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Spatio-temporal patterns in coastal zoobenthos under environmental stress

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ABSTRACT

The human exploitations of coastal marine systems have led to rapid degradation of the soft-sediment benthic ecosystems, variation of food supplies for the benthic assemblages, and loss of habitats and consequent reductions in species diversity. Sediments, in particular, act as a sink for organic matter and contaminants. Since benthic populations are directly exposed to dissolved contaminants, they constitute one of the most effective tools for assessing the state of environmental health of any given sedimentary habitat. Disturbances from natural or human influences result in shaping and structuring of the ecosystems to a degree that often exceeds what nature can cope with and hence threatens ecosystem resilience and thus also the functions (goods and services) the ecosystems provide.

Among the soft-sediment benthic communities, macrozoobenthos drives important ecosystem processes in marine coastal sediments such as nutrient cycling, sediment reworking, bio-irrigation, organic matter decomposition, and secondary production (*i.e.* acts as a trophic link). Environmental disturbances threaten macrofaunal communities in terms of biodiversity, functional expressions, and trophic structure, modifying their stability and ecosystem resilience.

The main aim of this doctoral research was to investigate the structural and functional variations of coastal marine macrofaunal communities subjected to diversified types of impact, from natural phenomena to anthropogenic pressures. For this reason, to study the natural fluctuations of the ecosystem, I chose a Marine Protected Area where the anthropogenic pressure is low. To investigate how the macrozoobenthos is affected by different stressors and contaminants, I selected three diversely impacted areas: the Po River delta, the harbour of Trieste and the Mar Piccolo of Taranto.

The benthic infaunal community was analysed along a temporal scale at a relatively undisturbed station located in the buffer zone of the Miramare-MPA (Gulf of Trieste). The aim of the study was to assess the temporal variability of the community structure (biodiversity, feeding- and reproductive traits) related to food sources and environmental variations. The community displayed an

intermediate level of stability from high diversity and unvarying feeding habits, to high turnover of diversity, and large variations in reproductive frequencies. The capability of the community to maintain its structure (number of individuals, species diversity and feeding strategies), despite the physical disturbance events, illustrates the resistance to natural stress exhibited by these macrofaunal invertebrates.

The coastal area nearby the Po River delta is characterized by a high amount of organic matter loads, derived from the terrigenous river outflow. The aim of the study was to analyse the proportions of various primary producers contributing to the plume of suspended organic matter and the extent to which subsidies from rivers may influence the macrobenthic community structure and biomass. An increase in biomass close to the tributary of the principal river was registered, as well as changes in the trophic composition and variation in food sources determined from the isotopic signal along the river gradient. Further, the study highlighted a close link between grain-size and macrofaunal structure and trophic pathways in a variable and highly hydrodynamic system such as this deltaic area.

The Port of Trieste represents an area with a diffuse sediment contamination deriving from several productive activities: shipbuilding, iron and steel plant, petroleum industry, and port activities. The aim of this study was to detect variation (deviation) in the structure of the macrofaunal community, such as number of individuals, biodiversity and trophic functional groups, as a response to anthropogenic impacts. The species composition varied among stations: species that are more tolerant to stress were observed in high numbers in vicinity of contaminated sites (*e.g.* the bivalve *Corbula gibba* and the polychaete *Lumbrineris latreilli*), highlighting a clear separation among stations along the studied gradients. Overall, despite the presence of stress-tolerant species, the environmental contamination within the harbour seems not too severe to affect the macrofaunal feeding structure.

As an example of an area severely contaminated by heavy metals and synthetic organic compounds, the Mar Piccolo of Taranto was chosen. The macrofaunal community was investigated and a marked influence of contamination on the abundance and biodiversity was observed. The presence of stress-resistant species and highly reduced abundance of some sensitive organisms were registered, indicating poor conditions for the benthic ecological status in the Mar Piccolo. To assess the influence of contamination on trophic pathways, stable isotope analysis was conducted on macrofaunal communities. These results showed that chemical contamination affected the trophic structure in the absence of intermediate consumers that link basal organic matter sources to higher predators. The high biomass of predators compared to that of deposit feeders resulted in a simplification of the food web that compromises the energy transfer efficiency.

The functional features of polychaetes associated with levels of contaminated sediments were analysed and compared in the harbour of Trieste and in the Mar Piccolo of Taranto, using biological trait analysis. Functional diversity and trait richness displayed fairly constant values despite varying taxa richness, highlighting the presence of functional redundancy among polychaetes. Functional identity illustrated the relationships and adaptation to contaminated sediments. In fact, high motility of the species seems to be an essential trait for living in highly contaminated sediments. This could be linked to the ability to avoid hotspots of contaminants through active movements, thus, influencing sediment reworking and especially the diffusion of contaminants towards the water column.

These findings confirmed that soft-sediment macrofaunal communities responded differently to natural and, more subtly, to anthropogenic stress in the different environments. These results would not have been obtained without applying the novel approach used in this work, *i.e.* using stable isotopes (trophic links) and functional trait analysis. Integrative approaches that consider the whole environmental features in relation to structural and functional macrofaunal patterns provide useful tools for understanding, monitoring and assessing the ecosystem functioning in coastal environments subjected to multiple stressors.

RIASSUNTO

Nel corso degli ultimi cinquanta anni, il molteplice e sempre più esteso utilizzo delle coste ha portato in alcuni casi ad una rapida degradazione degli ecosistemi bentonici, inducendo una frammentazione degli habitat costieri, alterando l'approvvigionamento di cibo delle comunità bentoniche con una conseguente riduzione della biodiversità specifica. I sedimenti, in particolare, costituiscono un deposito della sostanza organica ed in alcuni casi anche di agenti contaminati derivanti dalle attività antropiche che determinano l'esposizione di numerosi invertebrati bentonici a tali fattori di stress. Poiché gli organismi bentonici sono sessili o a ridotta mobilità risultano direttamente e costantemente esposti ai sedimenti contaminanti. Il loro studio perciò, costituisce uno degli strumenti più efficaci per la valutazione dello stato di salute di un ecosistema marino. Disturbi di origine naturale o antropica provocano un'alterazione del funzionamento dell'ecosistema e quindi una conseguente riduzione delle funzioni (beni e servizi) che tali ecosistemi forniscono all'uomo.

Tra le comunità bentoniche costiere di fondo mobile, il macrozoobenthos è coinvolto nei principali processi ecologici come il ciclo dei nutrienti, la mobilitazione dei sedimenti, la bio-irrigazione, la decomposizione della materia organica e la produzione secondaria. Alterazioni ambientali minacciano le comunità macrofaunali in termini di biodiversità, di cambiamenti nella struttura trofica e in generale nell'espressione dei tratti funzionali modificandone la stabilità, e conseguentemente la resistenza e resilienza.

L'obiettivo principale del presente progetto di dottorato è stato quello di valutare le variazioni strutturali e funzionali delle comunità macrofaunali costiere soggette a diverse tipologie d'impatto, dai fenomeni naturali alle pressioni antropiche. Per questo motivo, per valutare le fluttuazioni naturali dell'ecosistema, ho scelto una Riserva Marina Naturale (MPA), dove la pressione antropica è relativamente bassa. Mentre, per analizzare le comunità macrofaunali influenzate da diversi fattori di stress ho selezionato tre aree soggette a diversa tipologia e grado di impatto: il delta del Po, il porto di Trieste e il Mar Piccolo di Taranto.

Nell’area antistante alla riserva di Miramare-MPA (Golfo di Trieste), la comunità macrofaunale è stata analizzata in una stazione relativamente indisturbata lungo una scala temporale di tre anni. Lo scopo dello studio è stato quello di valutare la variabilità temporale della struttura della comunità indagata (biodiversità, abitudini trofiche e caratteristiche riproduttive) in relazione alle naturali variazioni ambientali, in particolar modo rispetto alle fluttuazioni delle diverse fonti di cibo. La comunità ha riportato un livello intermedio di stabilità, ovvero: un’elevata diversità media, abitudini alimentari tendenzialmente costanti, un elevato turnover e considerevoli variazioni nelle frequenze riproduttive. La capacità della comunità di mantenere la sua struttura (numero d’individui, numerosità delle specie e strategie di alimentazione), nonostante eventi di disturbo ambientale, ha evidenziato una certa resistenza allo stress naturale esibita da questi invertebrati macrofaunali.

La zona costiera prossima al delta del Po è tipicamente soggetta ad un elevato carico organico, principalmente derivante dagli apporti terrigeni del fiume. Quest’area è stata indagata allo scopo di analizzare l’influenza che gli apporti dei diversi rami deltizi e le principali fonti di cibo (terrigene e/o marine) hanno sulla struttura delle comunità macrobentoniche, sia in termini di abbondanza e biomassa, che in termini di abitudini trofiche. Un cambiamento della struttura della comunità legato alla variazione delle fonti di cibo (determinate con segnale isotopico) è stato osservato, in particolare, dalla bocca principale verso i rami deltizi minori. Inoltre, lo studio ha evidenziato uno stretto legame tra la composizione granulometrica del sedimento e lo sviluppo delle comunità all’interno di un sistema soggetto a variazioni idrodinamiche tipiche di un’area deltizia.

Il porto di Trieste rappresenta un'area soggetta a contaminazione diffusa e multipla che deriva da molteplici attività produttive che insistono nell’area portuale, quali: cantieri navali, l'impianto siderurgico della Ferriera di Servola, il terminale petrolifero marino del Transalpine Pipeline (TAL) e numerose altre attività portuali. Lo scopo di questo studio è stato quello di determinare le variazioni nella struttura delle comunità macrofaunali in termini di abbondanza, biodiversità e abitudini trofiche in risposta alla contaminazione dei sedimenti. La composizione delle specie è variata tra le stazioni indagate: specie considerate più tolleranti allo stress sono state osservate maggiormente in prossimità dei siti contaminati (ad esempio il bivalve *Corbula gibba* e il polichete *Lumbrineris latreilli*), evidenziando una certa separazione tra le stazioni campionate. Nel complesso, nonostante la presenza di specie stress-tolleranti, la contaminazione ambientale all'interno del porto non sembra alterare la struttura trofica delle comunità che rimane sostanzialmente invariata.

Un altro studio è stato eseguito in un'area fortemente contaminata da metalli pesanti e composti organici di sintesi (policlorobifenili-PCB), ovvero il Mar Piccolo di Taranto. La contaminazione ambientale risulta aver influenzato sia il numero di individui che la biodiversità delle comunità qui indagate. La concomitante presenza di specie resistenti allo stress con la ridotta abbondanza di taxa sensibili, hanno indicato un alterato stato ecologico del sistema bentonico in alcune aree del Mar Piccolo. Per valutare l'influenza della contaminazione a livello trofico, è stata eseguita un'analisi degli isotopi stabili su tali comunità. I risultati hanno reso evidente come la contaminazione chimica abbia influenzato la struttura trofica, poiché ha indotto una riduzione lungo la catena trofica dei consumatori intermedi che tipicamente congiungono le risorse primarie di energia alle componenti trofiche superiori. L'elevata biomassa di predatori rispetto a quella dei deposivori ha comportato una semplificazione della catena alimentare che ha compromesso un efficiente trasferimento di energia lungo la catena trofica.

Le caratteristiche funzionali dei policheti associati a diversi livelli di contaminazione sono state analizzate e contestualmente confrontate sia nel porto di Trieste che nel Mar Piccolo di Taranto, utilizzando l'analisi biologica dei tratti funzionali (BTA). La ricchezza dei tratti e la diversità funzionale hanno riportato valori costanti nonostante sia stata osservata una diversa numerosità delle specie in alcune delle stazioni campionate, evidenziando perciò un certo grado di ridondanza funzionale all'interno del taxon dei policheti. Tramite l'analisi dell'identità funzionale è emerso come l'elevato tasso di mobilità di una specie, sia probabilmente un tratto funzionale essenziale per vivere in sedimenti altamente contaminati. Questo potrebbe essere legato alla capacità di evitare zone ad alta concentrazione di contaminati, influenzando perciò anche la mobilitazione dei sedimenti e la diffusione dei contaminati verso la colonna d'acqua.

Questi risultati hanno confermato come le comunità di fondo mobile rispondano in modo differente alle variazioni naturali e allo stress antropico nei differenti ambienti analizzati. Tali risultati non sarebbero stati ottenuti senza l'utilizzo di nuovi approcci adottati in questo lavoro, ovvero l'analisi degli isotopi stabili (connessioni trofiche) e l'analisi dei tratti funzionali. Approcci alternativi ed integrati che considerano le caratteristiche ambientali accoppiate allo studio delle caratteristiche strutturali e funzionali della macrofauna, forniscono strumenti utili per comprendere, monitorare e valutare il funzionamento degli ecosistemi costieri soggetti a molteplici fonti di disturbo e stress.

CONTENTS

1. INTRODUCTION

Coastal environments provide high habitat heterogeneity and biodiversity. These areas house ecosystems which are extremely important since they play a key role in the carbon and nutrient cycling and biodiversity. Coastal ecosystems are one of the most productive zones on earth and are of global importance for nutrient budgets and primary productivity. High nutrient levels, multiple sources of primary and secondary production, shallow depths, organically rich sediments and tidal currents, and freshwater inflows, combine to establish the high natural productivity of near-shore areas (Livingston 2002). Despite covering less than 10 % of the surface of the Earth, coastal seas still harbour the highest marine biodiversity (MEA 2005). Humans also appreciate the coastal areas as they provide a variety of goods and services such as coastal protection against erosion, production of food and recreational activities (Worm et al. 2006; Barbier et al. 2011).

Today, approximately half of the human population lives within 200 kilometres from a coastline, and this trend is likely to double by 2025 (Creel 2003). Consequently, the coastal areas experience the largest threats to marine biodiversity (Lotze et al. 2006). The human exploitations of the coastal systems have led to rapid degradation, variation of food supplies and loss of habitats and consequent reductions in species diversity (MEA 2005). These large-scale and long-term effects have implications for the stability and resilience (*sensu* Holling 1973) of coastal ecosystem (Lotze et al. 2006). Therefore, it is of the outmost importance to safeguard the richness and diversity of organisms, as they are the ones who perform many functions sustaining the coastal system and in turn enable human well-being (Cardinale et al. 2012). Simultaneously, there is an urgent need to understand how these systems function and improve assessments and analysis tools that allow effective interpretation of these changes.

The habitats associated with the sediments are important ecological components of the coastal zone. The wide variety of biota associated with the sediment is commonly called "benthos" and prokaryotes, animals and plants are known to be involved in numerous processes affecting the entire coastal ecosystem. The benthic organism inhabiting the sediments can be divided according to their size into: macrobenthos (>1000 µm), meiobenthos (38–1000 µm), microbenthos (20–200 µm), nanobenthos (2-20 µm) and picobenthos (0.2–2 µm). Macrofaunal organisms due to their high biomass and species diversity (Snelgrove 1998), could be considered a key biological component, which drive important processes in marine sediments, such as nutrient cycling, sediment reworking, bio-irrigation, organic matter decomposition and secondary production (Widdicombe et al. 2004; Bremner et al. 2006; Olsgard et al. 2008).

