. Scientia Marina, 65(S2), 107-119.

Rouse, G. W., 2000. Polychaetes have evolved feeding larvae numerous times. Bulletin of Marine Science, 67, 391-409(19).

Rumolo, P., Barra, M., Gherardi, S., Marsella, E., Sprovieri, M., 2011. Stable isotopes and C/N ratios in marine sediments as a tool for discriminating anthropogenic impact. Journal Environmental Monitoring, 13 (12), 3399–3408.

Scheiner, S. M., Kosman, E., Presley, S. J., Willig, M. R., 2017. The components of biodiversity, with a particular focus on phylogenetic information. Ecology and Evaluation, 7(16), 6444-6454.

Simboura, N., & Zenetos, A., 2002. Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new biotic index. Mediterranean Marine Science , 3 (2):77-111.

Simonini, R., Ansaloni, I., Bonvicini Pagliai, A.M., Prevedelli, D., 2004. Organic enrichment and structure of the macrozoobenthic community in the northern Adriatic Sea in an area facing Adige and Po mouths. ICES Journal of Marine Science, 61, 871–881.

Solan, M., Cardinale, B. J., Downing, A. L., Engelhardt, K. A. M., Ruesink, J. L., Srivast, D. S., 2004. Extinction and Ecosystem Function in the Marine Benthos. Science, 306, 5699, 1177-1180.

Solís-Weiss, V., Aleffi, F., Bettoso, N., Rossin, P., Orel, G., 2007. The benthic macrofauna at the outfalls of the underwater sewage discharges in the Gulf of Trieste (northern Adriatic Sea). ANNALES. Ser. Hist. nat. 17, 1.

Souza, T.S.O., Ferreira, L.C., Sapkaite, I., Pérez-Elvira, S.I., Fdz-Polanco, F., 2013. Thermal pretreatment and hydraulic retention time in continuous digesters fed with sewage sludge: assessment using the ADM1. Bioresource Technology, 148, 317–324. https://doi.org/10.1016/J.BIORTECH.2013.08.161.

Taylor, L. A., Chapman, P. M., Miller, R. A., Pym, R. V., 1998. The effects of untreated municipal sewage discharge to the marine environment off Victoria, British Columbia, Canada. Water science and technology, 38(10), 285-292.

Tillin, H. M., Hiddink, J. G., Jennings, S., Kaiser. M. J., 2006. Chronic bottom trawling alters the functional composition of benthic inverteBRate communities on a sea-basin scale. Marine Ecology Progress Series, 318, 31–45.

Törnroos, A. & Bonsdorff, E., 2012. Developing the multitrait concept for functional diversity: lessons from a system rich in functions but poor in species. Ecological Applications, 22: 2221–2236.

Trainito, E & Doneddu, M., 2005. Conchiglie del Mediterraneo. Il Castello, Trezzano sul Naviglio.

Thrush, S.F., Hewitt, J.E., Cummings, V.J., Ellis, J.I., Hatton, C., Lohrer, A., Norkko, A., 2004. Muddy waters: elevating sediment input to coastal and estuarine habitats. Frontiers in Ecology and the Environment, *2*, 299–306.

Villéger, S., W. H. Mason, N., Mouillot, D., 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecological Society of America, 89(8), 2290-2301.

Villnäs, A., Hewitt, J., Snickars, M., Westerbom, M., Norkko, A., 2018. Template for using biological trait groupings when exploring large-scale variation in seafloor multifunctionality. Ecological Applications, 28(1), 78–94.

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., Garnier. E., 2007. Let the concept of trait be functional! Oikos 116, 882892.

Waldron, S., Tatner, P., Jack, I., Arnott, C., 2001. The impact of sewage discharge in a marine embayment: a stable isotope reconnaissance. Estuarine, Coastal and Shelf Science, 52(1), 111-115.

Weigel, B., Blenckner, T., Bonsdorff. E., 2016. Maintained functional diversity in benthic communities in spite of diverging functional identities. Oikos, 125, 1421–1433.

Wentworth, C. K., 1922. A scale of grade and class terms for clastic sediments. The journal of geology, 30 (5), 377-392.

Wild, C., Roy, H., Huettel, M., 2005. Role of pelletization in mineralization of fine-grained coastal sediments. Marine Ecology Progress Series, 291, 23–33.

Yao, Q., Wang, X., Jian, H., Chen, H., Yu, Z., 2015. Characterization of the particle size fraction associated with heavy metals in suspended sediments of the Yellow River. International Journal of Environmental Research and Public Health, 12 (6), 6725–6744.

Table 1: Traits and categories used in Biological Trait Analysis. Codes of categories are also presented.

Traits		Categories	Code
Adult size and shape	Maximum size (mm)	<5	S
		5-30	M
		30-80	M/L
		>80	L
	Body form	Vermiform	Vrm
		Dorso-ventral compressed	Drs
		Laterally compressed	Lat
		Globose	Glb
	Protection	No protection	Npr
		Tube	Tub
		Case	Cas
		Shell	Shl
Life history	Longevity	≤1 yr	A11
		1-3 yrs	A13
		3-6 yrs	A16
		6-10 yrs	A110
	Reproductive frequency	Semelparous	Sem
		Iteroparous	Iter
		Semi-continuous	Scon
	Adult feeding habitat	Suspension feeder	Susp
		Surface deposit feeder	Sdep
		Subsurface deposit feeder	Ssdep
		Herbivore	Herb
		Predation	Pred
		Scavenger	Scav
Adult activity	Environmental position	Endofauna	Endo
		Epifauna	Epif
		Interface	Inter
		Epibionte	Epib
	Mobility	Sessile	Sess
		Semi-motile	Smot
		Motile	Mot
	Life habit	No mov.	Nmo
		Swimmer	Swim
		Crawler	Craw
		Tube-builder	Tubl
		Burrower	Bur
	Bioturbation	None	Non
		Superficial modifier	Smod
		Biodiffuser	Bdif
		Regenerator	Regr
		Conveyor	Cnvy
Response to anthropogenic pressure	AMBI index	Group I	GrpI
		Group II	GrpII
		Group III	GrpIII
		Group IV	GrpIV
		Group V	GrV

Table 2: Physical-chemical and contaminants values measured at sampling stations. Corg (organic carbon); TN (Total Nitrogen); C:N (carbon and nitrogen ratio); Eh (redox potential); BR (Biogenic remains); PAHs (Polycyclic Aromatic Hydrocarbons).

Station	**Sand**	**Silt**	**Clay**	**Corg**	**TN**	**C:N**	**Eh**	**BR**	**Hg**	**PAHs**
	%			mg C g^{-1}	mg N g^{-1}		mV	mL	mg Kg^{-1}	
–2D	0.0	67.5	32.5	16.6	2.0	9.6	-116.0	2400.0	0.7	189.0
–1D	5.3	55.8	38.9	17.4	1.9	10.4	-136.0	9166.7	0.5	118.0
0D	7.9	56.2	35.9	14.4	1.5	11.1	-255.0	12166.7	0.1	31.0
1FD	5.6	62.1	32.3	16.1	1.9	10.0	-124.0	3333.3	0.6	174.0
2FD	4.8	63.3	31.9	14.7	1.8	9.6	-176.0	3500.0	0.8	107.0
1D	7.6	60.9	31.5	17.4	1.9	10.9	-113.0	5333.3	0.6	58.0
2D	5.0	61.3	33.7	14.9	1.7	10.5	-69.0	2333.3	0.8	166.0
–2M	20.3	52.4	27.3	12.1	1.3	10.5	-132.0	3500.0	0.4	27.0
–1M	24.8	49.9	25.3	11.5	1.3	10.3	-82.0	9333.3	0.5	47.0
0M	6.5	56.9	36.6	12.8	1.3	11.1	-137.0	13333.3	0.2	57.0
1M	2.5	61.3	36.3	14.7	1.7	10.3	-67.0	3333.3	0.6	105.0
2M	5.6	61.7	32.7	16.4	1.9	9.8	-133.0	7000.0	0.5	101.0
–2P	5.0	60.9	34.1	16.0	1.9	9.8	-112.0	4500.0	0.8	89.0
–1P	22.5	53.2	24.3	18.2	1.8	12.0	-162.0	5000.0	0.4	89.0
0P	12.3	57.5	30.2	15.9	1.8	10.4	-117.0	14166.7	0.4	119.0
1P	4.6	60.3	35.1	16.4	1.9	10.3	-97.0	2416.7	0.6	127.0
2P	4.7	63.6	31.7	16.1	1.7	10.8	-77.0	3666.7	0.7	137.0
RS	3.6	58.8	37.6	12.1	1.4	9.8	95.0	2833.3	0.5	5.0
–2D	7.0	54.6	38.4	16.2	1.8	10.3	-210.0	223.3	0.5	170.0
–1D	10.5	54.2	35.3	21.9	2.5	10.2	-101.0	1533.3	0.5	297.0
0D	39.6	38.0	22.4	60.5	3.8	18.6	-290.0	3756.7	0.4	229.0
1FD	9.0	54.3	36.7	16.7	1.9	10.1	-150.0	373.3	0.7	207.0
2FD	5.9	52.7	41.4	15.2	1.8	10.0	-130.0	383.3	0.7	154.0
1D	13.6	53.3	33.1	16.8	1.8	10.8	-210.0	466.7	0.6	306.0
2D	7.0	54.1	38.9	16.1	1.9	10.0	-150.0	226.7	0.7	189.0
–2M	21.3	48.9	29.8	13.3	1.6	9.9	8.0	500.0	0.4	86.0
–1M	34.9	40.8	24.3	12.3	1.3	11.3	70.0	1483.3	0.4	114.0
0M	39.4	37.3	23.3	13.5	1.7	9.4	-25.0	1160.0	0.4	115.0
1M	11.4	54.6	34.0	15.9	1.9	10.0	-117.0	406.7	0.4	118.0
2M	5.7	55.0	39.3	16.0	1.9	9.7	35.0	526.7	0.6	156.0
–2P	7.8	53.2	39.0	14.1	1.8	9.3	-115.0	246.7	0.4	109.0
–1P	17.2	50.1	32.7	16.8	2.3	8.5	-117.0	1266.7	0.5	187.0
0P	25.7	46.6	27.7	27.8	2.5	13.0	-204.0	1600.0	0.5	660.0
1P	12.1	54.3	33.6	17.6	2.5	8.2	-57.0	636.7	0.5	155.0
2P	8.0	54.5	37.5	16.7	2.1	9.2	-15.0	176.7	0.5	143.0
RS	7.0	50.5	42.5	10.7	1.2	10.5	54.0	660.0	0.7	126.0

Table 3: Biotic indices calculated at sampling stations and periods. Ecological status are indicated with colour code (green=high, light blue=good, yellow=moderate, brown=poor).

Stations	BENTIX	M-AMBI	M-bAMBI
-2D'16	2.39	0.71	0.57
-1D'16	2.68	0.71	0.66
OD'16	2.5	0.2	0.36
1FD'16	2.73	0.63	0.62
2FD'16	3	0.65	0.59
1D'16	2.7	0.78	0.76
2D'16	2.96	0.68	0.67
-2M'16	2.98	0.86	0.73
-1M'16	3.25	0.94	0.86
OM'16	3.01	0.89	0.82
1M'16	2.61	0.69	0.61
2M'16	2.23	0.68	0.51
-2P'16	2.52	0.73	0.66
-1P'16	2.65	0.72	0.71
0P'16	3.13	0.83	0.81
1P'16	2.73	0.77	0.63
2P'16	2.45	0.84	0.67
RS'16	2.3	0.61	0.42
-2D'18	2.68	0.72	0.61
-1D'18	2.49	0.68	0.8
0D'18	2.45	0.31	0.64
1FD'18	2.15	0.72	0.7
2FD'18	2	0.79	0.6
1D'18	2.6	0.74	0.72
2D'18	2.51	0.7	0.72
-2M'18	2.67	0.78	0.78
-1M'18	2.92	0.92	0.76
0M'18	2.68	0.88	0.88
1M'18	2.62	0.94	0.81
2M'18	2.65	0.72	0.62
-2P'18	2.95	0.82	0.84
-1P'18	2.8	0.79	0.82
0P'18	2.86	0.69	0.63
1P'18	2.63	0.85	0.67
2P'18	2.85	0.9	0.74
RS'18	1.64	0.76	0.49

Table 4: RLQ analysis based on species abundance. RLQ analyses and individual R-L-Q separate analysis.

Species abundance					
Cumulative projected inertia(%):					
Ax1	Ax1:2				
56.49	78.97				
Projected inertia (%):					
Ax1	Ax2				
56.48	22.49				
Eigenvalues decomposition:					
	eig	covar	sdR	sdQ	corr
eig1	0.58	0.76	1.82	1.60	0.26
eig2	0.23	0.48	1.38	2.07	0.16
Inertia & coinertia R (env):					
	inertia	max	ratio		
eig1	3.31	3.46	0.95		
eig1+2	5.22	5.94	0.88		
Inertia& coinertia Q(traits):					
	inertia	max	ratio		
eig1	2.56	7.59	0.34		
eig1+2	6.86	13.01	0.53		
Correlation L (CA on abundance):					
	corr	max	ratio		
eig1	0.26	0.56	0.46		
eig2	0.16	0.53	0.31		

Table S1: DistLM (Marginal test) results for species abundance and biomass in relation to environmental variables. SS: Sum of square. Prop: proportion of variance explained by each variable. p values having statistical significance are highlighted in bold. Corg (organic carbon); TN (Total Nitrogen); C:N (carbon and nitrogen ratio); Eh (redox potential); BR (Biogenic remains); PAHs (Polycyclic Aromatic Hydrocarbons).

Response variables	R^2 (DistLM)	Selected predictors	SS(trace)	Pseudo-F	P	Prop.
Species abundance	0.47	**Sand**	**7523.4**	**3.68**	**0.0001**	**0.10**
		Silt	**8944.4**	**4.47**	**0.0001**	**0.12**
		Clay	**5983.9**	**2.86**	**0.0004**	**0.08**
		TN	3255.4	1.50	0.0737	0.04
		Corg	**4505.6**	**2.11**	**0.0047**	**0.06**
		C:N	**5507.6**	**2.62**	**0.0005**	**0.07**
		Eh	**5005.4**	**2.36**	**0.0027**	**0.06**
		BR	**7162.6**	**3.49**	**0.0001**	**0.09**
		Hg	**8256.9**	**4.08**	**0.0001**	**0.11**
		PAHs	3027.8	1.39	0.1191	0.04
Species biomass	0.35	**Sand**	**7552.1**	**2.61**	**0.0030**	**0.07**
		Silt	**8146.8**	**2.83**	**0.0020**	**0.08**
		Clay	**5353.8**	**1.81**	**0.0260**	**0.05**
		TN	2314.8	0.76	0.7770	0.02
		Corg	3687.8	1.23	0.1870	0.03
		C:N	**5604.9**	**1.90**	**0.0050**	**0.05**
		Eh	3868.9	1.29	0.1600	0.04
		BR	**6900.4**	**2.37**	**0.0100**	**0.07**
		Hg	**7427.1**	**2.56**	**0.0020**	**0.07**
		PAHs	3531.7	1.17	0.2510	0.03

Table S2: DistLM (Marginal test) results for CWM values based on species abundance and WW biomass in relation to environmental variables. SS: Sum of square. Prop: proportion of variance explained by each variable. p values having statistical significance are highlighted in bold. Corg (organic carbon); TN (Total Nitrogen); C:N (carbon and nitrogen ratio); Eh (redox potential); BR (Biogenic remains); PAHs (Polycyclic Aromatic Hydrocarbons).

Response variables	R^2 (DistLM)	Selected predictors	SS(trace)	Pseudo-F	P	Prop.
Species abundance	0.49	**Sand**	**232.87**	**2.65**	**0.0390**	**0.07**
		Silt	**257.11**	**2.95**	**0.0330**	**0.08**
		Clay	204.97	2.31	0.0690	0.06
		TN	343.58	4.06	0.0270	0.11
		Corg	**524.06**	**6.61**	**0.0220**	**0.16**
		C:N	**485.85**	**6.04**	**0.0300**	**0.15**
		Eh	**519.74**	**6.54**	**0.0010**	**0.16**
		BR	**250.96**	**2.87**	**0.0370**	**0.08**
		Hg	**460.68**	**5.68**	**0.0050**	**0.14**
		PAHs	73.207	0.79	0.3910	0.02
WW biomass	0.18	Sand	401.79	1.01	0.3416	0.03
		Silt	334.47	0.83	0.4270	0.02
		Clay	285.45	0.71	0.4951	0.02
		TN	136.52	0.34	0.8243	0.01
		Corg	176.61	0.43	0.7824	0.01
		C:N	414.04	1.04	0.3550	0.03
		Eh	303.2	0.75	0.4622	0.02
		BR	642.5	1.64	0.1743	0.05
		Hg	386.82	0.97	0.3474	0.03
		PAHs	392.75	0.98	0.3695	0.03

Table S3: Pearson correlation with the two first PCO axes obtained by PCO analyses for CWM species abundance and biomass (Fig. 5).

Traits		**CWM species abundance**		**CWM WW biomass**	
		PCO1	PCO2	PCO1	PCO2
Adult size and shape	S	-0.39	-0.42	0.33	-0.26
	M	-0.19	-0.66	0.57	-0.77
	M/L	0.62	0.55	0.67	0.04
	L	-0.20	-0.08	-0.68	0.67
	Vrm	0.60	0.63	0.77	-0.01
	Drs	-0.51	-0.71	-0.82	-0.47
	Lat	-0.37	-0.22	0.25	-0.13
	Glb	-0.40	-0.10	0.13	0.63
	Npr	0.62	0.56	0.81	0.50
	Tub	-0.49	0.04	0.46	-0.30
	Cas	-0.09	-0.20	0.03	0.10
	Shl	-0.44	-0.65	-0.85	-0.42
Life history	A11	0.77	-0.38	0.29	0.06
	A13	0.79	-0.35	0.65	-0.26
	A16	-0.69	0.56	0.80	-0.21
	A110	-0.31	0.04	-0.96	0.15
	Sem	-0.22	0.29	0.29	0.30
	Iter	-0.88	0.36	-0.44	0.41
	Scon	0.91	-0.37	0.27	0.05
	Susp	-0.58	-0.57	-0.87	-0.34
	Sdep	-0.45	0.11	0.72	0.14
	Ssdep	0.78	-0.48	0.76	0.53
	Herb	-0.50	-0.04	0.59	0.08
	Pred	-0.42	0.83	0.74	-0.07
	Scav	-0.36	0.04	0.29	-0.27
Adult activity	Endo	0.61	0.32	0.81	-0.09
	Epif	-0.42	0.20	0.03	0.01
	Inter	0.24	0.73	-0.88	0.18
	Epib	-0.41	-0.52	0.01	0.17
	Sess	-0.65	-0.39	-0.84	-0.42
	Smot	0.65	0.27	0.86	0.11
	Mot	-0.59	0.68	0.80	0.45
	Nmo	-0.51	-0.68	-0.94	0.03
	Swim	-0.56	-0.08	0.58	-0.06
	Craw	-0.12	0.84	0.74	-0.16
	Tubl	-0.58	-0.09	0.50	-0.26
	Bur	0.74	0.31	0.78	-0.08
	Non	-0.49	-0.62	0.24	-0.41
	Smod	-0.54	-0.33	-0.84	-0.33
	Bdif	-0.51	0.79	0.25	0.52

	Regr	-0.19	-0.44	0.15	-0.12
	Cnvy	0.88	-0.22	0.55	0.19
Response to anthropogenic pressure	Grp I	-0.68	-0.08	0.58	-0.74
	Grp II	-0.48	0.67	0.50	-0.03
	Grp III	-0.14	-0.22	0.38	0.17
	Grp IV	-0.22	-0.22	0.10	0.16
	Grp V	0.93	-0.33	0.26	0.09

Figure captions

Figure 1: Location of sampling sites in the Gulf of Trieste.

Figure 2: Boxplots showing the variability of FDis-abu (a) and FDis-biom (b) at stations gathered by transects. The different groups are indicated with different colours

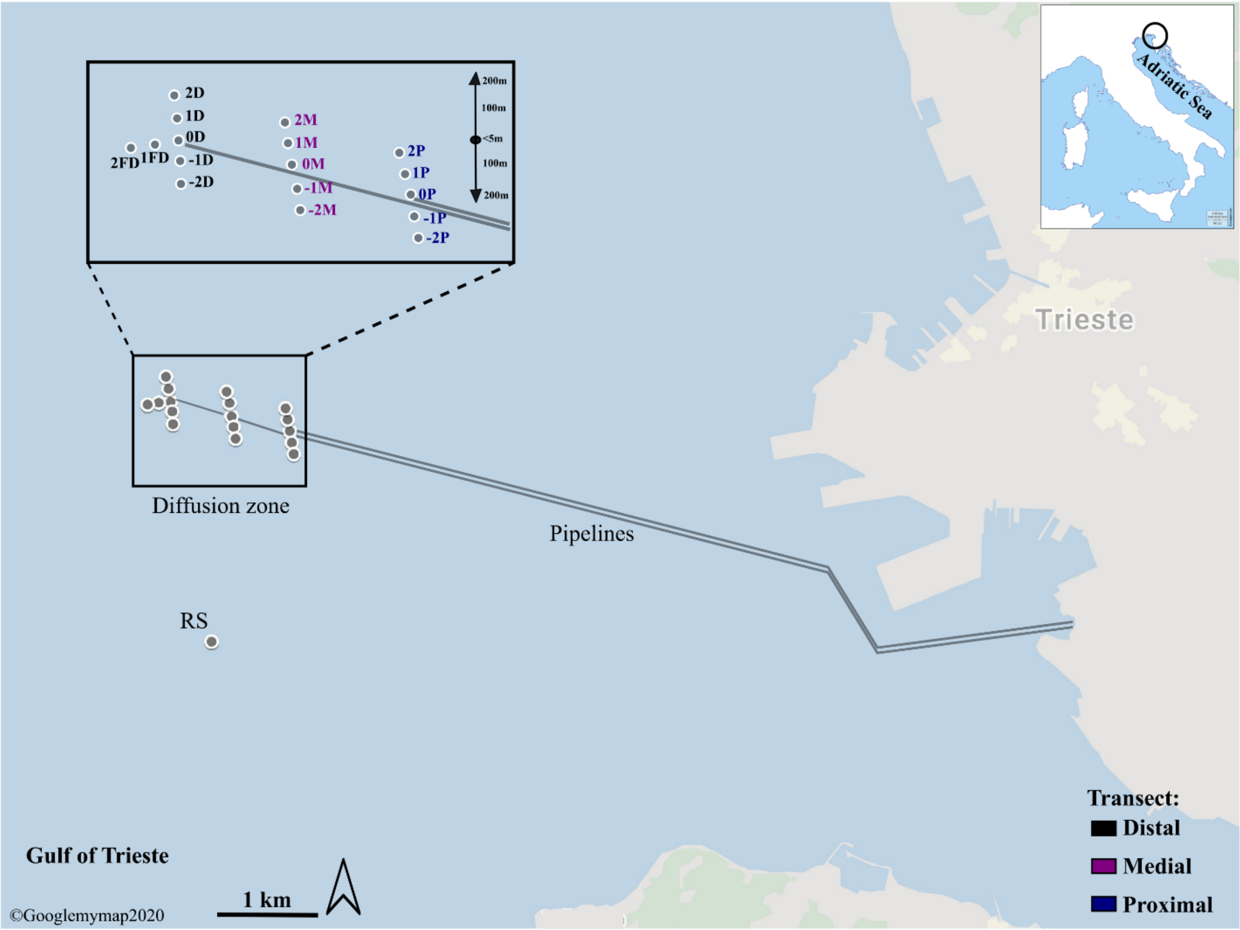
Figure 3: Relationship between functional diversity (abundance- and biomass-based), number of species and biotic indices

Figure 4: Correlation matrix between functional diversity, benthic indices, and environmental variables. Positive correlations are displayed in blue while negative correlations in red colour. Colour intensity and the size of the circle are proportional to the correlation coefficients. Asterisk and cross indicate significant correlations (0.05 and 0.01, respectively). Corg (organic carbon); TN (Total Nitrogen); C:N (carbon and nitrogen ratio); Eh (redox potential); BR (Biogenic remains); PAHs (Polycyclic Aromatic Hydrocarbons); BNTX (BENTIX); MAMB (M-AMBI); MbAM (Mb-AMBI); S (number of species); FDA (FDis-abu); FDB (FDis-biom).

Figure 5: PCO ordinations describing the variability in CWM values abundance- (a) and biomass-based (b) across sampling sites. Traits major correlated with PCO axes (>0.7) are overlaid. The distances from the pipe are indicated with different colours. See Table 1 for complete trait labels.

Figure 6: Results of RLQ analyses performed on traits occurrences abundance (a) and relationships between the RLQ environmental axes and individual traits (b) and between the RLQ trait axes and individual environmental variables (c). The d value in the upper right of the corner is the scale of the graph (a). The distance from the pipe and the sampling periods are indicating with different colour and shape, respectively. Significant associations with the first axis are represented in blue, with the second axis in orange, while variables with no significant association are in black. Corg (organic carbon); TN (Total Nitrogen); C:N (carbon and nitrogen ratio); Eh (redox potential); BR (Biogenic remains); PAHs (Polycyclic Aromatic Hydrocarbons). See Table 1 for complete trait labels

Figure 1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1D
0D
2FD
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-2D
2M
1M
0M
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0P
-1P
-2P
200m
100m
<5m
100m
200m
Adriatic Sea
Trieste
Diffusion zone
Pipelines
RS
Gulf of Trieste
1 km
©Googlemymap2020
Transect:
■ Distal
■ Medial
■ Proximal

Figure 2

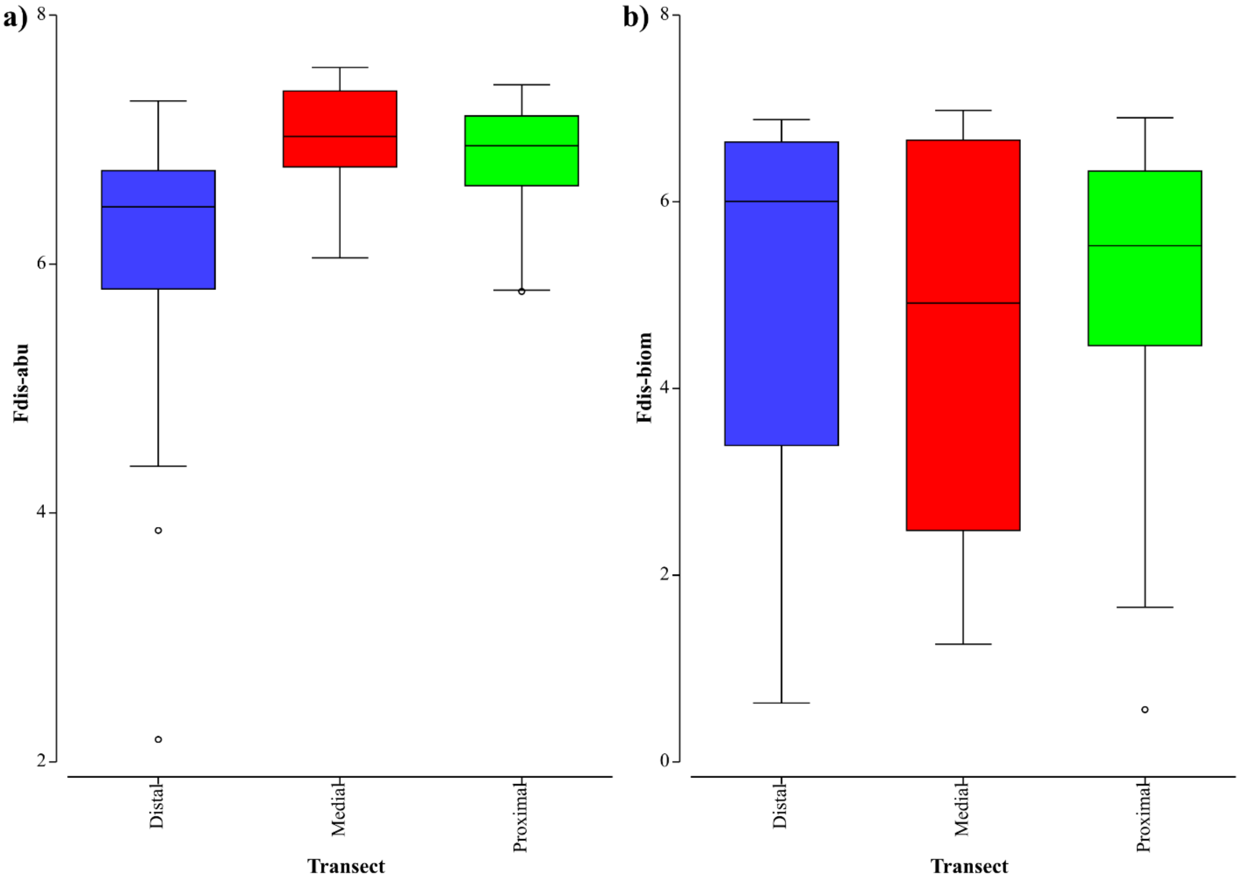

a)
8
6
4
2
Fdis-abu
Distal
Medial
Proximal
Transect
b)
8
6
4
2
0
Fdis-biom
Distal
Medial
Proximal
Transect

Figure 3

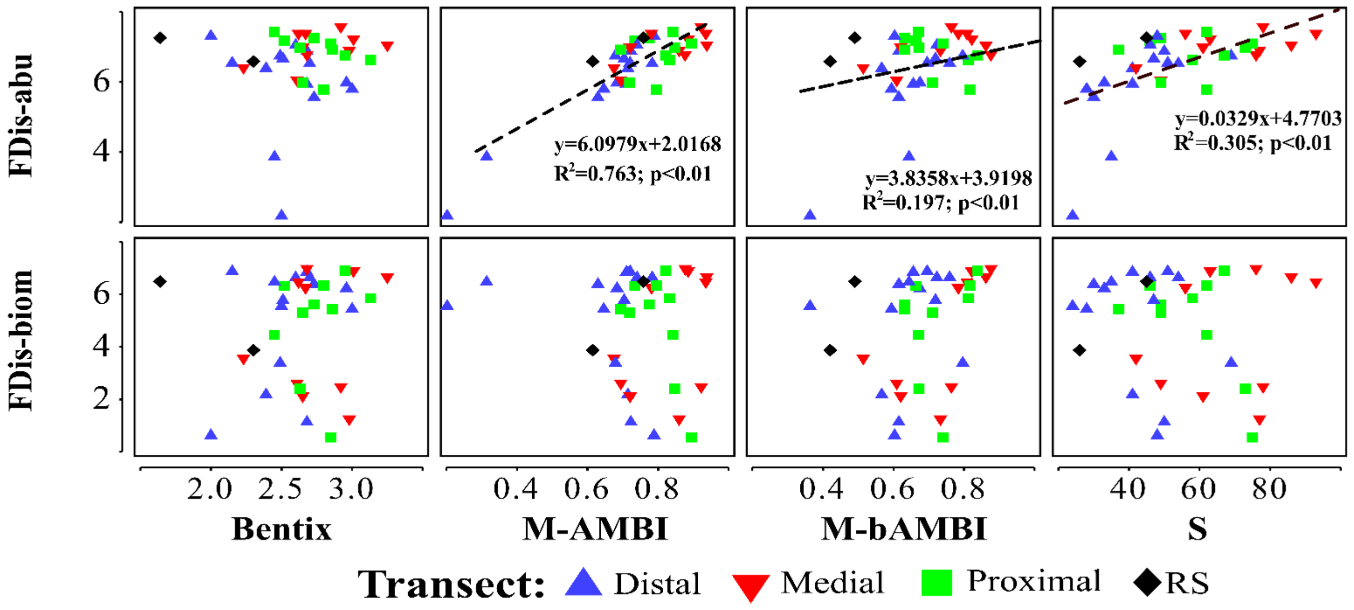

FDis-abu
FDis-biom
6
4
6
4
2
2.0 2.5 3.0
Bentix
0.4 0.6 0.8
M-AMBI
0.4 0.6 0.8
M-bAMBI
40 60 80
S
y=6.0979x+2.0168
R^2=0.763; p<0.01
y=3.8358x+3.9198
R^2=0.197; p<0.01
y=0.0329x+4.7703
R^2=0.305; p<0.01
Transect: ▲ Distal ▼ Medial ■ Proximal ◆ RS

Figure 4

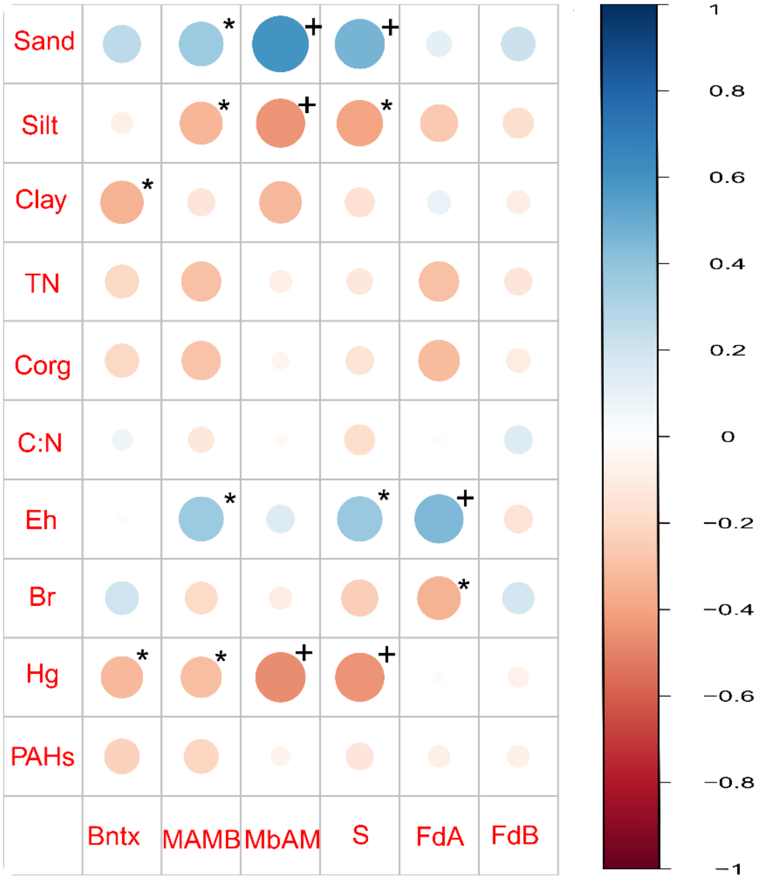

Sand
Silt
Clay
TN
Corg
C:N
Eh
Br
Hg
PAHs
Bntx
MAMB
MbAM
S
FdA
FdB
*
+
1
0.8
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
−0.8
−1

Figure 5

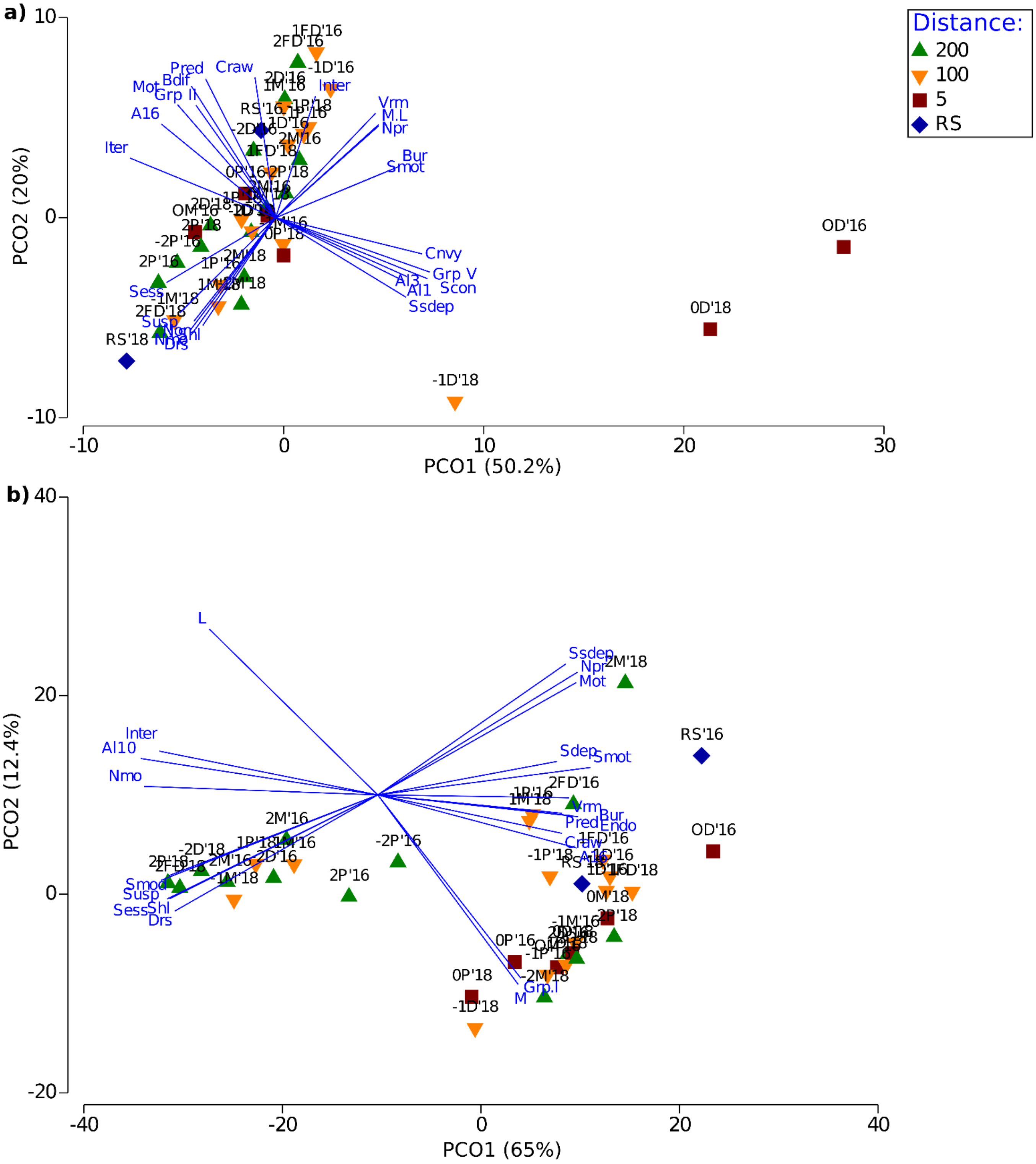
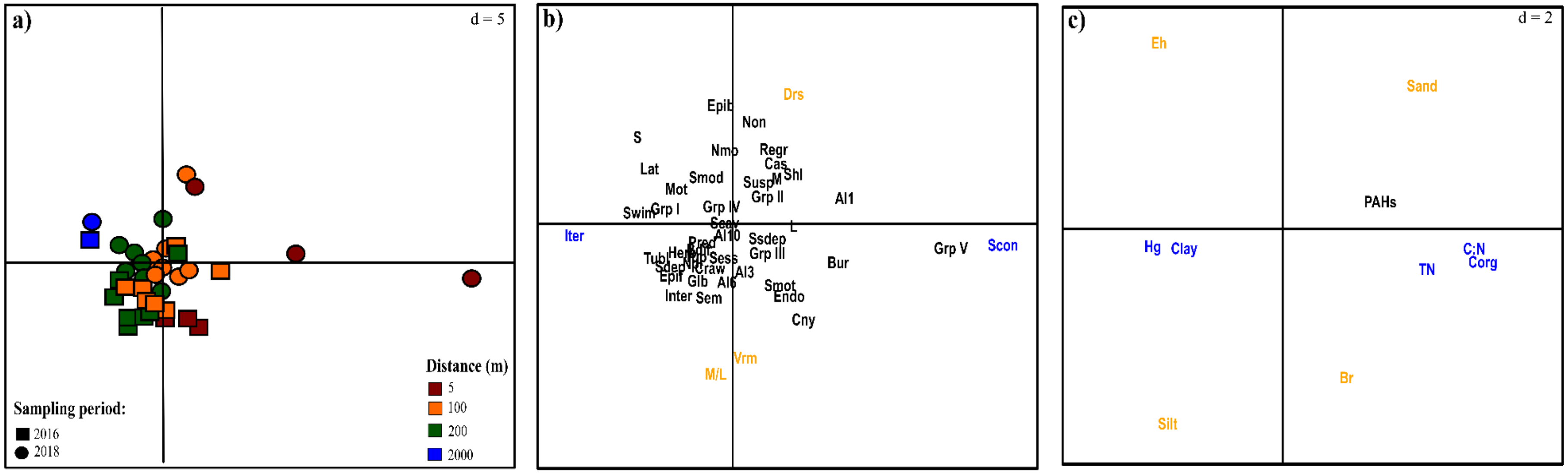

a)
b)
Distance:
▲ 200
▼ 100
■ 5
◆ RS
PCO1 (50.2%)
PCO2 (20%)
PCO1 (65%)
PCO2 (12.4%)
Pred
Craw
Bdif
Mot
Grp II
A16
Iter
Inter
Vrm
M.L
Npr
Bur
Smot
Cnvy
Grp V
Al3
Al1
Scon
Ssdep
Sess
Susp
Ngnl
Nm
Drs
OD'16
0D'18
-1D'18
RS'18
RS'16
1FD'16
2FD'16
-1D'16
L
Ssdep
Npr
Mot
Sdep
Smot
Al10
Nmo
Vrm
Bur
Pred
Endo
Craw
Smod
Susp
Sess
Shl
Drs
Grp.I
M
2M'18
RS'16
OD'16
0P'18
-1D'18
2P'16
-2P'16
2M'16

Figure 6


a)
d = 5
Sampling period:
■ 2016
● 2018
Distance (m)
5
100
200
2000
b)
Drs
Epib
Non
S
Nmo
Regr
Cas
Lat
Smod
Susp
M
Shl
Mot
Grp II
Al1
Swim
Grp I
Grp IV
Scav
L
Iter
Al10
Ssdep
Pred
Grp III
Grp V
Scon
Tubl
Sess
Bur
Sdep
Craw
Al3
Epif
Glb
Al6
Smot
Endo
Inter
Sem
Cny
Vrm
M/L
c)
d = 2
Eh
Sand
PAHs
Hg
Clay
C:N
Corg
TN
Br
Silt

3. Functional richness and β-diversity

This chapter is adapted as Paper II from: Federica Nasi, Seyed Ehsan Vesal, Larissa Ferrante, Federica Relitti, Matteo Bazzaro, Rocco Auriemma, Paola Del Negro. Taxonomic and functional macrofaunal diversity along a gradient of sewage contamination: a three year-study. Short communication, under preparation.