In my doctoral study, in the framework of the benthic ecosystem functioning, I implemented the tools and research approaches for the evaluation of macrofaunal communities and their response to diversified types of impact, disturb and stress events, from natural phenomena to anthropogenic pressures. Further, I considered their interaction with the other benthic and planktonic communities. In the following sections, I link these notions to topics such as biodiversity, ecosystem functioning, and food web ecology in marine ecosystems.

1.1. Biodiversity and ecosystem functioning

Coastal ecosystems are hotspots of environmental variability, biogeochemical transformations, and biological interactions, where dynamic exchanges of energy mass, and nutrients occur between benthic and pelagic habitats *via* diverse pathways. Consequently, they are among the world's most productive ecosystems that provide important ecosystem services, such as food provision and water filtration (Griffith et al. 2017). 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 (Reiss et al. 2009). In light of the global trend of decline in biodiversity (Sala & Knowlton 2006), ecologists, prompted a rapidly expanding field of research that strives to shed light on the relationship between biodiversity and ecosystem function (BEF). Biological diversity (*i.e.* biodiversity) was defined in 1992 by the Convention of Biological Diversity as "the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems". Thus the importance of biological diversity for ecosystem function is widely recognised today, both in terrestrial (*e.g*. Cardinale et al. 2002; Hooper et al. 2005; Balvanera et al. 2006) and marine systems (*e.g*. Solan et al. 2004; Ieno et al. 2006). BEF approach states that functioning of an ecosystem in not governed by the phylogeny of the biota, but by the characteristics of the organisms (*i.e.* traits) present in a system and their distribution (Naeem 2002). Species interact with and respond to their physical and chemical environment in various ways depending on their ability to do so. The maintenance and regulation of functions in an ecosystem is thus essentially dependent on the ecological roles and traits of the taxa present. BEF approach supposes that an increasing diversity translates into an increasing number of expressed biological traits, with greater effects on ecosystem functioning, compared to less diverse assemblages that have a poorer functional expression (Chapin et al. 2000; Hooper et al. 2005).

Ecosystem functions are the ecological processes that control changes in energy and matter over time and space, directed by biotic activities as well as by abiotic factors (*i.e.* physical and chemical), but can also be represented by measures of ecosystem resilience and stability (Srivastava & Vellend 2005). During the last decades, the relationship between biodiversity and ecosystem functioning has been explored experimentally, in the laboratory and using mesocosm and field experiments, often by manipulating the number of species and by creating random species assemblages. These studies have provided important insights into the nature of the BEF relationship and its underlying processes. Indeed, recent meta-analyses have concluded that biodiversity significantly contributes to a range of ecosystem functions such as biomass production, decomposition, biogeochemical cycling, and ecosystem stability (Hooper et al. 2005; Schmid et al. 2009; Cardinale et al. 2012).

1.2. Disturbance to ecosystems

Disturbance has been defined as "any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability or the physical environment" (White & Pickett 1985). Disturbances from natural or human influences are important in shaping and structuring ecosystems, often exceeding what nature can cope with, which threatens ecosystem resilience and thus the functions ecosystems provide (Thrush et al. 2009).

The (ecological) resilience of an ecosystem can be depicted as its domain of stability, which describes ecosystem behaviour as determined by its structure, functions and relationships. Ecological resilience can be defined by the amount of disturbance that an ecosystem can absorb before changing into an alternative state (Holling 1973). Transition into an alternative stable state is depicted by changes in mechanisms that maintain resilience, such as the presence of key species, the diversity within functional groups, the recovery potential of the biota after disturbance ceased and the functional redundancy (Thrush et al. 2009; Rosenfeld 2002). The concept of functional redundancy (the degree to which organisms have evolved to do similar things and so have an overlapping niche) is closely related to the maintenance of the principal ecosystem processes of community alternative state (Rosenfeld 2002). In fact, theory suggests that if there is evidence of functional redundancy, the system may be less susceptible to changes in ecosystem function caused by a species loss. Indeed the redundancy hypothesis states that several species perform functions and thus express similar trait values in case one being eliminated other remain to continue or potentially even expand the function (Yachi & Loreau 1999).

Despite the considerable amount of research that has focused on disturbance ecology, the generalization of disturbance effects is still challenging, as disturbances are highly variable in nature. Sources of variation include the magnitude of disturbance and its spatial (*e.g.* area, shape, distribution) and temporal characteristics (*e.g.* duration, frequency, seasonality: Coma et al. 2000; Reiss & Kröncke 2005). Different disturbance effects are also caused by variations in the physical and chemical environment, and by differences in species composition, sensitivities and adaptations among ecosystems (White & Jentsch 2001).

Great variations in chemical characteristics are due to the contamination by human activities, that severely affects the ecosystem structure and function (Kennish et al. 2014). The sediments act as a sink for contaminants and frequently contain higher concentrations of contaminants compared to the water column (Trannum et al. 2004). Benthic organisms living in or on the sediment surface may be exposed not only to contaminants in the overlying water or in 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). Due to their scarce ability to avoid different stressors, they are forced to react synergistically without any escape possibility (Paul et al. 2001). To some extent, the benthic ecosystem is able to adapt to anthropogenic pressure by trying to minimise its impact through the development of stress-resistant communities that occupy new ecological niches. Stress-resistant species can therefore replace the more sensitive ones within a community that consequently maintains its original functioning (Johnston et al. 2015). Overall the contamination affects the community diversity as it reduces the number of species but it does not affect ecosystem functioning, if it reduces those species that contribute little to function or that are functionally redundant in these communities (McMahon et al. 2012).

Another threat to coastal ecosystems is pollution due to organic enrichment. Excess organic matter causes physical effects such as smothering and also leads to reduced oxygen concentrations in the water column or pore-water in sediments. This leads to decreases in biodiversity and biomass values on the account of opportunistic species (*r*-strategist). These short-lived opportunistic species are the first ones respond disturbance and dominate the initial stages of succession. Their ability to respond quickly to disturbance and attain high densities with low biomass has been primarily attributed to their life-history features (*e.g.* wide dispersal ability, high reproductive rates), that fit them to take immediate advantage of a sudden environmental change. The diversity increases at an intermediate level of disturbance due to the gradual succession of species with high biomass and low abundance (*K*-strategist). However, the diversity decreases further along a gradient where the organic stress is low and the *K*-strategist species dominate (Odum 1969; Pianka 1970; Pearson & Rosenberg 1978).

Moreover, the environmental stress could have a natural origin. Estuaries have long been regarded as environmentally naturally stressed areas because of the high degree of variability in their physico-chemical characteristics, for example oxygen, temperature and salinity in the water column and bed sediment dynamics. However, their biota is well-adapted to cope with that stress and so the areas may be regarded as resilient because of that inherent variability; their ability to absorb stress without adverse effects is called "Environmental homeostasis" (Elliott & Quintino 2007).

1.3. Trait-based approach

Trait-based approaches are widely used in ecological and evolutionary research. Historically, the term "trait" has naturally moved from the common language to a more scientific one in different 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 of organism's performance (Violle et al. 2007). Over the last three decades, the trait concept has evolved firstly in terrestrial realm, from studies discussing its wider application for vegetation classifications (Grime 1974) and habitat heterogeneity (Southwood 1977) toward the introduction of the expression "functional traits" (Diaz & Cabido 2001; Petchey & Gaston 2002). A functional trait has been defined as component of an organism's phenotype that determines its effect on processes and its response to environmental factors (Reiss et al. 2009).

Trait approaches in marine benthic systems focused primarily on trophic functional groups. Generally the community was divided into groups of taxa (functional group) that share similar functional attributes or exploit common resource bases (guilds) using the following criteria: 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 & Rosenberg 1978; Bonsdorff & Pearson 1999; Jumars et al. 2015). This approach was implemented with the Biological Traits Analysis (BTA), introduced by Bermner et al. (2003; 2006). BTA is based on the habitat template theory, which states that species ʻcharacteristics evolve in response to habitat constraints (Southwood 1997). The community structure is governed by habitat variability and the biological traits exhibited by organisms will provide information about how they behave and respond 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.* movement method, environmental position, reproductive typology) thus it provides a link between species, environment and ecosystem processes (Bermner et al. 2006).

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 (van der Linden 2012; Zhang et al. 2015) and CO_2 vent-systems (Gambi et al. 2016). Functioning has also been assessed in relation to human-induced impact of climate changes (Neumann & Krönke 2011; Weigel et al. 2015), 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). This approach has also been developed in relation to the implementation of bioturbation (Solan et al. 2004) and functional indices (Laliberté et al. 2014).

In the terrestrial realm, measuring trait (*e.g.* plant height, leaf area and seed mass) is relatively easy to conduct for each individual 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). In fact, 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 macrofaunal community. This point highlights once again that macrofaunal organisms are useful in understanding the ecosystem functioning of coastal environments (Törnroos 2014).

1.4. Food web ecology and stable isotope variability

The ecological niche can be defended as "the *n*-dimensional hyper volume consisting of environmental condition, resource level and densities of other organisms, together allowing for the survival, growth and reproduction of a species" (Hutchinson 1957). To define species niches the effort is focused on either biotic or abiotic subunits of the dimension, such as the trophic characteristics of a species (Cohen 1977) or characteristics of an inhabited area (Heinänen & von Numbers 2009). The trophic niche of a species refers to the dimension regarding resource use and may include foraging strategies, trophic level, pray preferences, degree of omnivory or pray handling capability (Nordström 2009). Thus, the trophic connections, in characterising the species niches, were analysed and discovered in the food web ecology. The food web can be defined as a "network of consumer-resource interactions among a group of organisms, populations, or aggregate trophic units" and describes the exchange of organic matter among organisms of an ecosystem, and energy flow from basal sources to top predators (Winemiller & Polis 1996; Krumins et al. 2013).

Three basic approaches can be identified in the investigation of community trophic networks (Winemiller & Polis 1996). 1) A topological food web is a static description of feeding links, either observed or estimated, with binary link among species or guilds. 2) A bioenergetics web (flow web) quantifies the transport of energy and matter, via predation, among species or guilds. 3) A functional web (interaction web) identifies the species and feeding links that are most influential in the dynamics of community composition and structure. Consequently a food web can contain both direct interactions (feeding links) and indirect interactions (trophic cascades, competition). The food web attempts to holistically summarize ecological connections and the topological approach is the first step toward elucidating the web complexity, providing information on their general structure (*e.g.* web size, linkage density and connectance) (Raffaelli 2000).

Traditionally, the construction of qualitative and quantitative food web has relied on methods such as gut content analysis, direct observations and experimental trails. Two decades ago the use of stable isotopes in ecological research has been discovered as a useful method in food web ecology (Fry 2007).

Stable isotope analysis has been proven to be a powerful tool for the study of the trophic relationships in diverse coastal ecosystems (Michener & Schell 1994). Ratios of carbon (^{13}C:^{12}C, $\delta^{13}C$) and nitrogen isotopes (^{15}N:^{14}N, $\delta^{15}N$) are the ones most frequently used in food web studies. Since stable isotope ratios in an organism's tissues derive from all trophic pathways culminating in that individual, they can be used to depict the trophic niche. Hence, the stable carbon and nitrogen isotopic signature of an animal depends on its feeding sources stable isotopic compositions and isotopic fractionation during food assimilation.

$\delta^{15}N$ and $\delta^{13}C$ are typically enriched from prey to consumers by mean coefficients of 3.4% and 1% for $\delta^{15}N$ and $\delta^{13}C$, respectively (Fry 2006). Thus, the $\delta^{15}N$ shift between a consumer and its food allows $\delta^{15}N$ to be a reliable indicator of the trophic level of an organism within the classic food web, based on primary producers. However, recent reviews indicate that fractionation of $\delta^{15}N$ depends on multiple factors, such as N-content of the food (detrital matter or other animals), environmental conditions and even taxonomy. Animals retain ^{15}N preferentially over ^{14}N, which is excreted as urinary nitrogen (Zanden & Rasmussen 2001; McCutchan et al. 2003). The slight enrichment of ^{13}C over the trophic levels has been suggested to result from processes involving (1) loss of ^{12}C through respiration, (2) preferential uptake of ^{13}C from diet, and/or (3) fractionating occurring during metabolic synthesis of different tissues. $\delta^{15}N$ functions as a proxy for the trophic level as it increases in a step-wise manner along the food chain, whereas $\delta^{13}C$ provides information on the primary energy source(s) used by consumers. Overall, this method is useful to study the trophic web over time and space. When the stable isotope analysis is combined with the faunal biomass measurements this deepens the knowledge on the energy pathways based on the dynamics of species' feeding relationship (Quillien et al. 2016).

2. STUDY SITES AND THEIR MAIN ENVIRONMENTAL FEATURES AND ISSUES

2.1. Miramare- marine protected area

Miramare is a small Marine Protected Area (MPA) declared an MPA already in 1979, the Reserve is part of the Natura 2000-network, and represents the only completely protected area in the Italian part of the Gulf of Trieste (northern Adriatic Sea). Miramare-MPA is divided in two distinct zones: the inner part (30 ha), subjected to a regime of integral protection, is surrounded by a larger buffer zone (90 ha). The Reserve reaches a maximum depth of 18 meters and presents several types of sea-beds: from the rocky habitats, near the coast, to the sandy and muddy ones, off the coast. Overall, this MPA is characterised by high levels of biodiversity and is populated by a variety of flora and fauna species of high conservation value (Vallarola 2013).

The study site (St. C1), located nearby the Marine Reserve of Miramare, is a relatively undisturbed area because sheltered by boats, fishing and swimmers, representing the only completely protected area in the Italian part of the Gulf of Trieste (Fig. 1a). The earliest samplings carried out in this site are dated back to the 70s and included several physical, chemical and biological parameters of the water column. Since 2002 samplings have been extended to the sediments, too (www.nettuno.ogs.trieste.it/lter/). Having a several-year dataset at this site, St. C1 has been often chosen as a reference site of the Gulf of Trieste in studies on different types of impact induced by anthropogenic activities. Recently, this site has been included in the LTER-Italia (Long Term Ecological Research) network, as part of the macrosite “LTER-Adriatico Settentrionale”.

Most of the research focused on the macrozoobenthic communities in the Gulf of Trieste was carried out in relation to hypoxia and anoxia events that occurred in the 1980s and 1990s (*e.g.* Aleffi et al. 1992); anthropogenic pressures (industrial activities and sewage outfalls) (*e.g.* Solis-Weiss et al. 2004; Cibic et al.

2008); biodeposition from mussels farms (Aleffi et al. 2006), and riverine inputs (Faresi et al. 2012). The study included in this doctoral thesis represents the first attempt to investigate the role of the food supply and environmental factors in structuring the macrofaunal community during a three-year period.

2.2. Po River-deltaic environment

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 (Boldrin et al. 2005). The annual mean discharge of the Po River is 1511 m^3 s^{-1} and means sediment loads are in the range of 13–15 million tons y^{-1}. The river is characterized by two annual floods associated with rainfall in autumn and snowmelt in spring (Boldrin et al. 2005). The Po River supplies over 50% of the fresh water to the northern Adriatic basin via a large delta. This delta is composed of five main distributary mouths: Maestra, Pila, Tolle, Gnocca and Goro. Po di Pila' is the main distributary channel, it discharges 74% of the sediment loads, followed by Gnocca, Goro and Tolle with 11%, 8%, and 7%, respectively (Tesi et al. 2008) (Fig. 1b).

During normal flow conditions, fine-grained sediments in the Po River undergo rapid deposition close to the mouth. Subsequently, the sediments are principally transported southward along the shelf due to the predominant cyclonic current (WACC Western Adriatic Coastal Current) and Bora (north-easterly) winds. On the contrary, infrequently, Scirocco (south-easterly) wind events lead the river flow northward (Fain et al. 2007). Changes in macrofaunal communities (in terms of abundance, diversity and biomass) in response to riverine inputs have been deeply investigated in coastal areas nearest to the Po River delta (*e.g.* Ambrogi 1990; Crema et al. 1991; Occhipinti-Ambrogi et al. 2005). However, to the best of my knowledge only one previous research (Simonini et al. 2004) has examined the trophic structure of macrofaunal communities and the role of the river plume as a source for coastal benthic consumers.

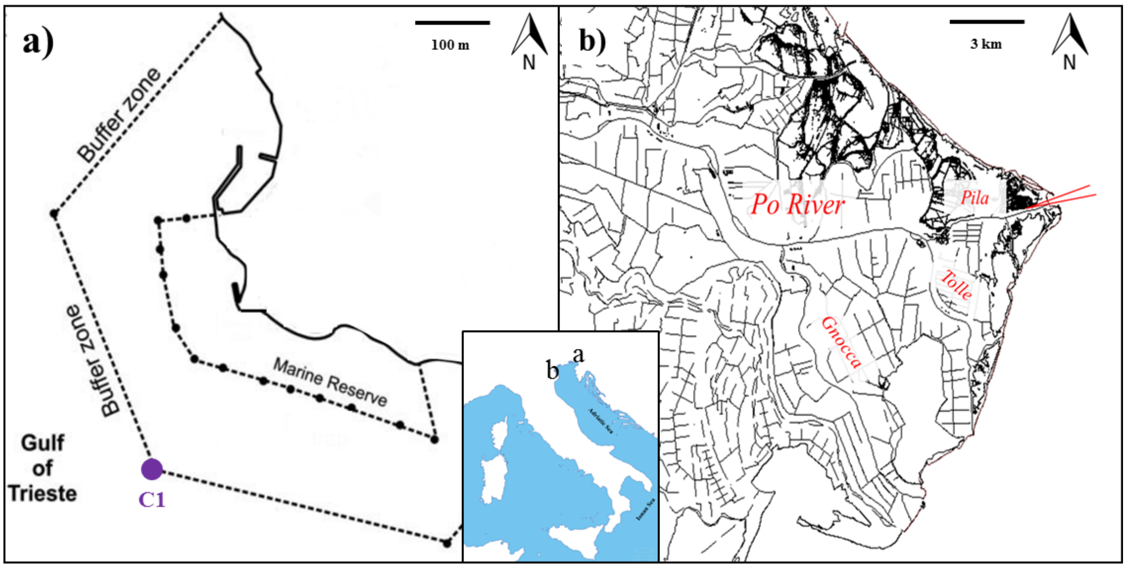

Fig 1. Investigated area in the Gulf of Trieste (a) and the coastal area nearby the Po River delta (b). The main river mouth is depicted with the red lines.

2.3. Harbour of Trieste - multiple and diffuse contamination

The harbour of Trieste is located in the Bay of Muggia (Gulf of Trieste-northern Adriatic Sea). It is a shallow embayment (8-20 m) about 7 km long and 4 km wide and oriented NW- SE (Ghirardelli & Pignatti 1968). Sedimentation is controlled by low hydrodynamics and fluvial inputs: two streams enter the Bay, Rosandra and Ospo that may discharge large amounts of fine sediments containing chemical fertilizers (www.porto.trieste.it). The morphology of the Bay makes it prone to the accumulation of contaminants from human activities carried out in the harbour (Fig. 2a). The development of the port dates back to the early 1900s with the construction of three external dams (1904-1909), the creation of large industrial structures in the area Gaslini (Drozina 1999) as well as the industrial complex for the manufacture of iron and steel. In the following decades other industrial structures were built, such as the industrial canal (completed in the 50s), the navigation channel (1966) as well as the construction of the pipeline terminal Trieste-Monaco of Bavaria (SIOT) (1967) (Russiani 1992), the most important pipeline that serves central Europe (about $36\text{x}10^6$ tons of crude oil discharged in 2001) and finally the expansion of the commercial docks. For these reasons, this area was declared a Site of National Interest (SIN) by Decree of the Italian Ministry of the Environment in 2003. The macrofaunal community structure in relation to contamination has been studied in this area (*e.g.* Solis-Weiss et al. 2004) but no previous research has focused on the functional expression and trophic interactions of macrofaunal invertebrates with other benthic communities (*i.e.* microphyto- and meiobenthos). Further, the study included in this thesis is the first one presenting data on macrofaunal functional diversity, functional expressions and adaptation to diffuse contamination.

2.4 Mar Piccolo of Taranto - severe contamination

The Mar Piccolo of Taranto is an inner and semi-enclosed shallow sea located in the northern area of the Taranto city (Ionian Sea). It has a surface area of 20.72 km^2 and is divided by a promontory in two smaller basins named First and Second Inlet. The Mar Piccolo is connected with the open sea (Mar Grande) through two channels: the 'Navigabile' and the 'Porta Napoli' and it is characterized by a confinement gradient from the First to the Second Inlet. The fluxes of water through these inlets are generally weak and depend on the difference of density between the two basins. Most of the water inputs derive from numerous small surface watercourses and 34 submarine freshwater springs, called 'Citri', which influence salinity and temperature of both inlets (Cardellicchio et al. 2016). The Mar Piccolo is an ecosystem that is strongly exploited for intensive mussel commercial fishery and is also affected by industrial, agriculture and sewage inputs (Caroppo et al. 2012). As more than 80% of the land in the Taranto province is used for farming, in particular for the cultivation of wheat, cereal crops and fodder, freshwater inputs contain chemicals drained from the surrounding agricultural soils in the basin (Di Leo et al. 2010). Further, the many industrial activities are considered a threat for this environment. Taranto hosts the largest steelwork plant in Europe (ILVA) and the largest navy arsenal in Italy, a major oil refinery, shipbuilding and other industrial activities that are responsible for severe environmental contamination, mainly due to heavy metals, asbestos, polycyclic aromatic hydrocarbons (PAHs), organic solvents, polychlorinated biphenyls (PCBs) and dioxins (Fig. 2b). These contaminants have heavily affected the coastal marine environment, especially the sediment quality: the sediments of the Mar Piccolo contain contaminants at concentrations that often exceed those of the overlying water column by several orders of magnitude (Cardellicchio et al. 2016). For these reasons, the Taranto area is classified as Site of National Interest, established by National Law 426 (1998), and is included in the list of "National Environmental Remediation and Restoration Projects". Previous studies in this area have been mainly focus on the chemical characterization of sediments, with an emphasis on contaminants (*e.g.* Cardellicchio et al. 2007). Others studies were based on the macroscopic fraction of pelagic and benthic communities (*i.e.* Prato & Biandolino 2005). The study presented in this paper collection implements the information on the macrozoobenthic structural changes in relation with contamination along a confinement gradient. This is also the first study in which a particular attention has been given to the macrofaunal trophic structure, its functional diversity and functional expression, and adaptation to severe contamination.

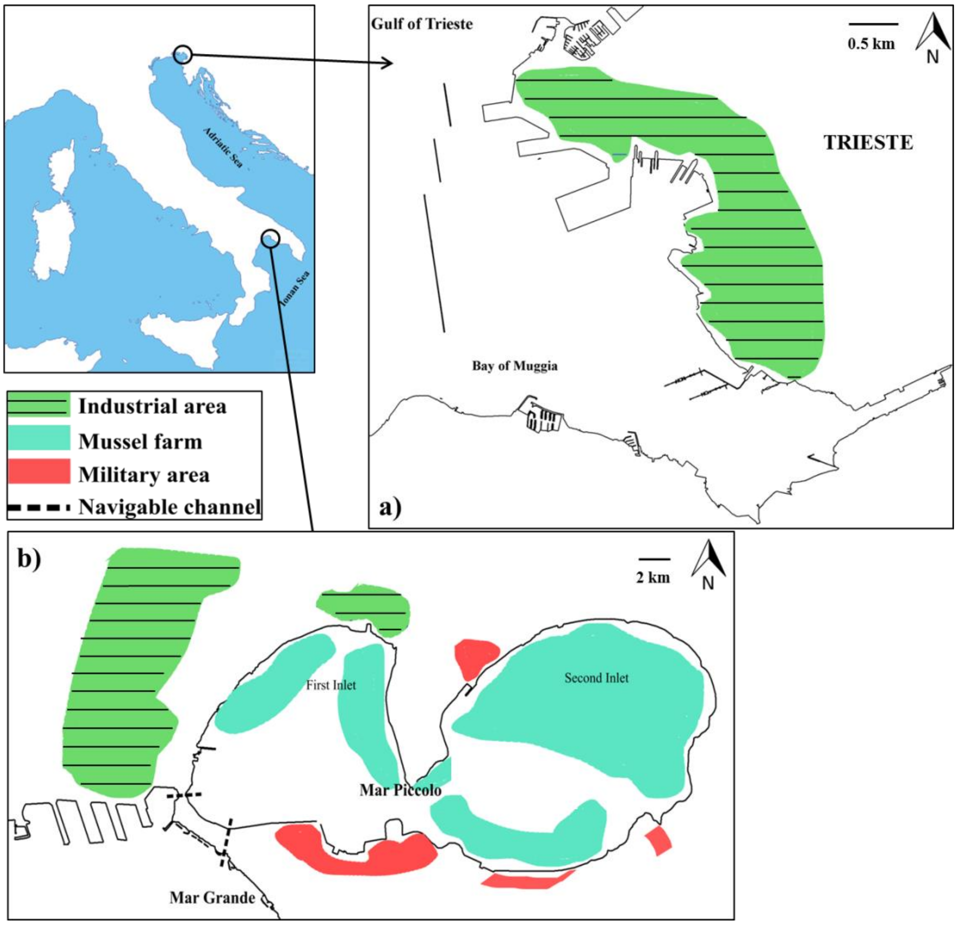

Fig. 2. Locations of the two impacted areas: the Mar Piccolo of Taranto (a) and the harbour of Trieste (b). The main anthropogenic pressures that insist along its coast are highlighted: the industrial area (green- lined), the military area (red), and the mussel farms (blue). The navigable channels are indicated by dotted lines.

3. AIMS OF THE THESIS

The main objective of this thesis was to investigate the response of soft-bottom macrofaunal communities inhabiting coastal areas characterized by different degrees of natural or human pressures. To achieve that, I have considered any structural and functional changes of coastal marine macrozoobenthos in terms of (1) biodiversity and species composition, (2) feeding guilds and trophic structures (3) functional diversity and trait expressions. Therefore, I have implemented the feeding-groups approach, stable isotope analysis and biological traits analysis to estimate the effects of environmental disturbances on these communities.

My doctoral thesis is a collection of scientific papers; each of them focuses on the macrofaunal structural and functional response but in different coastal environmental contexts, from relatively undisturbed areas to heavy polluted sites due to high human pressures.

In the six sections of this thesis, I assessed:

1. Temporal variability of the macrofaunal community structure (biodiversity, feeding habits and reproductive frequencies) at a relatively undisturbed site (C1 in the buffer zone of Miramare-MPA). More precisely, the scientific questions addressed in this paper were: i) How stable is the macrofaunal community over time? ii) Do the environmental variations observed in the three-year period induce resistance or resilience of this community? **Paper I**

2. Macrofaunal invertebrate incorporation of organic matter, derived from the terrigenous river outflow in the prodelta of the Po River. More specifically, I sought to answer the following questions: i) Are there differences in terms of macrofaunal community structure, isotopic composition and feeding habits in the adjacent coastal area directly affected by river floods? ii) Which is the contribution of freshwater and marine food sources to benthic primary consumer diets? **Paper II**

3. Spatial variability of macrofaunal community structure and feeding features in the harbour of Trieste subjected to multiple and diffuse contamination. In this study case, the following questions were addressed: i) How does the macrofaunal community respond to different types of pollution and environmental factors? ii) How is the feeding habits distribution in this basin? **Paper III**

4. Spatial variability of macrofaunal community structure and feeding habits in the severely contaminated Mar Piccolo of Taranto. Here, I focused on how the macrofaunal community responds to severe contamination by analysing its abundance, biodiversity, taxa composition and trophic habits. **Paper IV**

5. Spatial variability of the benthic food web in the impacted area of the Mar Piccolo of Taranto. The scientific questions I addressed were: i) Is there a spatial variability in the isotopic composition of organic sources and consumers? ii) Is it possible to link the isotopic composition to anthropogenic impacts and identify their effect on the food web? **Paper V**

6. Spatial variability of functional traits of polychaetes inhabiting contaminated sediments of two differently impacted areas: the Mar Piccolo of Taranto and the harbour of Trieste. Precisely, to what extent do the contaminated sediments affect the functional features of the macrofauna invertebrates? Which is the role of the environmental factors on functional trait patterns? **Paper VI**

4. ORIGINAL PUBBLICATIONS

Paper I

BIODIVERSITY, FEEDING HABITS AND REPRODUCTIVE STRATEGIES OF BENTHIC MACROFAUNA IN A PROTECTED AREA OF THE NORTHERN ADRIATIC SEA: A THREE-YEAR STUDY

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Data analyses: **FN**, RA

Manuscript preparation: **FN**, RA, EB, FIA, NB, TC, PDN

Project leader: PDN

Abstract

The macrozoobenthic community at a sublittoral station located in the Marine Protected Area of Miramare, Gulf of Trieste (Italy) was investigated monthly from June 2002 to July 2005. Community variables were studied and related to food sources (particulate, total and biopolymeric carbon contents, benthic microalgae and meiofauna). In addition, Univariate and multivariate analyses highlighted that the macrozoobenthic community structure shifted towards the end of the study. Diversity remained fairly stable throughout the study, despite the high turnover values. From a dominance of short-lived invertebrates related to irregular fresh organic matter inputs, the community shifted toward long-lived taxa, principally related to an increase of biopolymeric C and microalgal biomass. Semelparous invertebrates seem to be less resistant to high temperatures compared to iteroparous ones. The latter proved to be capable competitors since they prevailed over the semelparous species towards the end of the study.