3.1 Introduction

Half the world's population lives along the coastline and leads to multiplied inputs of freshwater, nutrients, and contaminants, consequently, the habitats located in those areas are under great human impacts (Cabral-Oliveira et al., 2014). As such, the biodiversity and ecological processes on coastal ecosystems are severely threatened (Törnroos et al., 2013; Zubikarai et al., 2014), which could have drastic effects on the structure and functioning of the marine ecosystem (Halpern et al., 2007; Bustamante et al., 2012). Among that pressures, sewage discharges have long been recognized as one of the most widespread threats affecting coastal ecosystems (Pearson and Rosenberg, 1978; Bustamante et al., 2012).

Drastic changes in the patterns of distribution of marine assemblages in time and space have been reported worldwide as a consequence of sewage discharges (Roberts et al., 1998; Smith et al., 1999). Under the stress of this contamination, especially in macrobenthic communities (Munda, 1993; Díez et al., 2010), the shift in community structure is frequently paralleled by a decline in diversity and species richness (Wear and Tanner, 2007), due to the dominance of few species (Roberts et al., 1998). This caused changes in the overall macrofauna structure (Terlizzi et al., 2002; Zubikarai et al., 2014).

Additionally, benthic stress levels from sewage discharges can vary considerably depending on effluent treatments and flow rates (Del Pilar Ruso et al., 2010), as well as on the physical environmental conditions in which the effluent is discharged (Pastorock and Bylard, 1985; Zubikarai et al., 2014). However, our understanding and predictive ability regarding the effects of environmental change on ecosystem function and services are still extremely limited, particularly in marine ecosystems (Loreau, 2001). Besides, analyses of changes in community functional diversity components in response to environmental variations have the potential to connect species loss to shifts in ecosystem function (Teixidó et al., 2018). High functional diversity (i.e., the breadth of species' ecological functions), functional redundancy (i.e., the number of species performing similar functions in the system), and functional vulnerability (i.e., decrease in functional diversity following the loss of species) are pivotal features for sustaining ecosystem function (Naeem et al., 2012; Tilman et al., 2014). Accordingly, in this study, we use a functional-trait approach to quantify different components of functional diversity of macrobenthic communities influenced by sewage-derived materials along a distance gradient from the main source of contamination. Moreover, we tested the community functional features influenced by upgrading treatments of the wastewater plant. The functional attributes were analyzed together with the study of α and β diversity. The α diversity refers to biodiversity, whereas the β-diversity (or turnover) study two distinct processes: the replacement and the loss (or gain) of species. The portioning of diversity and the analyses of its components are important to understand the community changes that take place following a forcing event (Baselga, 2012) both along with spatial and temporal scales. Changes in the occurrence of functional traits, which are defined as those traits that directly influence an organism's performance, subject to the different environmental conditions can provide insights into potential loss of ecosystem function in response to perturbations. Functional traits have proven to be an exceptionally versatile and sensitive approach to assess functional diversity and redundancy in terrestrial communities, but our understanding of how marine functional diversity and redundancy respond to global environmental change across taxa and ecosystems remains elusive (Teixidó et al., 2018).

Many studies analyzed the influence of sewage discharges on macrofaunal communities (e.g. Pearson and Rosemberg, 1978), whereas their changes have been less investigated in terms of the effectiveness of wastewater treatment variations, solely a few attempts have estimated the effect of sewage treatments on macrofauna of hard substratum assemblages (e.g., Zubikarai et al., 2014; Bustamante et al., 2012). In particular, to the best of our knowledge, this study is the first attempt where functional attributes and β-diversity changes were related to wastewater effluent discharge and treatment along a distance gradient from the main source of contamination.

In this study, we aim to investigate the pattern in changes taxonomic and functional diversity to variations of sewage-derived materials deposition along a distance gradient. In particular, we sought to answer the following questions: (1) Is functional diversity influenced by sewage effluent variations along a distance gradient? (2) Is functional diversity buffered by functional redundancy, such that the loss of functional diversity with changes in sewage deposition than taxonomic diversity? 3) Does biodiversity (α and β) vary following the variation of treatments?

To test these hypotheses, we characterized the ecology of each macrofaunal species using 9 functional traits describing life histories, feeding habits, and movement methods, which resulted in 188 different unique traits combination or functional entities (FEs). In addition, we calculated the functional richness as a volume filled by each assemblage in four dimensions of functional space to assess variation in functional space along with the temporal and spatial scales.

3.2 Material and methods

The Gulf of Trieste is a shallower basin of about 600 km^2 with a coastline of 100 km^2, located at the northernmost part of the Adriatic Sea. Geographical, hydrological, and sedimentological features were exhaustively described in Franzo et. al. (2016). The Gulf of Trieste hosts the main sewage discharge of Trieste city. The Servola wastewater treatment plant is located in the north-eastern part of the Gulf, serving up to 190.000 equivalent inhabitants. It started operations in 1992 and disposes of mix type plants (both meteoric and wastewaters for about 35 million m^3 per year). There are three treatment phases with an additional substitution of the new biological treatment plant since June 2018 (including pumping station, primary sedimentation, UV disinfection, and biofilters for pre-denitrification, nitrification/oxidation for post-denitrification, and phosphorous removal). After the chemical-physical treatments, the plant carries the residual waters through a submarine pipeline (7.5 km), which the last long of 1.5 km is characterized with specific turrets to facilitate the dispersion of wastewater. The conduct reaches an average depth of 22 m (www.acegasapsamga.it)

The macrofaunal community was collected in April 2018, December 2019, and January 2021 at 10 stations located at an increasing distance (5, 25, 100, 200 meters) from the main outfall (LAT: 45°38'36.30"; LON: 13°40'51.70"; Fig.1). At each sampling period, macrofaunal community was sampled with a van Veen grab (0.1 m^2) in three replicates of each station. The sediments for the macrofauna community were sieved on a 1.0 mm mesh to retain the fraction of macrofaunal organisms. The retained sediment and organisms were immediately fixed in ethanol 90°. Macrofaunal invertebrates were identified under a stereomicroscope (Zeiss Discovery V.12, 8-110× final magnification) and counted.

The functional diversity of benthic assemblages was assessed using 9 traits that described complementary facets of species ecology as illustrated in Table 1. Traits values for the 240 benthic species 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). Following we calculated the functional entities (FEs, unique trait combination), and in total, the 240 species were classified into 188 different FEs. We tested and quantified whether species richness and functional diversity were influenced by sewage-derived materials and thus observed along a distance gradient from the main outfall. Further, we analyze functional diversity variation along with temporal scales due to improvements in sewage treatments. Then for each selected group (i.e. distance from the duct for every sampling periods), we calculated (i) species richness, (ii) FEs present in the assemblage relative to the number from the global pool; and (iii) the functional richness as the volume inside the convex hull surrounding the FEs present. Species richness, number of FEs, and functional volume filled by each selected group were expressed as a relative percentage of the global pool. To build a functional space accounting for trait values of FEs, we calculated pairwise functional distances between species pairs based on the 9 functional traits using the Gower metric, which allows mixing different types of variables while giving them equal weight (Legendre and Legendre, 1988). Then, we used the first four principal axes of the principal coordinates analysis (PCoA) computed on the functional distance matrix to build a multidimensional functional space where the position of FEs represents their differences (Teixidó et al., 2018, and reference therein). We selected the number of axes to build a functional space accordingly to the mean squared deviation index (mSD) computed between initial functional distance among FEs (i.e. based on trait values) and final Euclidean distance in the functional space (Maire et al., 2015). Functional space with four dimensions had a low mSD (0.00191) and could be optimal to represent functional differences among species.

Further, we assessed the taxonomic α- (Shannon–Wiener Log_2 and evenness-Pelou) and β-diversity among benthic assemblages along with spatial and temporal scales. The β-diversity portioning framework (Velléger et al., 2013; Baselga et al., 2012) was based on the Jaccard'dissimilarity index (Baselga, 2010, Baselga et al., 2012). β-diversity equals 0 when communities are identical and equals 1 when communities are maximally dissimilar (e.g. no species shared for taxonomic β-diversity; Baselga, 2010). In addition, we investigated whether taxonomic temporal and spatial β-diversity associated with sewage-derived materials was mostly due to turnover (i.e. differences in species between sampling periods or along sewage distance gradient were due to a replacement) or due to nestedness resultant processes (i.e. species between two sampling periods or species at gathered stations that represented a subset of those found in the previous years or at the other group of stations). Turnover equals 0 when communities are functionally nested and equals 1 when no species are shared by the communities (Baselga and Leprieur, 2015).

In addition, differences in taxonomic diversity (i.e. number of species, α- and temporal β-diversity), FEs, and functional volumes were tested among sampling periods and the stations gathered based on the distance from the duct (i. e. 5, 25, 100, 200 meters), by a non-parametric test (Kruskal-Wallis H test).

Lastly, functional diversity analyses were computed using the R functions from the ‘FD', ‘tripack', ‘geometry', ‘matrixStats', and ‘betapart' R packages (version R v 4.1.0; Teixidó et al., 2018, and reference therein). The Shannon–Wiener diversity index was carried out using the PRIMER7 software. Also, the Kruskal-Wallis test was applied to analyze statistical differences on taxonomic and functional diversity among selected factors (i.e. sampling periods and distance gradient) were computed using STATISTICA 7.

3.3 Results and Discussion

The total numbers of macrofaunal species increased moving from the outfall toward the farther stations in 2018. Indeed, the lowest value of species richness was observed at the nearest zone to the pipe in 2018, whereas the major one was noticed at 200 meters from the outfall, representing 17.1% and 44.2% of the global pool, respectively. On contrary for the following years, the described increasing pattern from species richness was not detected. Both in 2019 and 2021, we observed the lower species percentages at 5 meters from the duct (17.1 and 31.3%, respectively), whereas the highest toward the farther zone (100 meters: 40.7 and 37.1%) (Fig.1). Many studied evidenced that for a macrofaunal community subjected to an allochthonous amount of organic enrichment, the species gradually increased moving far from the main source of contamination reaching the ecotone point (e.g. Pearson and Rosenberg, 1978; Nasi et al., 2020). Indeed, we infer that in 2018, at 200 meters we observed the transitional zone between the natural environmental condition, low organic matter input, and the stressed condition, where a huge reduction in species numbers occurred. In fact, in this zone, where the exclusion of sensitive species is no already occurred but the presence of some opportunistic species starts to attest, the species richness could be major (Cibic et al., 2017). We supposed that the ecotone point in 2019 and 2021 was observed at 100 meters from the pipe due to the highest richness values. Similar to species richness, Shannon-Wiener diversity (H’) evidenced increasing values from the station located nearby the outfall to farther one in particular during 2018 (Fig.3). Additionally, higher H’ values were detected in 2021 compared to the previous years. In fact, after improvement treatments, we noticed that increasing species richness and that specimen values were evenly distributed among species thus diversity values showed higher values. The latter was observed above all at stations gathers at 100 meters from the diffusing zone in 2021 (H' and evenness average values: 5.15±0.21 and 0.93±0.01). This was evident also from other studies where the reduction of sewage-derived materials due to the improvement of treatments led to a major structured community (Archambault et al., 2001). In addition, the FEs values followed the same increasing pattern of species richness toward the distance gradient from the pipe in 2018, whereas the highest values were detected at 100 meters in 2019 and 2021. Indeed, the lowest value of FEs was observed at the zone close to the pipe in 2018 (18.1%), whereas the higher one was noticed at 100 meters from the diffusion area, representing 50.0% (in 2019) of the global pool. Overall, as species richness, the reduction of sewage-derived materials deposition moving far from the outfall had positively affected the FEs. In addition, comparing the species richness and FEs values we could infer that community in the whole sampling area was not characterized by functional redundancy, thus few species performed for few functions meaning this area was likely more susceptible to species loss and function than others (Gamfeldt et al., 2008).

The most striking results were, however, the sharp increase in functional richness (defined as the volume of functional space delineated by traits filled by species) in the zone nearby the outfall (5 meters) along the temporal scale. On contrary, functional richness decreased moving far from the diffusion zone (25, 100, and 200) from 2019 to 2021 (Fig. 2). The excess of particulate matter, which tends to accumulate at sediments also at deeper layers, caused variations in the structure and functions of the community. However, following the improved effluent treatments, the reduced deposition could have led to enhancing environmental conditions and, therefore, to higher species richness and functions (Buscamante et al., 2012). Despite environmental quality improvement after wastewater treatment, the surrounding area resulted to be affected by the reduction of species, FEs, and thus functional richness. In particular, at farther zone (100 and 200 meters) we noticed a gradual decrease in functional richness among years. Hence, as observed by other authors after improving treatments, there was a small decline, followed by a progressive increase towards stabilization, following the successional stages within Pearson and Rosenberg's (1978) model for the recovery of benthic ecosystems (Del-Pilar-Ruso et al., 2010; Zubikarai et al., 2014).

On the one hand, the implementation of treatment enhanced the ecosystem process at the area nearby the pipe but on the other hand, in the other sampling areas, the reduction of some species and their function can result in loss of ecosystem properties (Teixidó et al., 2018). In addition, we observed, in particular from 2018 to 2019, a great variation in species. This was evidenced by analyzing the β-diversity values and their components along with the spatial scales. Higher values of turnover were detected at stations gathered at 100 and 200 meters from the pipe (average values: 0.71±0.10 and 0.60±0.06, respectively) compared to 2019-2021 (average values: 0.61±0.18 and 0.71±0.09, respectively; Fig. 3). Similarly, at the station closer to the pipe we calculated a great species turnover from 2018 *vs* 2019 if compared to 2019 *vs* 2021 (0.72 and 0.53, respectively). On contrary, the 25 meters area was characterized by lower turnover as opposed to high nestedness-resultant from 2018 to 2019, whereas from 2019 and 2021 we noticed an increase in turnover value. We infer that in particular at 25 meters zone, the components partitioning of the β-diversity, the turnover of species resulted higher than nestedness, underlining as, at sites with smaller numbers of species, the biotas are not subsets of the richer sites (Baselga, 2012), but the establishment of new species. In addition, in the area influence by the sewage-derived materials, the improvements of physical and chemical treatments had generally originated a temporal turnover of species, implying the replacement of some species by others leading to a great change of the community (Baselga, 2012). The latter was observed also along with spatial scale, we noticed promptly decreasing values of nestedness-resultants among stations collected in 2021, confirming the establishment of a new community for each sampling zones (Table 2). The site-scale heterogeneity of assemblages, following sewage discharges and the mitigation intervention, was also discussed by other authors (Terlizzi et al., 2005 and Buscamante et al., 2012). Our results support the idea that spatial heterogeneity of assemblages is sensitive to anthropogenic disturbance, regardless of the direction of change, and is, therefore, a suitable response variable for the assessment of both human impacts and mitigation interventions (Bevilacqua et al., 2012, Buscamante et al., 2012).

Our result showed that the impact of sewage discharges on the marine environment is reduced when wastewater treatment technologies were improved however this is not sufficient to mitigate the effects since habitat heterogeneity occurred and a prompt reduction of function was noticed. In addition, the understanding of which ecosystem properties will be reduced remains to discuss further. However, this first attempt to evidence the influence of wastewater effluents and their mitigation on structural and functional community evidenced how this integrative approach can give an accurate characterization of the association between traits and their sensitivity to environmental stressors thus could be useful to predict uncertain events.

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References

www.acegasapsamga.it

Archambault, P., Banwell, K., & Underwood, A. J., 2001. Temporal variation in the structure of intertidal assemblages following the removal of sewage. Marine Ecology Progress Series, 222, 51–62.

Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. Global Ecol. Biogeogr., 19, 134–143.

Baselga, A., 2012. The relationship between species replacement, dissimilarity derived from nestedness, and nestedness. Global Ecology and Biogeography, 21(12), 1223–1232.
DOI: 10.1111/j.1466-8238.2011.00756. x.

Baselga, A., Gómez-Rodríguez, C., Lobo, J. M. 2012. Historical legacies in world amphibian diversity revealed by the turnover and nestedness components of beta diversity. PLoS One, 7(2), e32341.

Baselga, A., Leprieur, F. 2015. Comparing methods to separate components of beta diversity. Methods in Ecology and Evolution, 6(9), 1069-1079.

Bevilacqua, S., Plicanti, A., Sandulli, R., Terlizzi A., 2012. Measuring more of beta diversity: quantifying patterns of assemblage heterogeneity. An insight from marine benthic assemblages. Ecological Indicators, 18: 140–148.

Bustamante, M., Bevilacqua, S., Tajadura, J., Terlizzi, A., Saiz-Salinas, J. I. J. L., 2012. Detecting human mitigation intervention: Effects of sewage treatment upgrade on rocky macrofaunal assemblages. Marine Environmental Research 80: 27–37.

Cabral-Oliveira, J., Dolbeth, M., Pardal, M.A., 2014. Impact of sewage pollution on the structure and functioning of a rocky shore benthic community. Marine and Freshwater Research, 65: 750–758.

Cibic, T., Acquavita, A., Aleffi, F., Bettoso, N., Blasutto, O., De Vittor, C., Falconi, C., Falomo, J., Faresi, L., Predonzani, S., Tamberlich, F., Fonda Umani, S., 2008. Integrated approach to sediment pollution: a case study in the Gulf of Trieste. Marine Pollution Bulletin. 56(9):1650–7. doi: 10.1016/j.marpolbul.2008.05.009. Epub 2008 Jun 24. PMID: 18579162.

Del-Pilar-Ruso, Y., de-la-Ossa-Carretero, J. A., Giménez-Casalduero, F., Sánchez-Lizaso, J. L., 2010. Sewage treatment level and flow rates affect polychaete assemblages. Marine Pollution Bulletin, 60(11), 1930–1938.

Díez, I., Santolaria, A., Gorostiaga, J.M., 2010. Different levels of macroalgal sampling resolution for pollution assessment. Marine Pollution Bulletin, 60: 1779–1789.
Franzo, A., Cibic, T., Del Negro, P. 2016. Integrated approach for the assessment of the benthic ecosystem functioning at a coastal site in the northern Adriatic Sea. Continental Shelf Research, 121, 35-47.

Gamfeldt, L., Hillebrand, H., Jonsson, P.R., 2008. Multiple functions increase the importance of biodiversity for overall ecosystem functioning. Ecology 89(5), 1223-1231. https://doi.org/10.1890/06-2091.1

Giangrande, A., 1997. Polychaete reproductive patterns, life cycles, and life histories: an overview. Oceanography and Marine Biology*:* An Annual Review, 35, 323–386.

Halpern, B., Selkoe, K., Micheli, F., Kappel, C., 2007. Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Conservation Biology 21, 1301e1315.

Jumars, P. A., Dorgan, K. M., Lindsay, S. M., 2015. Diet of worms emended: an update of polychaete feeding guilds. Annual review of marine science, 7, 497–520.

Legendre, P. & Legendre, L., 1988. Numerical ecology, 24. Dev. Environ. Model. 24, 870.

Loreau, M., 2001. Microbial diversity, producer–decomposer interactions and ecosystem processes: a theoretical model. Proceedings of the Royal Society of London, Series B 268: 303–309.

Maire, E., Grenouillet, G., Brosse, S., Villéger, S., 2015. How many dimensions are needed to accurately assess functional diversity? A pragmatic approach for assessing the quality of functional spaces. Glob. Ecol. Biogeogr. 24, 728–740.

www.marlin.ac.uk/biotic

Munda, I.M., 1993. Changes and degradation of seaweed stands in the Northern Adriatic. Hydrobiologia, 261: 239-253.

Naeem, S., Duffy, J. E., Zavaleta, E., 2012. The functions of biological diversity in an age of extinction. Science, 336(6087), 1401–1406.

Nasi, F., Ferrante, L., Alvisi, F., Bonsdorff, E., Auriemma, R., Cibic, T., 2020. Macrofaunal bioturbation attributes in relation to riverine influence: What can we learn from the Po River lagoonal system (Adriatic Sea)? Estuarine, Coastal and Shelf Science, 232, 106405.

Pastorock, R.A., Bylard, G.B., 1985. Effects of sewage pollution on coral reef communities. Marine Ecology Progress Series, 21: 175–189.

Pearson, T. H., & Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology: An Annual Review, 16, 229–311.

http://polytraits.lifewatchgreece.eu/

Roberts, D. E., Smith, A., Ajani, P., Davis, A. R., 1998. Rapid changes in encrusting marine assemblages exposed to anthropogenic point source pollution: A ‘Beyond BACI’ approach. Marine Ecology Progress Series 163: 213–224.

Rouse, G. W., 2000. Polychaetes have evolved feeding larvae numerous times. Bulletin of Marine Science, 67, 391-409(19).

Smith, A., Ajani, P.A., Roberts, D. E. 1999. Spatial and temporal variation in fish assemblages exposed to sewage and implications for management. Marine Environmental Research, 47: 241–260.

Teixidó, N., Gambi, M. C., Parravacini, V., Kroeker, K., Micheli, F., Villéger, S., Ballesteros, E., 2018. Functional biodiversity loss along natural CO_2 gradients. Nature Communications, 9(1), 5149.
https://doi. org/10.1038/s41467-018-07592-1

Terlizzi, A., Fraschetti, S., Guidetti, P., Boero, F., 2002. The effects of sewage discharge on shallow hard substrate sessile assemblages. Marine Pollution Bulletin, 44: 544–550.

Terlizzi, A., Benedetti-Cecchi, L., Bevilacqua, S., Fraschetti, S., Guidetti, P., Anderson, M.J., 2005. Multivariate and univariate asymmetrical analyses in environmental impact assessment: a case study of Mediterranean subtidal sessile assemblages. Marine Ecology Progress Series, 289: 27–42.

Tilman, D., Isbell, F., Cowles, J. M., 2014. Biodiversity and ecosystem functioning. Annual Review of Ecology. Evol. Syst. 45, 471–493.

Törnroos, A., Nordström, M.C., Bonsdorff, E., 2013. Coastal Habitats as Surrogates for Taxonomic, Functional and Trophic Structures of Benthic Faunal Communities. PLoS ONE, 8 (10): e78910.

Villéger, S., Grenouillet, G., & Brosse, S. (2013). Decomposing functional β-diversity reveals that low functional β-diversity is driven by low functional turnover in European fish assemblages. Global Ecology and Biogeography, 22(6), 671-681.

Wear, R.J., Tanner, J.E., 2007. Spatio-temporal variability in faunal assemblages surrounding the discharge of secondary treated sewage. Estuarine Coastal and Shelf Science, 73: 630–638.

Zubikarai, N., Borja, A., Muxika, I., 2014. Assessment of benthic hard substratum communities responses to changes in the management of anthropogenic pressures in the Basque coast. Revista de Investigación Marina, AZTI-Tecnalia, 21(3), 40–88.

Table 1: Biological traits used to measure functional diversity of benthic species. N= number of categories.

Trait	Trait Type	N	Categories
1) Morphology and protection	Categorical	6	a) vermiform with no protection b) dorso-ventral compressed with shell c) dorso-ventral compressed with no protection d) laterally compressed e) case/shell protection f) globose
2) Adult size	Ordinal	6	1) up to 5 mm 2) 5-15 mm 3) 15-35 mm 4) 35-60 mm 5) 60-80 mm 6) >80 mm
3) Longevity	Ordinal	4	1) ≤1 yr 2) 1-3 yrs 3) 3-6 yrs 4) 6-10 yrs
4) Reproductive frequency	Categorical	3	a) Semelparous b) Iteroparous c) Semi-continuous
5) Mechanism development	Categorical	2	a) Direct b) Planctotrophic
6) Adult feeding habit	Categorical	14	a) Suspension feeders b) Tentaculate suspension feeders c) Jawed suspension feeders d) Surface deposit feeders e) Tentaculate surface deposit feeders f) Jawed surface deposit feeders g) Subsurface deposit feeders h) Tentaculate subsurface deposit feeders i) Jawed subsurface deposit feeders j) Carnivores k) Tentaculate carbivores l) Jawed/toothed carnivores m) Omnivores n) Herbivores
7) Mobility	Categorical	6	a) Swimmer b) crawler/creeping c) burrower d) tube-builder e) temporary attachment f) permanent attachment
8) Adult environmental position	Ordinal	4	1) Epibionte/Epilithic 2) Epifauna 3) Demersal 4) Endofauna
9) Ecosystem engineering	Categorical	5	a) no b) biodiffusor c) conveyor d) regenerator e) habitat-building

Table 2: Taxonomic β-diversity comparisons among groups of stations along with sewage distance gradient for each sampling period. Values show β-diversity, its two components (turnover and nestedness-resultant), and relative contribution of turnover to β-diversity (%).

	2018			2019			2021		
Turnover	**5**	**25**	**100**	**5**	**25**	**100**	**5**	**25**	**100**
25	0.34			0.51			0.59		
100	0.30	0.53		0.54	0.51		0.62	0.57	
200	0.52	0.59	0.43	0.61	0.60	0.52	0.77	0.71	0.47
Nestedness-resultant									
25	0.38			0.12			0.05		
100	0.42	0.01		0.23	0.16		0.09	0.05	
200	0.26	0.01	0.03	0.19	0.12	0.00	0.03	0.01	0.05
β-diversity									
25	0.72			0.63			0.64		
100	0.72	0.55		0.77	0.66		0.71	0.62	
200	0.78	0.60	0.46	0.80	0.72	0.52	0.80	0.71	0.52
Turnover/ β-diversity (%)									
25	47.13			80.79			91.82		
100	41.51	97.33		69.96	76.28		87.71	91.89	
200	66.89	98.41	92.73	76.61	83.06	99.28	96.12	98.97	90.13

Figure captions

Fig.1.: Sampling area and stations in the Gulf of Trieste.

Fig. 2.: Species and functional diversity changes among groups of stations along with wastewater effluents gradient for each sampling period (5, 25, 100, and 200 meters). Plots show species richness (Sp), number of functional entities (unique trait combinations, FE), and functional richness (volume filled by each assemblage in the four dimensions of the functional space, Vol. 4D). Values are expressed as a relative percentage of the value for the total pool.

Fig. 3: Boxplots showing the variability of α-diversity for each sampling period (a, b, c), temporal β- diversity (d, g) turnover (e, h), and nestedness-resultant (f, i) of macrofaunal community between 2018-2019 and 2019-20210. Stations were gathered based on the distance gradient from the pipe.

Figure 1

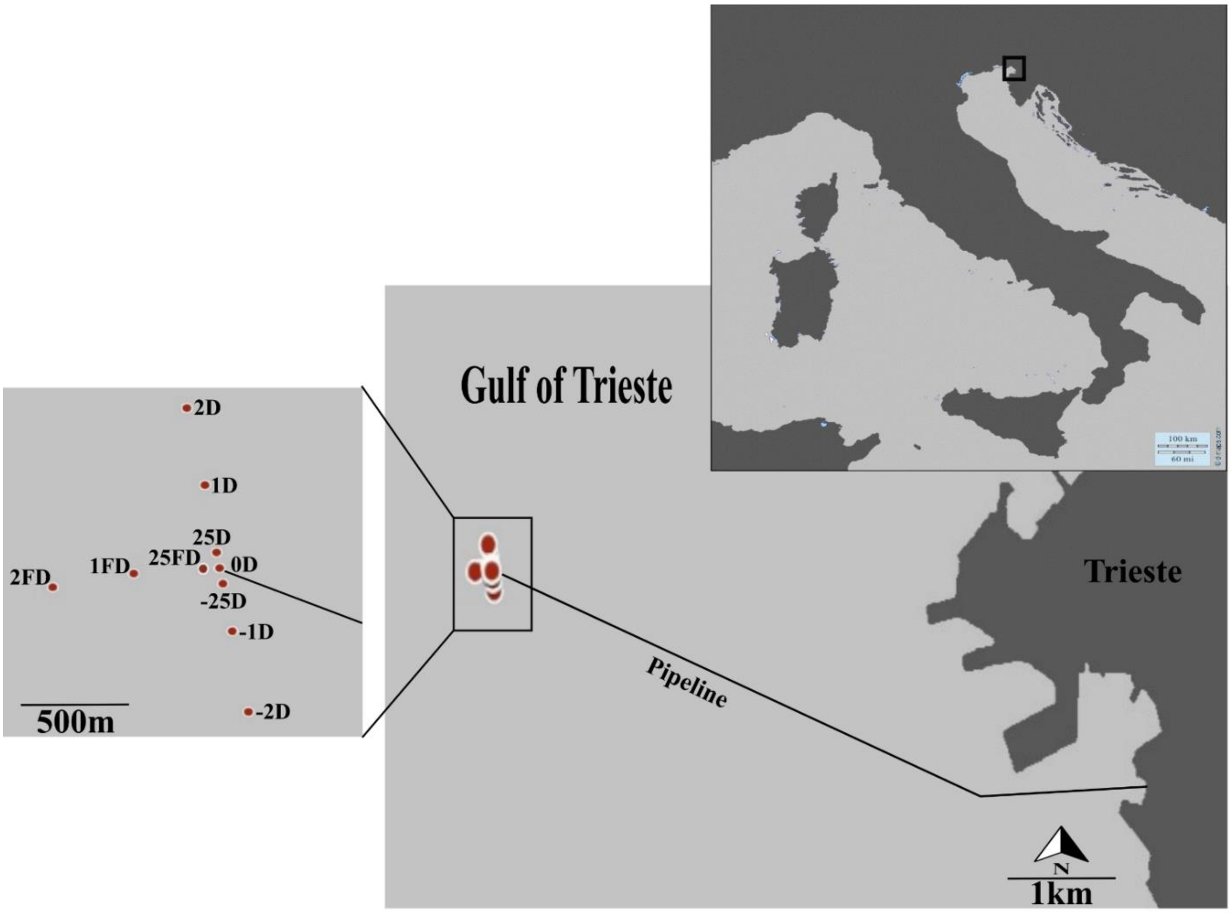

2D
1D
25D
2FD
1FD
25FD
0D
-25D
-1D
-2D
500m
Gulf of Trieste
Trieste
Pipeline
N
1km
100 km
60 mi