The community exhibited a certain degree of resistance to high temperature, due to the adaptation of the macrofaunal invertebrates to this particular stress, induced by the wide natural fluctuations in temperature that occur on a seasonal basis in the Gulf of Trieste. This study highlights the importance of long datasets to assess the state and ecological processes of the macrofaunal communities.

Keywords: Soft bottom macrozoobenthos, Community structure, Feeding habits, Reproductive strategies, Environmental factors, Northern Adriatic Sea.

Introduction

Coastal benthic macrofaunal communities are subjected to fluctuations in response to natural environmental drivers and anthropogenic pressures which vary in frequency and intensity, both on a temporal and spatial scale (Boero, 1994; Turner et al., 1995). As a consequence, the structure (i.e. abundance, biodiversity, species composition) of these communities is constantly changing, hence reducing their stability. Stability per se is not a simple characteristic of a community, but rather a multiplicity of distinct attributes (Putman & Wratten, 1984): persistence, variability, resilience and resistance. Persistence can be defined as the constancy in some parameter (e.g. number of species, taxonomic composition, size of a population) of the system over time; variability is the degree to which a parameter fluctuates over time; resilience refers to the ability of a system to recover and continue functioning after disturbance; and resistance describes the ability of the system to withstand or resist such perturbation in the first place (Turner et al., 1995 and references therein).

The changes in community structure are primarily influenced by seasonality (Marchini et al., 2004; Quijón et al., 2008) altering the seawater temperature, primary production and food availability. Besides the predictable cycle of seasonality, shallow coastal water sedimentary macrobenthos shows year-to-year variability (Warwick et al., 2002). Unpredictability of weather conditions (e.g. cold winters and heavy storms), variations in current direction or velocity, flood and drought events, and unusually high temperature are physical-chemical processes that can cause shifts in the community stability (Hermand et al., 2008; Munari, 2011; Somerfield et al., 2014). In addition to these physical variations, biological variability such as year-to-year differences in seasonal reproduction and recruitment, predation, species dominance, competition for food and space or inhibition of recruitment (Giangrande et al,. 1994; Rossi, 2003; Reiss & Kröncke, 2005; Quijón & Snelgrove, 2005) can be responsible for a high variability within the faunal assemblage. The detection of changes in specific drivers is of primary importance because they often alter the conditions of the system (especially food sources) in an apparently unpredictable way.

Food supply is a key factor structuring marine zoobenthic communities in terms of functional aspects of biodiversity, namely feeding habits and reproductive strategies. The organic material that benthos receives can have different effects depending on the concentration and timing of delivery (Widdicombe & Austen, 2001). The food supply to benthic organisms relies on the sedimentation of organic matter that is produced in the euphotic zone by primary producers. Particulate food is supplied to the seafloor by either horizontal transport of phytodetritus from terrestrial or vertical transfer of marine planktonic production (Desrosiers et al., 2000). In the Mediterranean, the most important energy input to the benthos occurs shortly after the spring and autumn blooms, which take only a few days to create a tight coupling between the pelagic and benthic system (Coma et al., 2000; Cibic et al., 2007b). The phytoplankton pulses of organic matter thus constitute labile directly available and highly nutritive food sources for the macrofaunal communities (Quijón et al., 2008 and reference therein). Also the benthic microalgal organisms are a preferred high-quality food sources for many soft-bottom benthic invertebrates especially for deposit feeders, due to a well-balanced content of essential fatty acids and amino acids (Sköld & Gunnarsson, 1996; Hardison et al., 2013). Both benthic microalgae and meiofauna represent a basic food source for the macrofaunal invertebrates (Van Colen et al., 2015).

The importance of changes in shallow macrobenthic communities in relation to environmental factors and food source supplies has been extensively studied over the past twenty years, both in field studies on spatial scales (Turner et al., 1995; Simonini et al., 2004; Occhipinti-Ambrogi et al., 2005; Hermand et al., 2008) and in experimental ones (Bolam & Fernandes, 2002; Rossi 2003; Levinton & Kelaher, 2004). However, the role of the food supply in structuring the community along a temporal scale has received less attention. The study of the macrofaunal community and its trophic interactions with other benthic communities (i.e. microalgal and meiofaunal organisms) can be clarified by using the functional traits analysis. The functional traits, in particular feeding habits and reproductive frequencies, reflect the components of functioning and are used as proxies for ecological processes such as their role in the trophic pathways and community development along the temporal scale (Törnroos & Bonsdorff, 2012).

The macrofaunal community dynamics in relation to environmental parameters are less known in the Adriatic Sea, especially in its north-western part. Indeed, to date most of the literature on the Gulf of Trieste has investigated the macrozoobenthic communities in relation to other aspects: hypoxia and anoxia events that occurred in the 1980s and 1990s (Brizzi et al., 1994; Aleffi et al., 1992;

Orel et al., 1993); anthropogenic pressures (industrial activities and sewage outfalls) (Solis-Weiss et al., 2004, Solis-Weiss et al., 2007; Cibic et al., 2008); biodeposition from mussels farms (Aleffi et al., 2006), and riverine inputs (Faresi et al., 2012). In contrast, the role of the food supply in structuring the macrofaunal community has not been considered yet.

Here we present the main results gained over a three-year study of the structural macrofaunal community changes (abundance, biodiversity, community composition, feeding habits and reproductive frequencies) in relation to environmental fluctuations (temperature and dissolved oxygen concentration) and food sources (organic matter from the water column and surface sediments, benthic microalgal biomass and meiofauna abundance) in a sublittoral site in the northern Adriatic Sea. The choice of the site, a virtually pristine area (the Marine Protected Area of Miramare), was driven by the need to attribute the observed response solely to the natural temporal variability without any evident anthropogenic disturbance. More precisely, the aim of the present study was to answer the following questions: 1) How stable is the macrofaunal community over time? 2) Are the feeding habits and reproductive frequencies of the macrobenthic community related to the food supply? 3) Are the resistance or resilience of the macrofaunal community consequent to the observed environmental variations over the three-year study?

Material and methods

Study site

The Gulf of Trieste, located at the north-western end of the Adriatic Sea, is a shallow basin of about 600 km2 and a coastline of about 100 km. It 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). The Gulf experiences annual fluctuations of temperature varying from 5°C to > 24°C at the surface and from 6 °C to >20°C at the bottom. The general pattern of currents in the Gulf may be rapidly modified in response to intense local atmospheric forcing (winds) and river plume (Querin et al., 2007; Malačič & Petelin, 2009). The region is highly influenced by the Bora, a north-easterly wind characterized by strong intensity that is able to mix the entire water column also due the shallow depth of the basin (Querin et al., 2007). The dominant sediment type in the Gulf is sandy-mud but the composition of soft bottoms can vary from sands with patches of rocks to detrital muds (Brambati & Catani, 1988). Sedimentation within the Gulf is mainly controlled by river inputs rather than marine currents and the main terrigenous sediments supply comes from the Isonzo River (Covelli & Fontolan, 1997). The annual average sedimentation rate is about 1 mm y-1 in the middle of the Gulf and increases up to 2.5 mm y-1 in front of the Isonzo River (Covelli et al., 1999 and references therein).

The study was carried out at a costal station (C1: 45.700830° N, 13.71000° W) (Fig. 1). C1 is located ca. 250 m offshore at a depth of 17 m, within the Marine Reserve of Miramare. This small marine protected area (MPA) is divided in two distinct zones: the inner part (30 ha), subjected to a regime of integral protection, is surrounded by a larger buffer zone (90 ha). Declared an MPA already in 1979, the Reserve is part of the Natura 2000-network, and represents the only completely protected area in the Italian part of the Gulf of Trieste. At C1, the grain size is clayey silt, with <10% of sand and organogenic detrital components (Cibic et al., 2007a).

Sampling

Monthly sampling was performed from June 2002 to July 2005. In August and October 2002, October 2004 and February 2005 sampling was not carried for logistic reasons. Sea bottom temperature, salinity and dissolved oxygen concentration were obtained with a CTD probe (Idronaut Ocean Seven 316 and SeaBird 19 Plus). Water samples for particulate organic carbon were collected with Niskin bottles at 15 m depth.

Sediments samples for the analyses of chemical parameters (total organic carbon, TOC and biopolymeric carbon, BPC) microalgal biomass (MPBB) and meiofaunal abundance (Meio) were collected by a KC Haps bottom corer (KC-Denmark, Silkeborg, Denmark). For the study of Meio, three replicate cores (polycarbonate tubes of 13.3 cm Ø with a sample area of 127 cm2) were taken down to the depth of 10 cm. Macrofauna was sampled collecting three replicates at each sampling occasion using a 0.1 m2 van Veen grab.

Food sources

The chemical and biological variables were considered as food sources to better understand the macrofaunal variations (abundance, biodiversity, community composition, feeding habits and reproductive frequencies) linked to environmental changes (seasonal and year-to-year variations of temperature, dissolved oxygen and organic matter availability). In particular, we considered as food sources: the particulate organic matter from the water column, total organic carbon in the sediments and especially its labile fraction composed of carbohydrates, proteins and lipids; and finally the microalgal biomass and meiofaunal abundance that were related to the different macrofaunal feeding habits.

Water subsamples for POC analysis of 0.5 l each were filtered on 25 mm Whatman GF/F pre-combusted filters and were stored frozen at -20 °C. Standard method of POC was used (Lipizer *et al.*, 2012) and their values were expressed as μmol l^{-1}.

The analyses of TOC and the determination of sediment carbohydrates, lipids, and proteins were performed on subsampled aliquots of oxic sediments as described by Cibic *et al.* (2008). TOC values were expressed as mg g^{-1} dry mass. The sum of carbohydrates, lipids, and proteins carbon was referred to as biopolymeric carbon (BPC) and their values were expressed as μg g^{-1}. The protein-carbohydrate ratio (PTR/CHO ratio) was used to indicate the "age" of the organic matter present in sediments (Pusceddu, 2002).

The microalgal abundance was analysed following the method described by Cibic *et al.* (2007a). Standard deviation of three replicate values was below 25%. MPBB was calculated to assess the carbon content of occurring algae varying in shape and size in accordance with the method proposed by Cibic *et al.* (2007a). MPBB, expressed as mg C cm^{-3}, was obtained multiplying the microalgal abundance (cells cm^{-3}) by the carbon content of each counted cell.

For Meio extraction, sediments were treated according to Cibic *et al.* (2009). The Meio density of the main taxa was expressed as number of individuals per 10 cm^2.

Macrofauna

Sediments were sieved through 1 mm sieve mesh to retain the fraction of macrozoobenthic organisms. The collected invertebrates were immediately fixed with neutralized formaldehyde solution (4% v/v final concentration in seawater). After washing, organisms were separated according to their main taxonomical groups and preserved in ethanol 70°. All the animals were identified to the lowest possible taxonomical level under a stereomicroscope at 7-80X final magnification and counted. For the organism identification, the taxonomical keys listed in Morri et al. (2004), were used. The abundance was expressed as number of individuals per m2.

In order to investigate the functional structure of the community related to the available food sources, two functional traits of paramount importance were considered: feeding habits and reproductive frequencies. Six different feeding habits were assigned to all individuals: surface deposit feeders (SDF), subsurface deposit feeders (SSDF), suspension feeders (SF), predators (P), omnivores (OMN) and grazers (G) using the following criteria: morphology of the feeding apparatus, feeding mode, nature and origin of the food. Three different types of reproductive frequencies based on their breeding were evaluated. Iteroparous (I), organisms breeding several times per lifetime; Semelparous (S), individuals that have only one brood during their life time and then the parent usually dies and Semi-continuous (C), reproduction occurs all year round or for the most part of the year.

Traits for each taxon were derived from literature sources and database, namely Giangrande et al., (1994); Roth & Whilson (1998); MarLIN (2006); Jumars et al. (2015) and Polytraits Team (2016). Taxonomical resolution was kept at the species level whenever possible but adjusted to genus or family when the information on traits were only available on a higher taxonomic level.

Statistical analyses

The diversity of the investigated macrofaunal community was evaluated using the following univariate community indices: abundance, species richness, diversity (H', Shannon & Weaver, 1949) and evenness (J', Pielou, 1966). The temporal variations of the macrofaunal diversity were calculated measuring the turnover diversity (βt) (Wilson & Shmida, 1984) that was calculated as follows:

$$\beta_t = \frac{[g(H)+l(H)]}{2\bar{\alpha}}$$

where g(H) is the number of species gained and l(H) the number of species lost, standardized by the average sample richness, and [$\bar{\alpha}$] is the average number of species found within the monthly samples.

Multivariate analysis of the macrobenthic community structure was performed on the species abundance matrix applying a non-metric multidimensional scaling analysis (nMDS) ordination model. Monthly samplings were gathered in four seasonal groups based on bottom temperature: January, February and March as winter; April, May and June as spring; July, August and September as summer; October, November and December as autumn. Square root was used to transform the data matrix and Bray-Curtis similarity was applied. In addition, to highlight the variations of macrofaunal trophic habits and reproductive frequencies on a temporal scale, two bubble plots were applied on the nMDS ordination of seasonal abundance.

To detect which taxa were mainly responsible for changing in species composition during this three-year study SIMPER (SIMilarityPERcentage) analysis was employed and factor (years) was assigned. A log (X+1) transformation and a percentage dissimilarity cut-off of 70% were applied. Similarity Profiles (SIMPROF) analysis was used to test whether taxa significant ($p < 0.05$) co-varied coherently, or rather to determine whether species were associated with each other in terms of numerical variation through the time-series. The taxa with an individual similarity contribute percentage >1 (SIMPER analysis) were used to perform SIMPROF analysis. All univariate and multivariate analyses were performed using PRIMER 7(PRIMER-E Ltd., Plymouth,UK) software (Clarke et al., 2014).

The sum of monthly taxa abundances highlighted by SIMPROF were used to construct a Bray-Curtis dissimilarity matrix and an nMDS ordination. The environmental (temperature and dissolved oxygen) and food sources (POC, TOC, BPC, MPBB and Meio) variables were overlaid as supplementary variables (vectors) onto ordination spaces to investigate their relations on this distribution.

This vector fitting ordination was performed using the package Vegan in the R program (version 3.1.3).