Figure 2

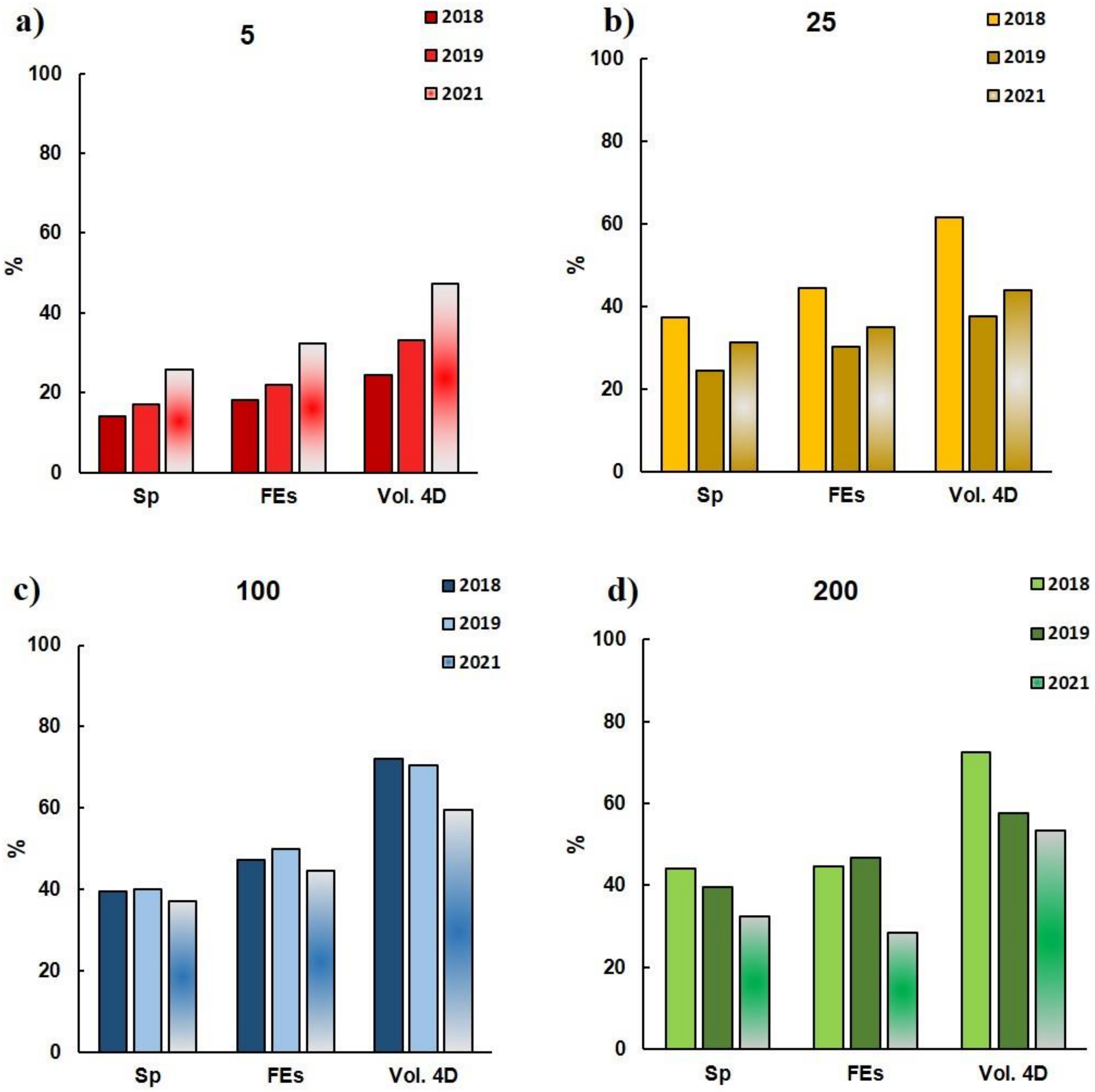
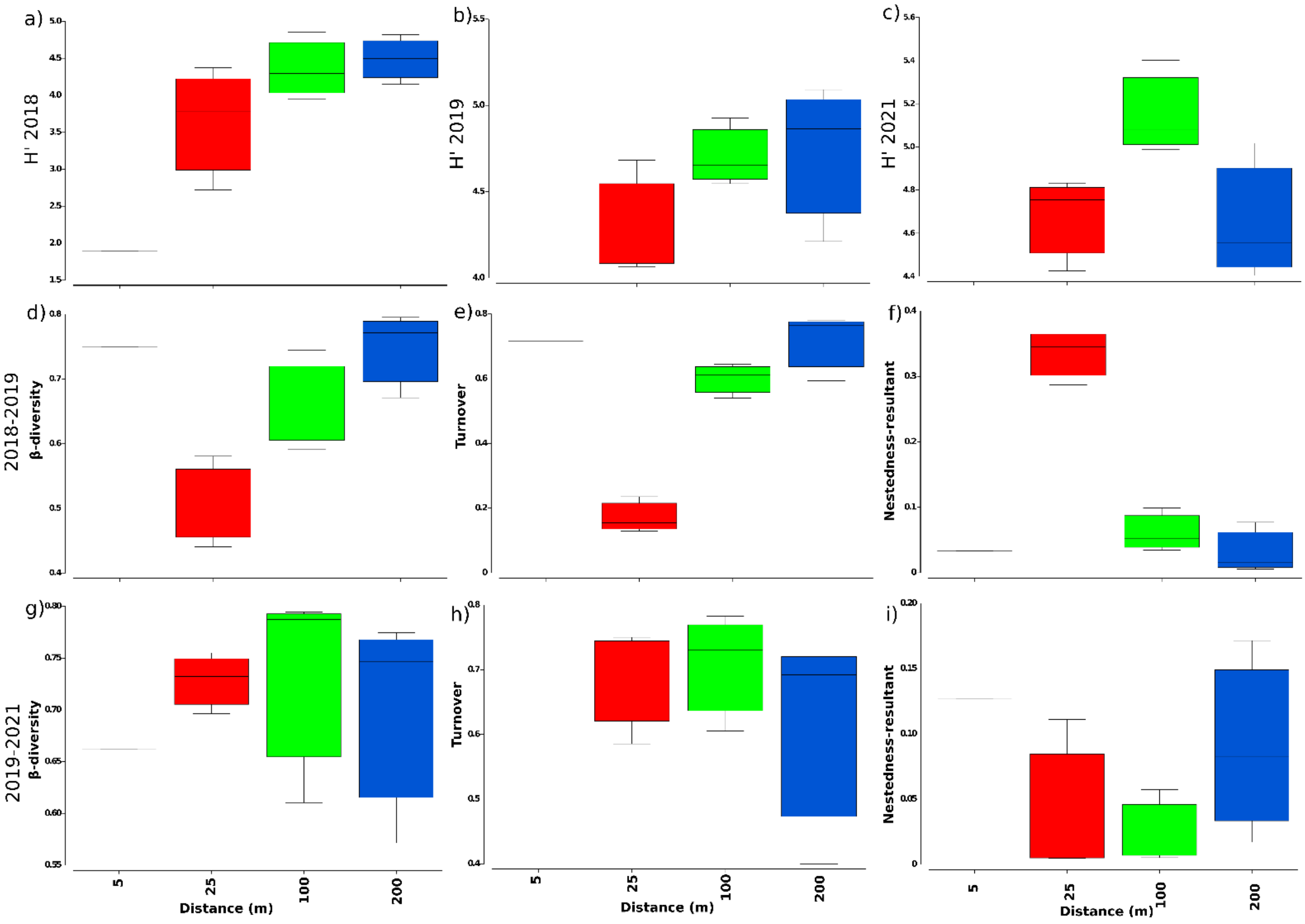

a)
5
2018
2019
2021
100
80
60
40
20
0
%
Sp
FEs
Vol. 4D
b)
25
2018
2019
2021
100
80
60
40
20
0
%
Sp
FEs
Vol. 4D
c)
100
2018
2019
2021
100
80
60
40
20
0
%
Sp
FEs
Vol. 4D
d)
200
2018
2019
2021
100
80
60
40
20
0
%
Sp
FEs
Vol. 4D

Figure 3


a)
H' 2018
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
b)
H' 2019
5.5
5.0
4.5
4.0
c)
H' 2021
5.6
5.4
5.2
5.0
4.8
4.6
4.4
d)
2018-2019
β-diversity
0.8
0.7
0.6
0.5
0.4
e)
Turnover
0.8
0.6
0.4
0.2
0
f)
Nestedness-resultant
0.4
0.3
0.2
0.1
0
g)
2019-2021
β-diversity
0.80
0.75
0.70
0.65
0.60
0.55
h)
Turnover
0.8
0.7
0.6
0.5
0.4
i)
Nestedness-resultant
0.20
0.15
0.10
0.05
0
5
25
100
200
Distance (m)

4. Bioturbation and bioirrigation potential

This chapter is adapted as Paper III from: Seyed Ehsan Vesal, Federica Nasi, Larissa Ferrante, Rocco Auriemma, Paola Del Negro. Assessing the effects of organic enrichment on bioturbation attributes of macrofaunal community. Original manuscript, under preparation.

4.1 Introduction

Macrofaunal organisms are considered a key biological component due to high biomass and biodiversity (Snelgrove, 1998), most of them are sessile and play a critical role in cycling nutrients, oxygenation of deeper sediment layers, and sediment reworking. Moreover, they show marked responses to environmental changes depending on their species-specific sensitivity/tolerance levels (Ferraro and Cole, 1995; Lancellotti and Stotz, 2004; Bremner et al., 2006). The benthic organisms' activities can alter the physical and chemical conditions of the sedimentary environment via several complex processes (Diaz and Rosenberg, 2008) and as the species vary in sensitivity, they undergo changes in composition corresponding to the degree of disturbances (Bilyard, 1987; Olsgard & Gray, 1995; Pearson &Rosenberg, 1978). Biogenic activities such as bioturbation and bioirrigation by benthic organisms are fundamental because not only cause mixing the substrate as sediment particle preservation and sediment reworkings such as burrow and mound construction, particle ingestion, food caching, prey excavation, etc. (i.e. bioturbation activities), but also burrow-dwelling can ventilate the sediments, creating a rapid exchange of water between the overlying water and subsurface sediment (i.e., bioirrigation activities) which relates directly to species activities, but food availability and geochemical composition within the substrate are all affected (Aller, 1977; Rhoads and Germano, 1982; Schaffner, 1990; Meysman et al., 2006).

These fundamental processes also affect sediment turnover and diffusive and advective processes that transport elements in dissolved and particulate among sediments (Remaili et al., 2016, 2017), consequently, have implications for ecosystem-related functions ranging from alteration of sediment biogeochemistry, organic matter regeneration, and nutrient cycling to the provision and maintenance of habitats for other organisms (Mermillod-Blondin, 2011; Birchenough et al., 2012).

The coastal marine ecosystems are subjected to many impacts from natural to anthropogenic origins, which deposal huge amount organic matter (OM). The major natural point of OM sources are rivers which largely contribute to accumulating allochthonous OM in the area interested by their plumes (Giani et al., 2009; Bongiorni et al., 2018). Moreover, the growing urban development of coastal areas entails increased anthropogenic pressures such as domestic and municipal wastewater disposal into marine environments. Sewage-derive materials present a widespread environmental problem in coastal waters which are often released a high amount of OM into shallow subtidal habitats (Koop and Hutchins, 1996). They contain organic contaminants, faecal sterols, heavy metals, bacteria, nutrients, and large amounts of suspended and particulate organic matter (Moon et al., 2008).

The coastal sediments act as a sink for the accumulation of allochthonous-OM (Wilkinson et al., 2018). Despite some studies that have documented the importance of allochthonous, terrestrial/riverine resource supply for marine communities (e.g. Darnaude et al., 2004; Savage et al., 2012). However, a high amount of allochthonous OM could cause the most pervasive threat to the diversity, structure, and functioning of marine coastal ecosystems (Lotze et al., 2006; Airoldi et al., 2008; Crain et al., 2009).

Macrofaunal communities adapt to environmental disturbances, and the anthropogenic impact factors have to be measured against the background of natural forces; an anthropogenic factor can be detected if its impact exceeds the intensity and frequency of natural physical disturbance (Kaiser et al., 2006). Detrimental effects of sewage discharge are evidenced (Borja et al., 2006; Borja et al., 2010), but it is challenging to disentangle and quantify the relative importance of the anthropogenic organic matter and natural ones in environments with competing activities, permanent alterations, and persistent usage (Kenny et al., 2009, 2018). Community responses to anthropogenic disturbances are rarely compared to natural disturbance patterns. Such comparisons increase our ability to predict the responses of organisms to future disturbances and help place human activity in a more realistic perspective of natural history (Lissner et al., 1991).

Hence, biogenic activities such as bioturbation and bioirrigation can diminish the possible negative effects of organic enrichment in the sediment, if contamination does not reach high levels causing partial or total defaunation (Pearson and Rosenberg, 1978). However, in some cases, these can be influenced by the different amounts of OM and its allochthonous origin (Nasi et al., 2020).

Bioturbation has been quantified by a series of modeled simulations and calculated with metrics from benthic quantitative data, such as bioturbation potential community-BP_c (Solan et al., 2004a) and irrigation potential community-IP_c (Wrede et al. 2018) calculations. Bioturbation potential calculations are linked to the adoption of a trait-based approach and can be quantitatively estimated from benthic quantitative data using the metric of bioturbation indices (BP_c and IP_c). This approach is useful when trying to categorize and understand ecosystem functions conducted by benthic communities (Solan et al., 2004a; Birchenough et al., 2012; Wrede et al., 2018). Community bioturbation potential (BP_c) is a metric first described by Solan et al., (2004a), which combines abundance and biomass data with information about the life traits of individual species or taxonomic groups. This information describes modes of sediment reworking and mobility of taxa in a dataset, two traits known to regulate biological sediment mixing, a key component of bioturbation (Solan, 2000; and references therein; Solan et al., 2004b). BP_c is thus not a direct measure of the process of bioturbation. Rather, BP_c provides an estimate of the potential of a community to bioturbate. Hence, where macrofauna abundance and biomass data are available, BP_c provides a means to estimate the extent to which benthic communities are likely to affect important ecosystem properties that underpin ecosystem functioning. The consequences of environmentally driven changes in biodiversity to BP_c, and its relation to ecosystem functioning, have been explored in this way in terrestrial (Bunker et al., 2005) and marine habitats (Solan et al., 2004a and b); at the local (Lohrer et al., 2010; Teal et al., 2013) and regional scales (Queirós et al., 2011; Birchenough et al., 2012; Solan et al., 2012); for different contexts (e.g., Queirós et al., 2011; Van Colen et al., 2012; Villnäs et al., 2012); and for a variety of ecosystem functions including productivity (Solan et al., 2012), nutrient cycling (Solan et al., 2004a), carbon storage (Bunker et al., 2005; Solan et al., 2012), and decomposition of plant pigments in surface sediments (Josefson et al., 2012).

Another important feature is the sediments irrigation derived by animals that affects the different processes of biogeochemical at the seafloor. Bioirrigation is mainly caused by burrow-dwelling organisms that can ventilate the sediments, creating a fast water interchange between the overlying water and subsurface sediments (Kristensen et al., 2012; Nasi et al., 2020). The latter process is mostly induced by suspension deposit-feeding activities and ventilation rates of benthic organisms (Aller, 1982). Accordingly, bioirrigation is predominantly related to body mass and feeding type (Christensen et al., 2000). Wrede et al., (2018) modified the BP_c index suggested by Solan et al., (2004a) into community irrigation potential (IP_c), as a new index, whereas in the bioturbation potential calculation-BP_c, the mobility trait presumably underrates the contribution of sessile organisms with low mobility rate but high biorrigation efficiency. In this context, Wrede et al., (2018) tried to replace the reworking and mobility traits with the feeding types, burrow, and depth pocket injection of burrows (as bioirrigation functional characteristics).

So far, the BP_c index has been usefully applied in many marine studies and by calculating BP_c over time, or for different locations or scenarios, changes in the efficiency of the organism-sediment couple can be monitored for compliance in support of management and policy objectives (Painting et al., 2012; Van Hoey et al., 2013), while the new IP_c index has been less adopted in ecological surveys (Renz et al., 2018; Nasi et al., 2020).

The environmental effects of OM enrichment depend on origin-specific conditions including the prevailing Physico-chemical and biological features of the receiving environment (Islam, 2005; Sweetman et al., 2014). We provide the different biological responses of macrofaunal community to perturbation impact by evaluating changes in the metric of bioturbation indices (BP_c and IP_c) caused by natural and anthropogenic organic enrichment in two different areas. Therefore, we focused on the Po River Delta (northern Adriatic Sea) and Servola pipelines (Gulf of Trieste, northern Adriatic Sea). The first one is considered one of the major transitional systems in Europe, which is characterized by multiple physical-chemical and biological processes favoring natural organic enrichment and sedimentation (Hedges and Keil, 1995), and wide seasonal, daily variability of chemical-physical parameters and fluvial inputs (Marchini et al., 2008). The latter one is the main sewage discharge of Trieste city carrying out organic enrichment from anthropogenic origin.

Specifically, this study aims to investigate the effects of organic enrichment by natural and anthropogenic impacts on the macrofaunal community applying bioturbation and bioirrigation indices (BP_c and IP_c) in two different impacted areas. We hypothesized that the macrofaunal community, inhabiting the coastal area in front of Po River Delta and nearby sewage outfalls, responds differently in terms of bioturbation attributes to uneven amounts of OM. We aimed to answer the following specific questions: (1) Does the structure of the benthic macrofauna community affect bioturbation processes in different sedimentary environments? (2) Do macrofaunal bioturbation attributes show spatial variability associated with different OM inputs of terrigenous/freshwater allochthonous and sewage-derived materials? (3) Are the bioturbation attribute patterns driven by specific sediment physicochemical parameters?

4.2 Material and methods

4.2.1 Study area

The study was performed in coastal areas located in the northern Adriatic Sea subjected to a high amount of organic enrichment from natural (Po River Delta) and anthropogenic origins (sewage discharges in the Gulf of Trieste) (Fig. 1 and Table S1).

The Po River, with a drainage basin of 71,000 km^2 and a length of 673 km, is the most important river in Italy and one of the largest in Europe. It extends over 685 km^2 and most of the drainage basin runs through a wide low-gradient alluvial plain, with seven river branches, several lagoons, and wetlands (Tesi et al., 2011). Characterized by two annual floods (>5000 m^{3}s^{-1}), associated with rainfall in autumn and snowmelt in spring (Boldrin et al., 2005). Its total discharge is not equally distributed along the coast of the delta. Only 20% flows into the northern coast, 30% to the Pila tip (then driven southward by coastal currents), and the remaining 50% into the southern coast. During normal flow conditions, transported fine-grained sediments undergo a relatively rapid deposition nearby the mouths (~6 cm year^{-1} near the Pila distributary; Tesi et al., 2011).

Conversely, during flood events, these particles may cover a wide distance before reaching the sea bottom. The plume is principally transported southward along the shelf due to the predominant cyclonic Western Adriatic Coastal Current –WAC (driven chiefly by the pressure gradient established between interior dense-water and coastal freshwater set up by the Italian rivers) and it is subjected to wind-induced resuspension events promoted principally by the north-easterly Bora wind (Frignani et al., 2005). Furthermore, the latter tends to confine the plume along the Italian coastline (Kourafalou, 1999), especially during winter when this katabatic wind is stronger while the south-easterly Scirocco drives riverine water northward (Pirazzoli and Tomasin, 2002).

The Gulf of Trieste is a shallow embayment of about 100 km coastline and vastness about 600 km^2, located at the northern Adriatic Sea (Italy) with an average depth of 17 m. The physical and chemical features of the Gulf are exhaustively described by Lipizer et al., (2012). The Gulf hosts the main sewage treatment plant of Trieste city. The plant is located at the foot of the Servola hill, serving up to 270,000 inhabitants of Trieste with a maximum flow of 6000 L sec^{-1}(Solis-Weiss et al., 2007). In this plant, the wastewaters are subjected to a treatment of physical-chemical type since 1992 (while for the sludge is provided anaerobic digestion heated with the recovery of the biogas produced). The Servola sewage disposal plant is composed of two adjacent pipelines (6.5 and 7.5 km) leading to the sea at a depth of 20 to 23 m with a numeral of diffusers and a length of dispersion zone about 1.5 km (1 km longest and 0.5 km shortest pipe, respectively) by type of mixture collected and treating both wastewaters and meteoric within 50 million m^3 per year (Novelli, 1996).

4.2.2 Sampling design and samples processing

Sediments in front of the Po River delta were collected in December 2014 (after flood events; Bongiorni et al., 2018); whereas the sampling nearby sewage outfalls (in the Gulf of Trieste) was performed in April 2018. Nine sampling sites in the Po River prodelta area were located at increasing depths (comprised between 9 and 21 m) and distance from the main distributary mouth (Po di Pila) along the southward river plume (Fig. 1a, Table S1). In the Gulf of Trieste, to expose the best coverage of the whole diffusion area, 15 stations were sampled following an increasing distance from the pipelines (5, 100, and 200 m) for each outfall, the shortest pipe ('proximal' transect) and the longest and main one ('distal' transect; Fig 1b, Table S1). Additionally, in 'distal' transect were sampling stations at 25 meters from the duct.

In both areas, sediments were collected by a van Veen grab (0.1 m^2). The macrofaunal were collected in three replicates and sieved with a 1 mm mesh and the organisms instantly fixed with ethanol (80%). At the laboratory, the organisms were separated from the sediment and identified into the lowest possible taxonomical level employing a stereomicroscope (Model; Zeiss Discovery V.12, 8-110× final magnification) and counted for each station, separately.%).

Weight estimate (Wet Weight-WW) was measured for each taxon. Subsequently, to obtain the Dry Weight (DW), samples were placed in an oven at 100°C for 24 hours, cooled in a lab desiccator to the normal temperature of the room, and then weighed. To obtain the ash quantity of the organisms, they were placed into an oven with 500°C for 24 hours**,** cooled to room temperature in a lab desiccator, and after that weighed. To obtain Ash Free Dry Weight (AFDW), ash-weight was subtracted from DW (Wetzel et al., 2005). The environmental variables considered in this study (i.e. shells, sand, silt and clay fractions, Total Organic Carbon-TOC, Total Nitrogen-TN and carbon and nitrogen molar ratio-C:N) were determined on the same samples, and are thoroughly described in Nasi et al. (2020) for Po River coastal area, whereas in Vesal et al., (Accepted) for the Gulf of Trieste.

4.2.3 Estimation of the bioturbation potential (BP) and irrigation potential (IP)

The bioturbation potential (BP-Solan et al., 2004a) was computed according to the following equation:

$$BP_c = \sum_{i=1}^{n} BP_i \text{, whereas } BP_i = (B_i/A_i)^{0.5} * A_i * M_i * R_i$$

where B_i and A_i metrics are biomass AFDW in (gr m^{-2}), and the number of individuals (m^{-2}), respectively. M_i and R_i are categorical scores of species that represent increasing mobility (M_i) and increasing sediment reworking (R_i). Community-level bioturbation potential (BP_c) and individual taxa (BP_i) were calculated across whole sampling species. The list of mobility (M) and reworking (R) scores was used in this study as have been introduced by Queirós et al., (2013) and Gogina et al., (2017). Moreover, coding was determined according to the expert knowledge for a small number of sampling species that were not allocated in the previous study (Supplemental Table S2 provides the categorical of species scores for M_i and R_i).

The irrigation potential (IP) of Wrede et al., (2018) is defined by traits that include burrow type (BT_i), feeding type (FT_i), injection pocket depth (ID_i) which are the irrigation behaviour of benthic macrofaunal species and their effects on ecosystem functioning. The irrigation potential equation of each species taking into account in the different sampled stations is given by:

$$IP_c = \Sigma^n\ i=1\ IP_i,\ \text{where}\ IP_i = (B_i/A_i)^{0.75} * A_i * BT_i * FT_i * ID_i$$

mean individual biomass of each species (i) is expressed by ratio B_i/A_i, where B_i is the biomass of species (AFDW) per 0.1 g^2 while A_i is abundance per 0.1 m^2. According to Wrede et al., (2018), the categorical trait demonstrates the species-specific occurrence of the relevant trait and is assigned by numerical scores. We considered a bit of modification for the categorical trait scores, due to the lack of the sub-suspension/funnel habit in the sampling area and prevailing occurrence of the deposit-feeding type. The categorical traits were adjusted by different scores, considering deposit feeder type in surface and subsurface deposit feeder moods (see Table S2 provides the categorical taxa scores for BT_i, FT_i and ID_i). The collection of data on BT_i, information was acquired from the previous studies (Kristensen et al., 2012; Renz et al., 2018; De-la-Ossa-Carretero et al., 2012). We obtained the scores on FT_i mood based on the literature (e.g., Jumars et al., 2015) and the databases (www.polytraits.lifewatchgreece.eu; www. marlin.ac.uk/biotic). Moreover, we implemented the information on ID_i moods from the literature (e.g., Levin et al., 1997; Atkinson et al., 1998; Morys et al., 2017). If no information could be available for injection depth scores at the species level, a score was used from the next highest taxonomic rank or indicated as not available information (n.a. in Table S2) and delete from the index calculation.

4.2.4 Statistical analyses

Before all the analyses, data were explored checked for normality and collinearity (Zuur et al., 2010). The differences in species richness, BP_c, IP_c, and environmental factors among groups of stations in both study areas (for factors see the 'transects' namely in Tabel S1) were computed by Mann-Whitney U tests. A one-way PERMANOVA test was used to check for significant differences in BP_i and IP_i values for every single species among groups of stations in both study areas, where factors (reported in Table S1) 'were selected as fixed factors. When significant differences were noticed, PERMANOVA pairwise tests were performed. Unrestricted permutation of raw data and 9999 permutations were performed.

To observe any spatial patterns in bioturbation attribute values, a non-metric multidimensional scaling analysis (nMDS) was applied for two matrices (i.e. BP_i and IP_i, for both study areas separately). The environmental (sand, silt, clay, TOC, and C:N) variables were overlaid as supplementary variables (vectors) onto ordination spaces to investigate their relations in this distribution.

In addition, to assess the variation of species score in sampling areas, the relative frequencies of scores for the factors 'north and south' and 'distance gradient' for the prodelta and diffusion zones were measured, respectively.

Further, to indicate the significance covaried coherently on the BP_i and IP_i values i in both study areas, Similarity Profiles (SIMPROF) analysis was applied. To detect which taxa were mainly responsible for bioturbation and irrigation activities (BP_i and IP_i data, respectively) at stations gathered into different transects in both study areas, SIMPER analysis was used and different factors (see Table S1) was determined. A cut-off at <70% was applied.

Additionally, distance-based redundancy analysis (dbRDA) was used to detect the relationships between bioturbation indices (BP_i and IP_i values) selected species by SIMPER test and environmental variables. Before analysis, environmental data were normalized.

To highlight the spatial relationship between predictor variables (the considered environmental parameters) and response variables (BP_c and IP_c values and scores frequencies), linear regression and Spearman's correlations were computed for each area separately. By doing so, the predictive power of environmental parameters for each bioturbation attribute was discriminated via the coefficient of determination r_s (Spearman's correlation) and R2 (linear regression).

For the multivariate analyses, the matrices BP_i and IP_i for sampling areas were square root, and the Bray-Curtis similarity was applied. The Mann-Whitney test was computed using STATISTICA 7 software and the multivariate analyses were performed using PRIMER 7 (PRIMER-E Ltd., Plymouth, UK) software.

4.3 Results

4.3.1. Taxonomic composition of the macrobenthic community

In both study areas, the macrofaunal abundance varied from 7.3±1.4 ind. m^2 at C23 to 532.3±62.0 ind. m^2 at C8, and ranged from 36.0±0.6 ind. m^2 at 0P to 343.6±9.0 ind. m^2 at -1D in the Po River delta and the Gulf of Trieste, respectively. Regarding biomass, the lowest value was observed at C12 (0.03±0.0 g m^{-2}), whereas at -1X the highest biomass was measured (2.6±0.1 g m^{-2}). Polychaetes were the dominant taxa (41.7%), molluscs (32.9%), crustaceans (10.1%), echinodermata (12.65%), and other groups (anthozoa and sipuncula together 2.5%) were found in the Po River delta, whereas polychaetes (74.3% of the total abundance) followed by molluscs (16.2%), crustaceans (7.0%), echinodermata (2.3%), and other groups (sipuncula <0.1%) in the Gulf of Trieste. In both sampling areas, a total of 253 taxa were found. In the Po River delta, the C23 had the lowest species richness (6 species) and C1 was the highest one (39 species), whereas, the species richness showed the minimum value at 0D (35 species) and maximum value at the 2P (75 species), in the Gulf of Trieste. Overall, in the diffusion zone area, significant higher species numbers were recorded if compared to Po River prodelta (U test, $z=3.8$; $p<0.01$) (Fig. 2).

4.3.2. Macrofaunal bioturbation attributes

The community bioturbation potential (BP_c) values were lower at C12 (12.8) and 0P (11.8), while the highest ones were estimated at C8 (468.01) and 1X (234.8) in the Po River delta and the Gulf of Trieste, respectively. Similarly, the irrigation potential (IP_c) showed lower values at C12 (13.2) and 0P (1.82), whereas this index was higher at C3 (250.0) and 1X (167.9) in the coastal area nearby Po River mouth and pipelines, respectively. In addition, either BP_c and IP_c did not follow variation patterns with species richness in both sampling areas (Fig. 2). Overall, higher values of irrigation potential were noticed at Po River prodelta if compared to the area nearby the pipelines, as corroborated by U test ($z = -2.9$; $p<0.01$). Further, higher values of the indices were noticed at the stations placed in the north part of the Po River (U test ‘north’ *vs* ‘south’: $z= 2.3$; $p<0.05$) for BP_C and IP_C (Fig. 3). In addition, significantly major values of BP_c and IP_c were measured at stations located at 25m far from the sewage pipeline ($H=7.8$; $p<0.05$ for both indices). No differences were measured between stations gathered in ‘distal’ and ‘proximal’ transects for diffusion area (Fig. 3)

The PERMANOVA tests on bioturbation attribute values (BP_i and IP_i) for every single species showed differences between both study areas. According to the PERMANOVA main-test, we observed a highly significant difference at increasing distance from the main distributary mouth (Po di Pila) along the southward river plume (Pseudo-F= 2.5 and 2.6; $p<0.05$ for BP_i and IP_i, respectively). Similarly, significant differences were noticed in bioturbation indices among stations placed at increasing distances from the pipes (Pseudo-F=3.02 and 3.03; $p<0.01$, respectively). In addition, the bioturbation indices of species significantly differed between stations gathered in ‘distal’ and ‘proximal’ transects (Pseudo-F=2.0; $p<0.05$ for BP_i and IP_i).

Additionally, SIMPER analysis performed on BP_i and IP_i values showed differences among the sampling stations in Po River prodelta area and the Gulf of Trieste. For the first sampling area, the higher BP_i values of the bivalve *Striarca lactea* (Contrib % = 7.4) and the polychaete *Owenia fusiformis* (Contrib % = 6.9) were responsible for the main difference from the stations placed on the northward part and southward of the Po River delta (north *vs* south: 77.8%, average dissimilarity). Similarly, *O. fusiformis* mostly contributed to the dissimilarity between ‘north *vs* south’ (Contrib% 11.8) due to high values of IP_i at norther sites (average dissimilarity=75.6). By SIMPER analysis on BP_i and IP_i values the dissimilarity in the Gulf of Trieste, between stations placed in 5m and 25m distance groups (5 *vs* 25: 77.8 and 87.8% average dissimilarity for BP_i and IP_i

values, respectively) were mainly due to the high BP_i and IP_i values of the mollusc *Polititapes aureus* sampled at 25 m from the main pipe (Contribution % = 5.7 and 8.6, respectively). The dissimilarity between stations located in 25m and 100m groups (25 *vs* 100: 77.1% and 84.9 average dissimilarity for BP_i and IP_i values, respectively) were mostly due to the high value of polychaetes *Capitella capitata* (25m group), with contribution % = 5.65 and 8.35 for BP_i and IP_i values, respectively. SIMPER analysis showed the dissimilarity between stations placed at 25 *vs* 200 meters (25 *vs* 200: 85.9 and 90.5%, average dissimilarity for BP_i and IP_i values, respectively) were characterized by polychaetes *C. capitata* that were highly present at 25m station group with contribution 6.1 and 8.7% for BP_i and IP_i values, respectively.

Considering the relative frequencies (%) of scores belong to the BP_c values showed higher occurrences for reworking scores representing the superficial modifiers (64.0%) for the stations located in the northern and southern part of the prodelta (64.0 and 49.0%, respectively). In addition, towards the southern stations, a higher % of biodiffusers were calculated (29.0%). In the diffusion area, we measured slightly increasing percentages of 'superficial modifiers' score coupled with decreasing values of 'biodiffusors' from the stations located nearby the main outfall towards the farther ones (Fig. 4a). Regarding the mobility scores (Fig. 4b), in the coastal area of Po River, major values of relative frequencies of organisms with 'limited movements' were measured in northern stations compared to southern ones. In addition, in the Gulf of Trieste, we observed great changes of score % belong to 'movements through the sediment matrix' and 'free movements via burrow types'. The latter was noticed with a higher value nearby the outfalls (34.5%) and remarkably decreased towards the stations far from the diffusion zone. On contrary, we measured increasing values of 'movements through the sediment matrix' and 'organism that lives in fixed tubes' scores at stations placed at 100 and 200 meters from the main pipe. According to the IP_c values, relative frequencies (%) indicated the scores belong to burrow type varied in the coastal area of prodelta. A higher % of 'infauna with internal irrigation' score in northern stations (34.0%), whereas the increasing value of 'blind-ended irrigation' score (41.8%) was noticed at southern ones. In the Gulf of Trieste, we observed decreasing value of 'open irrigation' score moving from the stations nearby diffusion zone towards the farther ones (Fig. 5a). 'Surface deposit feeder', and 'injection depth from 2 to 5 cm' were highly expressed in northward and 25 m distance front of the main outfall in prodelta and Gulf of Trieste, respectively (Fig. 5b and c). Lastly, the stations gathered in 25 meters were characterized by a higher % of 'depth over 10 cm' score if compared to the other ones were observed in later stations in both study areas.

4.3.3 Relation between bioturbation indices and environmental factors

Considering both sampling areas, TN and TOC were highly covaried (r_s=0.096), therefore we deleted TN from the subsequent analyses. The linear regression was computed using environmental parameters as predictor and bioturbation indices (BP_c and IP_c) as response variables and was summarized in Table 1.

The nMDS performed on BP_i and IP_i values of Po River coastal area further confirmed the PERMANOVA results (Fig. 6a and b). Also, the differences among groups of stations (i.e. 'north and south') were enhanced by SIMPROF analyses, particularly for IP_i values. The stations located in the northern part of prodelta (C1, C3, and C8) were placed on the right side of the nMDS plot-based BP_i (Fig. 6a) and on the left side of the nMDS plot-based IP_i values (Fig. 6b). Fig. 6a and b explained that clay was only responsible for the difference among the stations mainly those increasing away from the main distributary mouth (Po di Pila) along the southward river plume (C12, C19, C22, and C23). In addition, the nMDs performed on BPi and IPi composition in the Gulf of Trieste did not follow (by SIMPROF test) the same results obtained by PERMANOVA test. Furthermore, the nMDS showed the stations located at 25 meters distance from the sewage duct (-25D, 25FD, and 25D stations) were placed on the left side of the nMDS plot-based BP_i (Fig. 7a) and the right side of the nMDS plot-based IP_i (Fig. 7b) values at the maximum distance (Bray-Curtis maximum dissimilarity) from stations placed at 100 and 200 meters. These results evidenced a different composition in reworking and bioirrigation attributes of species that inhabiting the stations 25 meters far from the duct also if compared to whom at 5 meters. The latter differences could due to higher values of sand and TOC at stations gathered at 25 meters from the duct, whereas a major % of silt and clay was noticed at 100 and 200 meters group of stations.

The distance-based redundancy analysis (dbRDA) performed using BP_i and IP_i values of species selected by SIMPER and environmental variables for both sampling areas, explained the 54.9% and 52.2% of the total variation, respectively (Fig. 8a and b). Overall, both analyses plotted the stations separately according to sampling areas and factors. Regarding both dbRDA the negative part of dbRDA1 (left side of Fig. 8a), higher percentages of silt corresponded to high occurrences of the polychaete *Sternaspis scutata, O. fusiformis,* and *Heteromastus filiformis* in prodelta area. Clay was the predominant element of the positive axis of dbRDA2, related principally with the polychaetes *Lumbrinereis lusitanica* and *Hilbigneris gracilis* that occurred in the further stations from the diffusion zone. In addition, C:N, TOC, and sand were the predominant elements of the negative part of dbRDA2, strictly related to some bivalves species in prodelta area (e.g. *Varicorbula gibba, S. lactea,* and *Peronidia albicans* and polychaetes such as *Glycera trydactila* and *Eunice vittata*). Regarding the dbRDA analyses performed on IP_i values, some different species were related to second axis (e.g. the bivalve *Moerella distorta*, and the polychaetes *C. capitata* and *Maldane sarsi*).

4.4 Discussion

This study shows that the variations in macrofaunal bioturbation attributes among stations in both study areas were likely due to the effects of grain-size composition and the degree of organic enrichment more than the differences in the type of organic matter inputs (terrigenous/freshwater allochthonous and sewage derive). In addition, our results show that macrofauna invertebrates with high burrowing depths and blind-ended burrows, abundant at sandier stations, enhanced bioirrigation. Regardless of the type of organic matter sources, the high density of superficial modifiers and biodiffusors (belonging to 'reworking' traits categories) enhance the bioturbation activities, increasing the entire ecosystem functioning in both study areas, where a higher % of biodiffusers were found towards the southern stations at the coastal area of Po River, while a slightly increasing percentage of 'superficial modifiers' score coupled with decreasing values of 'biodiffusors' were observed from the stations located nearby the main outfall towards the farther ones in the diffusion area.

In both sampling areas was not evident a variation pattern of species richness and macrofaunal attributes. In the Gulf of Trieste, the highest values of species richness were observed toward the increasing distance from the pipe, whereas the maximum of BP_c and IP_c was noticed at stations located at 25 meters from the main outfall. Similarly, in the prodelta area, where despite the higher numbers of species at northern stations if compared to southern ones, the major values of BP_c and IP_c at norther sites did not follow richness values. Our results evidenced that the structure of the benthic macrofauna community did not affect bioturbation processes in different sedimentary environments. Our finding is in contrast with what was observed by Nasi et al., 2020. They discovered that the structure of macrobenthic community deeply influenced the bioturbation attributes. However, they study macrofaunal invertebrates from brackish environments. The latter community is *per se* less structure if compared to one from coastal areas, thus few species could be able to sustain the bioturbation processes (Nasi et al., 2020).