In order to detect any changes on a temporal scale, the monthly data of different food sources, feeding habits, reproductive frequencies and taxa abundances gathered by SIMPROF ($p < 0.05$) were split into two groups (2002-2003 and 2004-2005). The difference in the considered variables between these two periods were tested by Kruskal-Wallis ANOVA test.

The Spearman rank correlation test (rs) was used to investigate relationships between food sources/feeding habits, reproductive frequencies and the taxa abundances gathered by SIMPROF ($p < 0.05$). Kruskal-Wallis ANOVA and Spearman tests were conducted using STATISTICA 7 (StatSoft, Inc., USA) and only statistically significant data ($p < 0.05$, < 0.01) are presented.

Results

Environmental variables and food sources

The seawater temperature at the bottom varied from 6.5°C in February 2003 to 25.1°C in July 2003. The salinity ranged from 37.2 in June 2002 to 38.3 in February 2004. The lowest value of dissolved oxygen concentration was measured in August 2004 (59.1%) and the highest in April 2004 (120.2%). Over the study period, the lowest oxygen values were consistently observed in summer and autumn whereas they considerably increased from winter to spring due to the vertical mixing and high primary production in the water column. Hypoxic conditions of the seabed were not recorded in this time frame (Table 1).

At the water column bottom layer, POC concentrations varied from 123.8 $\mu g\ l^{-1}$ in December 2004 to 358.5 $\mu g\ l^{-1}$ in June 2005. A seasonal pattern with a slight increase towards the end of the whole period was observed (Fig. 2a).

In the surface sediments, the lowest TOC contents were measured in November and September 2003 (7.4 ± 1.1 and 7.9 ± 0.2 mg g^{-1}, respectively), whereas TOC increased at the end of the study period (13.8 ± 0.2 mg g^{-1} in July 2005). Indeed, significant differences in TOC content were observed between 2002-2003 vs 2004-2005 ($H = 6.8$; $p < 0.01$) (Fig. 2a).

BPC values were very low in 2002 and 2003 (minimum in June 2002 with 794.1 µg g^{-1}) while toward the end of the study period a major content was recorded (maximum in June 2005 with 2550.5 µg g^{-1}). Statistically significant differences in BPC content between 2002-2003 vs 2004-2005 ($H = 10.6$; $p < 0.01$) were observed (Fig. 2b). The PRT/CHO ratio values varied from 1.5 (June 2003) to 6.6 (October 2003). Amplitude (indicated by the standard deviation) of this ratio was obtained by comparing data of the first part of the study (2002-2003, mean PRT/CHO = 3.4 ± 1.5) with the more stable values from 2004 to the end of the sampling period (mean PRT/CHO = 3.3 ± 0.8).

The MPBB varied between 5.7 µg C cm-3 in November 2002 and 39.6 µg C cm^{-3} in July 2005 and seasonal variations were noticed (Fig. 2b). Diatoms were the dominant microalgal group, representing up to 96.7% of the total MPBB. At the end of the three-year study MPBB increased, mainly due to the development of the large diatom genus *Gyrosigma* spp. that peaked in July 2005 (10.3 µg C cm^{-3}).

The Meio-density ranged between 542 ± 83 ind.10 cm-2 in January 2003 and 3345 ± 407 ind.10 cm-2 in July 2003. Meio were dominated by Nematoda (72.0% of the total abundance), followed by Copepoda with their naupliar stages (18.0%) and Kinorhyncha (2.6 %) (Fig. 2b).

As POC, MPBB and Meio showed a seasonal pattern over the study period, with a slight increase of MPBB and decrease in Meio abundance towards the end of the three-year study.

Macrofaunal community structure

The total abundance of benthic macrofauna at C1 did not show any seasonal pattern over the three-year study. Abundance peaked in January 2002 (3283 ± 1753 ind. m^{-2}), and high abundances (≥1700 ind. m^{-2}) were also recorded in May 2003, March and August 2004, and in January 2005. The lowest values were measured in July 2003 (490 ± 533 ind. m^{-2}) and in July 2002, March and June 2005 (<550 ind. m^{-2}) (Fig. 3). Polychaetes were by far the dominant group, reaching 73.1% of the total abundance over the whole study period, followed by molluscs (11.5%), crustaceans with 9.8%, echinoderms with 4.7% and "others" (pooling the remaining groups which were rarely found: anthozoans, sipunculids and nemertines) with 0.7% of the total abundance.

Up to 225 taxa were observed during the three-year study. The number of taxa ranged from a minimum of 38 (July 2002) to a maximum of 81 (January 2003 and August 2004) (Fig. 4a). No specific seasonal pattern was noticed in the study period and values remained fairly stable (average number of taxa 56.7±11.1), though a large difference between the gained and lost taxa was observed between the monthly samplings, indicating a high turnover ratio in the benthic community structure. In the three-year study, the turnover diversity (β_t) related to the variation in taxa composition, ranged from 0.3 (May 2003) to 0.6 (July 2002). Although the average values of the turnover diversity between the first (2002-2003) and second (2004-2005) part of the study period remained stable (0.39 ± 0.10 and 0.38 ± 0.04, respectively), a major amplitude of β_t during the first years was observed (Fig. 4b). The values of Shannon's diversity and Pielou's evenness indices remained quite steady throughout the study period with an average of $H' = 4.77 \pm 0.25$ and $J' = 0.80 \pm 0.05$, respectively. Their lowest values were recorded in June 2002 ($H' = 4.03$ and $J' = 0.71$) whereas the highest ones were calculated in August 2003 ($H' = 5.20$) and July 2002 ($J' = 0.93$).

Functional characteristics of macrobenthos

The macrozoobenthic community was characterized by SDF (34.3%), followed by P (31.1%), SF (18.5%) and SSDF (13.1%). OMN and G were the less represented, with 3% of the total community. All the trophic guilds, except for G, were recorded each month of the entire sampling period.

The community feeding habits did not show any clear seasonal variation during the three-year study (Fig. 5a). SDF peaked in January (1336 ± 517 ind. m^{-2}) and May 2003 (976 ± 587 ind. m^{-2}), in March (680 ± 318 ind. m^{-2}) and August 2004 (636 ± 432 ind. m^{-2}). A clear relationship between P and SDF was found over the study period, with a highly significant positive correlation between these two feeding habits ($r_s = 0.8$; $p < 0.01$).

In contrast, low values were recorded for SF and SSDF. SF varied from 83 ± 86 ind. m^{-2} in December 2004 to 510 ± 392 ind. m^{-2} in January 2003. The lowest value of

SSDF was recorded in July 2002 (20 ± 34 ind. m^{-2}) and the highest in August 2004 (290 ± 234 ind. m^{-2}). Both feeding habits pattern did not change over the study period.

For the reproductive strategies (traits) of the macrofaunal community, the I category was predominant (61.6% of the total abundance) followed by S (36.5%), while the C frequencies displayed a negligible share (1.9%) (Fig. 5b). Similarly to the feeding habits, the reproductive traits showed an unclear seasonal variation. However, similar dynamics of I and S abundance were observed during the study period as confirmed by a positive correlation ($r_s = 0.7$; $p < 0.01$). I abundances varied from 153 ± 301 ind. m^{-2} in July 2002 to 1296 ± 731 ind. m^{-2} in January 2003. S invertebrates peaked in January and May 2003 (1326 ± 470 and 960 ± 486 ind. m^{-2}, respectively). Further, significant differences of S abundance between the period 2002-2003 *vs* 2004-2005 ($H = 10.56$; $p < 0.01$) were observed. A highly significant relation was observed between I and P ($r_s = 0.9$; $p < 0.01$) as well as between S and SDF ($r_s = 0.9$; $p < 0.01$). Considering the relations among the food sources and reproductive frequencies, similar patterns were observed between S density and PRT/CHO ratio values on a temporal scale, as corroborated by a positive correlation ($r_s = 0.4$; $p < 0.05$) (Fig. 6).

The nMDS analysis based on the macrozoobenthic species composition (Fig. 7a,b) highlighted a higher variability of the community among years than seasons. In this analysis spring and autumn 2002, and winter 2003 were positioned on the left side of the plot at the maximum distance (Bray-Curtis maximum dissimilarity) from summer 2004 and summer 2005, indicating that during the three-year study the community was subjected to significant temporal variations. These variations could not be attributed to the feeding habits (bubble plot) that did not remarkably vary during the study period (Fig. 7a).

In contrast to feeding habit-traits, the reproductive traits displayed a clear inter-annual pattern on a temporal scale (Fig. 7b). Most of the seasons of 2002 and 2003 were characterised by a higher abundance of S invertebrates compared to I ones, which decreased during the seasons of 2004 and 2005.

Similarity Profiles analysis (SIMPROF) performed on the selected taxa with a percentage contribution >1 (highlighted by SIMPER analysis), illustrated four assemblages that significantly co-varied coherently during the three-year study. The abundances of the taxa gathered in the four groups represented on average 54.3 ± 8.3% of the total macrofaunal density.

These four groups were characterized by the following taxa (Table 2 and Fig. 8):

1) the polychaetes *Prionospio cirrifera, Spirobranchus triqueter, Spiophanes kroyeri* and Syllidae and the crustaceans *Pisidia longimana* and *Phtisica marina* (Fig. 8a). Their abundance was higher in the first part of the study and then decreased towards the end of the period. *S. triqueter* was the most abundant taxon of this group, displaying higher values in August 2003 and January 2003 (226 ± 253 and 210 ± 200 ind. m^{-2}, respectively).

2) the polychaetes *Aricidea* sp., *Eunice vittata*, *Hilbigneris gracilis* and *Magelona* sp. (Fig. 8b). Considering the total density of *Aricidea* sp. over the study period (100%), they were divided as follows: 62.7% ('02-'03) to 31.1% (2004) and only 5.9% in 2005, remarkably decreasing towards the end of the study period. High values of *H. gracilis* were recorded over the period with an average of 180 ± 116 ind. m^{-2}. *E. vittata* showed decreasing values during the three-year study and significant differences in its abundances between '02-'03 *vs* '04-'05 ($H = 7.9$; $p < 0.01$).

3) the polychaetes *Labioleanira yhleni*, *Lumbrineris latreilli*, *Marphysa sanguinea*, *Melinna palmata* (Fig. 8c). High densities were observed from summer 2003 to spring 2004, particularly of the most abundant species *M. palmata* that reached its maximum in February 2004 (70 ± 45 ind. m^{-2}).

4) the polychaetes Maldanidae nd., *Pseudoleiocapitella fauveli* and *Scoletoma impatiens*, the crustacean *Ampelisca* sp., the scaphopoda *Antalis inaequicostata* and the ophiuroid *Amphiura chiajei* (Fig. 8d). Their abundances increased towards the end of the study period. *Ampelisca* sp. was the most abundant taxon in this group reaching its maximum in January 2005 (216 ± 25 ind. m^{-2}). *S. impatiens* and *A. chiajei* were abundant with highest density in August 2004 (80 ± 43 and 60 ± 47 ind. m^{-2}, respectively). Statistically significant differences between '02-'03 *vs* '04-'05 were recorded for *Ampelisca* sp and *S. impatiens* ($H = 4.7$ and

$H = 6.6$; $p < 0.05$, respectively) and for *A. chiajei* ($H = 10.0$; $p < 0.01$). This latter taxon was also related to an increase of BPC and MPBB ($r_s = 0.6$; $p < 0.01$ and 0.3; $p < 0.05$, respectively). Also other positive correlations were obtained between different food sources and taxa gathered in this assemblage. *A. inaequicostata* was correlated with POC, BPC and MPBB (r_s = 0.4, 0.5; $p < 0.01$ and 0.4; $p < 0.05$, respectively). Maldanidae nd. was related with TOC and BPC (r_s = 0.4 and 0.4; $p < 0.05$).

The vector fitting *n*MDS analysis based on the monthly abundances of the four groups of taxa and the superimposed environmental parameters (temperature, oxygen concentration and food sources) showed a variation in taxa composition on a temporal scale. The community composition differed to a higher extent among years than seasons. In the ordination plot, a temporal pattern of monthly taxa was observed. Precisely, a variation in species distribution of the gathered taxa from the lower left corner of the graph (2002 and 2003, except for July 2002) (G.1 and G.2. in Fig. 9) to the upper right one (2004 and 2005) (G.3 and G.4 in Fig. 9) was displayed (dotted line). The environmental parameters as TOC, POC, BPC and MPBB were plotted spatially close to the G4 (toward the upper part of the ordination), conversely Meio and above all temperature, were located in the opposite direction, relatively close to G.2 and G.3 (toward the lower part of the plot).

Discussion

Our results revealed a higher variability of the macrozoobenthic community structure among years rather than seasons. This is in disagreement with the general pattern for the Mediterranean Sea, where most of the benthic species exhibit a seasonal pattern, with reproduction in spring or autumn and growth from autumn to spring (Coma *et al.*, 2000).

The primary forcing of changes in benthic communities often occurs during a distinct period of seasonal cycles, e.g. caused by food supply due to sedimentation of the spring phytoplankton bloom (Cibic *et al*. 2007b) or by oxygen deficiency due to high temperatures in late summer (Reiss & Kröncke, 2005). Although our results do not follow this general pattern dictated by seasonality, they are in accordance with those reported by Clarke & Frid (2001); Warwick *et al.* (2002); Rousi *et al.* (2013) who also observed major yearly differences in the macrofaunal structure rather than seasonal ones. According to these authors' findings the macrofaunal development was strictly linked to particular environment variations such as changes in grain size, sedimentation rate and high temperature.

The taxonomic composition of the macrofaunal community during the study period revealed a typical community of sandy-mud and detrital sediments, as previously reported for the Gulf of Trieste (Mavrič *et al.*, 2010; Faresi *et al.*, 2012), for the northern Adriatic Sea (Simonini *et al.*, 2004; Occhipinti-Ambrogi *et al.*, 2005; Franzo *et al.*, 2015), and worldwide (Warwick *et al.*, 2002; Rousi *et al.*, 2013; Somerfield *et al.*, 2014).

Focusing on the food sources for the macrofaunal community, a clear seasonal pattern was observed for POC, MPBB and Meio. POC slightly increased towards the end of the study period probably following a phytoplankton bloom occurred in summer 2004 and spring 2005 (Cabrini *et al.*, 2012). In contrast, TOC and BPC contents exhibited high variability between the two periods ('02-'03 *vs* '04-'05) as corroborated by the Kruskal-Wallis analysis (H = 10.6; $p < 0.01$). TOC generally exhibits a more conservative nature. This suggests that changes in the quantity of BPC might respond to changes in the trophic status more promptly than TOC (Pusceddu *et al.*, 2009). In fact in our study, the macrofaunal community was subjected to high organic matter availability in 2002 that gradually decreased towards autumn 2003. Afterwards, TOC values increased again reaching their maxima in June 2005. In contrast, this was not observed for BPC that did not show a remarkable decrease in autumn 2003 but higher values were displayed towards the end of the three-year study in correspondence with major MPBB. The increase of MPBB in the sediment surface layer might have modified the quality of organic matter, rendering it more palatable, as already highlighted by Hardison *et al.* (2013). The PRT/CHO <1 suggests the presence of aged organic matter (Dell'Anno *et al.*, 2002). In our study the ratio ranged from 1.5 (June 2003) to 6.6 (October 2003) indicating the availability for benthic organisms of freshly produced organic matter over the entire period, even though wide fluctuations of the ratio were observed especially in the first part of the study (2002-2003, see Fig. 6).