We inferred that physical environmental features, mainly different grain-size fractions, determine the broad pattern of benthic organism distribution, instead of the origin of organic matters (i.e. terrigenous or sewage-derived materials). Our findings are in line with previous studies that have reported grain-size as the main driver of the spatial distribution pattern of the bioturbation attributes (Gogina et al., 2017; Morys et al., 2017). In addition, despite the lower values of species richness, the bioturbation attributes in BP_c and IP_c in prodelta area, resulted higher if compared to the diffusion zone (Gulf of Trieste), also at the stations farther from the main point of contamination (the principal distributary mouth). This is principally linked to the presence of the highest dominance of surface deposit feeders at norther sites whereas sub-surface deposit feeders and biodiffuors in southern ones. The feeding strategy of macrofaunal invertebrates is fundamental to determine the type of burrows and the modes of locomotion and defecation (Kristensen and Kostka, 2005; Kristensen et al., 2012; Jumars et al., 2015).

Our results, as observed by other authors (Gogina et al., 2017, Breine et al., 2018) indicated that surface and subsurface deposit feeders mostly contributed to BP_c and IP_c of coastal marine environments. The huge amount of riverine organic matter deeply influenced the bioturbation features in prodelta areas. The continuous and high load of terrigenous material from a river is known to affect suspension-feeding animals by clogging feeding structures, interfering with particles selection, and require the use of energy to clear away unvented particles (Thrush et al., 2004). In fact, in the central part of the Po delta system (i.e. C8), where high current velocities and low turbidity were reported (Braga et al., 2017; Maicu et al., 2018), we observed a high dominance of suspension feeder on the account of *O. fusiformis* and the bivalve *Varicorbula gibba*. The majority of these suspension feeders are superficial modifiers. Those can rework sediments but not so much as biodiffusers. The majority of these suspension feeders are superficial modifiers. Superficial modifiers are considered weak bioturbators since these animals can rework only the most superficial sediments and thus have a low impact on bioturbation processes compared to the other reworking modalities (i.e. conveyors, biodiffusors, and regenerators) (Queirós et al., 2013). The higher values of BP_c in this station (i.e. C8) were principally due to the huge amount of *O. fusiformis*.

Southern stations were characterized by high expression of modalities biodiffuser and deposit feeders, on the account of *S. scutata* and the echinoderm *Amphiura chiajei*. Biodiffusers are dominant in muddy sediments since they can constantly and randomly biomix (both horizontally and vertically) the local sediments over a short distance, which results in particle transport (Queirós et al., 2015). Among them, gallery biodiffusers often occur in finer sediments in which they are promoters of diffusive local biomixing primarily due to burrowing activities within the upper 10–30 cm of sediments (Kristensen et al., 2012). These animals, in particular polychaetes, are considered non-selective deposit feeders (Lopez and Levinton, 1987) able to feed on both fresh and aged organic matter, promoting nutrient cycling within sediment layers (Törnroos and Bonsdroff, 2012; Nasi et al., 2020). Commonly, *S. scutata* is a deep burrowing subsurface deposit feeder living below 4 cm of depth and can use the food resource provided by river floods later on and over a longer period (Selen-Picard et al., 2003).

Regarding IP_c, higher values of bioirrigation, were noticed at both groups of the site in prodelta areas, if compared to diffusion zone, however major bioturbation activities were reported in the southern station if compared to sediment reworking ones. These results confirmed the importance of grain-size distribution for bioirragtion features. In finer sediments, the permeability rate is low, and therefore, open irrigation (U- or Y-shaped burrows with two or more openings to the sediment surface and radial diffusion mode) are prevalent and enhance the water interchange. These differences reflect the fact that bioirrigation rates are species-specific and depend on the physical mechanism used (Kristensen, 2001; Shull et al., 2009; Kristensen et al., 2014). This is supported by a shift from anoxia to air-saturated conditions at the burrow opening and increases microbial respiration faster than possible by the molecular diffusion system (Mermillod-Blondin and Rosenberg, 2006).

The bioturbation processes in the Gulf of Trieste were lower if compared to Po River prodelta area. We inferred that this difference could be strictly linked to the biomass but above all to the abundance of species found. In the diffusion zone, we have higher values of species but also not so many individuals for species (higher evenness values, data not reported), excepted for *Capitella capitata* that was observed with higher abundance but low biomass at only 0D station. On contrary, in prodelta coastal area, we noticed in many stations major specimen density (Nasi et al., 2020) so the values of BP_c and IP_c were higher. The highest values of BP_c and IP_c latter, confirmed by U test, were observed at stations placed 25 m distance away from the main sewage outfall. Regarding bioturbation processes, the latter results were due to the dominance of conveyors.

The dominance of *C. capitata* at 25 meters from the pipe, could have influenced the distribution of organic matter within sediments (i.e. TOC and TN), promoting the unexpected decreasing of organic matter at surface layers nearby the duct. It is known that the high presence of *C. capitata* may promote the mineralization of organic matter deposited in the sea bottom within a relatively short period (Chareonpanich et al., 1993). Reworking and ingestion of sediment particles may have contributed to modify sediment properties and so promoted microbial population resulting in accelerated degradation of organic matter (Kinoshita et al., 2008). Additionally, the latter was confirmed by Braeckman et al. (2014), suggesting that BP_c values could be a good predictor of oxygen consumption, denitrification, alkalinity and ammonium fluxes in fine sandy sediments. What was observed by the authors might explain the hypoxic conditions at the area nearby the outfall despite high sediments reworking. Furthermore, the higher values of IP_c at stations gathered at 5 and 25 meters from the pipes were principally due to the presence of open irrigation system at sandier sites. In fat, the main role in determining how pore water exchanged within sediments is linked to the morphology of burrows (Kristensen and Kostka, 2005; Nasi et al., 2020), and burrow irrigation is characterized by the advection of pore water in more penetrable sandy sediments, causing the building of blind-ending burrows (Kristensen et al., 2012). Moreover, at the stations nearby the diffusion zone, we observed a more injection depth. The burrowing depth is important for the pore water exchange and local input of oxygen into anoxic sediments. In particular, the effects of deep burrowing organisms are enhanced in oxic or suboxic sediments compared to those of shallow burrowers (Aller, 1988).

The present study represents a contribution to the growing body of bioturbation research, especially in the scantily investigated coastal river lagoons. The use of BP_c and IP_c could be matched with experimental data to corroborate our findings and help introduce the application of functional traits in the assessment of the benthic ecosystem functioning. The deepening of the knowledge on macrofaunal bioturbation attributes concerning their sediment reworking and ventilation abilities, after anoxic and dystrophic events, is of paramount importance in the framework of efficient management and sustainable use of coastal resources, especially areas deeply influence by human impacts.

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References

Airoldi, L., Balata, D., Beck, M.W., 2008. The Gray Zone: Relationships between habitat loss and marine diversity and their applications in conservation. Journal of Experimental Marine Biology and Ecology 366, 8–15.

Aller, R. C., 1977. The influence of macrobenthos on chemical diagenesis of marine sediments. Ph.D. dissertation, Yale University, New Haven, Conn., 600p.

Aller, R.C., 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water. In: McCall, P.L., Tevesz, M.J.S. (Eds.), Animal Sediment Relations. Plenum Press, New York, pp. 53–102.

Aller, R. C., 1988. Interactions between bioturbation and Mn cycling in marine sediments. EOS Transact. Am. Geophys. Union 69:1106.

Atkinson, R.J.A., Froglia, C., Arneri, E., Antolini, B., 1998. Observations on the burrows and burrowing behaviour of Brachynotus gemmellari and on the burrows of several other species occurring on Squilla grounds off Ancona, Central Adriatic. Sci. Mar. 62 (1–2), 91–100.

Bilyard, G. R., 1987. The value of benthic infauna in marine pollution monitoring studies. Mar. Pollut. Bull. 18,581-585.

Birchenough, S., Parker, R., McManus, E., Barry, J., 2012. Combining bioturbation and redox metrics: potential tools for assessing seabed function. Ecol. Ind. 12:8–16.

Boldrin, A., Langone, L., Miserocchi, S., Turchetto, M., Acri, F., 2005. Po River plume on the Adriatic continental shelf: dispersion and sedimentation of dissolved and suspended matter during different river discharge rates. Mar. Geol. 222, 135–158.

Bongiorni, L., Nasi, F., Fiorentino, F., Auriemma, R., Rampazzo, F., Nordstr€om, M.C., Berto, D., 2018. Contribution of deltaic wetland food sources to coastal macrobenthic consumers (Po River Delta, north Adriatic Sea). Sci. Total Environ. 643, 1373–1386.

Borja, A., Galparsoro, I., Solaun, O., Muxika, I., Tello, E.M., Uriarte, A., Valencia, V., 2006. The European Water Framework Directive and the DPSIR, a methodological approach to assess the risk of failing to achieve good ecological status. Estuarine, Coastal and Shelf Science 66, 84–96.

Borja, A., Dauer, D.M., Elliott, M., Simenstad, C., 2010. Medium and long-term recovery of estuarine and coastal ecosystems: patterns, rates and restoration effectiveness. Estuaries and Coasts 33, 1249–1260.

Braeckman, U., Foshtomi, M. Y., Van Gansbeke, D., Meysman, F., Soetaert, K., Vincx, M., et al. 2014. Variable importance of macrofaunal functional biodiversity for biogeochemical cycling in temperate coastal sediment. Ecosystems (in press) doi:10.1007/s10021-014-9755-7.

Braga, F., Zaggia, L., Bellafiore, D., Bresciani, M., Giardino, C., Lorenzetti, G., et al., 2017. Mapping turbidity patterns in the Po river prodelta using multi-temporal Landsat 8 imagery. Estuar. Coast Shelf Sci. 198, 555–567.

Breine, N. T., De Backer, A., Van Colen, C., Moens, T., Hostens, K., Van Hoey, G., 2018. Structural and functional diversity of soft–bottom macrobenthic communities in the Southern North Sea. Estuarine, Coastal and Shelf Science, 214: 173–184. https://doi.org/10.1016/j.ecss.2018.09.012

Bremner, J., Rogers, S. I., Frid, C. L. J., 2006. Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). Ecological Indicators, 6(3), 609–622.

Bunker, D. E., DeClerck, F., Bradford, J. C., Colwell, R. K., Perfecto, I., Phillips, O. L., Naeem, S., 2005. Species loss and aboveground carbon storage in a tropical forest. Science, 310 (5750), 1029–1031.

Chareopanich, C., Montani, S., Tsutsumi, H., Matsuoka, S., 1993. Modification of chemical characteristics of organically enriched sediment by Capitella sp. I. Mar Poll Bull 26(7): 375–379

Christensen, B., Vedel, A., Kristensen, E., 2000. Carbon and nitrogen fluxes in sediment inhabited by suspension-feeding (*Nereis diversicolor*) and non-suspension feeding (*N. virens*) polychaetes. Mar. Ecol. Prog. Ser. 192, 203–217.

Crain, C. M., Halpern, B. S., Beck, M. W., Kappel, C.V., 2009. Understanding and managing human threats to the coastal marine environment. Ann. NY Acad. Sci. 1162, 39 – 62. doi:10.1111/j.1749-6632.2009. 04496.x

Darnaude, A. M., Salen-Picard, C., Polunin, N. V. C., HarmelinVivien, M. L., 2004. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138: 325−332.

De-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F., Sánchez-Lizaso, J. L., 2012. Assessing reliable indicators to sewage pollution in coastal soft-bottom communities. Environ. Monit. Assess. 184, 2133–2149.

Diaz, R. J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science 321:926– 29.

Ferraro, S. P., Cole, F. A., 1995. Taxonomic level sufficient for assessing pollution impacts on the Southern California bight macrobenthos—revisited. Environmental Toxicology and Chemistry 14:1031–1040.

Frignani, M., Langone, L., Ravaioli, M., Sorgente, D., Alvisi, F., Albertazzi, S., 2005. Fine-sediment mass balance in the western Adriatic continental shelf over a century time scale. Mar. Geol. 222, 113–133.

Giani, M., Berto, D., Rampazzo, F., Savelli, F., Alvisi, F., Giordano, P., et al., 2009. Origin of sedimentary organic matter in the north-western Adriatic Sea. Estuar. Coast Shelf Sci. 84 (4), 573–583.

Gogina, M., Morys, C., Forster, S., Gräwe, U., Friedland, R., Zettler, M.L., 2017. Towards benthic ecosystem functioning maps: quantifying bioturbation potential in the German part of the Baltic Sea. Ecol. Indicat. 73, 574–588.

Hedges, J.I., Keil, R.G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. Mar. Chem. 49, 81–115.

Islam, S., 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. Mar. Pollut. Bull. 50:48–61

Josefson, A. B., Norkko, J., Norkko, A., 2012. Burial and decomposition of plant pigments in surface sediments of the Baltic Sea: role of oxygen and benthic fauna. Mar. Ecol. Prog. Ser. 455:33–49.

Jumars, P.A., Dorgan, K.M., Lindsay, S.M., 2015. Diet of worms emended: an update of polychaete feeding guilds. Annu. Rev. Mar. Sci. 7, 497–520.

Kaiser, M. J., Clarke, K. R., Hinz, H., Austen, M. C. V., Somerfield, P. J., Karakassis, I., 2006. Global analysis of response and recovery of benthic biota to fishing. Marine Ecology Progress Series, 311, 1–14. https://doi.org/10.3354/meps311001

Kenny, A. J., Skjoldal, H. R., Engelhard, G. H., Kershaw, P. J., Reid, J. B., 2009. An integrated approach for assessing the relative significance of human pressures and environmental forcing on the status of Large Marine Ecosystems. Prog Oceanogr 81: 132−148.

Kenny, A.J., Jenkins, C., Wood, D., Bolam, S.G., Mitchell, P., Scougal, C., Judd, A., 2018. Assessing cumulative human activities, pressures, and impacts on North Sea benthic habitats using a biological traits approach. ICES J. Mar. Sci. 75, 1080e1092.

Kinoshita, K., Wada, M., Kogure, K., Furota, T., 2008. Microbial activity and accumulation of organic matter in the burrow of the mud shrimp, *Upogebia major* (Crustacea: Thalassinidea). Mar Biol 153: 277–283.

Koop, K., Hutchins, P., 1996. Disposal of sewage to the ocean--a sustainable solution? Mar. Pollut. Bull. 33, 121–123.

Kourafalou, V. H., 1999. Process studies on the Po River plume, North Adriatic Sea, J. Geophys. Res., 104(C12), 29,963–29,985.

Kristensen, E., 2001. Impact of polychaetes (Nereis spp. and *Arenicola marina*) on carbon biogeochemistry in coastal marine sediments. Geochem Trans 2: 92−103.

Kristensen, E., Kostka, J. E., 2005. Macrofaunal burrows and irrigation in marine sediment: microbiological and biogeochemical interactions. In: Kristensen E, Haese RR, Kostka JE (eds) Interactions between macro- and micro - organisms in marine sediments. American Geophysical Union, Washington, DC, p 125−157

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C.O., Banta, G. T., 2012. What is bioturbation? The need for a precise definition for fauna in aquatic sciences. Mar. Ecol. Prog. Ser. 446, 285–302.

Kristensen, E., Delefosse, M., Quintana, C. O., Flindt, M. R., Valdemarsen, T., 2014. Influence of benthic macrofauna community shifts on ecosystem functioning in shallow estuaries. Front. Mar. Sci. 1, 1–14.

Lancellotti, D.A., Stotz, W.B., 2004. Effects of shoreline discharge of iron mine tailings on a marine softbottom community in northern Chile. Marine Pollution Bulletin 48, 303–312.

Levin, L., Blair, N., DeMaster, D., Plaia, G., Fornes, W., Martin, C., Thomas, C., 1997. Rapid subduction of organic matter by maldanid polychaetes on the North Carolina slope. J. Mar. Res. 55 (3), 595–611.

Lipizer, M., De Vittor, C., Falconi, C., Comici, C., Tamberlich, F., Giani, M., 2012. Effects of intense physical and biological forcing factors on CNP pools in coastal waters (Gulf of Trieste, Northern Adriatic Sea). Estuarine, Coastal and Shelf Science, 115, 40–50.

Lissner, A. L., Taghorn, G. L., Diener, D. R. Schroeter, S. C., Dixon, D., 1991. Recolonization of deep-water hard substrate communities: potential impacts from oil and gas development. Ecological Applications, 1, 258–267.

Lohrer, A., Halliday, N., Thrush, S., Hewitt, J., Rodil, I., 2010. Ecosystem functioning in a disturbance-recovery context: contribution of macrofauna to primary production and nutrient release on intertidal sandflats. J. Exp. Mar. Biol. Ecol. 390:6–13.

Lopez, G. R., Levinton, J. S., 1987. Ecology of deposit feeding animals in marine sediments. Q. Rev. Biol. 62: 235- 260.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., Jackson, J. B. C., 2006. Depletion, Degradation, and Recovery Potential of Estuaries and Coastal Seas. Science 312:1806–1809.

Maicu, F., De Pascalis, F., Ferrarin, C., Umgiesser, G., 2018. Hydrodynamics of the Po River-Delta-Sea system. J Geophys Res Oceans. 123:6349–6372.

Marchini, A., Munari, C., Mistri, M., 2008. Functions and ecological status of eight Italian lagoons examined using biological traits analysis (BTA). Mar. Pollut. Bull. 56, 1076–1085. https://www.marlin.ac.uk/biotic.

Mermillod-Blondin, F., Rosenberg, R., 2006. Ecosystem engineering: the impact of bioturbation on biogeochemical processes in marine and freshwater benthic habitats. Aquatic Sciences 68:434–442.

Mermillod-Blondin, F., 2011. The functional significance of bioturbation and biodeposition on biogeochemical processes at the water-sediment interface in freshwater and marine ecosystems. J N Am Benthol Soc 30: 770−778.

Meysman, F.J., Middelburg, J.J., Heip, C.H., 2006. Bioturbation: a fresh look at Darwin’s last idea. Trends Ecol. Evol. 21, 688–695.

Moon, H-B., Yoon, S-P., Jung, R-H., Choi, M., 2008. Wastewater treatment plants (WWTPs) as a source of sediment contamination by toxic organic pollutants and fecal sterols in a semi-enclosed bay in Korea. Chemosphere; 73:880–9.

Morys, C., Powilleit, M., Forster, S., 2017. Bioturbation in relation to the depth distribution of macrozoobenthos in the southwestern Baltic Sea. Mar. Ecol. Prog. Ser. 579, 19–36.

Nasi, F., Ferrante, L., Alvisi, F., Bonsdorff, E., Auriemma, R., Cibic, T., 2020. Macrofaunal bioturbation attributes in relation to riverine influence: What can we learn from the Po River lagoonal system (Adriatic Sea)? Estuarine, Coastal and Shelf Science, 232, 106405.

Novelli, G., 1996. Gli scarichi a mare nell`alto Adriatico. Rassegna tecnica del Friuli Venezia Giulia, 3, 11–19.

Olsgard, F., Gray, J. S., 1995. A comprehensive analysis of the effects of offshore oil and gas exploration and production on the benthic communities of the Norwegian continental shelf. Mar. Ecol. Prog. Ser. 122: 277–306.

Painting, S. J., Van der Molen, J., Parker, E., Coughlan, C., Birchenough, S., Bolam, S., Aldridge, J. N., Forster, R. M., Greenwood, N., 2012. Development of indicators of ecosystem functioning in a temperate shelf sea: a combined fieldwork and modelling approach. Biogeochemistry 113:1–21.

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

Pirazzoli, P. A., Tomasin, A., 2002. Recent evolution of surge-related events in the northern Adriatic area, J. Coast. Res., 18(3), 537– 554.

Queirós, A. M., Hiddink, J. G., Johnson, G., Cabral, H. N., Kaiser, M. J., 2011. Context dependence of marine ecosystem engineer invasion impacts on benthic ecosystem functioning. Biological Invasions, 13(5), 1059-1075.

Queirós, A. M., Birchenough, S. N., Bremner, J., Godbold, J. A., Parker, R. E., Romero-Ramirez, A., Reiss, H., Solan, M., Somerfield, P. J., Van Colen, C., Van Hoey, G., Widdicombe, S., 2013. A bioturbation classification of European marine infaunal invertebrates. Ecol. Evol. 3, 3958–3985.

Queirós, A. M., Fernandes, J. A., Faulwetter, S. et al. 2015. Scaling up experimental ocean acidification and warming research: from individuals to the ecosystem. Global Change Biology, 21, 130–143.

Remaili, T. A. Simpson, S. L., Amato, E. D., Spadaro, D. A., Jarolimek, C. V., Jolley, D. F., 2016. The impact of sediment bioturbation by secondary organisms on metal bioavailability, bioaccumulation and toxicity to target organisms in benthic bioassays: implications for sediment quality assessment. Environ. Pollut. 208, 590−599.

Remaili, T. M., Simpson, S. L., Jolley, D. E. F., 2017. Effects of enhanced bioturbation intensities on the toxicity assessment of legacy-contaminated sediments. Environ Pollut 226:335–345.

Renz, J.R., Powilleit, M., Gogina, M., Zettler, M.L., Morys, C., Forster, S., 2018. Community bioirrigation potential (BIPc), an index to quantify the potential for solute exchange at the sediment-water interface. Mar. Environ. Res. 181, 214–224.

Rhoads, D. C., and Boyer, L. F., 1982. The effects of marine benthos on physical properties of sediments. In Animal-sediment relations (3–52). Springer, Boston, MA.

Salen-Picard, C., Arlhac, D., Alliot, E., 2003. Responses of a Mediterranean soft bottom community to short-term (1993–1996) hydrological changes in the Rhone river. Mar. Environ. Res. 55, 409–427.

Savage, C., Thrush, S. F., Lohrer, A. M., & Hewitt, J. E., 2012. Ecosystem services transcend boundaries: estuaries provide resource subsidies and influence functional diversity in coastal benthic communities. PloS one, 7(8), e42708.

Schaffner, L., 1990. Small-scale organism distributions and patterns of species diversity: evidence for positive interactions in an estuarine benthic community. Mar. Ecol. Prog. Ser. 61, 107 – 117.

Shull, D. H., Benoit, J. M., Wojcik, C., Senning, J. R., 2009. Infaunal burrow ventilation and pore-water transport in muddy sediments. Estuarine, Coastal and Shelf Science 83:277–286.

Snelgrove, P. V., 1998. The biodiversity of macrofaunal organisms in marine sediments. Biodiversity and Conservation, 7(9), 1123–1132.

Solan, M., 2000. The concerted use of 'traditional' and Sediment Profile Imagery (SPI) methodologies in marine benthic characterisation and monitoring. Unpublished PhD thesis, Department of Zoology, National University of Ireland, Galway.

Solan, M., Cardinale, B. J., Downing, A. L., Engelhardt, K. A., Ruesink, J. L., Srivastava, D. S., 2004a. Extinction and ecosystem function in the marine benthos. Science, 306(5699), 1177–1180.

Solan, M., Wigham, B. D., Hudson, I. R., Kennedy, R., Coulon, C. H., Norling, K., Rosenberg, R. 2004b. In situ quantification of bioturbation using time-lapse fluorescent sediment profile imaging (f-SPI), luminophore tracers and model simulation. Marine Ecology Progress Series, 271, 1–12.

Solan, M., Scott, F., Dulvy, N. K., Godbold, J. A., Parker, R., 2012. Incorporating extinction risk and realistic biodiversity futures: implementation of trait-based extinction scenarios. Marine biodiversity and ecosystem functioning: frameworks, methodologies, and integration. Oxford University Press, Oxford, UK, 127–148.

Solis-Weis, V., Aleffi, I.F., Bettoso, N., Rossini, P., Orel, G., 2007. The benthic macrofauna at the outfalls of the underwater sewage discharges in the Gulf of Trieste (Northern Adriatic Sea, Italy). Annales Series Historia Naturalis, 17 (1), 1–16.

Sweetman, A. K., Norling, K., Gunderstad, C., Haugland, B. T., Dale, T., 2014. Benthic ecosystem functioning beneath fish farms in different hydrodynamic environments. Limnol. Oceanogr. 59, 1139–1151.

Teal, L., Parker, E., Solan, M., 2013. Coupling bioturbation activity to metal (Fe and Mn) profiles in situ. Biogeosciences 10:2365–2378.

Tesi, T., Miserocchi, S., Goñi, M. A., Turchetto, M., Langone, L., De Lazzari, A., Albertazzi, S., Correggiari, A., 2011. Influence of distributary channels on sediment and organic matter supply in event-dominated coastal margins: the Po prodelta as a study case. Biogeosciences, 8(2), 365.

Thrush, S.F., Hewitt, J.E., Cummings, V.J., Ellis, J.I., Hatton, C., Lohrer, A., Norkko, A., 2004. Muddy waters: elevating sediment input to coastal and estuarine habitats. Front. Ecol. Environ. 2, 299–306.

Tornroos, A., Bonsdorff, E., 2012. Developing the multitrait concept for functional diversity: lessons from a system rich in functions but poor in species. Ecol. Appl. 22 (8), 2221–2236.

Van Colen, C., Rossi, F., Montserrat, F., Andersson, M.G.I., Gribsholt, B., Herman, P.M.J., Degraer, S., Vincx, M., Ysebaert, T., Middelburg, J.J., 2012. Organism-sediment interactions govern post-hypoxia recovery of ecosystem functioning. PLoS One 7, e49795.

Van Hoey, G., Permuy, D. C., Vandendriessche, S., Vincx, M., Hostens, K., 2013. An ecological quality status assessment procedure for soft-sediment benthic habitats: weighing alternative approaches. Ecol. Ind. 25:266–278.

Vesal, S. E., Nasi, F., Pazzaglia, J., Ferrante, L., Auriemma, R., Relitti, F., Bazzaro, M., Del Negro, P. The effect of sewage discharges on soft-bottom macrofauna thoroughly traits-based approach. Accepted to Marine Pollution Bulletin.

Villnäs, A., Norkko, J., Lukkari, K., Hewitt, J., Norkko, A., 2012. Consequences of increasing hypoxic disturbance on benthic communities and ecosystem functioning. PLoS One 7(10), e44920.

Wetzel, M. A., Leuchs, H., Koop, J. H. E., 2005. Preservation effects on wet weight, dry weight, and ash-free dry weight biomass estimates of four common estuarine macro-invertebrates: no difference between ethanol and formalin. Helgoland Marine Research 59:206–213.

Wilkinson, G. M., Besterman, A., Buelo, C., Gephart, J., Pace, M. L. 2018. A synthesis of modern organic carbon accumulation rates in coastal and aquatic inland ecosystems. Sci. Rep. 8, 15736.

Wrede, A., Beermann, J., Dannheim, J., Gutow, L., Brey, T., 2018. Organism functional traits and ecosystem supporting services. A novel approach to predict bioirrigation. Ecol. Indicat. 91, 737–743.

www. marlin.ac.uk/biotic

www.polytraits.lifewatchgreece.eu

Zuur, A. F., Ieno, E. N., Elphick, C. S., 2010. A protocol for data exploration to avoid common statistical problems. Method Ecol Evol 1:3–14.

Table 1: Linear regression of bioturbation indices (BP_c and IP_c) as response variables and environmental parameters as predictor. R^2: coefficient of determination; df: degrees of freedom; F and P give the calculated F value (F-test) and the corresponding probability associated with the null hypothesis. In bold the negative linear regressions. TOC-Total Organic Carbon; C:N-carbon and nitrogen ratio.

Area	Response variables	Predictor variables	R^2	df	F	P
Po River prodelta	*BPc*	**Clay**	**0.74**	**1.7**	**20.71**	**<0.01**
	IPc	Sand	0.75	1.7	21.12	<0.01
		Clay	**0.79**	**1.7**	**26.65**	**<0.01**
		C:N	0.49	1.7	6.77	0.03
Diffusion zone	*BPc*	Sand	0.56	1.13	17.07	<0.01
		Silt	**0.57**	**1.13**	**17.28**	**<0.01**
		Clay	**0.53**	**1.13**	**14.94**	**<0.01**
		TOC	0.29	1.13	5.44	0.03
	IPc	**Clay**	**0.55**	**1.13**	**16.3**	**<0.01**
		TOC	0.31	1.13	5.96	0.02
		Silt	**0.61**	**1.13**	**20.78**	**<0.01**
		Sand	0.6	1.13	19.62	<0.01

Table S1: Coordinates and depth for both sampling stations (a) along the coastal Po River Pro delta and (b) in the Gulf of Trieste along with the sewage discharge. Indication of the transect to which the station belongs as well as the distance from the sewage pipelines are reported

Station	**Latitude**	**Longitude**	**Depth (m)**	**Transect**	**Distance from the main duct (m)**
C1	44°59'34.80"	12°33'21.60"	12.5	north, nearshore	
C3	44°58'4.75"	12°34'15.58"	8.1	north, nearshore	
C8	44°55'44.37"	12°33'28.79"	11.3	north, nearshore	
C10	44°53'27.60"	12°32'16.80"	14.7	south, nearshore	
C12	44°52'1.20"	12°31'8.40"	14	south, nearshore	
C16	44°47'47.71"	12°27'22.59"	14	south, nearshore	
C19	44°45'49.03"	12°27'42.52"	19	south, offshore	
C22	44°45'3.96"	12°30'20.99"	24.5	south, offshore	
C23	44°42'29.46"	12°33'18.74"	28	south, offshore	
0D	45°38'36.30"	13°40'51.70"	22.6	distal	5
0P	45°38’28.53’’	13°41’36.67’’	22	proximal	5
25FD	45°38'36.23"	13°40'51.79"	22.4	distal	25
–25D	45°38'35.66"	13°40'51.86"	22.5	distal	25
25D	45°38'36.95"	13°40'51.53"	22.3	distal	25
1P	45°38’31.58’’	13°41’35.94’’	21.9	proximal	100
1D	45°38'39.75"	13°40'50.93"	23	distal	100
1FD	45°38'36.07"	13°40'47.26"	22.7	distal	100
–1D	45°38'33.68"	13°40'52.34"	22.3	distal	100
–1P	45°38’25.43’’	13°41’37.43’’	22	proximal	100
2P	45°38’34.56’’	13°41’35.17’’	22	proximal	200
2D	45°38'42.95"	13°40'50.02"	23.5	distal	200
–2D	45°38'30.34"	13°40'53.17"	21.9	distal	200
–2P	45°38’22.40’’	13°41’38.19’’	21.4	proximal	200
2FD	45°38'35.51"	13°40'43.10"	23.1	distal	200

Table S2: Bioturbation Potential (BPi) (Queirós et al., 2013) and Irrigation Potential (IPi) (Wrede et al., 2018) categorical scores of taxa observed in both sampling areas. Ri (Reworking) scores: 1-epifauna, 2-superficial modifiers, 3-upward and downward conveyors, 4-biodiffusors, 5-regenerators; Mi (Mobility) scores: 1-organism that lives in fixed tubes, 2-organism with limited movement, 3-movements through the sediment matrix, 4-free movements via burrow types. BTi (Burrow type) scores: 1-infauna with internal irrigation (e.g. siphons), 2-open irrigation (U- or Y- shaped burrows), 3- blind ended irrigation (blind-ended burrows, no burrows systems); FTi (Feeding type) scores: 1-surface filter feeder, 2-predator, 3-surface deposit feeder, 4-subsurface deposit feeder; IDi (Injection depth) scores:0-epibiont; 1-depth from 0 to 2 cm, 2-depth from 2 to 5 cm, 3-depth from 5 to 10 cm and 4-depth over 10 cm. From Wrede et al., (2018) the calculation of FTi was modified by dividing deposit feeder habit in surface and subsurface deposit feeder modalities, with different categorical scores.

	BPi		**IPi**		
	Ri	Mi	BTi	FTi	IDi
Abra alba	2	2	1	3	1
Abra longicallus	2	2	1	3	1
Abra prismatica	2	2	1	3	1
Abra tenuis	2	2	1	3	1
Abyssoninoe hibernica	4	3	3	2	3
Acanthocardia paucicostata	2	2	1	1	2
Aclis ascaris	1	2	1	1	1
Aclis minor	1	2	1	1	1
Acrocnida brachiata	4	3	2	4	3
Aequipecten opercularis	2	3	1	1	0
Ampelisca diadema	2	1	2	3	1
Ampelisca gibba	2	1	2	3	1
Ampelisca intermedia	2	1	2	3	1
Ampelisca ledoyeri	2	1	2	3	1
Ampelisca sarsi	2	1	2	3	1
Ampelisca spinipes	2	1	2	3	1
Ampelisca tenuicornis	2	1	2	3	1
Ampelisca tipica	2	1	2	3	1
Ampharete acutifrons	3	2	2	3	2
Amphibalanus eburneus	1	1	1	1	1
Amphibalanus improvisus	1	1	1	1	1
Amphipolis squamata	4	3	2	4	3
Amphiura chiajei	4	3	2	4	3
Amphiura filiformis	4	3	2	4	3
Anadara corbuloides	4	3	1	1	1
Anadara gibbosa	4	3	1	1	1
Anadara inaequivalvis	4	3	1	1	1

Anapagurus adriaticus	1	4	1	3	0
Anomia ephippium	1	1	3	1	0
Antalis inaequicostata	3	2	3	4	n.a.
Aonides oxycephala	3	2	1	3	1
Aphelochaeta filiformis	2	2	3	3	2
Aponuphis bilineata	2	3	3	4	n.a.
Aponuphis brementi	2	3	3	3	n.a.
Aporrhais pespelecani	2	3	3	1	1
Arcopella balaustina	4	3	2	3	3
Arcuatula senhousia	4	3	3	1	0
Aricidea (acmira) assimilis	2	3	3	3	1
Aricidea claudiae	2	3	2	3	1
Aricidea Strelzovi	2	3	3	3	1
Astropecten irregularis	2	3	1	2	1
Athanas nitescens	2	2	1	2	n.a.
Atrina fragilis	4	3	3	1	0
Azorinus chamasolen	2	2	3	1	n.a.
Balanus trigonus	1	1	1	1	2
Bela fuscata	1	2	1	3	n.a.
Bolinus brandaris	1	2	1	3	n.a.
Bradabyssa villosa	3	2	3	3	n.a.
Calyptraea chiliensis	1	2	3	1	1
Capitella capitata	3	2	2	4	2
Ceratonereis costae	4	3	3	2	4
Chaetopterus variopedatus	3	1	2	4	4
Chaetozone caputesocis	2	2	3	3	2
Chaetozone carpenteri	2	2	3	3	2
Chaetozone gibber	2	2	3	3	2
Chamelea gallina	2	2	1	1	3
Chondrochelia savignyi	2	1	n.a.	n.a.	n.a.
Coracuta obliquata	4	3	2	1	n.a.
Cylichna cylindracea	2	3	3	2	2
Dasybranchus caducus	3	4	3	4	2
Deflexilodes acutipes	2	3	3	2	0
Dexamine spinosa	2	3	1	3	1
Diodora graeca	1	2	3	2	1
Diplocirrus glaucus	3	2	3	3	n.a.
Dosinia lupinus	2	2	1	1	3
Epitonium celesti	2	2	1	2	n.a.
Epitonium clathrus	2	2	1	2	n.a.
Eriphia verrucosa	5	4	3	2	1
Ethusa mascarone	2	1	3	2	1
Eualus cranchii	2	4	1	2	1
Euclymene lumbricoides	3	1	3	4	3

Euclymene oerstedi	3	1	3	4	3
Euclymene palermitana	3	1	3	4	3
Eunice vittata	4	3	3	2	n.a.
Euspira nitida	2	3	1	2	1
Galathowenia oculata	2	1	3	3	1
Gallardoneris iberica	2	3	3	2	3
Gari fervensis	2	2	2	3	n.a.
Glycera Rouxii	4	3	2	2	4
Glycera tridactyla	4	3	2	2	4
Glycera unicornis	4	3	2	2	4
Gnathia oxyurea	2	3	1	2	n.a.
Harpinia crenulata	2	3	3	3	3
Harpinia dellavallei	2	3	3	3	3
Heteromastus filiformis	3	2	2	4	2
Hiatella artica	1	2	3	1	0
Hiatella rugosa	1	2	3	1	0
Hilbigneris gracilis	4	3	3	2	3
Iphinoe serrata	2	3	1	3	1
Joeropsis brevicornis	2	1	3	n.a.	n.a.
Kellia suborbicularis	4	3	2	4	3
Kirkegaardia dorsobranchialis	2	2	2	3	2
Kurtiella bidentata	2	2	2	4	3
Labioleanira yhleni	4	3	3	2	1
Lagis koreni	3	1	3	3	2
Laonice cirrata	3	1	3	3	2
Laonice cochilega	3	1	3	3	2
Lentidium mediterraneum	2	2	1	1	1
Leptocheirus pectinatus	2	4	2	1	n.a.
Leptopentacta tergestina	2	3	1	1	1
Leptopentacta elongata	2	3	1	1	1
Leptosynapta macrankyra	2	3	3	4	n.a.
Leuchote pacnycera	2	3	1	2	1
Leucothoe incisa	2	3	1	2	1
Leucothoe lillyeborgi	2	3	1	2	1
Leucothoe oboa	2	3	1	2	1
Levinsenia gracilis	2	3	2	3	1
Liljeborgia psaltrica	2	3	2	3	n.a.
Liocarcinus depurator	5	4	3	2	n.a.
Loripes orbiculatus	4	4	2	1	3
Loripinus fragilis	4	3	2	1	3
Lucinella divaricata	2	2	2	3	3
Lumbrineris latreilli	4	3	3	4	3
Lumbrineris luciliae	4	3	3	4	3
Lumbrineris lusitanica	4	3	3	4	3