Feeding habits and reproductive frequencies in relation to food sources

During the entire study period, biological traits related to feeding habits were not influenced by the naturally occurring physical fluctuations at the sampling site (St. C1), showing an unclear variability over the study period (see Fig. 5a and 7a). SDF remained the most common trophic trait category, probably due to the even and stable grain size-distribution (Cibic *et al.*, 2012 and Franzo *et al.*, 2016) This is in accordance with Simonini *et al.* (2004); Occhipinti-Ambrogi *et al.* (2005); Faresi *et al.* (2012), who found deposit feeding invertebrates to be numerically dominant in sandy-mud or muddy sediments. Much of the detrital food for these invertebrates is associated with high proportion of particles within the silt-clay range. The high surface-area-to volume ratios of small particles provide a large expanse for the attachment and growth of microbial populations that produce mucopolysaccharide exudates, very palatable for these invertebrates (Donald & Larry, 1982 and reference therein).

Some SDF showed an opportunistic behaviour. Favourable environmental conditions, following an increase of food supply, could rapidly stimulate the growth, reproduction rate and survivorship of individuals. After consumption of the peaks in food and/or being consumed by P, SDF abundance could decrease considerably, suggesting wide temporal fluctuations (Rossi, 2003 and references therein).

The SDF are not bulk ingestors of surface sediments, but rather they are able to modify their behaviour and choose to feed on fresh organic matter at very small spatial scale (Cruz-Rivera & Hay, 2000a). Experimental studies (Bolam, 2002; Rossi, 2003; Levinton & Kelaher, 2004) highlighted that the presence of food patches in the sediments could increase the frequency at which they move and stay in those patches because of a more suitable micro-habitat, with a consequent increase in individual abundances.

In this macrofaunal community, P as a feeding category followed the pattern of SDF (r_s=0.6; p<0.01). P probably prayed on the numerically dominant species, as suggested by Quijón & Snelgrove (2005). In fact, SDF aggregated in large abundance soon after the establishment of patches, and thus the subsequent arrival and feeding by mobile predator could be a real possibility.

In the first part of the study period (2002 and 2003) the majority of SDF individuals belonged also to S species, as corroborated by the significant correlation between these two traits. These species are considered *r*-strategist with an opportunistic response or rather, they are short-lived invertebrates with fast growth, early maturity and high fecundity that increase in abundance when fresh food supply is available (Gray & Elliott, 2009). The S polychaetes such as *Aricidea* sp., *P. cirrifera*, *S. kroyeri* and *S. triqueter* were numerically dominant in the first part of the study period. These taxa displayed unpredictable changes in abundance in response to high and irregular input of organic matter. In fact high individual density and irregular peaks were observed in correspondence with wide variations of PRT/CHO ratio in the first part of the study period that might have favoured the growth of opportunistic/*r*-strategist species (also corroborated by S and PRT/CHO positive correlation). Our findings are in accordance with other studies from coastal areas which have reported wide variations of food supply due to the intrinsic features of these sublittoral environments (i.e. highly variable hydrodynamics, runoff, sedimentation and turbidity) (Ellis *et al.*, 2000 and reference therein).

Over the three-year study, a higher density of P was observed compared to the other feeding habits; the majority of P were also I organisms ($r_s = 0.9$; $p < 0.01$), indicating that this trophic guild presented different reproductive features compared to the majority of SDF invertebrates. For instance, I is a reproductive trait typical of *K*-strategist, they breed several times during their lifetime, have a delayed reproduction, slow growth and longer life span with a less pronounced numerical fluctuation than S invertebrates (Giangrande, 1997). Towards the end of the 2004-2005 period, the monthly abundances of P were generally higher than those of deposit and suspension feeders. This may indicate that macrofaunal P preyed on Meio invertebrates. The potential top-down control on meiobenthic invertebrates by macrofaunal P has been often reported (Van Colen *et al.*, 2015). In fact, as found by Jumars *et al.* (2015), many polychaetes observed at our study site belonged to the families Lumbrineridae and Eunicidae (mainly *Lumbrineris gracilis* and *Eunice vittata,* respectively). They have paired mandibles and complex sets of maxillae in a strongly muscular and eversible pharynx and are able crawlers and borrowers in muddy sediments and therefore they could be potential predators on the Meio.

As highlighted by the vector fitting nMDS analysis, the last part of the study period was characterized by a community shift likely related with the increase of food supply, particularly POC, BPC and MPBB. The species observed during this time frame were principally SDF and I. While the feeding strategies did not remarkably change during the three-year study, the quantity and quality of food supply did. We infer that the high abundances of *A. Chiajei* and *A. Inequicostata* observed in last part of the study were a result of the high availability of BPC ($r_s = 0.7, 0.5$; $p < 0.01$). Sköld & Gunnarsson (1996) and Nilsson (1999) previously observed an increase of *A. chiajei* in response to food pulses. Their findings showed that brittle stars are capable of increasing their growth and gonad development in response to short-term fresh organic matter-enrichment and even more with a diet based on diatoms. Indeed, in our study A. *chiajei* was correlated with the MPBB ($r_s = 0.4$; $p < 0.05$). MPBB is the preferred food source for many soft sediment benthic invertebrates. In marine systems a high amount of energy (C flow) is directly transferred from primary producers to consumers (Cruz-Rivera & Hay, 2000b). The high amount of lipids (e.g. triglycerides and long fatty acid chains) contained inside the microalgal cells makes them very palatable to deposit feeder invertebrates (Goedkoop *et al.*, 2011). Similarly, the Scaphopoda *A. inequicostata* was correlated with MPBB and POC ($r_s = 0.4$; $p < 0.05$; $r_s = 0.4$; $p < 0.01$, respectively). A major fraction of this POC at St. C1 was probably ascribable to phytoplankton cells. The occurrence of high abundance of SDF has been related to the sinking of phytodetritus (Quijón *et al.*, 2008 and reference therein). According to the review of Reynolds *et al.* (2002) on the Scaphopoda class, *A. inequicostata* has been often classified as a predator, but it has been also reported as a deposit or suspension feeder, feeding on foraminifers and other microorganisms (diatoms) by using its tentacles.

Macrofaunal community development on a temporal scale

Persistent and stable benthic communities have generally been related to environments that are free of disturbances with relatively stable physical parameters (Buchanan *et al.*, 1986). These communities are not characterised by remarkable variability in their structure (biodiversity and functional characteristics of species) and further the absolute abundance of each species remains constant over time (Turner *et al.*, 1995). On the contrary, unstable communities usually display high biodiversity variability, changes in species composition and especially wide fluctuations in species richness and J′ values (Worm & Duffy, 2003).

In this three-year study, the macrofaunal community showed quite high and fairly stable diversity indices values (species richness, H′ and J′), although several changes in the community composition were observed during this period that were revealed by the high amplitude of the turnover biodiversity. The turnover values obtained towards the end of the study were high compared to those reported in the literature (Wilson & Shmida, 1984; Quillien *et al.*, 2015). In detail, the turnover biodiversity values ($\beta_t = 0.39 \pm 0.10$) displayed a wide range in amplitude in the first period (2002 and 2003) compared to the second one ($\beta_t = 0.38 \pm 0.04$) (2004 and 2005), indicating a great variability in the species turnover with a slight stabilization towards the end of the study. Overall, the high biodiversity and monthly turnover diversity suggest an intermediate level of stability. Indeed, although changes in the community structure were observed by the vector fitting nMDS analysis, some species were present throughout the study period. According to Huston (1994) and Sommer & Worm (2002) the highest biodiversity often occurs at intermediate levels of disturbance intensity or frequency, and, by inference, at an intermediate level of community stability. In addition, at this level of stability, the macrofauna displayed a minor variability in species composition but larger populations fluctuations that primarily involved relatively short-lived invertebrates (with year-to-year variability) (Gray & Elliott, 2009 and references therein).

We believe that the variations in dominant species composition and reproductive frequencies were mostly a consequence of the heatwave observed in summer 2003, rather than ascribable to the concomitant high values of BPC and MPBB. In fact, anomalous high sea bottom temperatures were observed in summer 2003 (25°C in July), more than 3°C above the average summer values recorded over a 12-year study in the Gulf of Trieste (Malačič *et al.*, 2006). This was the warmest summer recorded in the Mediterranean Sea in the last few decades (Schär *et al.*, 2004). This physical stress might have led to long-term changes in the macrofaunal individual abundance, biodiversity and taxa composition and their functional traits. As reported by Crisci *et al.* (2011) this heatwave caused an anomalous seawater warming, reaching temperatures between 1 and 3 °C above the climatic values in several areas of the Mediterranean Sea and consequent mass mortality events of benthic macroinvertebrate species occurred (Garrabou *et al.*, 2009). In spite of these high temperatures in summer 2003, the dissolved oxygen concentrations at St. C1 were not under-saturated, and therefore no mass mortality was observed that might have been expected following this heatwave.

The macrofaunal community biodiversity seemed not to be affected by this drastic pulse of environmental change. The H′ values remained within the same range of the study period (H′ = 4.9 and 5.0, in July and August 2003, respectively). The community resistance to this physical stressor could be attributed to its adaptation to the highly variable local environmental conditions. In the Gulf of Trieste, sea bottom temperatures display wide annual fluctuations (from 6°C to >20°C) that have likely selected macrofaunal communities with adaptation mechanisms in response to temperature stress. Communities of unstable environments are more pre-adapted to fluctuations (Munari, 2011). The same finding was obtained both from field studies (Fitt *et al.*, 2001) and experimental ones (Torrents *et al.*, 2008) carried out on benthic invertebrates. The authors concluded that thermo-tolerance can vary among populations of the same species and the upper thermal limits are correlated with their different temperature environment and previous stress history. In fact, in 2003 the highest (25.1 °C in July) and the lowest (6.5 °C in February) sea bottom temperatures were measured at St. C1 over the three–year study. However, the high temperatures in summer 2003 might have strongly affected the macrozoobenthic reproductive frequencies. The abundance of I invertebrates, although presenting numerous picks of density, remained relatively stable during the entire study period (see Fig. 5b and 7b). On the other hand, S organisms were very abundant in the first part of this study (2002-2003), reaching higher abundances than the I ones (i.e. January, February and April 2003). After the summer 2003, a significant decline was observed and even low density was noticed towards the end of the study period as showed by Kruskal-Wallis analysis (H= 10.6; $p < 0.01$).

Overall, low abundances of S organisms were recorded in each summer, although the heatwave of 2003 might have modified their fitness (i.e. the ability of a population to maintain or increase its abundance in succeeding generations). In fact, after these extreme events they did not reach comparable densities to those observed in the previous periods (2002 and winter-spring of 2003) (see Fig. 7b). Even I organisms were negatively influenced by high temperatures recorded in summer 2003. Their density slightly decreased in July 2003 but then they recovered towards the end of study. Since the heatwave lasted only for a short period of time, it is likely that the high temperatures hit principally the S species (*r*-strategist) that were less stress-resistant. In contrast, the long-lived iteroparous organism (*K*-strategist) are better buffered from environmental extreme events, their population size do not vary as those of smaller, shorter-lived organisms. Furthermore, their competitive relationships are presumably more predictable and constant (Pianka, 1970; Ranta *et al.*, 2002; Gray & Elliott, 2009). We infer that I invertebrates at St. C1 were able to resist to the temperature stress due to the ecological features of *K*-strategist species that allow them to prevail over the *r*-strategist in an inter-species competition also when the heatwave was over.

Conclusion

During the three-year study the macrofaunal community at St. C1 displayed a higher variability among years than seasons. A remarkable temporal variation in taxa composition was observed due to a shift in their reproductive frequencies: from the dominance of S invertebrates to I ones. In spite of an increasing food supply over time, the variations in the macrofaunal structure were likely linked to the heatwave occurred in 2003. S organisms were less resistant to high temperatures compared to I ones. The latter were able to resist to the temperature stress that allow them to prevail over the S species in an inter-species competition also when the heatwave was over.

Overall, the community displayed an intermediate level of stability from high diversity and unvarying feeding habits to a great turnover diversity and variations in reproductive frequencies. The capability of the community to maintain a similar structure (abundance, biodiversity and feeding strategies), despite remarkable physical disturbance events, further testifies the resistance exhibited by the macrofaunal invertebrates. The functional traits approach applied in this study allowed to identify the role of environmental factors and food supply on the temporal development of the macrofaunal community.

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References

Aleffi, F., Orel, G., Del Piero, D., Vio E., 1992. Oxygen condition in the Gulf of Trieste (High Adriatic). p. 431-440. In: *Marine Coastal Eutrophicatio.* Vollenweider, R.A., Marchetti, R., Viviani, R., (Eds). Elsevier.

Aleffi, I.F., Bettoso, N., Celio, M., Tamberlich, F., Predonzani, S., et al., 2006. Effects of suspended mussel culture on the macrozoobenthos in the Gulf of Trieste (northern Adriatic Sea). *Annales Series Historia Naturalis*, 16 (2), 135–146.

Boero, F., 1994. Fluctuations and variation in coastal marine environment. *Marine Ecology*, 15 (1), 3–25.

Bolam, S.G., Fernandes, T.F., 2002. Dance aggregation of tube-building polychaetes: response to small-scale disturbances. *Journal of Experimental Marine Biology and Ecology*, 269 (2), 197–222.

Brambati, A., Catani, G., 1988. Le coste e i fondali del Golfo di Trieste dall’Isonzo a Punta Sottile: aspetti geologici, geomorfologici, sedimentologici e geotecnica. *Hydrores*, 6, 13–28.

Brizzi, G., Orel, G., Aleffi, I.F., Landri, P., Goriup, F., et al., 1994. Evoluzione del popolamento macrobentonico in una stazione soggetta ad ipossia ed anossia del Golfo di Trieste (Alto Adriatico). *Biologia Marina Mediterranea*, 1, 249-253.

Buchanan, J.B., Branchi, R., Christie, G., Moore, J.J., 1986. An analysis of stable period in the Nourthumberland benthic fauna -1973-80. *Journal of the Marine Biological Association of the United Kingdom*, 66, 659-670.

Cabrini, M., Fornasaro, D., Cossarini, G., Lipizer, M., Virgilio, D., 2012. Phytoplankton temporal changes in a coastal northern Adriatic site during the last 25 years. *Estuarine, Coastal and Shelf Science*, 115, 113-124

Cibic, T., Blasutto, O., Falconi, C., Fonda Umani, S., 2007a. Microphytobenthic biomass, species composition and nutrient availability in sublittoral sediments of the Gulf of Trieste (northern Adriatic Sea). *Estuarine, Coastal and Shelf Science*, 75 (1), 50–62.

Cibic, T., Blasutto, O., & Umani, S. F. 2007b. Biodiversity of settled material in a sediment trap in the Gulf of Trieste (northern Adriatic Sea). *Hydrobiologia*, 580(1), 57-75.

Cibic, T., Acquavita, A., Aleffi, I.F., Bettoso, N., Blasutto., 2008. Integrated approach to sediment pollution: A case study in the Gulf of Trieste. *Marine Pollution Bulletin*, 56, 1650–1667.

Cibic, T., Blasutto, O., & Bettoso, N., 2009. Microalgal–meiofaunal interactions in a sublittoral site of the Gulf of Trieste (northern Adriatic Sea, Italy): a three-year study. *Journal of Experimental Marine Biology and Ecology*, 370(1), 144-154.

Cibic, T., Comici, C., Bussani, A., Del Negro, P., 2012. Benthic diatom response to changing environmental conditions. *Estuarine, Coastal and Shelf Science*, 115, 158-169.

Clarke, K.R., Frid, C.L.J., 2001. Long-term changes in the North Sea ecosystem. *Environmental Review*, 9, 131–187.

Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M., (Eds), 2014. *Change in marine communities: An Approach to Statistical Analysis and Interpretation.* PRIMER-E, Plymouth, 260 pp.

Coma, R., Ribes, M., Gili, J.M., Zabala, M., 2000. Seasonality in coastal benthic ecosystems. *Trends in Ecology and Evolution*, 15 (11), 448–453.

Covelli S., Fontolan, G., 1997. Application of a normalization procedure in determining regional geochemical baselines. *Environmental Geology*, 30 (1-2), 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, 415–428.

Crisci, C., Bensoussan, N., Romano, J.C., Garrabou, J., 2011. Temperature anomalies and mortality events in marine communities: insights on factors behind differential mortality impacts in the NW Mediterranean. *PLoS ONE*, 6, e23814.

Cruz-Rivera, E., Hay, M.A., 2000a. Can quality replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology,*81, 201–219.

Cruz-Rivera, E., Hay, M.A., 2000b. The effects of diet mixing on consumer fitness: macroalgae, epiphytes, and animal matter as food for marine amphipods. *Oecologia*, 123, 252-264.

Dell'Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine system: a new approach based on the biochemical composition of sediment organic matter. *Marine Pollution Bulletin*, 44 (7), 611-622.

Desrosiers, G., Savenkoffb, C., Oliviera, M., Storac, G., Juniperd, K., et al., 2000. Trophic structure of macrobenthos in the Gulf of St. Lawrence and on the Scotian Shelf. *Deep Sea Research Part II: Topical Studies in Oceanography,*47, 663–697.

Donald, C.R., Larry, F.B., 1982. The effects of Marine Benthos on Physical Properties of sediments A successional Perspective. p 3-52. In: *Animals-sediment Relations*. McCall, P.L., Tevesz, M.J.S. (Eds). Springer Science Business Media, New York.

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.

Faresi, L., Bettoso, N., Aleffi, I.F., 2012. Vertical distribution of soft bottom macrozoobenthos in the Gulf of Trieste (northern Adriatic Sea). *Annales, Series Historia Naturalis*, 22, 123-132.

Fitt, W.K., Brown, B.E., Warner, M.E., Dunne, R.P., 2001. Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs*, 20 (1), 51–65.

Franzo, A., Cibic, T., Del Negro, P., De Vittor, V., 2015. Spatial distribution of microphytobenthos, meiofauna and macrofauna in the north-western Adriatic Sea: a synoptic study. *Advances in Oceanography and Limnology*, 6 (1/2), 58-75.

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.

Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., et al., 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global Change Biology*, 15 (5), 1090–1103.

Giangrande, A., Geraci, S., Belmonte, G., 1994. Life-cycle and life-history diversity in marine invertebrates and the implications in community dynamics. *Oceanography and Marine Biology: An Annual Review*, 32, 305–333.

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

Gray, J.S., Elliott, M., 2009.*Ecology of Marine Sediments -From Science to Management.*

2nd ed. Oxford University Press. New York, 243 pp.

Goedkoop, W., Sonesten, L., Ahlgren, G., Boberg, M., 2011. Fatty acids in profundal benthic invertebrates and their major food resources in Lake Erken, Sweden: seasonal variation and trophic indications.*Canadian Journal of Fisheries and Aquatic Sciences*, 57 (11), 2267-2279.

Hardison, A.K., Canuel, E.A., Anderson, I.C., Tobias, C.R., Veuger, R., Waters, N.M., 2013. Microphytobenthos and benthic macroalgae determine sediment organic matter composition in shallow photic sediment. *Biogeosciences*, 10, 5571–5588.

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). *Estuarine, Coastal and Shelf Science*, 79, 1–10.

Huston, M.A., 1994. *Biological diversity: The coexistence of species on changing landscapes*. Cambridge University Press, pp 681.

Jumars, P.A., Dorgan, K.M., Lindsay, M.S., 2015. Diet of worms emended: an update of polychaete feeding guilds. *Annual Review of Marine Science*, 7, 497–520.

Levinton, J., Kelaher, B., 2004. Opposing organizing forces of deposit-feeding marine communities. *Journal of Experimental Marine Biology and Ecology*, 300, 65–82.

Lipizer M., De Vittor C., Falconi C., Comici C., Tamberlich F., et al., 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

Malačič, V., Celio, M., Cermelj, B., Bussani, A., Comici, C., 2006. Interannual evolution of seasonal thermohaline properties in the Gulf of Trieste (northern Adriatic) 1991-2003. *Journal of Geophysical Research*, 111, 1-16.

Malačič, V., Petelin, B., 2009. Climatic circulation in the Gulf of Trieste (northern Adriatic). *Journal of Geophysical Research*, 114, 1-15.

Marchini, A., Gauzer, K., Occhipinti-Ambrogi, A., 2004. Spatial and temporal variability of hard-bottom macrofauna in a disturbed coastal lagoon (Sacca di Goro, Po River Delta, Northwestern Adriatic Sea). *Marine Pollution Bulletin*, 48, 1084–1095.

MarLIN, 2006. BIOTIC - Biological Traits Information Catalogue. https://www.marlin.ac.uk/biotic

Mavrič, B., Orlando, M., Bettoso, N., Lipej, L., 2010. Soft-bottom macrozoobenthos of the sourthern part of the Gulf of Trieste: faunistic, biocoenotic and ecological survey. *Acta Adriatica*, 51 (2), 203–216.

Morri, C., Bellan-Santini, D., Giaccone, G., Bianchi, C., 2004. Principles of bionomy: definition of assemblages and use of taxonomic descriptors (macrobenthos). *Biologia Marina Mediterranea*, 11 (1), 573–600.

Munari, C., 2011. Effects of the 2003 European heatwave on the benthic community of a severe transitional ecosystem (Comacchio Saltworks, Italy). *Marine Pollution Bulletin*, 62 (11), 2761–2770.

Nilsson, H.C., 1999. Effects of hypoxia and organic enrichment on growth of the brittle stars *Amphiura filiformis* (O.F. Müller) and *Amphiura chiajei* Forbes. *Journal of Experimental Marine Biology and Ecology*, 237, 11–30.

Occhipinti-Ambrogi, 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.

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

Orel, G., Fonda Umani, S., Aleffi, I.F.,1993.*Ipossie ed anossie di fondali marini-L 'Alto Adriatico ed il Golfo di Trieste*. Regione Autonoma Friuli-Venezia Giulia, Direzione regionale dell'Ambiente, 104 pp.

Pianka, E.R., 1970. On r- and K-Selection. *The American Naturalist*, 104, 592–597.

Pielou, E. C., 1966. The measurement of diversity in different types of biological collections. *Journal of theoretical biology*, 13, 131-144.

Polytraits Team,2016. Polytraits: A database on biological traits of polychaetes. http://polytraits.lifewatchgreece.eu (1 September 2016).

Pusceddu A., Dell'Anno A., Fabiano M., 2002. Organic matter composition in coastal sediments at Terra Nova bay (Ross Sea) during summer 1995. *Polar biology*, 23 (4), 288–293.

Pusceddu, A., Dell'Anno, A., Fabiano, M., Danovaro, R., 2009. Quantity and bioavailability of sediment organic matter as signatures of benthic trophic status. *Marine Ecology Progress Series*, 375, 41–52.

Putman, R.J., Wratten, S.D., 1984.*Principles of ecology*. University California Press, Berkeley, CA, 399 pp.

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.

Quijón, P.A., Snelgrove, P.V.R., 2005. Predation regular of sedimentary fauna structure: potential effects of a fishery-induced switch in predators in a Newfoundland sub Artic fjord. *Oecologia*, 144, 125–136.

Quijón PA, Kelly MC, Sneglrove PVR, 2008. The role of sinking phytodetritus in structurig shallow-water benthic communities. Journal of Experimental Marine Biology and Ecology 366, 134–145.

Quillien, N., Nordström, M.C., Gauthier, O., Bonsdorff, E., Paulet, Y.M., et al., 2015. Effects of macroalgal accumulations on the variability in zoobenthos of high-energy macrotidal sandy beaches. *Marine Ecology Progress Series*, 522, 97–114.

Ranta, E., Tesar, D., Kaitala, V., 2002. Environmental Variability and Semelparity vs. Iteroparity as Life Histories. *Journal of Theoretical Biology*, 217, 391–396.

Reiss, H., Kröncke, I., 2005. Seasonal variability of benthic indices: An approach to test the applicability of different indices for ecosystem quality assessment. *Marine Pollution Bulletin*, 50, 1490–1499.

Reynolds, D.P., 2002. The Scaphopoda. *Advances in Marine Biology*, 42, 137-236.

Rossi, F., 2003. Short-term response of deposit-feeders to an increase of the nutritive value of sediment trough season in an intertidal mudflat (western Mediterranean, Italy). *Journal of Experimental Marine Biology and Ecology*, 290 (1), 1–17.

Roth, S., Wilson, J.G., 1998. Functional analysis by trophic guilds of macrobenthic community structure in Dublin Bay, *Ireland. Journal of Experimental Marine Biology and Ecology*, 222 (1), 195-217.

Rousi, H., Laine, O.A., Peltonen, H., Kangas, P., Andersin, A.B., Rissanen, J., et al., 2013. Long-term changes in coastal zoobenthos in the northern Baltic Sea: the role of abiotic environmental factors. *ICES Journal of Marine Science*, 70, 440-451.

Schär, C., Vidale, P., Lüthi, D., Frei, C., Häberli, C., et al., 2004. The role of increasing temperature variability in European summer heat waves. *Nature*, 427, 332–336.

Shannon, C. E., Weaver, W., 1949. The mathematical theory of information. Illinois Press, pp117.

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.

Simpson, J.H., (1997) Physical processes in the ROFI regime. *Journal of Marine Systems*, 12, 3–15.

Sköld, M., Gunnarsson, G., 1996. Somatic and germinal growth of the infaunal brittle stars *Amphiura filiformis* and *A. chiajei* in response to organic enrichment. *Marine Ecology Progress Series*, 142, 203–214.

Solis-Weis, V., Aleffi, I.F., Bettoso, N., Rossini, P., Orel, G., et al., 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, 247–263.

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.

Somerfield, P., Burton, M., Sanderson, W., 2014. Analyses of sublittoral macrobenthic community change in marine nature reserve using similarity profiles (SIMPROF). *Marine Environmental Research*, 102, 51–58.

Sommer, U., Worm, B.(Eds), 2002. *Competition and coexistence*. Ecological study, 221 pp.

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 (8), 2221–2236.

Torrents, O., Tambutte, E., Caminiti, N., Garrabou, J., 2008. Upper of thermal thresholds shallow vs. deep population of the precious Mediterranean red coral *Corallinum rubrum* (L.): assessing the potential effects of warming in the NW Mediterranean. *Journal of Experimental Marine Biology and Ecology*, 357, 7–19.

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

Van Colen C., Thrush S.F., Parkes S., Harris R., Woodin S.A., et al., 2015. Bottom–up and top–down mechanisms indirectly mediate interactions between benthic biotic ecosystem components. *Journal of Sea Research*, 98, 42–48.

Warwick, R.M., Ashman, C.M., Brown, A.R., Clarke, K.R., Dowell, B., et al., 2002. Inter-annual changes in the biodiversity and community structure of the macrobenthos in Tees Bay and the Tees estuary, UK, associated with local and regional environmental events. *Marine Ecology Progress Series*, 234, 1–13.

Widdicombe, S., Austen, M.C., 2001. The interaction between physical disturbance and organic enrichment: An important element in structuring benthic communities. *Limnology and Oceanography*, 47 (7), 1720-1773.

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

Worm B., Duffy E., 2003. Biodiversity, productivity and stability in real food webs. *Trends in Ecology and Evolution*, 18, 628–632.

Table 1: Temperature (°C), salinity and oxygen (%) of the water column at the sea bottom over the three-year study.