Lumbrineris nonatoi	4	3	3	4	3
Lygdamis muratus	1	1	3	1	n.a.
Lysianella dellavallei	3	2	2	3	n.a.
Lysidice unicornis	4	3	3	3	n.a.
Maera grossimana	2	3	1	3	n.a.
Maera sodalis	2	3	1	3	n.a.
Magelona rosea	2	2	3	3	4
Magilona minuta	2	2	3	3	4
Maldane glebifex	3	1	3	4	3
Maldane sarsi	3	1	3	4	3
Marphysa sanguinea	4	3	3	4	n.a.
Medicorophiium runcicorne	2	4	2	4	n.a.
Medicorophium rotundirostre	2	4	2	4	n.a.
Megastomia conoidea	1	2	1	1	n.a.
Melanella pseudoglabra	4	3	3	4	n.a.
Mellinna palmata	3	1	3	3	2
Microcassiope minor	5	4	3	1	0
Microdeutopus anomalus	1	3	1	3	1
Microdeutopus versiculatus	1	3	1	3	1
Moerella distorta	2	2	2	3	3
Musculus subpictus	4	3	3	1	0
Myrtea spinifera	2	2	2	1	3
Mysia undata	2	2	3	1	3
Mysta picta	4	3	2	2	3
Neanthes fucata	4	4	2	2	4
Nepinnotheres pinnotheres	2	2	2	1	0
Nepthys hystricis	4	3	2	2	1
Nereis lamellosa	4	4	2	2	4
Nereis rava	4	4	2	2	4
Notomastus aberans	3	2	3	3	4
Notomastus latericeus	3	2	3	3	4
Nucula nitidosa	2	3	3	3	1
Nucula nucleus	2	3	3	3	1
Oestergrenia digitata	2	3	3	4	n.a.
Ondina dilucida	1	2	1	3	n.a.
Ophiothrix quinquemaculata	2	2	3	3	1
Ophiothrix fragilis	2	2	3	3	1
Ophiura albida	2	2	1	3	1
Ophiura grubei	2	2	1	3	1
Ophiura ophiura	2	2	1	3	1
Orchomene humilis	2	3	2	3	n.a.
Ova canaliferus	4	2	3	4	2
Owenia fusiformis	2	1	3	3	1
Paguristes eremita	1	4	1	2	0

Pagurus prideaux	1	4	1	2	0
Pandora inaequivalvis	4	3	3	3	n.a.
Papillicardium papillosum	4	3	3	1	2
Paragnathia formica	2	3	n.a.	n.a.	n.a.
Paralysianopsis parthenopeia	2	1	2	3	n.a.
Parthenopoides massena	2	2	3	2	1
Parvicardium exiguum	2	2	1	1	1
Parvicardium scabrum	2	2	1	1	1
Paucibranchia bellii	4	3	3	4	n.a.
Pectinaria (Amphictene) auricoma	3	1	3	3	3
Peronidia albicans	2	2	2	3	3
Phascolosoma granulatum	4	3	1	3	n.a.
Phaxas adriaticus	2	2	3	1	1
Pherusa monilifera	3	2	3	3	n.a.
Philocheras monacanthus	2	4	3	2	n.a.
Phtisica marina	2	2	2	3	n.a.
Phyllophorus urna	4	3	1	3	n.a.
Phylo foetida	1	3	3	2	n.a.
Pilargis verrucosa	4	3	2	2	n.a.
Pilumnus hirtellus	2	3	3	2	n.a.
Pilumnus minutus	2	3	3	2	n.a.
Piromis eruca	4	4	3	3	n.a.
Pisidia bluteli	1	3	1	2	n.a.
Pista lornensis	3	1	3	1	2
Pitar rudis	1	4	1	1	1
Poecilochaetus fauchaldi	2	2	2	3	1
Polititapes aureus	2	2	3	1	2
Polycirrus auranhjticus	3	1	3	1	2
Polydora ciliata	3	1	1	1	2
Prionospio cirrifera	3	2	3	3	2
Prionospio multibranchiata	3	2	3	3	2
Processa macrophthalma	1	4	1	2	1
Protodorvillea kefersteini	4	3	1	2	1
Psammechinus microtuberculatus	1	3	1	2	0
Pseudoleiocapitella fauveli	3	2	2	4	2
Pseudolirius kroyeri	4	1	3	3	3
Rocellaria dubia	4	3	3	1	0
Sabella pavonina	2	1	1	1	1
Saxicavella jeffreysi	1	2	3	1	n.a.
Scalibregma inflatum	4	4	2	3	3
Schistomeringos rudolphi	4	3	1	2	1
Sclerocheilus minutus	2	4	3	4	3
Scoletoma emandibulata mabiti	4	3	3	3	3
Scoletoma laurentiana	4	3	3	3	3

Scrobicularia plana	2	2	1	1	2
Serpula vermicularis	2	1	3	1	0
Serratina serrata	4	3	3	3	3
Sicyonia carinata	2	3	3	2	0
Sigambra tentaculata	4	3	3	2	n.a.
Solemya togata	4	3	3	1	n.a.
Spiochaetopterus costarum	3	1	3	1	4
Spiophanes bombyx	3	1	3	3	4
Spirobranchus triqueter	1	1	3	1	0
Spisula subtruncata	2	2	1	1	2
Squilla mantis	4	4	2	2	1
Sternaspis scutata	4	3	3	4	3
Sthenelais limicola	4	3	1	2	1
Striarca lactea	4	3	3	1	n.a.
Tellimya ferruginosa	2	2	3	4	4
Terebellides stroemii	3	1	3	1	2
Therochaeta flabellata	2	3	3	3	1
Thyone fusus	2	3	1	3	n.a.
Thysanocardia catharinae	4	3	1	3	n.a.
Timoclea ovata	2	2	1	1	1
Tritia incrassata	2	3	1	2	1
Tritia mutabilis	2	3	1	2	1
Tritia reticulata	2	3	1	2	1
Tritia varicosa	2	3	1	2	1
Turritellinella tricarinata	4	3	1	1	1
Upogebia deltaura	3	4	2	4	4
Varicorbula gibba	2	2	1	1	1
Virgularia mirabilis	2	2	1	1	1
Websterinereis glauca	4	4	2	2	4
Westwodila rectirostris	2	3	3	2	n.a.

Figure captions

Figure 1: Map showing the location of both study areas: a) the Po River prodelta and b) the area nearby the two outfalls of Servola sewage plant (Gulf of Trieste).

Figure 2: Total bioturbation potential (BP_c) and irrigation potential (IP_c) of community and number of species in different sampled areas.

Figure 3: Boxplots showing the variability of bioturbation potential (BP_c) and irrigation potential (IP_c) of community at north and south for Po River coastal area (a) and at increasing distance from pipelines (5, 25, 100, and 200m) in the Gulf of Trieste (b).

Figure 4: Relative frequencies (%) of scores belonging to reworking (a) and mobility (b) of bioturbation activities of community at north and south for Po River coastal area and at increasing distance from pipelines (5, 25, 100, and 200m) in the Gulf of Trieste. For scores see Table S2.

Figure 5: Relative frequencies (%) of scores belonging to burrow type (a), feeding type (b), and injection depth (c) of bioirrigation activities of community at north and south for Po River coastal area and at increasing distance from pipelines (5, 25, 100, and 200m) in the Gulf of Trieste. For scores see Table S2.

Figure 6: nMDS ordination plot based on BPi (a) and IPi (b) values at sampled stations in the Po River prodelta area. The significant covaried groups of stations (by SIMPROF test) are indicated. The environmental variables vectors are overlaid.

Figure 7: nMDS ordination plot based on BPi (a) and IPi (b) values at sampled stations nearby the diffusion zone in the Gulf of Trieste. The significant covaried groups of stations (by SIMPROF test) are indicated. The environmental variables vectors are overlaid.

Figure 8: Distance-based redundancy analysis (dbRDA) performed using BPi (a) and IPi (b) values of species selected by SIMPER and environmental variables for both sampling areas. Arrows indicate environmental variables: sand, silt, Total Organic Carbon (TOC), Total Nitrogen (TN), and the ratio between carbon and nitrogen (C:N). For species, names see Table S2.

Figure 1

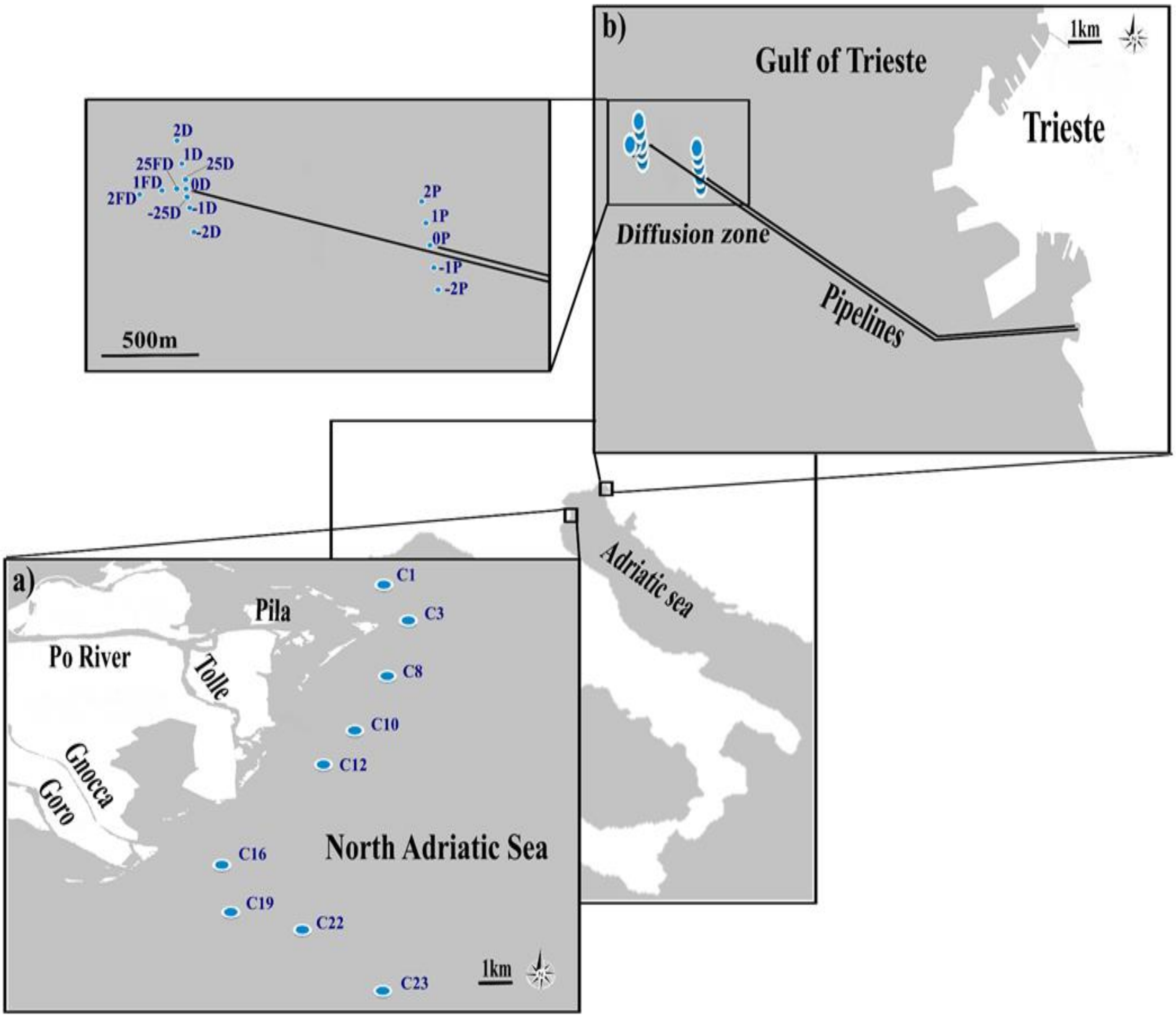

b)
Gulf of Trieste
1km
Trieste
Diffusion zone
Pipelines
2D
1D
25FD
25D
1FD
0D
2FD
-25D
-1D
-2D
2P
1P
0P
-1P
-2P
500m
Adriatic sea
a)
Pila
Po River
Tolle
Gnocca
Goro
C1
C3
C8
C10
C12
C16
North Adriatic Sea
C19
C22
C23
1km

Figure 2

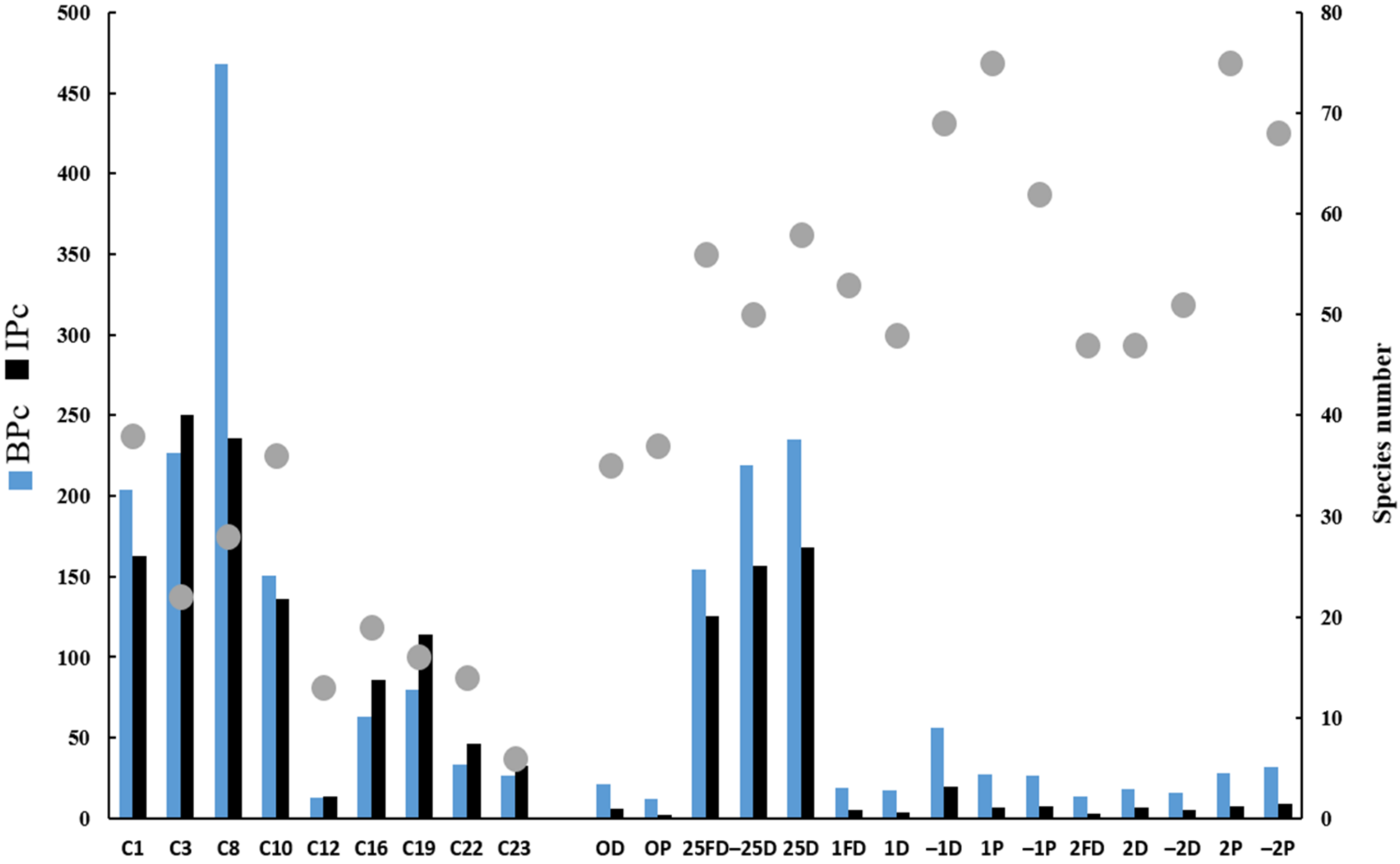

BPc
IPc
500
450
400
350
300
250
200
150
100
50
0
80
70
60
50
40
30
20
10
0
Species number
C1
C3
C8
C10
C12
C16
C19
C22
C23
OD
OP
25FD
−25D
25D
1FD
1D
−1D
1P
−1P
2FD
2D
−2D
2P
−2P

Figure 3

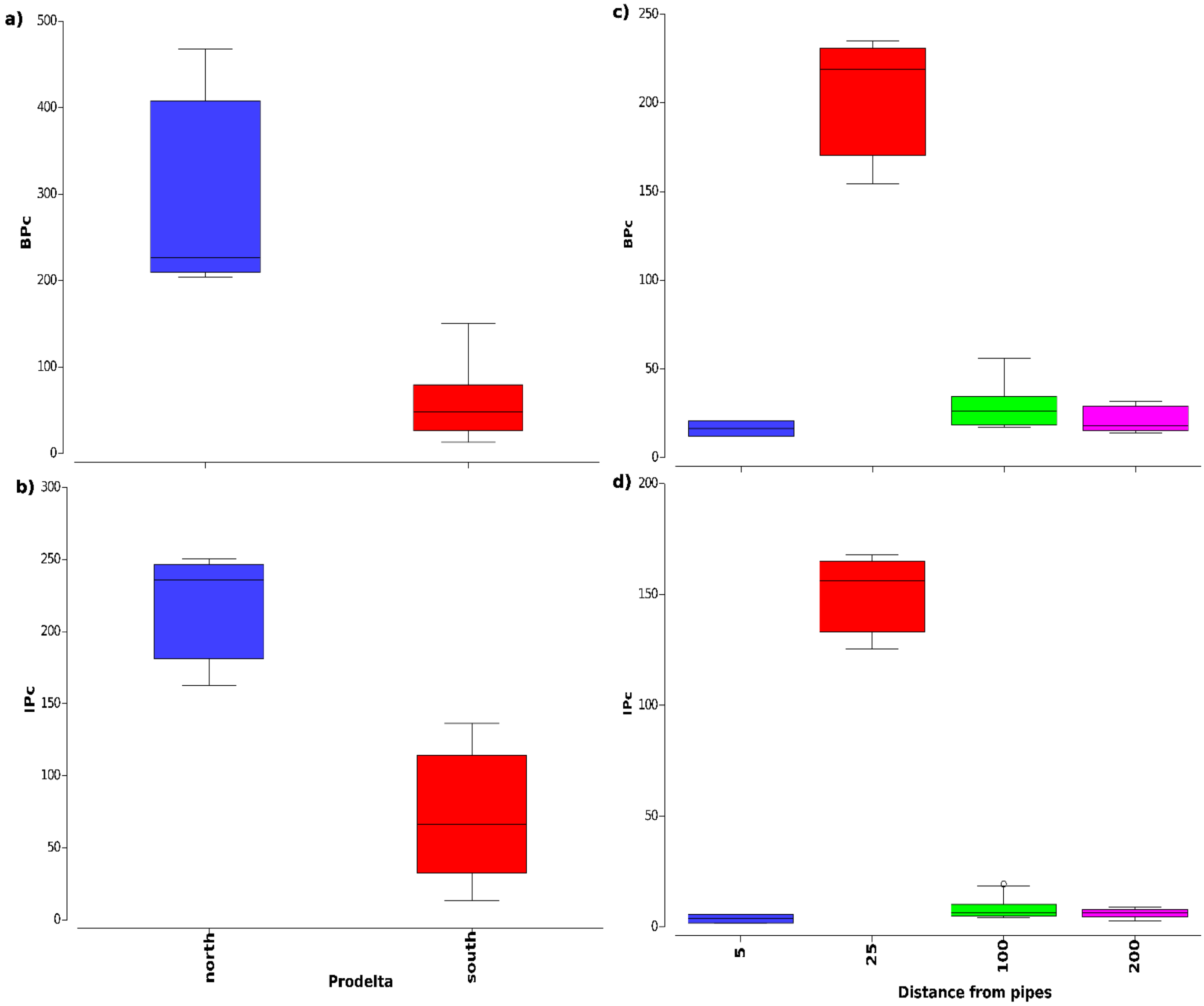

a)
500
400
300
200
100
0
BPc
b)
300
250
200
150
100
50
0
IPc
north
south
Prodelta
c)
250
200
150
100
50
0
BPc
d)
200
150
100
50
0
IPc
5
25
100
200
Distance from pipes

Figure 4

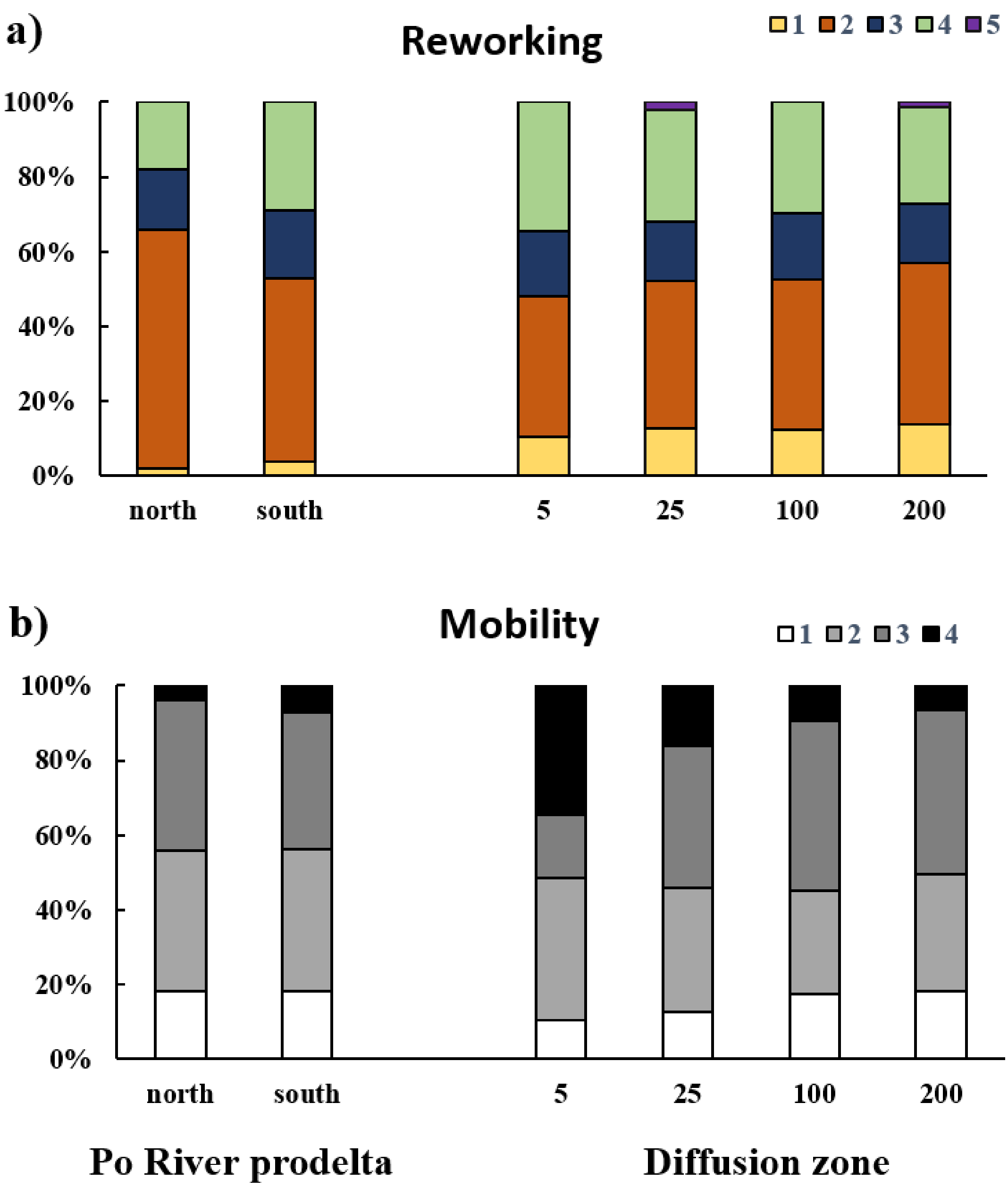

a)
Reworking
🟨1 🟧2 ■3 🟩4 🟪5
100%
80%
60%
40%
20%
0%
north
south
5
25
100
200
b)
Mobility
□1 ▨2 ▨3 ■4
100%
80%
60%
40%
20%
0%
north
south
5
25
100
200
Po River prodelta
Diffusion zone

Figure 5

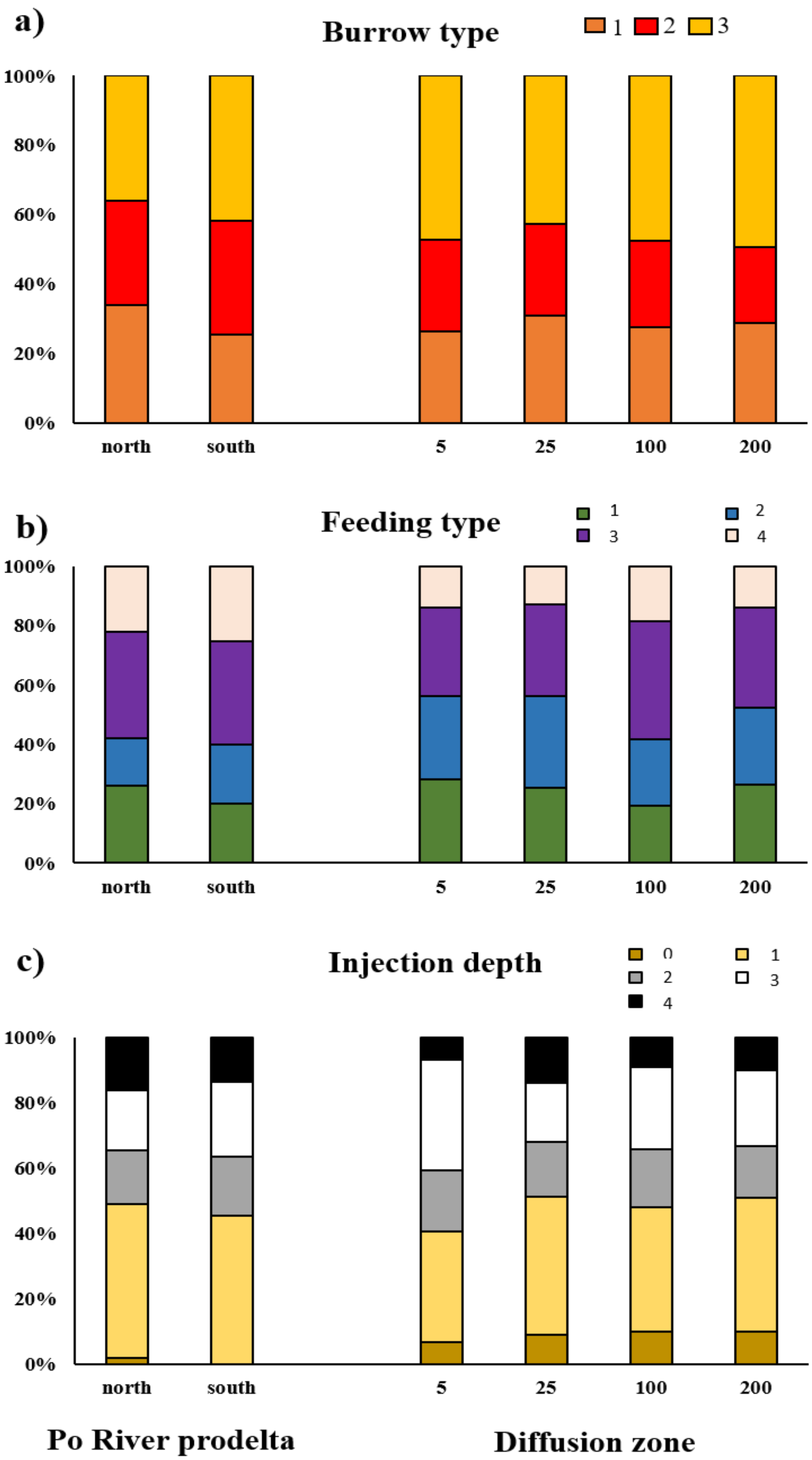

a)
Burrow type
1
2
3
100%
80%
60%
40%
20%
0%
north
south
5
25
100
200
b)
Feeding type
1
2
3
4
100%
80%
60%
40%
20%
0%
north
south
5
25
100
200
c)
Injection depth
0
1
2
3
4
100%
80%
60%
40%
20%
0%
north
south
5
25
100
200
Po River prodelta
Diffusion zone

Figure 6

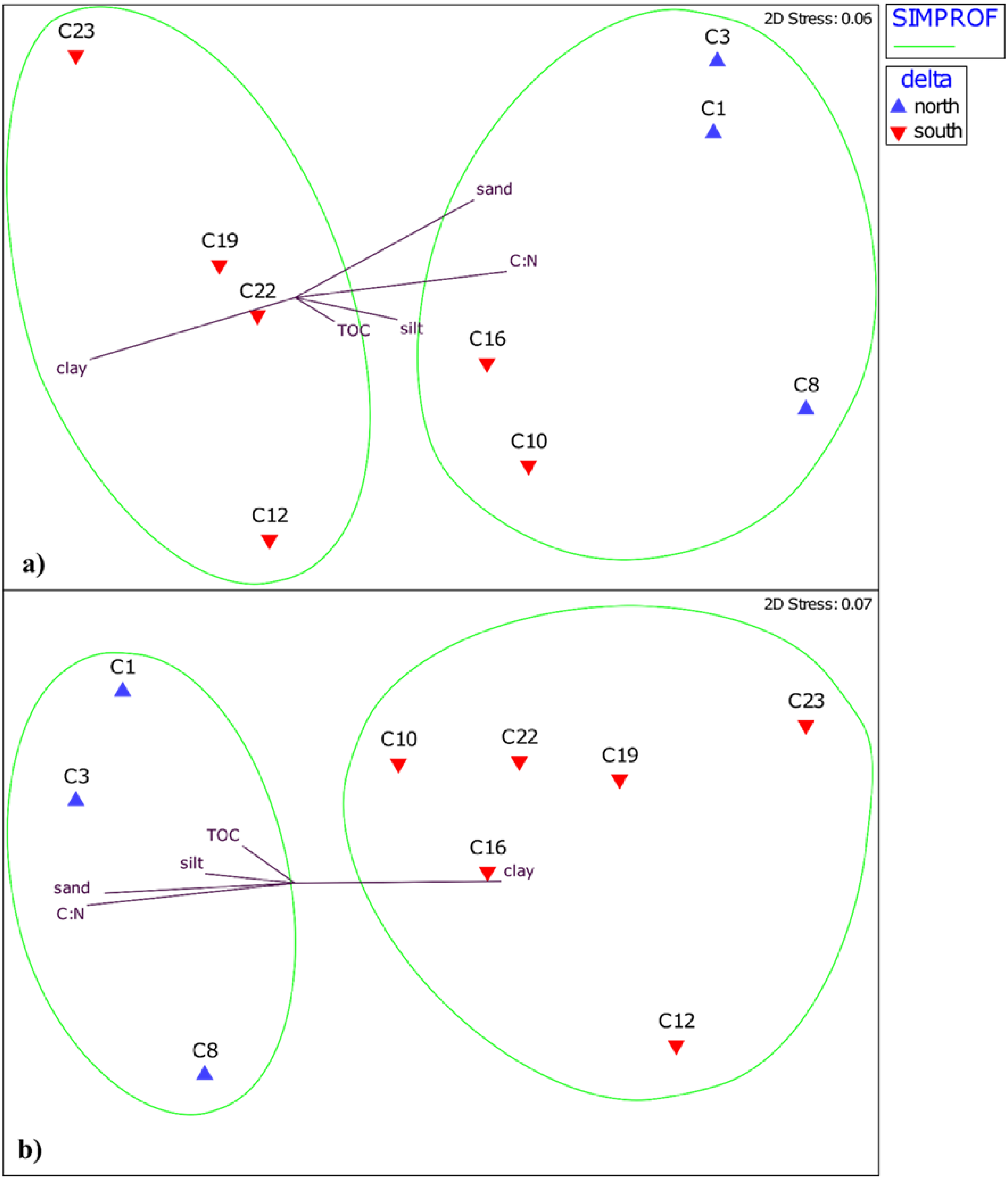

2D Stress: 0.06
SIMPROF
delta
▲ north
▼ south
C23
C3
C1
sand
C19
C:N
C22
TOC
silt
clay
C16
C8
C10
C12
a)
2D Stress: 0.07
C1
C23
C10
C22
C19
C3
TOC
silt
C16
clay
sand
C:N
C12
C8
b)

Figure 7

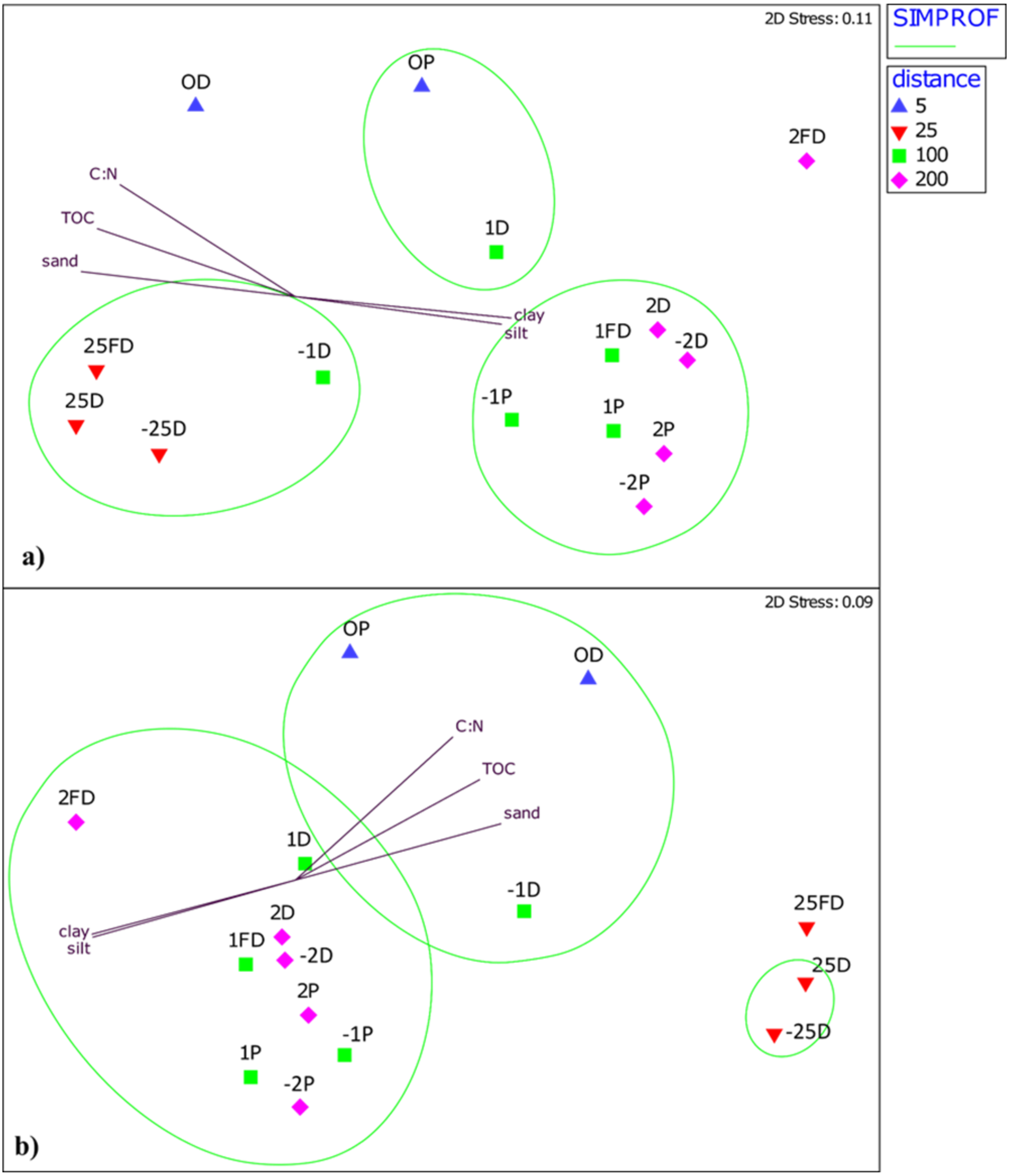
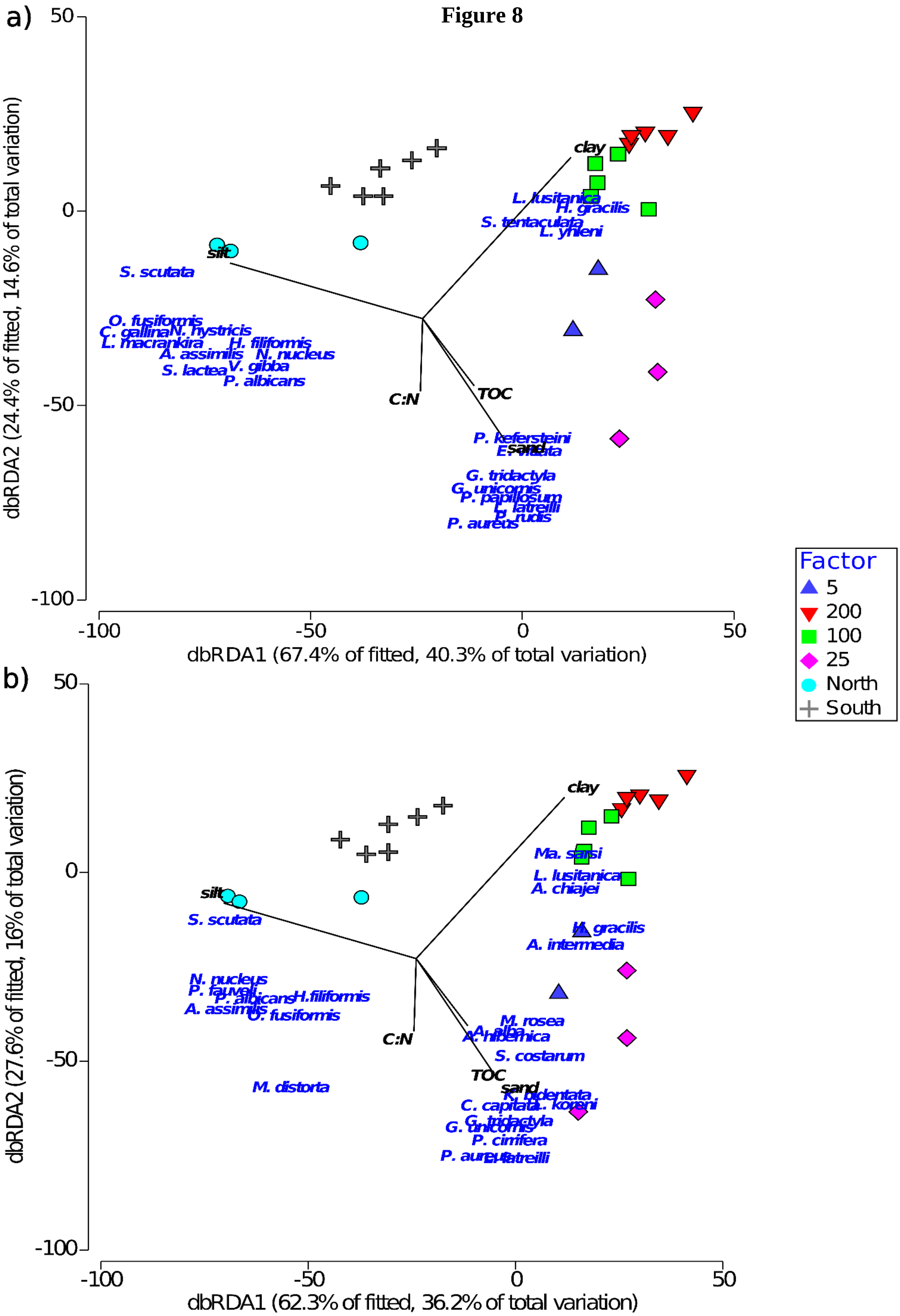

2D Stress: 0.11
SIMPROF
distance
5
25
100
200
OD
OP
2FD
C:N
TOC
sand
1D
clay
silt
2D
1FD
-2D
25FD
-1D
25D
-25D
-1P
1P
2P
-2P
a)
2D Stress: 0.09
OP
OD
C:N
TOC
sand
2FD
1D
-1D
25FD
2D
clay
silt
1FD
-2D
25D
2P
-1P
-25D
1P
-2P
b)

Figure 8


a)
50
0
-50
-100
-100
-50
0
50
dbRDA1 (67.4% of fitted, 40.3% of total variation)
dbRDA2 (24.4% of fitted, 14.6% of total variation)
clay
silt
C:N
TOC
sand
L. lusitanica
H. gracilis
S. tentaculata
L. yhleni
S. scutata
O. fusiformis
C. gallina
N. hystricis
L. macrankira
H. filiformis
A. assimilis
N. nucleus
S. lactea
V. gibba
P. albicans
P. kefersteini
E. vittata
G. tridactyla
G. unicornis
P. papillosum
L. latreilli
P. rudis
P. aureus
b)
50
0
-50
-100
-100
-50
0
50
dbRDA1 (62.3% of fitted, 36.2% of total variation)
dbRDA2 (27.6% of fitted, 16% of total variation)
clay
silt
C:N
TOC
sand
Ma. sarsi
L. lusitanica
A. chiajei
H. gracilis
A. intermedia
S. scutata
N. nucleus
P. fauveli
P. albicans
H.filiformis
A. assimilis
O. fusiformis
M. rosea
A. alba
A. hibernica
S. costarum
M. distorta
K. bidentata
C. capitata
L. koreni
G. tridactyla
G. unicornis
P. cirrifera
P. aureus
L. latreilli
Factor
▲ 5
▼ 200
■ 100
◆ 25
● North
+ South

5. Production, Productivity, and Transfer Efficiency

This chapter is adapted as Paper IV from: Seyed Ehsan Vesal, Rocco Auriemma, Simone Libralato, Federica Nasi, Paola Del Negro. Impacts of organic enrichment on macrobenthic production, productivity, and transfer efficiency: What can we learn from a gradient of sewage effluents? Original manuscript, under preparation.

5.1 Introduction

Coastal environments, which are the brittle ecotones at the boundary between marine and terrestrial ecosystems, host a high variety of benthic macrofauna species, that provide a fundamental food source for higher trophic levels through the food webs (Kabat et al., 2012). Besides, food webs, which consists of all of the interconnected and overlapping trophic interactions, is a useful framework to assess the magnitude and importance of trophic levels relationships and energy move in an ecosystem, and its linkages eventually specify the flux of every population in an ecosystem (Pimm, 1982; Link, 2002).

Living marine resources worldwide are sustaining high levels of anthropogenic impact; consequently, many habitats are extremely threatened and several species have declined (NRC 1995; Botsford et al., 1997; Jackson et al., 2001; Watson and Pauly, 2001; Pranovi et al., 2003). During the last decades, the demographic expansion, as well as the increase of agricultural, industrial, fishing, fish farming, and tourism activities, have increasingly threatened coastal marine ecosystems (Bouchet and Sauriau, 2008; Frontalini et al., 2009). Besides, demersal trawl fishing activities change the seafloor, modify habitats and influence the structure and functioning of benthic invertebrate communities (Reiss et al., 2009).

Benthic macrofaunal invertebrates hold up key ecological functions (Reiss and Kröncke, 2005; Bolam and Eggleton, 2014) and studies on macrozoobenthos communities are often used for monitoring marine realms and evaluating anthropogenic impacts (Nasi et al., 2018). Each species can play a significant role in the functioning of marine ecosystems, and therefore any changes in the composition of macrozoobenthic communities can affect not only ecosystem functioning (Gray et al., 2006; Tillin et al., 2006; Schratzberger et al., 2007) but also the food webs. Therefore, estimating the potential of benthic invertebrates in the food webs is fundamental because, for instance, small benthic invertebrates are eaten by larger benthic invertebrates and then move through the diet of demersal fish, which are considered as fish food (Petersen and Jensen, 1911), also eaten by mammals (Hjelset et al., 1999) and birds (Johansen et al., 1999). In addition, benthic species are important for the remineralization and redistribution of sediment organic matter (Wassmann, 1998). In this regard, several studies have noted that coastal eutrophication, in particular sewage discharges as a lately known phenomenon (Nixon, 1995; Dell'Anno et al., 2002; Yeleliere et al., 2018), significantly impacts the structural features of the macrozoobenthos community causing a remarkable decrease in biodiversity and variations in species composition (Short and Wyllie-Echeverria, 1996; Patrício et al., 2009; Tadir et al., 2017). Besides, benthic secondary production which indicates the assimilation of organic matter's energy per unit of time and area (Cusson and Bourget, 2005), is an important ecological parameter and considered as an indicator of both population dynamics (biomass, life span, and body size) and also biotic interactions and environmental variability within ecosystems (Waters and Crawford, 1973; Dolbeth et al., 2012). In addition, the information on biomass and production helps to understand ecosystem dynamics as it allows energy flow estimates within ecosystems and represents the formation of community biomass by growth through time across the trophic levels (TLs; the number of energy levels from the primary producers to the consumers by transferring biomass) (Dolbeth et al., 2005; Nilsen et al., 2006; Libralato and Solidoro, 2010; Dolbeth et al., 2012). Considering the way that energy is utilized as it is transferred between levels, the total biomass of organisms on each TL decreases from the bottom-up, and solely around 10% of the energy consumed is converted into biomass, whereas the rest is lost as heat, as well as to movement and other biological functions (Agrawal and Gopal, 2013).

Accordingly, benthic production can be used to show functional responses of assemblages subjected to long-term environmental and local anthropogenic impacts (Benke, 2010; Dolbeth et al., 2011; Dolbeth et al., 2012), and how this production is influenced by external factors and distributed through TLs (Odum and Heald, 1975; Pauly and Watson, 2005) is important for understanding the dynamics of the ecosystem and its function under environmental and anthropogenic influences (Dolbeth et al., 2012).

The production of macrobenthic can be measured either using classic methods, relying on calculating abundance and mean body mass of sampled groups (Sprung, 1993), or using empirical models which can predict production that can be expressed as the production-to-biomass ($P/\overline{B}$-ratio) (yr^{-1}), namely also productivity (Dolbeth et al., 2005). The benthic $P/\overline{B}$-ratio patterns are universally recognized to be mostly influenced by life-history characteristics such as population density, body mass, recruitment, age, and trophic conditions (Waters, 1977, Rigler and Downing, 1984). The $P/\overline{B}$-ratio for a given species was proposed by Sanders, (1956) as a proxy of the population turnover time of an organism and lifespan has been recognized as its main predictor (Robertson, 1979). Recent studies have successfully predicted $P/\overline{B}$ values using empirical models (Cusson and Bourget, 2005; Zhang et al., 2011; Bolam and Eggleton 2014). The Brey's (2012) model (Modified Version 01-2012) was an assumption to better estimate than any other's empirical ones the prediction of $P/\overline{B}$ or P values due to the further requirement inputs such as feeding mode and motility (Fuhrmann et al., 2015). Furthermore, the relationship between productivity and biomass (i.e., weight) (Brey, 1990) suggests a real shorter alternative route for the prediction of productivity. Besides, to represent the structure of ecosystem food webs (Libralato and Solidoro, 2010), has long been used the pyramid of biomass and productions over the trophic levels (TLs), which provides an understanding of energy flows (Stergiou and Karpouzi, 2002) and can be applied as an empirically based synthetic index to compare species feeding habits (Badalamenti et al., 2000). Moreover, another important concept related to the TLs is the transfer efficiency of energy (TE), which represents the fraction of energy transferred from one TL to another in a food web. TE is calculated as the ratio between production at two successive trophic levels (Lindeman, 1942; Baumann, 1995; Pauly and Christensen, 1995; Libralato et al., 2008). The TE between trophic levels is a central concept and related to the mean individual growth efficiency and standard metabolism (Kerr, 1974; Andersen et al., 2008) in an ecosystem. The higher value of TE means that a greater proportion of production at lower trophic levels is converted to production at the upper trophic ones (Eddy et al., 2020). Moreover, TE is a critical factor that shapes marine ecosystems because even intangible changes in TE can pool across trophic levels, and cause intense differences moving in the upper TLs until it affects the top predator abundances (Ryther, 1969; Stock et al., 2017; Moore et al., 2018; Link and Watson, 2019).

The estimation of biomass, production, and catches over TLs, termed the trophic spectrum, and their variations has been reported by Gascuel et al., (2005). The authors evidenced how their estimations are important to provide a deep understanding of the ecological effects of exploitation on marine ecosystems. In this study, we applied these approaches to macrofaunal communities influenced by organic enrichment from sewage-derived materials by implementing the dispersion-based trophic spectra model, which is based on the distribution of ecological data (i.e. production and biomass) according to density distribution function (ddf; the dispersion-based method), proposed by Libralato and Solidoro, (2010).

Research on benthic macrofaunal communities has traditionally focused on species diversity and composition (e.g., Washington, 1984; Mouillot et al., 2006; Ieromina et al., 2016), and little attempts have been made to assess the ecological importance of benthic invertebrate productivity and in particular TE (e.g., Pranovi et al., 2005). Some studies evidenced the variation of $P/\overline{B}$ due to temperature and depth (e.g., Degen et al., 2015), some others linked to areas at higher latitudes (e.g., Nilsen et al., 2006) and also estuaries influences (e.g., Bissoli et al., 2018). However, less attention was paid to $P/\overline{B}$ and TE estimations in coastal areas due to anthropogenic impacts.

Therefore, based on our knowledge, this is the first study to investigate the variation in the production (P), productivity ($P/\overline{B}$ yr^{-1}), and the transfer efficiency (TE) of coastal macrobenthic communities subjected to sewage discharge. We hypothesized that the macrobenthic communities, influenced by sewage-derived materials respond to the sewage discharge with spatial variations, not only in terms of numbers and species composition but also in terms of biomass, production, $P/\overline{B}$, and TE. More specifically, we answered the following questions: 1) What are the spatial differences in the biological factors of macrobenthos (biomass, production, $P/\overline{B}$, and TE) along a gradient of sewage effluents discharge? 2) Is there any relationship between the spatial variability of biological factors with the environmental variables (grain-size, TN, Corg, C/N, and Eh)? 3) Does TE reflect the variations in benthic communities' compositions and structure along the gradient of sewage discharges? To test the hypotheses, we applied Brey's and dispersion-based trophic spectra models to evidence the macrobenthic production and productivity processes and how sewage-derived materials could affect the fraction of energy transferred by the macrofaunal community through the food web.

5.2 Material and methods

5.2.1 Study area

The Gulf of Trieste, which is a shallower basin along the northwestern side of the Adriatic Sea, Italy (Fig.1), reaches about 17 m average depth, whereas maximal depth is about 25 m in the middle of the Gulf, and has a coastline of 100 km with a total area of 25×30 km from the Tagliamento River mouth to Savudrija/Punta Salvore (Croatia) (Celio et al. 2002; Fonda Umani et al., 2012; Barago et al., 2020). Bottom temperatures change from 6°C to >20°C, whereas the temperatures range from 5°C to >24°C at the surface.

Sedimentation within the Gulf is mainly controlled by river inputs rather than marine currents, in particular, Isonzo River, the main contribution of freshwater and sediments, leads to pycnocline which raises during summer due to mixture with the high temperature of surface layer, whereas the sedimentation rate reaches about 2.5 mm y^{-1} in front of Isonzo stream and a rate up to 1 mm y^{-1} in the central part of the Gulf (Malačič, 1991; Covelli et al., 1999).

The Servola disposal plant is the main urban sewage discharge plant of Trieste city, which is a mixed type plant, collecting and treating both meteoric and wastewaters serves up to 200,000 inhabitants and has a maximum flow of 6000 L sec^{-1} (Solis-Weiss et al., 2007). Since 1992, the Servola pipelines dispose of sewage after mechanical and chemical treatments. The sewage discharge flow is released through two submarine pipelines of 6.5 and 7.5 km in length, which includes several numbers of sewage diffusion towers at the end of both pipes, leading to the sea up to a depth of 22 m. These diffusion towers are placed in the last 500 m of the shortest pipe and the last 1000 m in the longest one, for a total of 1,5 km diffusion zone. Moreover, the pipelines have a capacity varying from 206 L/s during the dry to 618 L/s during the rainy seasons, respectively (Novelli, 1996).

5.2.2 Sampling design and processes

The sediment and macrobenthic monitoring were carried out in April 2018 through 18 sampling stations placed in a way to consider the distance from the diffusion zone, the distance from the ending part of the pipelines, and the direction to the average annual bottom current in the area (SSE 170 °). Thus 15 sampling stations were distributed along 3 transects: Proximal-P (at the end of the shortest pipeline); Medial-M (in the middle part of the 1km sewage diffusion area); Distal-D (at the end of the longest and main pipeline). For each transect one station (0P, 0M, 0D) was placed nearby the diffusion area, other stations were placed over current and undercurrent (indicated with "-") at 100 (1P, 1M and 1D; -1P, -1M and -1D) and 200 meters from the pipelines (2P, 2M and 2D; -2P, -2M and -2D). Two additional stations were placed in front of the main outfall at 100 and 200 m (1FD, 2FD, respectively). Additionally, a reference station was located at 2 km from the distal end of the pipeline in the opposite direction to the average annual bottom current (station RS) (Fig. 1 and Table 1). In this study, we considered Zero, One, and Two groups of stations for those stations placed at <5, 100, and 200 meters distance from the pipes, respectively.

In each sampling station, we estimated the water column temperature at the bottom using CTD Probe (SBE 16plus V2 SeaCAT). Sediments for physical and chemical analyses (grain-size, Total Organic Carbon-Corg, Total Nitrogen-TN contents, and redox potential-Eh) and macrofauna communities were collected by van

Veen grab (0.1 m^2). The macrofaunal communities were sampled in three replicates for each station and sieved through a mesh (1mm). The retained sediment and organisms were immediately fixed in ethanol 70°. At the laboratory, taxonomic identification of benthic macrofaunal was carried out to the lowest possible taxonomic level and species abundance was counted. Species names were cross-checked against the World Register of Marine Species (https://marinespecies.org/).

5.2.3 Environmental variables

The grain-size analysis which includes sand, silt, and clay fractions (%) was determined by sieving sediments at 2 mm, and then it was first pre-treated with 10% hydrogen peroxide ($60^{℃}$ for 24 hours) and afterward analyzed with Malvern Mastersizer 2000 equipped with Hydro 2000s. Total organic carbon (Corg) and nitrogen (TN) were also measured on freeze-dried sediment samples which were milled using a pestle and mortar and a fraction > 250 µm was isolated from the rest of the specimen.

Subsamples triplicates (~8–12 mg) were straightforwardly weighed in capsules (5×9 mm) on a micro-ultra balance Mettler Toledo model XP6 (precision of 0.1 µg). Tin and silver capsules were utilized for TN and Corg measurement, sequentially. The values of Corg and TN (represented as mg g^{-1}) were estimated utilizing an elementary analyzer CHNO-S Costech model ECS 4010. Before Corg quantification, based on Sharp methods (1974), subsamples were treated with expanding HCl concentrations (0.1 and 1 N) to eliminate the carbonate (Nieuwenhuize et al., 1994).

The redox potential (Eh) allows inferring the depth of oxygen permeation from surface sediments (Hargrave et al., 2008), determines the Physico-chemical state of marine sediments, and indicates the amount of organic matter. Eh measurements were estimated on the cores on board. Estimations were made utilizing electrodes from the undisturbed superficial layer (0-1 cm) (Pearson and Stanley, 1979). The platinum electrode was standardized (CRISON 5265) in a light solution and then, the analysis was carried out with Metrohm 704 voltimet (Clesceri et al., 1996).

5.2.4 Estimation of community biomass, production, and $P/\overline{B}$ ratio

For biomass measurements, individuals were blotted dry for a few seconds (Nilsen et al., 2006) and then wet weight (WW) was measured using a digital laboratory scale with high precision and accuracy. In case of the presence of tubicolous polychaetes, the tubes were removed before weighing. Subsequently, biomass within each species was assessed as Ash-Free Dry Weight (AFDW) in different stations applying conversion factors provided by Rumohr et al., (1987), Brey, (1988), Ricciardi and Bourget, (1998), and Brey, (2001). Moreover, the biomass of shell-organisms was transformed in Shells- Free Dry Weight (SFDW) before calculating their AFDW, using conversion factors mentioned above.

A modified multi-parameter model based on Artificial Neural Network (ANN) and developed by Brey, (2012; Version 01-2012 at http://www.thomas-brey.de/science/virtualhandbook), was used to estimate the Production-to-Biomass ratio ($P/\overline{B}$) of sampled invertebrates.

To implement the model and as one of the required input data, the biomass of each species (AFDW) belong to each species for each station was converted to energy Joule values using energy densities (kJ g^{-1}) referring to a global "Conversion factors" data published by Brey et al., (1988, 2010). If no conversion factors could be available at the species level, a factor was used from the next highest taxonomic rank. The model allows determining estimates of annual $P/\overline{B}$ ratios for each taxon at each station with a 95% confidence interval based on three main input parameters: individual body mass (Joules), the average temperature (°C), and the depth of the sampling station (meters). We added the average depth and temperature of the sampling area in April (21 m and 9.5°C, respectively) and we considered this month as the right sampling period for obtaining annual estimates of $P/\overline{B}$. Furthermore, the ANN uses functional traits which consisted in motility classes (infauna, sessile, crawler, and facultative swimmer), taxon (Mollusca, Annelida, Crustacea, Echinodermata, and Insecta), feeding type (herbivore, omnivore, and carnivore), habitat (lake, river, marine, subtidal, and exploited). Since the species were collected in a marine coastal environment with no commercially exploited in our study area, the other indices were always zero. The functional traits are described by binary inputs (0 or 1) to indicate belonging to categories. Additionally, we measured the productivity for each station by summing the $P/\overline{B}$ values of the species found in each station.

We obtained the information on data inputs and biological traits from literature (i.e. Giangrande, 1997; Rouse, 2000; Jumars et al., 2015), databases (https://www.itis.gov/; http://www.polytraits.lifewatchgreece.eu; https://www.marinespecies.org) and expert knowledge. We also calculated the average production for each station by multiplying $P/\overline{B}$ and biomass of all taxon found at each station.

5.2.5 Trophic spectra and transfer efficiency (TE) calculation

Data on AFDW biomass, $P/\overline{B}$-ratio, trophic levels (TL_i; species i), and dispersion of the TL_i (OI_i; quantified as the variance) for each species at sampling stations, were used for obtaining trophic spectra of production based on the dispersion-based method proposed by Libralato and Solidoro (2010). We collected the data on TL_i and OI_i for each species from the database (https://www.sealifebase.ca/search.php). When no information was available, we used the data for the family level.

The trophic spectra of production determined based on macrobenthic communities' data allowed the calculation the transfer efficiency (TE; see Libralato and Solidoro, 2010) as a measure between productions at two adjacent integer trophic levels and varies between 0 to 1 (Lindeman, 1942). We used the trophic spectra in the range between $2<TL<4$ to estimate TE values.

5.2.6 Data analysis

Firstly, all data were tested for normality following Shapiro Wilk's test. A dissimilarity matrix from square root transformed abundance data were calculated using the Bray–Curtis coefficient, and a non-metric multidimensional scaling analysis (nMDS) plot was created to visualize the ordering of the samples in reduced (2D) space.

To test for macrobenthic communities' differences along the gradient of sewage discharge an ordered one-way ANOSIM test was performed using 'distance from the diffusion zone'. Stations placed at <5, 100, and 200 meters were gathered in Zero, One and Two groups, respectively. Reference station was included in the Two group.

Multivariate analysis (Principal Component Analysis, PCA) on log-transformed and normalized data were used to investigate the spatial variations in biological factors (biomass, production, $P/\overline{B}$, and TE) among sampling stations. Additionally, redundancy analysis (RDA) was performed to determine the relationships between biological and environmental data. Both the PCA and RDA analyses were conducted using R version 3.6.0 (R Development Core Team, 2018). Statistical tests in SPSS v. 20 were also carried out to indicate the significant levels of observed environmental variables and biological factors. A Spearman–rank correlation was performed to test relationships between the biological factors and environmental variables, and Jonckheere-Terpstra test was used to detect significant differences in biological factors and environmental variables among the stations along the gradient of sewage effluents discharge.

5.3 Results

5.3.1 Spatial variation in environmental variables

The variation of environmental variables measured at each station e.g., sediment grain size, TN (Total Nitrogen); Corg (organic carbon); C/N (carbon and nitrogen ratio); Eh (redox potential) are displayed in (Table 1). The sediments of the whole study area were mainly characterized by the high percentage of fine particles. A higher value of silt was observed at stations 2M, whereas a higher percentage of clay was noticed at stations RS. The mean value of sand fraction (%) was 15.7±11.6 and the highest percentages were detected at the stations 0D and 0M. As corroborated by Jonckheere Terpstra test, significantly higher sand fractions were detected at stations nearby the ducts ($z = -3.60$, $p < 0.01$). On contrary, silt ($z = 1.90$, $p < 0.05$) and clay ($z = 3.60$, $p < 0.01$) values significantly increased toward the farther stations.

Furthermore, Corg and TN significantly increased nearby the pipelines (Jonckheere Terpstra test, $z = -2.23$; $p < 0.05$). For both Corg and TN, the highest contents were observed at 0D (60.5 and 3.8 mg g^{-1} respectively), whereas the lowest ones were measured at RS (10.7 and 1.2 mg g^{-1} respectively). Also for C/N ratio, the highest values were observed in the sewage diffusion zone compared to RS. Especially, higher ratio values were estimated at stations close to the two outfalls (18.6 and 13.0 at 0D and 0P, respectively).

Lower Eh values were observed in the whole sewage diffusion area (average: -105.0±97.1 mV) compared to RS (54 mV). In particular, the lowest values of Eh were observed in the D and P transects, both influenced by the two sewage pipeline outfalls with an average of -177.0±63.8 mV and -101.6±71.4 mV, respectively.

5.3.2 Macrobenthic communities' distributions along the gradient of sewage effluents discharge

The total abundance ranged between 366.6 ind. m^{-2} to 3580 ind. m^{-2} at sampling stations 0P and -1D, respectively. Species composition varied following the gradient of distance from the main pipeline (Fig. 2) The nMDS plot divided the station close to the pipes (Zero group) at the right side of the plot, to those farthest away at the left side (One and Two groups). The global ordered one-way ANOSIM test confirmed what was highlighted by nMDS analysis. The species composition significantly differed among groups of stations ($R=0.52$; $P<0.01$). In addition, the pairwise tests evidenced the similarly higher-range values of R (0.55 and 0.83) for the Zero *vs* One and Zero *vs* Two comparisons respectively, whereas with a lower value (of 0.19) for One *vs* Two. These results implied that the explanation for the global test results in Zero group differed from One and Two groups, but the latter ones were less distinguishable. Therefore, the pairwise test mirrored a clear pattern of decreasing differences in the macrofauna community composition with the increasing distance from the pipelines.

5.3.3 Biomass, production, and productivity ($P/\bar{B}$-ratio)

The biomass (B) of the macrofaunal communities in the whole sampling area was quite variable among the sampling stations, measured in 24.0±22.9 g m^{-2} based on wet weight. The total biomass, expressed in energy content, was 37.2±26.0 kJ m^{-2}, while production (P) and productivity ($P/\bar{B}$) were 66.7±51.8 kJ m^{-2} yr^{-1} and 1.7±0.6 yr^{-1}, respectively (Table 2). The highest biomass occurred at the station -1M, with a value of 99.9 kJ m^{-2}, whereas the lowest one occurred at the station -2P with a value of 10.5 kJ m^{-2}. The production values ranged from a minimum of 12.7 kJ m^{-2} yr^{-1} (0P) to a maximum of 189.9 kJ m^{-2} yr^{-1} (-1M), whereas the $P/\bar{B}$ varied from a minimum of 0.7 yr^{-1} (0D and 0P) to a maximum of 2.7 yr^{-1} (-2D) (Table 2). Additionally, both B and P values followed the same pattern. The highest values were observed at the stations placed 100 m far from the pipelines (B and P: 54.6±30.8 kJ m^{-2}, 101.5±61.7 kJ m^{-2} yr^{-1}, respectively) compared to farther ones (B: 27.1±16.5 kJ m^{-2}; P: 49.2±29.2 kJ m^{-2} yr^{-1}), whereas lower values were calculated at stations nearby the pipes (B: 28.0±16.1 kJ m^{-2}; P: 36.6±40.8 kJ m^{-2} yr^{-1}). On contrary, $P/\bar{B}$ followed the increasing distance pattern from the pipes. Lower values were measured at stations gathered in Zero group ($P/\bar{B}$: 1.1±0.6 yr^{-1}) compared to those in One ($P/\bar{B}$: 1.8±0.4 yr^{-1}) and Two groups ($P/\bar{B}$: 1.9±0.6 yr^{-1}) (Fig. 3).

The estimated average $P/\overline{B}$ for the main taxonomical groups Polychaeta, Crustacean, Mollusca, Echinodermata were 1.00, 0.46, 0.22, 0.06 yr^{-1}, respectively. Polychaeta was the only group present in high number and high biomass, despite the low individual medium weights, at the majority of stations.

High dominance of the polychaete *Capitella capitata* with low estimated $P/\overline{B}$ ratios and biomass resulted at the stations located nearby the main underwater outfall (0D) indicated a lower contribution of this species to the total production compared to biomass at these stations (Table 2). Polychaeta showed the highest value of $P/\overline{B}$ at stations -2D (76.5%) and -2M (78.3%) (2.09 and 1.9 yr^{-1}, respectively), whereas Crustacea and Mollusca were the major contributors to $P/\overline{B}$ at 1M. Echinodermata generally had a small contribution to total productivity due to their low abundance and biomass.

Spearman's rank correlation coefficient tests indicated that there was a positive significant correlation between B and P ($rs=0.858$, $p<0.01$) while there was no correlation between B and $P/\overline{B}$. Moreover, $P/\overline{B}$ was positively correlated with production ($r_s=0.605$, $p<0.01$). The result showed that there was no significant correlation between TE and other biotic factors (i.e., biomass, production, and productivity) (Table 3).

5.3.4 Transfer efficiency (TE)

TE showed the lowest values at the stations located 100 meters far from the pipes (One group), whereas the highest TE value was observed at stations placed <5 m away from the pipelines (Zero group) (Fig. 4). Moving away from the Zero to One groups, TE decreased (23.5±13.6% and 12.8±3.1%, respectively), whereas biomass increased (28.0±16.1 and 50.2±30.8 kJ m^{-2}, respectively) (Fig. 4). However, in One group, 0M showed a high TE value compared to other stations in front of the outfalls (i.e., 0P and 0D) (Table 2).

In the Principal component analysis (PCA), all stations were distributed along the gradient of sewage effluents discharge. The cumulative variance was 71.2%, which showed stations plotted along the PCA1 (50.7%) were separated by distinctions in B, P, and abundance. The PCA2 (20.5%) separated stations correspond to differences in TE and $P/\overline{B}$. Stations on the left side of the plot higher values of B, P, and abundance were noticed (i.e. the stations located at 100 m distance from the pipelines), whereas stations draft on the right side of the plot showed low in that biological factors. Besides, stations on the top side of the plot were plotted since high values of TE and low values of in $P/\overline{B}$ such as 0D and 0M were observed. Lastly, stations located in the bottom part of the plot showed high $P/\overline{B}$ values and low TE values (Fig. 5).

5.3.5 Relationships between benthic macrofaunal biological factors and environmental variables

The RDA, performed on biological factors and environmental variables of the sampling stations, showed 75.8% of the total variance, accounting for the 1st (47.4%) and 2nd (28.4%) axes (Fig. 6). Clay was removed due to the high self-related with silt. TN, Corg, and C/N were correlated with the negative part of the first axes and plotted on the right side, nearby the station in front of the main outfall (0D). The sand was positively related with TE, where 0M, 2M, and 2P were plotted in the bottom part of the plot, while silt had a negative correlation with TE, where -2D was plotted on the top side of the plot. The Eh vector points in the opposite direction of TN, Corg, and C/N contents, showing that stations with higher Eh tend to have the higher B and P with lower TN, Corg, and C/N values (i.e., -1D, -1M, 1M and 1P). Moreover, TN, Corg, and C/N values were positively associated with TE. RDA analysis showed that the distribution of stations nearby the pipeline in the plot (i.e., 0D, and 0M) was mainly related to the high values of TE and sand fraction. According to Spearman's rank correlation coefficient, P, $P/\overline{B}$ and TE showed a positive correlation with Eh (r_s=0.586, 0.505 and 0.477; $p<0.05$, respectively). The results highlighted that $P/\overline{B}$ had a negative correlation with Corg and TN (r_s= -0.545 and -0.509, $p<0.05$, respectively) (Table 3).

5.4 Discussion

The knowledge contribution of the stress effects on benthic assemblages has been already evaluated, especially in the last 40 years (e.g., Pearson and Rosenberg 1978; Peeters et al. 2001; Souza et al., 2013). Many authors found that changes in abundance of small-sized species and dominance of opportunists were caused by stressors, and have led to a reduction in macrofaunal species richness and diversity (Gray et al., 1990). To date, most of the attention has been focused on changing in terms of structural features of benthic communities (e.g. abundance, biomass, and species composition) but little is known about the production (P), productivity ($P/\overline{B}$), and transfer efficiency (TE) of macrofaunal communities. In this study for the first attempt, we evaluated the influence of underwater sewage discharges on P, $P/\overline{B}$, and TE. In addition, we considered the variation of biological factors (B, P, $P/\overline{B}$, and TE) related to Physico-chemical (grain-size, TN, Corg, C/N, and Eh) variables. This study used a sampling design including stations placed along with the increasing distance from the main source of contamination, to assess the spatial extent of increased organic enrichment, and biological effects on structures of macrobenthic communities and as a consequence their secondary production and productivity along the trophic pathway.

The sediments of the study areas are deeply influenced by wastewater discharges. Our results showed that the sand fraction at stations nearby the pipelines significantly increased in comparison to farther ones. On contrary, we found increasing patterns for silt and clay percentages toward the station placed 200 meters away from the pipes. Generally, the part of the Gulf of Trieste is characterized by fine sediments, mainly coming from the Isonzo river (Nasi et al., 2017). The higher sand fraction nearby the outfalls was expected due to the high value of deposited discharge by sewage pipelines that comprises also meteoric waters (Diaz et al., 1995, Melis et al., 2019).

Higher Corg contents observed close to the pipelines and their decreasing values toward the farther stations indicate the effects of organic enrichment derived from sewage discharge as also observed in other studies (e.g., Filgueiras et al., 2007; Martins et al., 2010; Souza et al., 2013). The lower redox potential with negative

Eh values were observed at the stations gathered in D and P transects, and indicate a probable lack of oxygen and hypoxic conditions at sediments nearby the end of the two pipes. The microbial decomposition of organic matter in the sediments severely evacuates dissolved oxygen and therefore the negative Eh values are due to a direct effect of wastewater discharge at the diffusion area (Matijević et al., 2007; Arend et al., 2011).

Considering the faunal composition in the whole area, our results indicated changes in benthic communities among stations sampled along the sewage effluents gradient. The nMDS plot showed a distinct gradient from the source of organic matter and suggests the presence of clear effects on the benthic macrofauna. As was previously observed for the same area by Auriemma et al., (2016), the monotonic changes in communities' compositions with increasing distance from the sewage ducts were also corroborated by the ordered ANOSIM analysis. These changes in benthic communities, highlighted by multivariate analysis, were related to a shift in macrofaunal species composition where there were both an increased abundance of some species and a change in the presence/absence of others ones. Along the observed gradient, these changes seemed to be smooth between the Two and One groups but became stronger between One and Zero ones (by ANOSIM pairwise tests). Therefore, the effect of sewage-derived materials was mirrored on the structure of macrobenthic communities nearby the diffusion zone. In particular, the stations gathered in <5 meters groups were affected by the sludges by the remarkable variation in species composition were noticed nearby the pipes (0P and 0D). Thus following the initial 'disturbance effect', if organic enrichment proceeds then reduced oxygen concentrations can lead to changes in trophodynamics, which in turn shift the species composition and the abundance of benthic organisms (Gray et al., 2002). Ultimately, in accord with the intermediate disturbance hypothesis, almost any disturbance gradients will display an analogous pattern (Connell and Slatyer, 1977).

5.4.1 Macrofaunal production, productivity, and trophic role along the gradient of sewage effluents

The finding that benthic biomass was an important factor in determining benthic production is consistent with other studies (Tumbiolo and Downing, 1994, Cusson and Bourget, 2005; Fuhrmann et al., 2015). Moreover, stations gathered in One group did tend to show an increased number of species and abundance relative to other stations with high B and P, these responses confirm the classical model of Pearson and Rosenberg, which is the prediction of responses of abundance, biomass, and species richness for different levels of anthropogenic impact on benthic communities (Pearson and Rosenberg, 1978; Rosenberg, 2001) (see Fig.3)

In our study, we provided the biomass and production in kJ to allow for more comparisons between this study and others ones. Biomass is also to be considered as an expression of the stored energy in a system, reflects production (Carstensen et al., 2012). Furthermore, benthic invertebrate biomasses depend on some elements linked to the quality and quantity of food, substrate, and top-down control from predators (Tumbiolo and Downing, 1994).

Production was affected by several anthropogenic disturbances to the seabed such as dredged material disposal (Rhoads et al., 1978; Wilber and Clarke, 1998), fish farming (Kutti et al., 2008), and bottom fishing and trawling (Jennings et al., 2001a, 2002; Hiddink et al., 2006). In accord with Burd et al., 2012), our results showed that production of macrofaunal communities had a relative increase with distance away from the pipelines toward the stations located 100 m away from the source of organic matter (One group), probably due to the reduced organic matter flow. This study illustrated that biomass was, as expected, significantly positively correlated with production, however, although biomass increased in a similar pattern, they stabilized off at moderate modified organic flux toward a normal condition and did not increase at the Two group, where the distance was 200 meters from the pipelines. This suggests that most probably at 100 m (One group) from the source of organic matter we are in the middle of the transition zone, where, as postulated by Pearson and Rosenberg (1978) species numbers and biomass are usually higher. This is also in accord with the intermediate disturbance hypothesis (Connell and Slatyer, 1977). In this transitional zone between the natural environmental condition, with reduced organic matter input and the severe environmental condition, with high organic matter and a huge reduction in species numbers, there is an ecotonal zone where the exclusion of sensitive species is no already occurred but the presence of some opportunistic species start to attest. Accordingly, our results indicate that stations close to pipelines (Zero group) can most likely be considered as an ecotonal zone that presents the maximum values of species richness, biomass, and consequently diversity, due to the proximity to the maximum value of organic enrichment where macrobenthic community can utilize before starting to decrease in species numbers and diversity. The results of this study were similar to those of other studies regarding infauna species dominance concerning a gradient of organic enrichment, albeit on a much larger spatial scale (e.g., Brown et al., 1987; Weston, 1990).

In our study, the community $P/\overline{B}$ ratios displayed a different pattern compared to biomass and production. Accord to the ANN model prediction, $P/\overline{B}$ increased along the gradient of sewage effluents discharge with increasing distance from the pipelines, where was lower at Zero group than One and Two ones. Additionally, in the whole study area polychaetes dominated the benthic macroinvertebrate communities in numbers, biomass, and $P/\overline{B}$ despite the low average individual weights, compared to the other macrofaunal taxa. The dominance of polychaetes is well documented (e.g., Nasi et al., 2017) in particular in silty-clay sediments as our study area. Besides, previous studies reported that polychaetes were the most productive group, mainly due to their density combined with a high $P/\overline{B}$ ratio (e.g., Rhoads et al., 1978; Moller, 1985; Mistri and Ceccherelli, 1994; Nilsen et al., 2006). In agreement with our results, the higher estimates of productivity in the stations with lower Corg (such as the stations at 200 meters from the pipes) were observed by Lin et al., (2016). The authors evidenced that the nature of the variations in the benthic communities (taxonomic groups) among stations with different organic matter amounts may lead to general productivity variations. Additionally, the highest values of $P/\bar{B}$ were observed at stations placed far away from the pipes in this study due to a higher proportion of polychaetes at these stations, as r-strategist species with high $P/\bar{B}$ (Moller, 1985). Surprisingly, we found the low values of $P/\bar{B}$ ratios at the Zero group. Accord to the model of Pearson and Rosenberg (1978), we observed no peak of opportunistic species (e.g., Polychaeta *C. capitata*) at Zero group as an ecotonal point, while other species with low $P/\bar{B}$ ratio (i.e., k-strategist) (Mistri and Ceccherelli, 1994; Cusson and Bourget, 2005) were also found at the same stations which influenced the total productivity at these stations with a low value compared to the other stations along the organic enrichment gradient derived from sewage discharge. To support our finding and predicted by the P-R model, Weston (1990) investigated the benthic fauna under and at distances up to 450 m from a fish farm and did not find the ‘peak of opportunists’.

In our study and accord with Burd et al., (2012), the positive correlation between P and $P/\bar{B}$ ratios based on Spearman's rank correlation coefficient tests, where the lowest $P/\bar{B}$ and P were close to the pipelines, suggesting that the benthic organisms were not able to utilize most of the considerable organic matter present at Zero group. Moreover, the average $P/\bar{B}$ estimated (1.7 y^{-1}) in our study area was considerably higher than previous estimates for the South-western Barents Sea (0.25 y^{-1}, Denisenko, 2001) the Sørfjord, North Norway (0.29 y^{-1}, Nilsen et al., 2006), the Barents Sea (0.3 y^{-1}, Denisenko and Titov, 2003) and the Baltic Sea (0.32 y^{-1}, Harvey et al., 2003) (Table 4). Due to the relatively high $P/\bar{B}$ ratios in our study area were often accompanied by low mean biomass of whole sampling stations, therefore they tended to result in high total production, indicating that the effects of organic enrichment derived from the sewage pipelines were not so negative on the impacted study area.

In this study, macrofauna invertebrates were sampled in April and the assessments of biotic variables did not reflect seasonal changes and the sampling period was before the settling of juveniles for most macrofaunal organisms. However, $P/\bar{B}$ values may differ due to seasonal variations in environmental variables and the relative contribution of juveniles (with thin and small body size and high $P/\bar{B}$ ratios) (Fuhrmann et al., 2015). Hence, estimates given in our result were likely not subject to large seasonal changes. Therefore, we could infer that $P/\bar{B}$ represents the average annual values and principally mirrored the impact of the sewage discharge along the sampling gradient.

Regarding the stations 0M, located close to the pipeline (<5 m away) in the middle part of the longest pipe, but not in front of the outfalls, we observed high value of biomass, production, $P/\bar{B}$ than other stations placed nearby of the outfalls (i.e. 0D and 0P). This was probably associated with the reduction of organic enrichment from stations 0D and 0P to 0M along the pipeline. Indeed, these features indicate that probably the environmental contest at 0M is quite similar to that observed at the stations placed 100 m away from the pipes, and must expect the same pattern of organic meters from the pipes. That also is reflected in increasing species richness such as station gathered in Zero group, as observed also by other studies of the same areas (Auriemma et al., 2016; Vesal et al., Accepted).

In addition, our results support the hypothesis that increased organic enrichments due to the sewage discharges could decrease the biomass and increased the transfer efficiency at the gathered stations located <5 m away from the pipelines (Zero group) compared to other groups of stations. The mechanisms accounting for these responses are somewhat difficult to discern. This approach is currently ensuring transfer efficiency is important to study macrofaunal communities not only influenced by sewage discharge given in the present study outcomes. Transfer efficiency is shaped by a diversity of food web interconnections and energy fluxes of organic material. However, fluctuations in species abundances can control energy pathways through food webs, and systems dominated by a small number of species may have limited resilience (Steneck et al., 2011). In our case, the sewage discharge causes the maintenance of the tolerated species with smaller individual bodies, fast turnover, with the lowest value of biomass (i.e., Polychaeta, *Capitella capitata* with a thin body and small-sized) that can explain the locally high TE. Moving away from pipelines the TE diminished and the accumulation of biomass was taking place and the TE and B moved toward normal values at larger distances (Two group). Therefore, we hypothesized *a priori* that sewage discharges would drive a decrease in production and productivity rates for macroinvertebrates near the pipelines thus resulting in a maximum of biomass at an intermediate disturbance level.

Besides, while TE emanates from diverse metabolic, such as life cycle, food web processes, and the biomass of all consumers, estimating transfer efficiency requires knowledge of the trophic level of organisms within an ecosystem determined by their diets, and the production at each trophic level (Ullah et al., 2018; Eddy et al., 2020). In our study, the macrofaunal species found with low trophic levels, low biomass, and as well as increased food availability at Zero group, might have higher overall TE between these stations compared to the farther stations under sewage discharge.

A wide range of processes and scales that affect transfer efficiency results can be challenging in its estimation (Eddy et al., 2020). Production-based transfer efficiency estimate average was equal to TE of 13% for trophic levels 1–2 (phytoplankton to herbivorous mesozooplankton and benthic organisms) for temperate Northern hemisphere marine ecosystems and an average of 10% for trophic levels 2–3 (zooplankton and benthic organisms to fish) (Harrison et al., 2000). We evaluated an average value of TE equal to 15.33% for macrofaunal communities in the whole sampling area.

5.4.2 Relation among biological factors and environmental variables

Similarly, the macrofaunal P, $P/\overline{B}$, and TE were influenced by the environmental variations due to the sewage effluents. In this study, we attempted to identify the relationships between environmental variables and biological factors of benthic macrofaunal.

The RDA analysis showed that all the stations grouped by distance (<5, 100, and 200 m) were distinctly different from each other according to their biological factors and environmental variables. The analysis showed that the influence of Eh and the grain size characteristics were most important for biological factors of macrofaunal communities. Environmental conditions typically structure benthic communities (see e.g., Nilsen et al., 2006; Gray and Elliott, 2009).

$P/\overline{B}$ contents showed a positive correlation with Eh so that the values of $P/\overline{B}$ and Eh increased with increasing distance from the pipelines where there were the stations with less impacted from sewage discharge. However, it is possible that while certain environmental conditions enhance productivity for one taxa, others species may show an increase in productivity under a different set of environmental variables (Bolam et al., 2010)**,** as higher $P/\overline{B}$ ratios suggest higher population resilience to environmental perturbations (Tumbiolo and Downing, 1994). We observed that Corg and TN contents along the gradient of sewage effluents discharge showed no correlation with biomass and production, but there was a notable negative correlation with benthic productivity.

In our study, the proportion of TE made up of the macrofaunal, increased at stations located near the pipelines with increasing sand fraction and low Eh. That is, the impact of sediment deposition tends to be more taxon-selective; ultimately it favors those taxa that have an inherent ability to vertically migrate through the disposed of sediments (e.g., Hinchey et al., 2006; Bolam, 2011; Last et al., 2011; Burd et al., 2012).

Eh content was the only environmental variable that showed a positively significant correlation with richness, production, productivity, and TE estimates, suggesting that the sewage discharge directly influences environmental (sediment) conditions, and biological factors may follow the environmental strata, even at small spatial scales. Kutti et al., (2008) reported that the station close to high loading of organic matter was characterized by negative redox potential. However, the direct correlations between the macrobenthos and sediment characteristics clearly showed that variation in environmental variables was responsible for the observed declines in biomass, production, and $P/\overline{B}$ of the macrofaunal. Environmental variables influence community compositions and $P/\overline{B}$ are largely a function of the intrinsic characteristics of its members, rather than a direct result of environmental conditions (Cusson and Bourget, 2005; Bolam et al., 2010). In general, sediment composition and the quality and quantity of available organic matter, are known to structure benthic communities and determine the distribution of benthic infauna (e.g. Pearson & Rosenberg 1978, Wieking and Kröncke 2003, Kröncke et al., 2004; van Hoey et al., 2004).

B, $P/\overline{B}$, and TE as well as all other parameters investigated here, are known to vary over time, to be influenced by external environmental factors, and also might be deeply affected by unquantified spatial exchanges and flows (because of transport and active movement of species). Therefore, although estimates are necessarily considering sampling areas as closed, in following considerations might include also possible lateral flows and influences that can only be assumed.

5.5 Conclusion

This study highlights the importance of adjusting the organic matter loading from sewage discharge to the coastal marine environment and its influence on biomass, production, productivity, and transfer efficiency of macrofaunal communities in the given areas. On the local scale, we observed a clear pattern of macrofaunal community's distributions along the sewage gradient and among station positions. Among the environmental variables, the influence of Eh and the grain size characteristics were most important for biological factors of macrofaunal communities. Our results showed minimum B, P, and $P/\bar{B}$ nearby the pipelines and maximum B and P for intermediate distance (stations located at 100 m from the pipelines), and $P/\bar{B}$ increasing with distance. This study showed a negative relationship between TE and B of benthic macrofaunal at the sampling stations. TE showed the highest value at stations close to the pipeline where benthic communities are characterized by smaller individuals with low biomass values and fast turnover species (i.e., Polychaeta, *Capitella capitata*). Therefore, this suggests that the stations placed at 100m distance (One group) from the source of organic matter most probably could be considered as the middle of the transition zone, where species numbers and biomass are usually higher, while close to the pipelines, there is an ecotonal zone (Zero group) where the exclusion of sensitive species is no already occurred but the presence of some opportunistic species start to attest. Overall, our results indicate a clear influence of the sewage discharge on the biological features of macrofaunal communities and show surrounding bottoms influenced by moderate organic impact. However, the effects of the sewage pipeline were not so dramatic and in fact, the comparison with other sites indicates on average a contribution to productivity, and local (proximity) effects were never too negative.

Additionally, to reduce the effects of sewage discharge on macrofaunal communities, we suggest designing the sewage discharge pipelines placing the maximum possible numbers of diffusion towers to reduce the impact at the end of the main outfalls. Besides, due to the lack of information on TE of macrobenthic communities, not only in stressed conditions, we strongly recommend applying this approach in further studies to better understand the behaviour of TE and the related role of the energy fluxes among the macrobenthic trophic webs concerning to different environmental conditions and macrofaunal compositions.

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References

Agrawal, A., Gopal, K., 2013. Biomass Production in Food Chain and Its Role at Trophic Levels. In Biomonitoring of Water and Waste Water, Springer, 59-70.

Aller J. Y., Aller, R. C., Green, M. A., 2002. Benthic faunal assemblages and carbon supply along the continental shelf/shelf break-slope off Cape Hatteras, North Carolina. Deep-Sea Res II 49:4599–4625.

Andersen, K. H., Beyer, J. E., Lundberg, P., 2008. Trophic and individual efficiencies of size-structured communities. Proceedings of the Royal Society B 276:109–114.

Arend, K.K., Beletsky, D., Depinto, J.V., Ludsin, S.A., Roberts, J.J., Rucinski, D.K., Scavia, D., Schwab, D.J., Hook, T.O., 2011. Seasonal and interannual effects of hypoxia on fish habitat quality in central Lake Erie. Freshwater Biology, 56, 366-383. https://doi.org/10.1111/j.1365-2427.2010.02504.x

Auriemma, R., Nasi, F., Del Negro, P., 2016. The macrozoobenthic fauna of the bottoms nearby the underwater sewage duct of Trieste (Northern Adriatic Sea). Biologia Marina Mediterranea,23(1), 90.

Badalamenti, F., Pinnegar, J. K., Polunin, N. V. C., D'Anna, G., 2000. Estimates of trophic level in the red mullet Mullus barbatus: comparison between gut-contents and stable isotope data. Commission Internationale pour l'Exploration Scientifique de la Mer Mediterranee, Workshop Series 12: 19–21.

Barago, N., Floreani, F., Acquavita, A., Esbrí, J. M., Covelli, S., Higueras, P., 2020. Spatial and temporal trends of gaseous elemental mercury over a highly impacted coastal environment (Northern Adriatic, Italy). Atmosphere, 11(9). https://doi.org/10.3390/atmos11090935.

Baumann, M., 1995. A comment on transfer efficiencies. Fish. Oceanogr. 4, 264–266.

Benke, A. C., 2010. Secondary production as part of bioenergetic theory–contributions from freshwater benthic science. River Research and Applications, 26, 36–44.

Bissoli, L. B., Bernardino, A. F., 2018. Benthic macrofaunal structure and secondary production in tropical estuaries on the Eastern Marine Ecoregion of Brazil. PeerJ 6: e4441. doi: 10.7717/peerj.4441.

Bolam, S. G., Barrio-Frojan, C. R. S., Eggleton, J. D., 2010. Macrofaunal production along the UK continental shelf. Journal of Sea Research, 64(3), 166–179. https://doi.org/10.1016/j.seares.2010.02.003

Bolam, S. G., Barry, J., Bolam, T., Mason, C., Rumney, H. S., Thain, J. E., Law, R. J., 2011. Impacts of maintenance dredged material disposal on macrobenthic structure and secondary productivity. Marine Pollution Bulletin, 62(10), 2230–2245. https://doi.org/10.1016/j.marpolbul.2011.04.012

Bolam, S. G., Eggleton, J. D., 2014. Macrofaunal production and biological traits: Spatial relationships along the UK continental shelf. Journal of Sea Research 88, 47–58.

Botsford, W., Castilla, J. C., Peterson, C. H., 1997. The management of fisheries and marine ecosystems. Science 277:509–515.

Bouchet, V. M. P., Sauriau, P. G., 2008. Influence of oyster culture practices and environmental conditions on the ecological status of intertidal mudflats in the Pertuis Charentais (SW France): a multi-index approach. Marine Pollution Bulletin 56, 1898–1912.

Brey, T., Rumohr, H., Ankar, S., 1988. Energy content of microbenthic invertebrates: general conversion factors from weight to energy. J Exp Mar Biol Ecol 117:271–278.

Brey, T., 1990. Estimating productivity of macroinvertebrates from biomass and mean individual weight. Meeresforschung 32, 329–343.

Brey, T., 2001. Population Dynamics in Benthic Invertebrates. A Virtual Handbook. Version 01.2. Institute for Polar and Marine Research, Germany. Version 01.2, 2001. Available at http://www.thomas-brey.de/science/virtualhandbook/navlog/index.html.

Brey, T., Müller-Wiegmann, C., Zittier, Z.M.C., Hagen, W., 2010. Body composition in aquatic organisms — a global data bank of relationships between mass, elemental composition and energy content. J. Sea Res. 64, 334–340. http://dx.doi.org/10.1016/j.seares.2010.05.002.

Brey, T., 2012. A multi-parameter artificial neural network model to estimate macrobenthic invertebrate productivity and production. Limnol. Oceanogr. Methods 10, 581–589. http://www.thomas-brey.de/science/virtualhandbook

Brown, J. B., Gowen, R. J., McLusky, D. S., 1987. The effect of salmon farming on the benthos of a Scottish sea loch J. Exp. Mar. Biol. Ecol., 109 pp. 39-51.

Burd, B. J., Macdonald, T. A., van Roodselaar, A., 2012. Towards predicting basin-wide invertebrate organic biomass and production in marine sediments from a coastal sea. PLoS ONE, 7(7). https://doi.org/10.1371/journal.pone.0040295.

Carstensen, J., Dahl, K., Henriksen, P., Hjorth, M., Josefson, A., Krause-Jensen, D., 2012. Coastal Monitoring Programs. In Treatise on Estuarine and Coastal Science (Vol. 7, Issue December). Elsevier Inc. https://doi.org/10.1016/B978-0-12-374711-2.00712-9

Celio, M., Comici, C., Bussani, A., 2002. Thermohaline anomalies in the spring and early summer of 2000 in the Gulf of Trieste. Marine Ecology, an Evolutionary Perspective 23, 101–110.

Clesceri, L. S., Eaton, A. D., Greenberg, A.E., Franson, M. A. H., American Public Health, A., American Water Works, A., Water Environment, F., et al. 1996. Standard Methods for the Examination of Water and Wastewater: 19th Edition Supplement. American Public Health Association, Washington, DC.

Connell, J. H., Slatyer, R. O., 1977. Mechanisms of succession in natural communities and their role in community stability and organization. Am Nat 111:1119–1144.

Cooper, K. M., Barrio Froján, C. R. S., Defew, E., Curtis, M., Fleddum, A., Brooks, L., Paterson, D. M., 2008. Assessment of ecosystem function following marine aggregate dredging. J. Exp. Mar. Biol. Ecol. 366, 82–91.

Covelli, S., Faganeli, J., Horvat, M., Brambati, A., 1999. Porewater Distribution and Benthic Flux Measurements of Mercury and Methylmercury in the Gulf of Trieste (Northern Adriatic Sea). Estuarine, Coastal and Shelf Science, 48(4), 415-428.

Cusson, M., Bourget, E., 2005. Global patterns of macroinvertebrate production in marine benthic habitats. Marine Ecology Progress Series 297, 1–14.

Degen, R., Vedenin, A., Gusky, M., Boetius, A., Brey, T., 2015. Patterns and trends of macrobenthic abundance, biomass and production in the deep Arctic Ocean. Polar Res 34:24008.

Dell'Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: A new approach based on the biochemical composition of sediment organic matter. Marine Pollution Bulletin, 44(7):611-622. doi:10.1016/S0025- 326X (01)00302-2

Denisenko, S., 2001. Long-term changes of zoobenthos biomass in the Barents Sea. Proc Zool Inst Russ Acad Sci 2001: 59–66

Densienko, S. G., Titov, O. V., 2003. Distribution of zoobenthos and primary production of plankton in the Barents Sea.Oceanology 43:78–88.

Diaz, R. J., Rosenberg, R., 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr Mar Biol Annu Rev 33: 245–303.

Dolbeth, M., Lillebo, A.I., Cardoso, P.G., Ferreira, S.M., Pardal, M.A., 2005. Annual production of estuarine fauna in different environmental conditions: an evaluation of the estimation methods. J. Exp. Mar. Biol. Ecol. 326, 115–127.

Dolbeth, M., Cardoso, P.G., Grilo, T.F., Bordalo, M.D., Raffaelli, D., Pardal, M.A., 2011. Long-term changes in the production by estuarine macrobenthos affected by multiple stressors Estuar. Coast. Shelf Sci., 92. 10–18.

Dolbeth, M., Cusson, M., Sousa, R., Pardal M.A., 2012. Secondary production as a tool for better understanding of aquatic ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 69, 1230–1253.

Duineveld, G. C. A., Künitzer, A., Niermann, U., De Wilde, PAWJ., Gray, J. S., 1991. The macrobenthos of the North Sea. Neth J Sea Res 28:53–65.

Eddy, T. D., Bernhardt, J. R., Blanchard, J. L., Cheung, W. W. L., Colléter, M., du Pontavice, H., Fulton, E. A., Gascuel, D., Kearney, K. A., Petrik, C. M., Roy, T., Rykaczewski, R. R., Selden, R., Stock, C. A., Wabnitz, C. C. C., Watson, R. A., 2020. Energy Flow Through Marine Ecosystems: Confronting Transfer Efficiency. Trends in Ecology and Evolution, 36(1), 76–86. https://doi.org/10.1016/j.tree.2020.09.006

Filgueiras, V.L., Campos, L.S., Lavrado, H.P., Frensel, R., Pollery, R.C.G., 2007. Vertical distribution of macrobenthic infauna from the shallow sublittoral zone of Admiralty Bay, King George Island, Antarctica. Polar Biol. 30, 1439–1447.

Fonda Umani, S., Malfatti, F., Del Negro, P., 2012. Carbon fluxes in the pelagic ecosystem of the Gulf of Trieste (Northern Adriatic Sea). Estuarine, Coastal and Shelf Science 115, 170–185.

Frontalini, F., Buosi, C., Da Pelo, S., Coccioni, R., Cherchi, A., Bucci, C., 2009. Benthic foraminifera as bio-indicators of trace element pollution in the heavily contaminated Santa Gilla lagoon (Cagliari, Italy). Marine Pollution Bulletin, 58, 858–877.

Fuhrmann, M. M., Pedersen, T., Ramasco, V., Nilssen, E. M., 2015. Macrobenthic biomass and production in a heterogenic subarctic fjord after invasion by the red king crab. J. Sea Res. 106: 1–13. doi: 10.1016/j.seares.2015.09.003

Gascuel, D., Bozec, Y-M., Chassot, E., Colomb, A., and Laurans, M., 2005. The trophic spectrum: theory and application as an ecosystem indicator. ICES Journal of Marine Science, 62: 443–452.

Giangrande, A., 1997. Polychaete reproductive patterns, life cycles and life histories, an overview. Oceanography and marine Biology, 35, 323–386.

Gray, J. S., Wu, R. S. S., Or, Y. Y., 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. Marine ecology progress series, 238, 249–279.

Gray, J., Clarke, K., Warwick, R., Hobbs, G., 1990. Detection of initial effects of pollution on marine benthos: an example from the Ekofisk and Eldfisk oilfields, North Sea. Marine Ecology Progress Series, 66, 285–299. https://doi.org/10.3354/meps066285

Gray, J.S., Dayton, P., Thrush, S., Kaiser, M.J., 2006. On effects of trawling, benthos and sampling design. Marine Pollution Bulletin, *52* (8), 840–843.

Gray, J.S., Elliott, M., 2009. Ecology of Marine Sediments -From Science to Management. 2nd ed. Oxford University Press. New York, 243 pp.

Hargrave, B. T., Holmer, M., and Newcombe, C. P., 2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators, Mar. Pollut. Bull., 56, 810–824.

Harrison, P.J., Parsons, T.R., 2000. Fisheries oceanography: an integrative approach to fisheries ecology and management. Book Review, Association for the Sciences of Limnology and Oceanography (ASLO) 46 p. 1844.

Harvey, C. J., Cox, S. P., Essington, T. E., Hansson, S., Kitchell, J. F., 2003. An ecosystem model of food web and fisheries interactions in the Baltic Sea. ICES J Mar Sci 60:939–950.

Hiddink, J. G., Hutton, T., Jennings, S., Kaiser, M. J., 2006. Predicting the effects of area closures and fishing effort restrictions on the production, biomass, and species richness of benthic invertebrate communities. ICES J Mar Sci 63:822–830.

Hinchey, E. K., Schaffner, L. C., Hoar, C. C., Vogt, B.W., Batte, L. P., 2006. Responses of estuarine benthic invertebrates to sediment burial: the importance of mobility and adaptation. Hydrobiologia; 556:85–98.

Hjelset, A. M., Andersen, M., Gjertz, I., Lydersen, C., Gulliksen, B., 1999. Feeding habits of bearded seals (Erignathous barbatus) from the Svalbard area, Norway. Polar Biology 21: 186–193.

Ieromina, O., Musters, C. J. M., Bodegom, P. M., Peijnenburg, W. J. G. M., & Vijver, M. G., 2016. Trait modality distribution of aquatic macrofauna communities as explained by pesticides and water chemistry. Ecotoxicology, 25(6), 1170–1180. https://doi.org/10.1007/s10646-016-1671-5

Jackson, J. B. C., Kirby, M. X., Berger, W. H., Bjorndal, K. A., Botsford, L. V., Bourque, B. J., Bradbury, R. H., Cooke, R., Erlandson, J., Estes, J. A., Hughes, T. P., Kidwell, S., Lange, C. B., Lenihan, H. S., Pandolfi, J. M., Peterson, C. H., Steneck, R. S., Tegner, M. J., Warner, R. R., 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science 293, 629–638.

Jennings, S., Dinmore, T. A., Duplisea, D. E., Warr, K. J., Lancaster, J. E., 2001a. Trawling disturbance can modify benthic production processes. J Anim Ecol 70:459–475.

Jennings, S., Pinnegar, J. K., Polunin, N. V. C., Warr, K. J., 2002. Linking size-based and trophic analyses of benthic community structure. Mar Ecol Prog Ser 226:77–85.

Johansen, R., Pedersen, T., Barrett, R., 1999. Cormorants (*Phalacrocorax carbo carbo*) as predators in a cod (*Gadus morhua L.*) enhancement area in North Norway. In: Howell B, Moksness E, Svåsand T (eds) Stock enhancement and sea ranching. Fishing News Books, Oxford, p 334–349.

Jumars, P. A., Dorgan, K. M., Lindsay, S. M., 2015. Diet of worms emended, an update of polychaete feeding guilds. Annual review of marine science, 7, 497-520.

Kabat, P., Bazelmans, J., van Dijk, J., Herman, P. M. J., and others 2012. The Wadden Sea region: towards a science for sustainable development. Ocean Coast Manage 68: 4−17.

Kerr, S. R., 1974. Theory of size distribution in ecological communities. J Fish Res Board Can 31:1859–1862.

Kröncke, I., Stoeck, T., Wieking, G., Palojärvi, A., 2004. Relationship between structural and trophic aspects of microbial and macrofaunal communities in different areas of the North Sea. Mar Ecol Prog Ser 282:13–31.

Kutti, T., Ervik, A., Hoisaeter, T., 2008. Effects of organic effluents from a salmon farm on a fjordic system. III. Linking deposition rates of organic matter and benthic productivity. Aquaculture 282, 47–53.

Last, K.S., Hendrick, V.J., Beveridge, C.M., Davies, A.J., 2011. Measuring the effects of suspended particulate matter and smothering on the behaviour, growth and survival of key species found in areas associated with aggregate dredging. Report for the Marine Aggregate Levy Sustainability Fund, Project MEPF 08/P76, 69p.

Libralato, S., Coll, M., Tudela, S., Palomera, I., Pranovi, F., 2008. Novel index for quantification of ecosystem effects of fishing as removal of secondary production. Marine Ecology Progress Series, 355, 107–129. https://doi.org/10.3354/meps07224

Libralato, S., Solidoro, C., 2010. Comparing methods for building trophic spectra of ecological data. ICES Journal of Marine Science, 67(3), 426–434. https://doi.org/10.1093/icesjms/fsp249.

Lin, H., Wang, J., Liu, K., He, X., Lin, J., Huang, Y., Zhang, S., Mou, J., Zheng, C., Wang, Y., 2016. Benthic macrofaunal production for a typical shelf-slope-basin region in the western Arctic Ocean. Continental Shelf Research, 113, 30–37. https://doi.org/10.1016/j.csr.2015.12.001

Lindeman, R. L., 1942. The trophic-dynamic aspect of ecology. Ecology, 23: 399–417.

Link, J. S., 2002. Does food web theory work for marine ecosystems? Mar. Ecol. Progr. Ser., 230, 1–9.

Link, J.S., Watson, R.A., 2019. Global ecosystem overfishing: clear delineation within real limits to production. Sci. Adv. 5, eaav0474

Malačič, V., 1991. Estimation of the vertical eddy diffusion coefficient of heat in the Gulf of Trieste (Northern Adriatic). Oceanologica Acta, 14 (1): 23–32.

Martins, C.C., Braun, J.A.F., Seyffert, B.H., Machado, E.C., Fillmann, G., 2010. Anthropogenic organic matter inputs indicated by sedimentary fecal steroids in a large South American tropical estuary (Paranaguá estuarine system, Brazil). Marine Pollution Bulletin 60, 2137–2143.

Matijević, S., Kušpilić, G., & Kljaković-Gašpić, Z., 2007. The redox potential of sediment from the Middle Adriatic region. Acta Adriatica, 48(2), 191–204.

McLusky, D. S., McIntyre, A. D., 1988. Characteristics of the benthic fauna. In: Potsma H, Zijlstra JJ (eds) Continental shelves. Elsevier, Amsterdam, 131–154.

Melis, R., Celio, M., Bouchet, V. M., Varagona, G., Bazzaro, M., Crosera, M., Pugliese, N., 2019. Seasonal response of benthic foraminifera to anthropogenic pressure in two stations of the Gulf of Trieste (northern Adriatic Sea, Italy): the marine protected area of Miramare versus the Servola water sewage outfall. Mediterranean Marine Science, 20(1), 120-141.

Mistri, M., Ceccherelli, V. U., 1994. Growth and secondary production of the Mediterranean gorgonian Paramuricea clavata. Marine Ecology Progress Series 103, 291–296.

Möller, P., 1985. Production and abundance of juvenile *Nereis diversicolor*, and orogenic cycle of adults in shallow waters of western Sweden. J Mar Biol Assoc UK 65:603–616.

Moore, J. K., Fu, W., Primeau, F., Britten, G. L., Lindsay, K., Long, M., 2018. Sustained climate warming drives declining marine biological productivity. Science, 359 (6380), 1139–1143. https://doi.org/10.1126/science.aao6379.

Mouillot, D., Spatharis, S., Reizopoulou, S., Laugier, T., Sabetta, L., Basset, A., Do Chi, T., 2006. Alternatives to taxonomic-based approaches to assess changes in transitional water communities. Aquat Conserv 16:469–482.

Nasi, F., Auriemma, R., Bonsdorff, E., Cibic, T., Aleffi, I. F., Bettoso, N., Del Negro, P., 2017. Biodiversity, feeding habits and reproductive strategies of benthic macrofauna in a protected area of the northern Adriatic Sea: a three-year study. Mediterranean Marine Science,18(2), 292-309.

Nasi, F., Nordström, M.C., Bonsdorff, E., Auriemma, R., Cibic, T., Del Negro. P., et al., 2018. Functional biodiversity of marine soft-sediment polychaetes from two Mediterranean coastal areas in relation to environmental stress. Marine Environmental Research, 137, 121-132.

Nieuwenhuize, J., Maas, Y. E. M., Middleburg, J. J., 1994. Rapid analysis of organic carbon and nitrogen in particulate materials. Marine Chemistry 44, 217–224.

Nilsen, M., Pedersen, T., Nilssen, E. M., 2006. Macrobenthic biomass, productivity (P/B̄) and production in a high-latitude ecosystem, North Norway. Marine Ecology Progress Series, 321(September), 67–77. https://doi.org/10.3354/meps321067

Nixon, S. W., 1995. Coastal marine eutrophication: A Definition, social causes, and future concerns. OPHELIA 41: 199–219.

Novelli, G., 1996. Gli scarichi a mare nell`alto Adriatico. Rassegna tecnica del Friuli Venezia Giulia, 3, 11-19.

NRC (National Research Council)., 1995. Understanding marine biodiversity: a research agenda for the nation. National Academy Press, Washington, DC.

Odum, W. E., Heald, E. J., 1975. The detritus-based food web of an estuarine mangrove community. In Estuarine Research, 1, 265–286. Ed. by L. E. Cronin. Academic Press, New York.

Patrício, J., Neto, J.M., Teixeira, H., Salas, F., Marques, J.C., 2009. The robustness of ecological indicators to detect long-term changes in the macrobenthos of estuarine systems. Mar. Environ. Res. 68, 25–36.

Pauly, D., Christensen, V., 1995. Primary production required to sustain global fisheries. Nature, 374: 255–257.

Pauly, D., Watson, R. 2005. Background and interpretation of the “marine trophic index” as a measure of biodiversity. Philosophical Transactions of the Royal Society B: Biological Sciences, 360: 415–423.

Pearson, T. H., Stanley, S. O., 1979. Comparative measurement of the redox potential of marine sediment as a rapid means of assessing the effects of organic pollution. Mar Biol 53: 371–379.

Pearson, T. H., Rosenberg, R., 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology, Annual Review, 16(January), 229–311.

Peeters, E. T. H. M., Dewitte, A., Koelmans, A. A., Van Der Velden, P. J. Den Besten, J. A., 2001. Evaluation of bioassays versus contaminant concentrations in explaining the macroinvertebrate community structure in the Rhine-Meuse delta, the Netherlands. Environmental Toxicology and Chemistry 20:2883–2891.

Petersen C. G. J., Boysen-Jensen, R., 1911. Valuation of the sea. Rep Dan Biol Stat 20:3–81.

Pimm, S., 1982. Food webs. New York: Chapman & Hall. 219 pp.

Pranovi, F., Libralato, S., Raicevich, S., Granzotto, A., Pastres, R., Giovanardi, O., 2003. Mechanical clam dredging in Venice lagoon: Ecosystem effects evaluated with a trophic mass-balance model. Marine Biology, 143(2), 393–403. https://doi.org/10.1007/s00227-003-1072-1

Pranovi, F., Raicevich, S., Libralato, S., Da Ponte, F., & Giovanardi, O., 2005. Trawl fishing disturbance and medium-term microfaunal recolonization dynamics: a functional approach to the comparison between sand and mud habitats in the Adriatic Sea (Northern Mediterranean Sea). In American Fisheries Society Symposium, 41(545). American Fisheries Society.

R Core Team., 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org

Reiss, H., Kröncke, I., 2005. Seasonal variability of infaunal community structures in three areas of the North Sea under different environmental conditions. Estuar. Coast. Shelf Sci. 65: 253–274. doi: 10.1016/j.ecss.2005.06.008

Reiss, J., Bridle, J. R., Montoya, J. M., Woodward, G., 2009. Emerging horizons in biodiversity and ecosystem functioning research. Trends in ecology and evolution, 24(9), 505–514.

Rhoads, D. C., McCall, P. L., Yingst, J. Y., 1978. Disturbance and production on the estuarine seafloor. American Scientist 66:577–586.

Ricciardi, A., Bourget, E., 1998. Weight-to-weight conversion factors for marine benthic macroinvertebrates. Mar Ecol Prog Ser, 163:245–251.

Rigler, F. H., Downing J. A., 1984: The calculation of secondary productivity. – In: Dowing, J. A. and F. H. Rigler (eds.), A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters, 19–58. Blackwell Scientific Publications, Oxford.

Robertson, A. I., 1979. The relationship between annual production ratio and lifespans for marine macrobenthos. Oecologia 38, 193–202.

Rosenberg, R., 2001. Marine benthic faunal successional stages and related sedimentary activity. Sci Mar 65: 107–119.

Rouse, G. W., 2000. Polychaetes have evolved feeding larvae numerous times. Bulletin of marine Science, 67(1), 391-409.

Rumohr, H., Brey, T., Ankar, S., 1987. A compilation of biometric conversion factors for benthic invertebrates of the Baltic Sea. Balt Mar Biol Publ 9:1–56

Ryther, J. H., 1969. Photosynthesis and fish production in the sea. Science 166, 72–76.

Sanders, H. L., 1956. Oceanography of Long Island sound, 1952–1954. X. The biology of marine bottom communities. Bull. Bingham Oceanogr. Collect. 15, 345–414.

Schratzberger, M., Warr, K., Rogers, S.I., 2007. Functional diversity of nematode communities in the southwestern North Sea. Marine Environmental Research 63(4), 368-389.

Sharp, W. E., 1974. The dilution capacity of small streams in South Carolina, Rep. 48, Water Resour. Res. Inst., Clemson Univ., Clemson, S.C.

Short, F. T., Wyllie-Echeverria, S., 1996. Natural and human induced disturbance of seagrasses. Environ Conserv 23: 17–27.

Solis-Weis, V., Aleffi, I.F., Bettoso, N., Rossini, P., Orel, G., 2007. The benthic macrofauna at the outfalls of the underwater sewage discharges in the Gulf of Trieste (Northern Adriatic Sea, Italy). Annales Series Historia Naturalis, 17 (1), 1-16.

Souza, F. M., Brauko, K. M., Lana, P. C., Muniz, P., Camargo, M. G., 2013. The effect of urban sewage on benthic macrofauna: A multiple spatial scale approach. Marine Pollution Bulletin, 67(1–2), 234–240. https://doi.org/10.1016/j.marpolbul.2012.10.021

Sprung, M., 1993. Estimating secondary production from body weight and mass—a field test in a non-boreal intertidal habitat. Marine Ecology Progress Series 100, 103–109.

Steneck, R.S., Hughes, T.P., Cinner, J.E. et al. (2011). Creation of a gilded trap by the high economic value of the Maine lobster fishery: gilded trap of Maine’s lobster fishery. Conserv. Biol. 25, 904–912.

Stergiou, K. I., Karpouzi, V. S., 2002. Feeding habits and trophic levels of Mediterranean fish. Reviews in Fish Biology and Fisheries, 11: 217–254.

Stock, C. A., John, J. G., Rykaczewski, R. R., Asch, R. G., Cheung, W. W. L., Dunne, J. P., Friedland, K. D., Lam, V. W. Y., Sarmiento, J. L. & Watson, R. A., 2017. Reconciling fisheries catch and ocean productivity. Proceedings of the National Academy of Sciences of the U.S.A. 114, E1441–E1449.

Tadir, R., Benjamini, C., Almogi-Labin, A., Hyams-Kaphzan, O., 2017. Temporal trends in live foraminiferal assemblages near a pollution outfall on the Levant shelf. Mar. Pollut. Bull. 117 (1–2), 50–60. https://doi.org/10.1016/ j. marpolbul.2016.12.045

Tillin, H. M., Hiddink, J. G., Jennings, S., Kaiser. M. J., 2006. Chronic bottom trawling alters the functional composition of benthic invertebrate communities on a sea-basin scale. Marine Ecology Progress Series, 318, 31–45.

Tumbiolo, M.L., Downing, J.A., 1994. An empirical model for the prediction of secondary production in marine benthic invertebrate populations. Marine Ecology Progress Series 114, 165–174.

Ullah, H., Nagelkerken, I., Goldenberg, S. U., Fordham, D. A., 2018. Climate change could drive marine food web collapse through altered trophic flows and cyanobacterial proliferation. PLoS Biology, 16(1), 1–21. https://doi.org/10.1371/journal.pbio.2003446

Van Hoey, G., Degrear, S., Vincx, M., 2004. Macrobenthic community structure of soft-bottom sediments at the Belgian Continental Shelf. Estuar Coast Shelf Sci 59: 599−613.

Vesal, S. E., Nasi, F., Pazzaglia, J., Ferrante, L., Auriemma, R., Relitti, F., Bazzaro, M., Del Negro, P. The effect of sewage discharges on soft-bottom macrofauna thoroughly traits-based approach. Accepted to Marine Pollution Bulletin.

Washington, H. G., 1984. Diversity, biotic and similarity indices. A review with special relevance to aquatic ecosystems. Wat. Res., 18: 653-694.

Wassmann, P., 1998. Retention versus export food chains: processes controlling sinking loss from marine pelagic systems. Hydrobiologia, 363, 29–57.

Waters, T. P., Crawford, G. W., 1973. Annual production of a stream mayfly population: a comparison of methods. LimnoL Oceanogr. 18:286-296.

Waters, T.F., 1977. Secondary production in Inland waters. Advances in Ecological Research 10, 91–164.

Watson R, Pauly, D., 2001. Systematic distortion in world fisheries catch trends. Nature 414:534–536.

Weston, D.P., 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient Mar. Ecol., Prog. Ser., 61. 233-244.

Wieking, G., Kröncke, I., 2003. Macrofauna communities of the Dogger Bank (central North Sea) in the late 1990s: spatial distribution, species composition and trophic structure. Helgol Mar Res 57: 34−46.

Wilber, D.H., Clarke, D.G., 1998. Estimating secondary production and benthic consumption in monitoring studies: a case study of the impacts of dredged material disposal in Galveston Bay, Texas. Estuaries 21, 230–245.

Yeleliere, E., Cobbina, S. J., Duwiejuah, A. B., 2018. Review of Ghana’s water resources: the quality and management with particular focus on freshwater resources. Applied Water Science, 8(3), 1–12.

Zhang, C.L., Xu, B.D., Ren, Y.P., Xue, Y., Ji, Y.P., 2011. The spatio-temporal change in the secondary production of macrozoobenthos in the intertidal zone of Jiaozhou Bay. Acta Ecologica Sinica 31, 5071–5080. (in Chinese).

https://marinespecies.org/

https://www.itis.gov/

https://www.polytraits.lifewatchgreece.eu

Table 1. Sample stations, depth range, coordinates, sampling stations distance from the pipelines, and physical-chemical parameters measured at sampling stations in April 2018. Sediment grain-size, TN (Total Nitrogen); Corg (organic carbon); C/N (carbon and nitrogen ratio); Eh (redox potential).

Station	**Depth** (m)	**Latitude**	**Longitude**	**Distance from the pipeline** (m)	**Sand** (%)	**Silt** (%)	**Clay** (%)	**TN** (mg N g^{-1})	**Corg** (mg C g^{-1})	**C/N**	**Eh** mV
0D	22.6	45°38'605"	13°40'862"	5	39.6	38	22.4	3.8	60.5	18.6	-290
0M	20.1	45°38'601"	13°41'788"	5	39.4	37.3	23.3	1.7	13.5	9.4	-25
0P	22	45°38’435’’	13°41’600"	5	25.7	46.6	22.7	2.5	27.8	13	-204
1FD	22.7	45°38'601"	13°40'788"	100	9	54.3	36.7	1.9	16.7	10.1	-150
1D	23	45°38'662"	13°40'849"	100	13.6	53.3	33.1	1.8	16.8	10.8	-210
–1D	22.3	45°38'561"	13°40'872"	100	10.5	54.2	35.3	2.5	21.9	10.2	-101
1M	20.6	45°38'599"	13°41'233"	100	11.4	54.6	34	1.9	15.9	10	-117
–1M	18.3	45°38'493"	13°41'258"	100	34.9	40.8	24.3	1.3	12.3	11.3	70
1P	21.9	45°38’483"	13°41’588"	100	12.1	54.3	33.6	2.5	17.6	8.2	-57
–1P	22	45°38’989"	13°41’612"	100	17.2	50.1	32.7	2.3	16.8	8.5	-117
2FD	23.1	45°38'592"	13°40'718"	200	5.9	52.7	41.4	1.8	15.2	10	-130
2D	23.5	45°38'716"	13°40'834"	200	7	54.1	38.9	1.9	16.1	10	-150
–2D	21.9	45°38'506"	13°40'886"	200	7	54.6	38.4	1.8	16.2	10.3	-210
2M	22,00	45°38'649"	13°41'215"	200	5.7	55	39.3	1.9	16	9.7	35
–2M	18.8	45°38'441"	13°41'271"	200	21.3	48.9	30	1.6	13.3	9.9	8
2P	22	45°38’531"	13°41’576"	200	8	54.5	37.5	2.1	16.7	9.2	-15
–2P	21.4	45°38’344"	13°41’623"	200	7.8	53.2	39	1.8	14.1	9.3	-115
RS	22.5	45°37'540"	13°41'118"	2000	7	50.5	42.5	1.2	10.7	10.5	54

Table 2. Total Biomass-B, Production-P, Productivity-$P/\overline{B}$, transfer efficiency-TE, number of Species-Sp. and Abundance-Abu from different stations. RS was excluded to calculate the mean of each variable.

Station	**B** (kJ m^{-2})	**P** (kJ m^{-2} yr^{-1})	**$P/\overline{B}$** (yr^{-1})	**TE** (%)	**Sp.** N°	**Abu** (ind m^{-2})
0D	19.2	13.4	0.7	28	43	1640
0M	46.5	83.7	1.8	34	94	1573
0P	18.1	12.7	0.7	8	42	366
1FD	20.4	32.7	1.6	14	63	980
1D	17.6	22.9	1.3	6	54	723
–1D	86.7	156.1	1.8	13	82	3580
1M	50.1	125.3	2.5	11	108	1856
–1M	99.9	189.9	1.9	17	86	1433
1P	58.6	105.5	1.8	12	87	1646
–1P	48.9	78.2	1.6	13	74	1146
2FD	23.8	42.8	1.8	20	48	456
2D	19.3	19.3	1.0	6	53	906
–2D	19.8	53.4	2.7	8	62	750
2M	54.4	81.6	1.5	22	71	1403
–2M	15.6	40.6	2.6	17	68	836
2P	45.9	91.9	2.0	21	86	1153
–2P	10.5	14.7	1.4	12	82	1853
RS	13.8	35.9	2.6	15	56	883
Means	37.2±25.9	66.7±51.7	1.7±0.6	15.3±7.5	69.9±18.7	1288.2±729.9

Table 3. Spearman's rank correlation coefficient (rs) for the relationship between biological factors and environmental variables. **$p < 0.01$; *$p < 0.05$

	Eh	C/N	Corg	TN	Clay	Silt	Sand	Sp.	Abu.	TE	$P/\bar{B}$	P
B	0.333	-0.247	0.126	0.279	-0.168	0.260	0.075	0.595**	0.496*	0.232	0.189	0.858**
P	0.586*	-0.342	-0.199	-0.065	-0.057	0.291	0.013	0.794**	0.501*	0.259	0.605**	
$P/\bar{B}$	0.505*	-0.172	-0.545*	-0.509*	0.198	0.230	-0.181	0.460	0.027	0.175		
TE	0.477*	-0.154	-0.275	-0.127	-0.131	-0.299	0.141	0.195	0.228			
Abu.	0.299	-0.313	0.053	0.289	-0.156	0.135	0.183	0.716**				
Sp.	0.583*	-0.594**	-0.278	-0.095	-0.133	0.231	0.144					

Table 4. Total production (kJ m^{-2} yr^{-1}), average production/ biomass (P/$\overline{B}$) ratio (yr^{-1}) and biomass (g ww m^{-2}) of benthic macrofaunal from different study areas.

Study area	**Biomass** (g ww m^{-2})	**Total Production** (kJ m^{-2} yr^{-1})	**P/$\overline{B}$ ratio** (yr^{-1})	**References**
Gulf of Trieste	24	66.7	1.7	Present study
Sørfjord, North Norway	307	nd	0.29	Nilsen et al., (2006)
Tyne/Tees	nd	19.8	1.2	Bolam et al., (2010)
Anglia	nd	99.6	1.6	Bolam et al., (2010)
Barents Sea	59.5	nd	0.3	Denisenko & Titov, (2003)
Humber/Wash	nd	47.1	1.9	Bolam et al., (2010)
Southwestern Barents Sea	nd	nd	0.25	Denisenko, (2001)
Eastern Channel	nd	180.4	1.4	Bolam et al., (2010)
Baltic Sea	53.8	nd	0.32	Harvey et al., (2003)
Western Channel	nd	94.3	1.3	Bolam et al., (2010)
Cardigan Bay	nd	196.6	1.7	Bolam et al., (2010)
North Sea, 57°N	20–90	nd	0.1–5.0	McLusky & McIntyre(1988)
North Sea, 51–57°N	76	nd	1.9	Duineveld et al., (1991)
Severn	nd	86.5	1.3	Bolam et al., (2010)
Irish Sea	nd	157	1.2	Bolam et al., (2010)
Cape Hatteras, USA	540	nd	1.3	Aller et al., (2002)
Minches and Malin Sea	nd	66.2	1.4	Bolam et al., (2010)
Chukchi Sea	nd	0.5–1603.1	0.2–1.1	Lin et al., (2016)
North Scotland Coast	nd	67.5	1.3	Bolam et al., (2010)
Beaufort Sea	nd	0.5–278.7	0.4–0.9	Lin et al., (2016)
English Channel	nd	75.0–350.0	nd	Cooper et al., (2008)
Porsangerfjord, North Norway	65	1744	1.02	Fuhrmann et al., (2015)

Figure captions

Figure 1: Study area and location of sampling sites in the Gulf of Trieste, Italy.

Figure 2: nMDS of the 18 stations from square-root transformed abundances of 270 species and Bray-Curtis similarities with the three distance groups and the reference station from the sewage discharge area indicated by different symbols and colors.

Figure 3: Bar plots showing the macrobenthic abundance (ind. m^{-2}) (a), number of species (N°) (b), biomass (kJ m^{-2}) (c), production (kJ m^{-2} y^{-1}) (d), $P/\overline{B}$ (yr^{-1}) (e) and transfer efficiency (TE, %) (f), along distance gradient from the pipelines (5, 100 and 200 meters). The data are presented as means (±SD) for each of the 3 gathered-group of sampling stations.

Figure 4: Relationship between the values (±SE) for transfer efficiency (TE) and biomass (B) along the sewage gradient with increasing distance from the pipelines (5, 100, and 200 meters).

Figure 5: Two-dimensional PCA plot of the sampling stations include stations placed at <5 m from the Pipelines-Zero group of stations (0D, 0M and 0P), stations placed at 100 m from the Pipelines-One group of stations (1FD, 1D, –1D, 1M, –1M, 1P and –1P), stations placed at 200 m from the Pipelines-Two group of stations (2FD, 2D, –2D, 2M, –2M, 2P and –2P) and RS, according to biological factors. The different groups are indicated with different symbols and colors.

Figure 6: RDA ordination diagram of biological factors (B, P, $P/\overline{B}$, and TE), abundance, and environmental variables (Sand, Silt, TN, Corg, C/N, and Eh) among the stations. The different groups are indicated with different symbols and colors.

Figure 1.

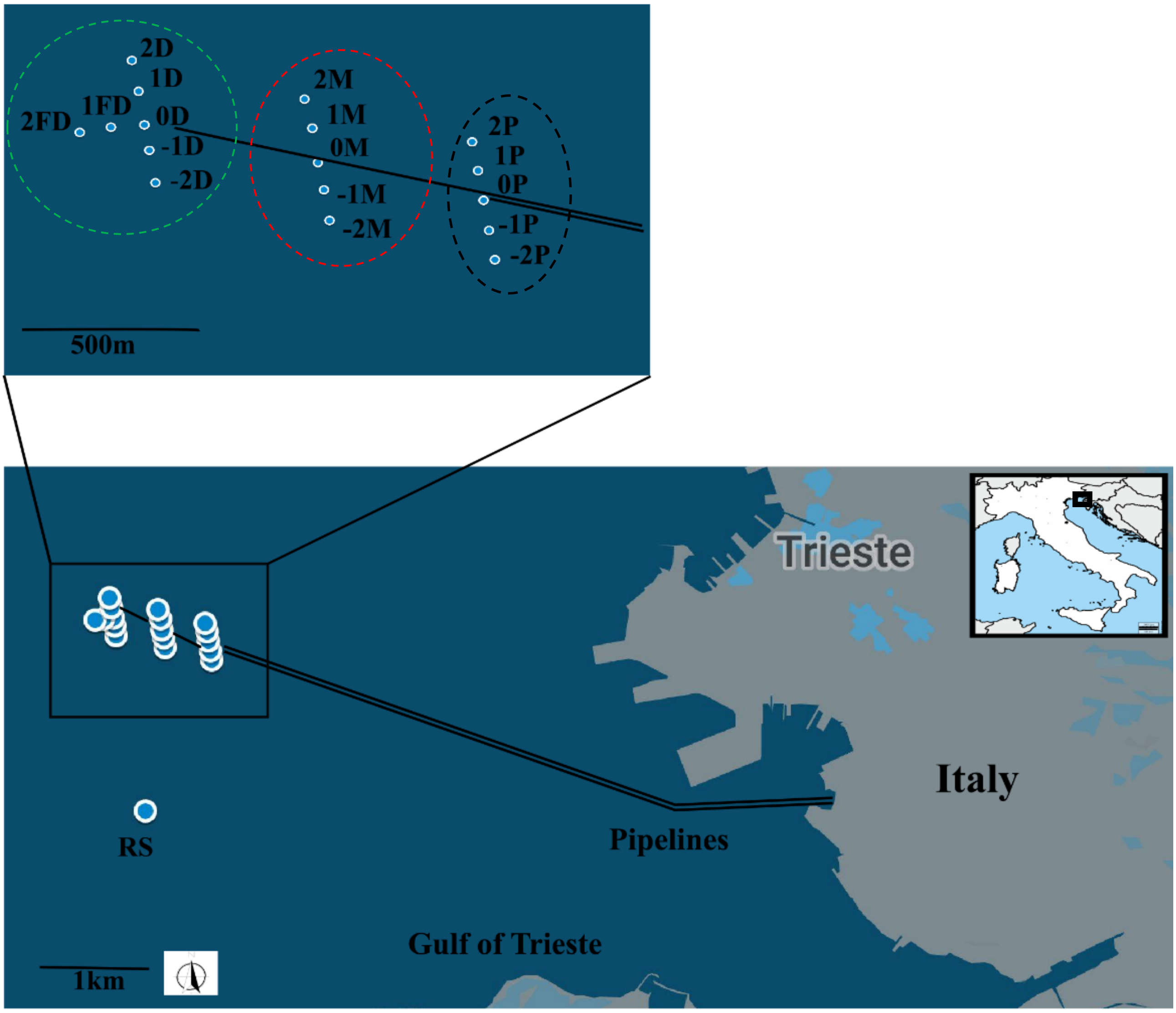

2D
1D
2FD
1FD
0D
-1D
-2D
2M
1M
0M
-1M
-2M
2P
1P
0P
-1P
-2P
500m
Trieste
Italy
RS
Pipelines
Gulf of Trieste
1km

Figure 2.

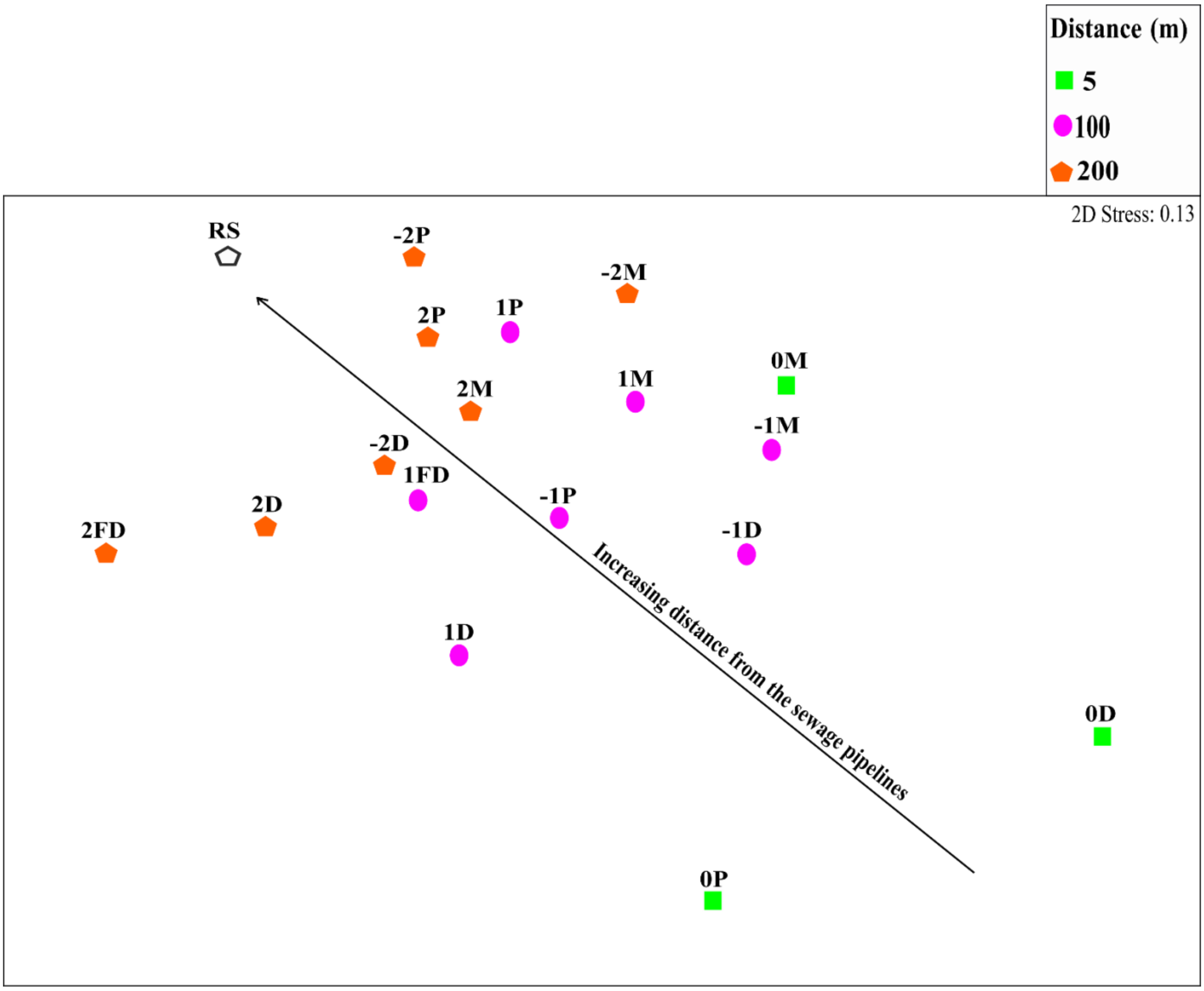

Distance (m)
5
100
200
2D Stress: 0.13
RS
-2P
-2M
2P
1P
0M
1M
2M
-1M
-2D
1FD
-1P
2D
-1D
2FD
Increasing distance from the sewage pipelines
1D
0D
0P

Figure 3.

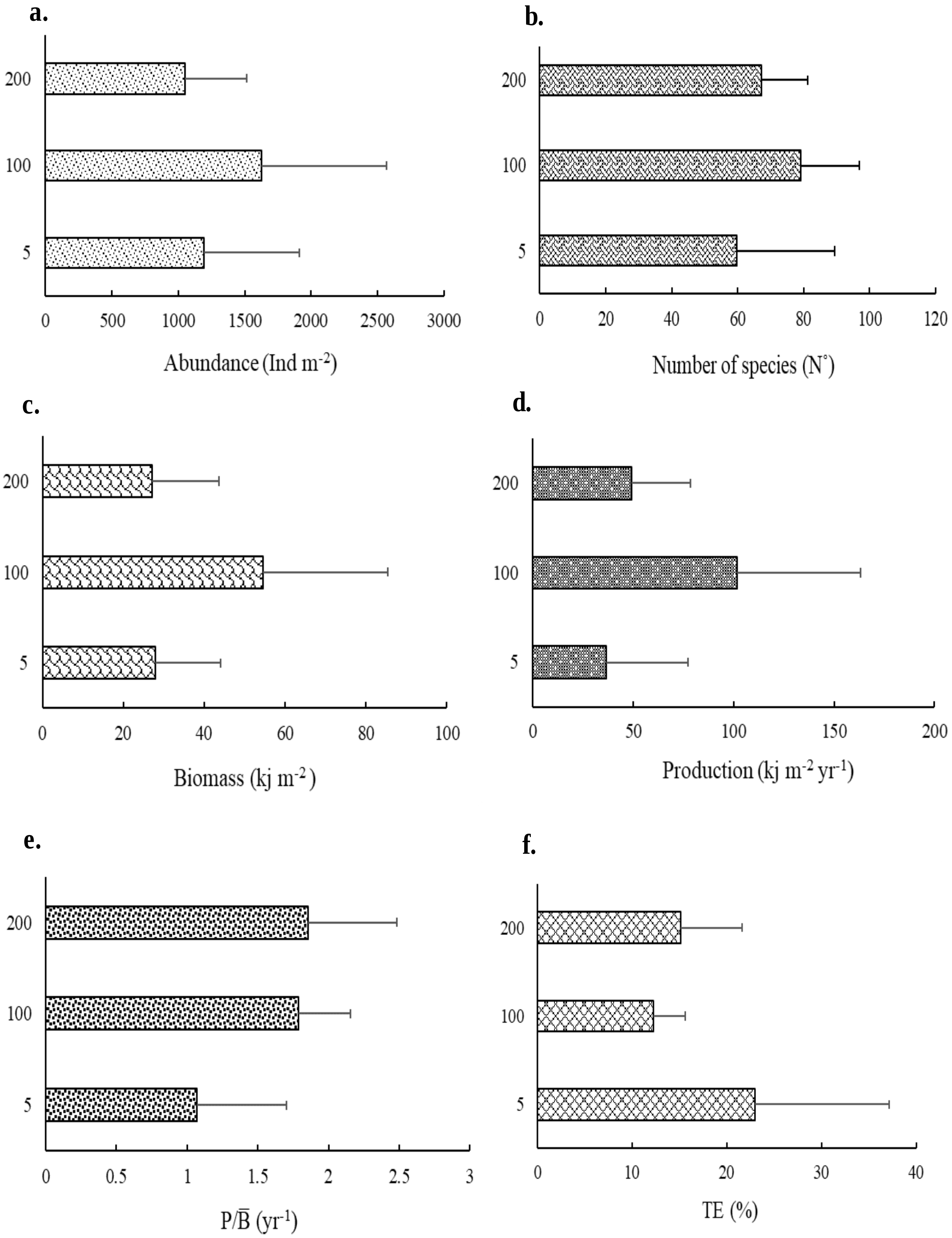

a.
200
100
5
0
500
1000
1500
2000
2500
3000
Abundance (Ind m-2)
b.
200
100
5
0
20
40
60
80
100
120
Number of species (N°)
c.
200
100
5
0
20
40
60
80
100
Biomass (kj m-2)
d.
200
100
5
0
50
100
150
200
Production (kj m-2 yr-1)
e.
200
100
5
0
0.5
1
1.5
2
2.5
3
P/B̄ (yr-1)
f.
200
100
5
0
10
20
30
40
TE (%)

Figure 4.

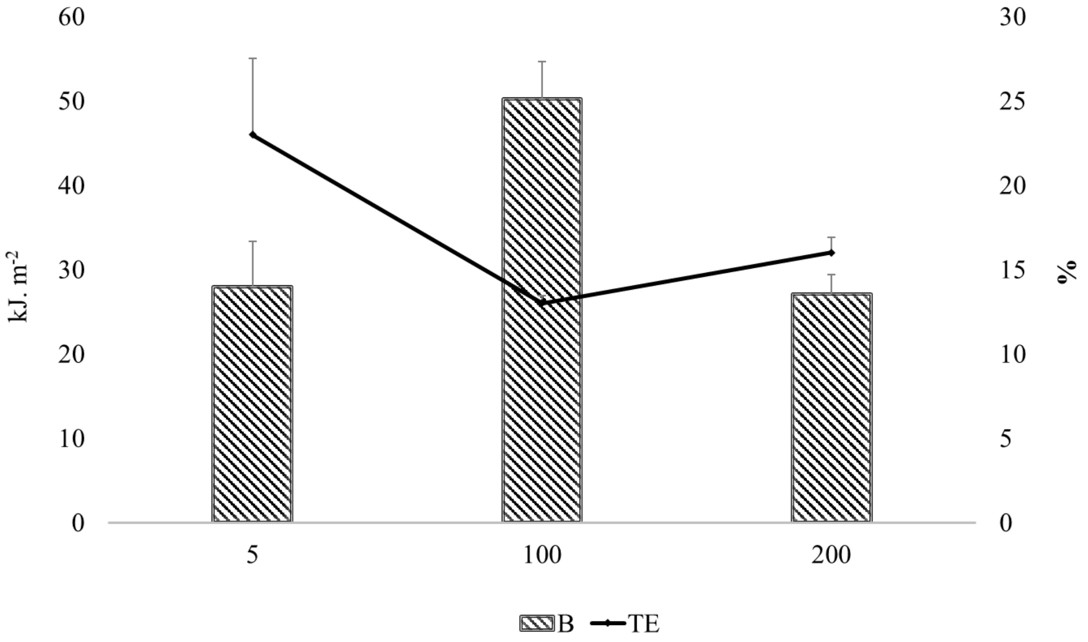


Figure 5.

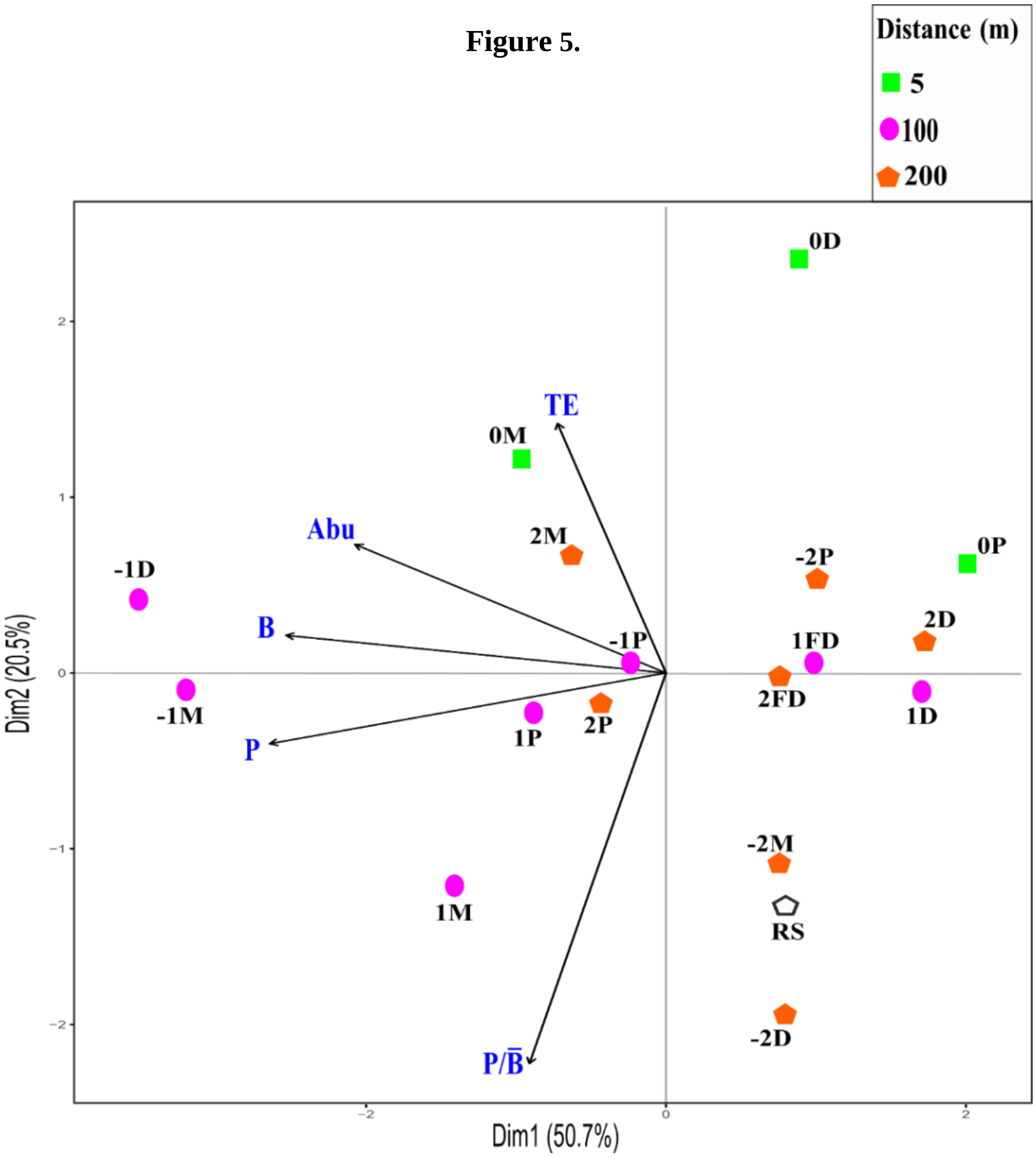
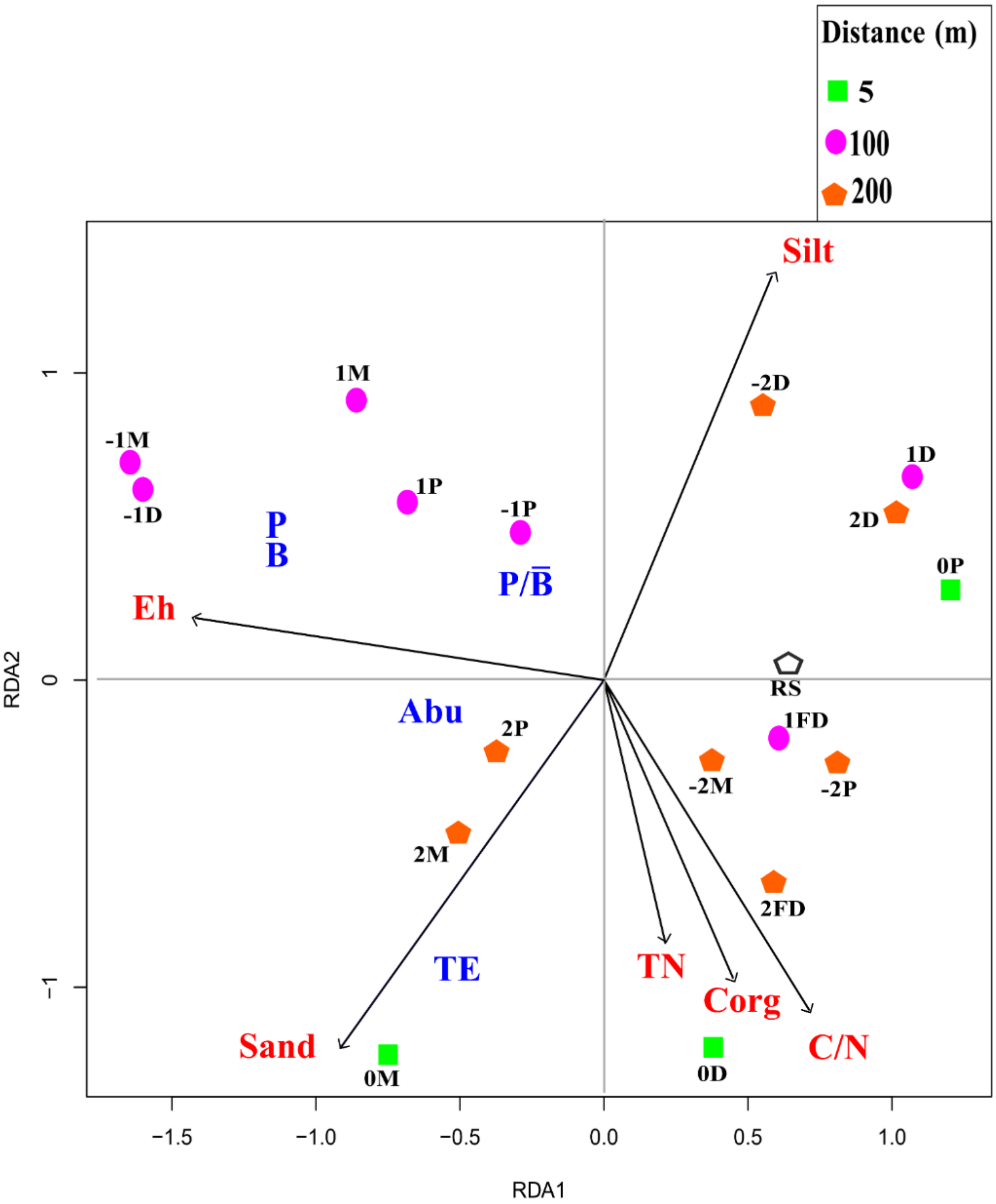

Distance (m)
5
100
200
0D
TE
0M
Abu
2M
-2P
0P
-1D
B
-1P
1FD
2D
2FD
1D
-1M
1P
2P
P
-2M
RS
1M
-2D
P/B̄
Dim2 (20.5%)
Dim1 (50.7%)
2
1
0
−1
−2
−2
0
2

Figure 6.


Distance (m)
5
100
200
Silt
Eh
Sand
TN
Corg
C/N
P
B
P/B̄
Abu
TE
1M
-1M
-1D
1P
-1P
-2D
1D
2D
0P
RS
1FD
-2M
-2P
2P
2M
2FD
0M
0D
RDA1
RDA2
−1.5
−1.0
−0.5
0.0
0.5
1.0
1
0
−1

6. Conclusion

The overall aim of this thesis was to assess the effects of sewage discharges on macrofaunal community, inhabiting the soft-sediment of the Gulf of Trieste (northern Adriatic Sea). The findings indicated that macrofaunal community responds to sewage discharges by a variation in structure and functional features as well as the secondary production, productivity, and transfer efficiency to higher trophic levels. However, the macrofaunal community displayed a high resilience due to the adaptation to long-lasting and continuous anthropogenically-induced contamination. This community minimized the impacts through the success of stress-resistant species that occupy new ecological niches, thus trying to keep the ecosystem functioning unchanged.

Overall, the results presented in this thesis highlight the importance of applying an integrated approach to study the impacts of organic enrichment derived from sewage effluents discharge on the macrobenthic communities. Considering only structural variables (species, abundance, and biomass) can cause misgiving outcomes. Therefore, it is possible to gain a more detailed and ecologically correct insight into the functioning of the benthic macrofaunal communities and their ecological functions by combining the traditional approaches with trophic features (productivity and transfer efficiency measurements), functional trait information (biological trait analysis), and information obtained from the community bioturbation attributes (BP_c and IP_c).

Lastly, the results of this thesis show that integrative approaches that consider sets of environmental features considering structural and functional macrofaunal patterns can provide important information on how an ecosystem can potentially withstand the loss of species without losing in function and ecological services, and it is useful tools for adequate monitoring and assessments of ecosystem functioning in impacted areas.

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