	Temperature	Salinity	Oxygen
Jn'02	17.7	37.2	114.9
Jl'02	20.5	38.0	77.1
Se'02	23.0	38.2	81.6
Nv'02	15.9	37.7	94.9
Ja'03	10.2	38.1	97.8
Fe'03	6.5	38.1	94.4
Mr'03	8.3	38.1	101.2
Ap'03	9.9	37.9	98.1
My'03	11.3	38.0	111.0
Jn'03	15.3	38.0	120.1
Jl'03	25.1	38.2	114.8
Au'03	20.6	38.2	80.7
Se'03	20.8	38.2	85.0
Oc'03	17.0	38.1	85.4
Nv'03	13.9	38.2	91.9
De'03	11.4	38.1	90.8
Ja'04	8.6	38.3	90.6
Fe'04	7.5	38.3	92.7
Mr'04	8.0	37.9	95.5
Ap'04	10.5	38.0	101.6
My'04	14.2	37.6	104.9
Jn'04	16.9	37.3	95.5
Jl'04	21.0	37.6	77.4
Au'04	22.6	37.4	59.1
Se'04	22.0	37.3	94.8
Nv'04	15.0	37.1	95.0
De'04	14.1	37.9	94.1
Ja'05	7.3	38.2	98.8
Mr'05	6.7	38.3	104.7
Ap'05	10.2	37.9	96.8
My'05	12.9	37.8	99.0
Jn'05	18.6	37.9	94.9
Jl'05	19.9	38.0	93.6

Table 2: Minimum and maximum abundance (ind. m^{-2}) of the four groups of taxa highlighted by SIMPROF analysis and their feeding habits and reproductive frequencies

Taxa	Range min-max (ind. m^{-2})	Feeding habit	Reproductive frequency
First group (G.1)			
Phtisica marina	0 - 63.3	Omnivorus	Semelparus
Pisidia longimana	0 - 56.6	Predator	Iteroparus
Prionospio cirrifera	0 - 90	Surface deposit feeder	Semi-continuous
Spiophanes kroyeri	0 - 126.3	Surface deposit feeder	Semi-continuous
Spirobranchus triqueter	0 - 226.6	Suspension feeder	Semelparus
Syllidae nd.	0 - 56.6	Omnivorus	n/d
Second group (G.2)			
Aricidea sp.	0 - 790.6	Surface deposit feeder	Semelparus
Eunice vittata	3.3 - 183.3	Predator	Iteroparus
Hilbigneris gracilis	6.6 - 443.3	Predator	Iteroparus
Magelona sp.	0 - 53.3	Surface deposit feeder	Semelparus
Third group (G.3)			
Labioleanira yhleni	0 - 36.6	Predator	Iteroparus
Lumbrineris latreilli	0 - 36.6	Subsurface deposit feeder	Iteroparus
Marphysa sanguinea	0 - 23.3	Surface deposit feeder	Iteroparus
Melinna palmata	3.3 - 70.3	Surface deposit feeder	Iteroparus
Fourth group (G.4)			
Ampelisca sp.	0 - 216.6	Suspension feeder	Iteroparus
Amphiura chiajei	3.3 - 60.6	Surface deposit feeder	Iteroparus
Antalis inaequicostata	0 - 26.6	Predator	Iteroparus
Maldanidae nd.	0 - 10	Subsurface deposit feeder	Iteroparus
Pseudoleiocapitella fauveli	0 - 30	Subsurface deposit feeder	Semelparus
Scoletoma impatiens	0 - 80	Surface deposit feeder	Iteroparus

Figure legend

Figure 1. Location of the study site (45.700830° N, 13.71000° W; 17 m depth) in the coastal area of the Gulf of Trieste. (8x9 cm)

Figure 2. Temporal variability of the food sources over the study period (2002-2005). a) POC= particulate organic carbon; TOC=total organic carbon. b) BPC = biopolymeric carbon; MPBB= microalgal biomass; Meio= meiofauna. (18 x17)

Figure 3. Macrofaunal abundance per m^2 (mean of three replicates and standard deviation) during the study period (2002-2005). (12x16 cm)

Figure 4. Number of taxa and turnover rates (taxa gained/lost at each sampling) (a) and β_t diversity (turnover diversity) (b) of soft sediment infauna over the study period (2002-2005). (8x14)

Figure 5. Macrofaunal feeding habits (a): SDF=surface-deposit feeders; SSDF=subsurface-deposit feeders; SF=suspension feeders and P=predators (Omnivores and grazers are not reported) and (b) macrofaunal reproductive frequencies over the study period (2002-2005): C=semi-continuous; I=iteroparous; S=semelparous. (18x16 cm)

Figure 6. Co-variation of semelparous invertebrates and the protein/carbohydrates ratio over the three-year study (r_s value = 0.36;$p < 0.05$). The black triangle indicates the heat-weave event. (8x14 cm)

Figure 7. Non-metric multidimensional scaling (nMDS) ordination plot of the biological matrix represented as segmented bubble plots.(a) Seasonal abundances of taxa with different feeding habits (omnivores and grazers are not reported) and (b) of reproductive frequencies (semi-continues are not reported). Spr=spring; Sum=summer; Aut= autumn; Win=winter. (13.8x14.4 cm)

Figure 8. Temporal variation of taxa gathered by SIMPROF (p<0.05) over the study period (2002-2005). (14.37x24.98 cm)

Figure 9. nMDS ordination plot based on the taxa abundance gathered by SIMPROF. Temperature, dissolved oxygen and food sources (blue arrows) were plotted using vector fitting with ordination scores (stress value 0.13). Temp= temperature; Oxy= dissolved oxygen; POC= particulate organic carbon; TOC = total organic carbon; BPC = biopolymeric carbon; PRT/CHO=protein-carbohydrate ratio; MPBB= microalgal biomass; Meio= meiofauna. Years are indicated with different colours and seasons with different shapes. Temporal variation (from 2002 to 2005 indicated as a dotted line) in species distribution of the gathered taxa. TOC and POC are overlaid. For groups description see Table 2. (10x13 cm).

Figure 1

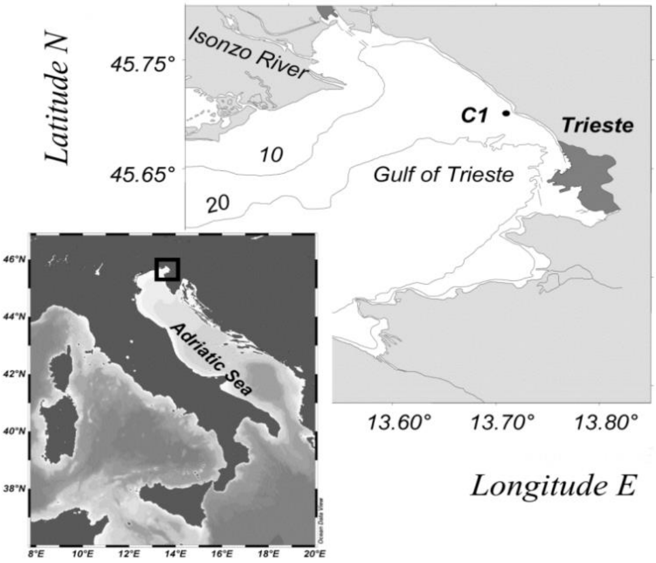

Latitude N
45.75°
45.65°
Isonzo River
C1
Trieste
10
20
Gulf of Trieste
13.60°
13.70°
13.80°
Longitude E
Adriatic Sea
46°N
44°N
42°N
40°N
38°N
8°E
10°E
12°E
14°E
16°E
18°E
20°E
Ocean Data View

Figure 2

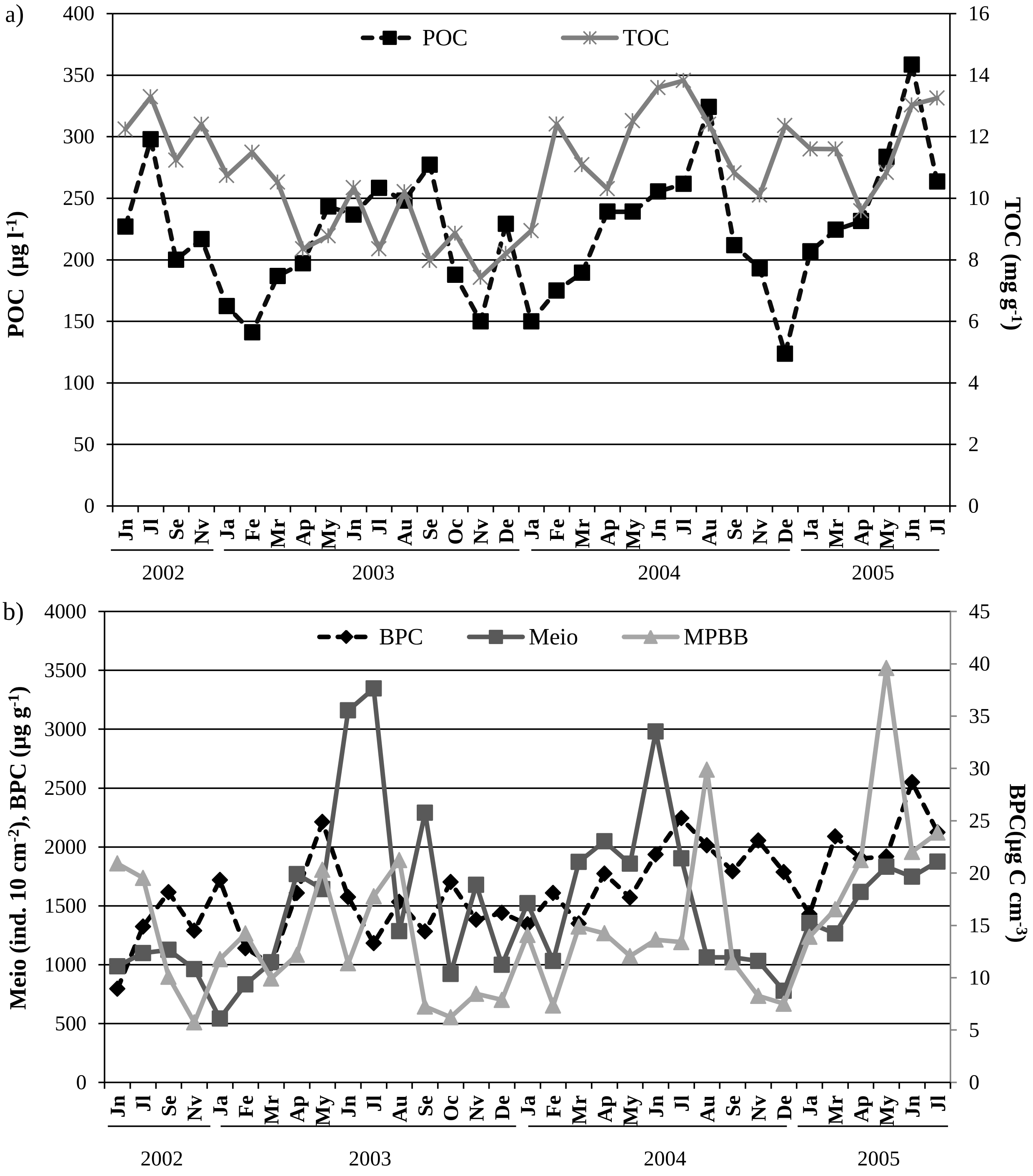
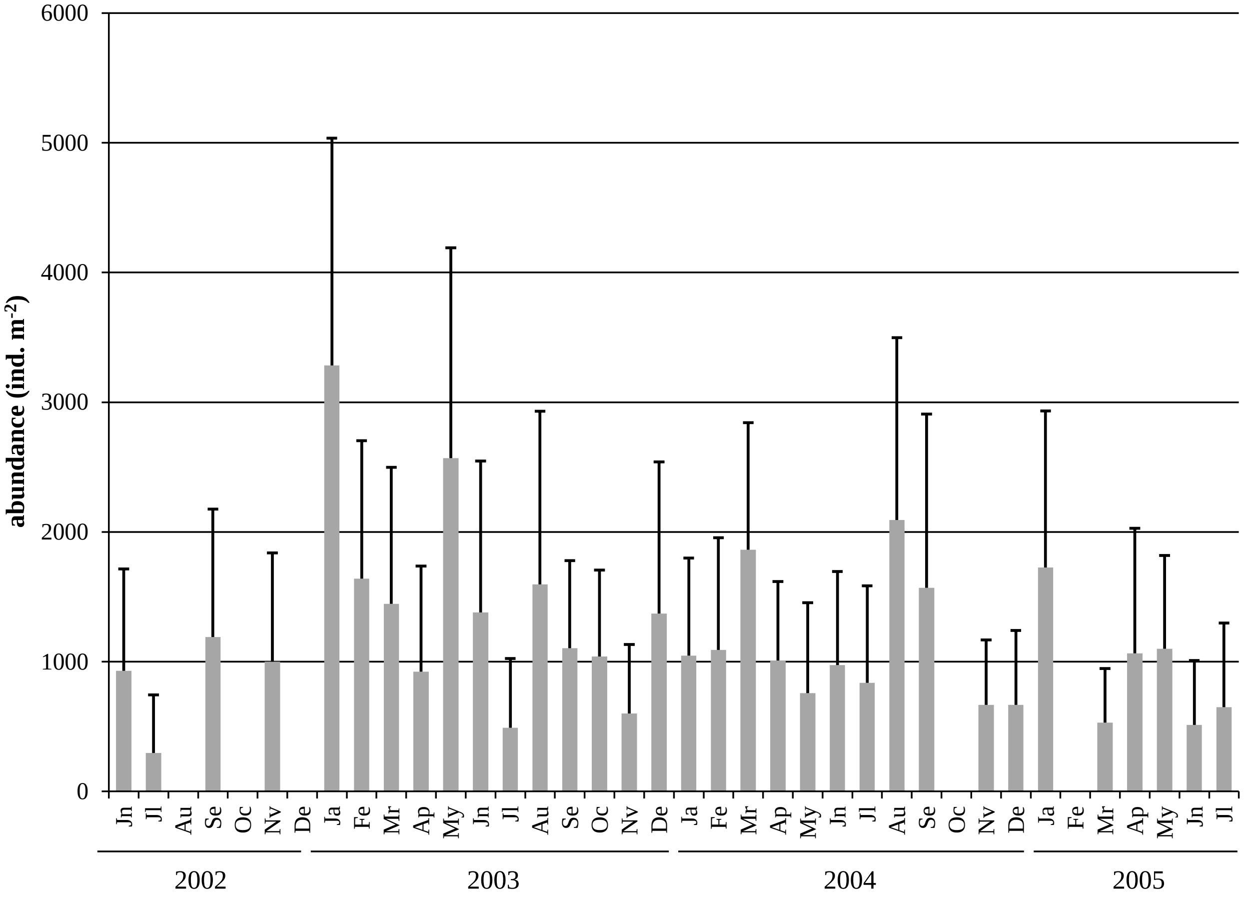

a)
POC
TOC
POC (µg l-1)
TOC (mg g-1)
0
50
100
150
200
250
300
350
400
0
2
4
6
8
10
12
14
16
Jn Jl Se Nv Ja Fe Mr Ap My Jn Jl Au Se Oc Nv De Ja Fe Mr Ap My Jn Jl Au Se Nv De Ja Mr Ap My Jn Jl
2002
2003
2004
2005
b)
BPC
Meio
MPBB
Meio (ind. 10 cm-2), BPC (µg g-1)
BPC(µg C cm-3)
0
500
1000
1500
2000
2500
3000
3500
4000
0
5
10
15
20
25
30
35
40
45
Jn Jl Se Nv Ja Fe Mr Ap My Jn Jl Au Se Oc Nv De Ja Fe Mr Ap My Jn Jl Au Se Nv De Ja Mr Ap My Jn Jl
2002
2003
2004
2005

Figure 3



6000
5000
4000
3000
2000
1000
0
abundance (ind. m^{-2})
Jn
Jl
Au
Se
Oc
Nv
De
Ja
Fe
Mr
Ap
My
Jn
Jl
Au
Se
Oc
Nv
De
Ja
Fe
Mr
Ap
My
Jn
Jl
Au
Se
Oc
Nv
De
Ja
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Mr
Ap
My
Jn
Jl
2002
2003
2004
2005

Figure 4

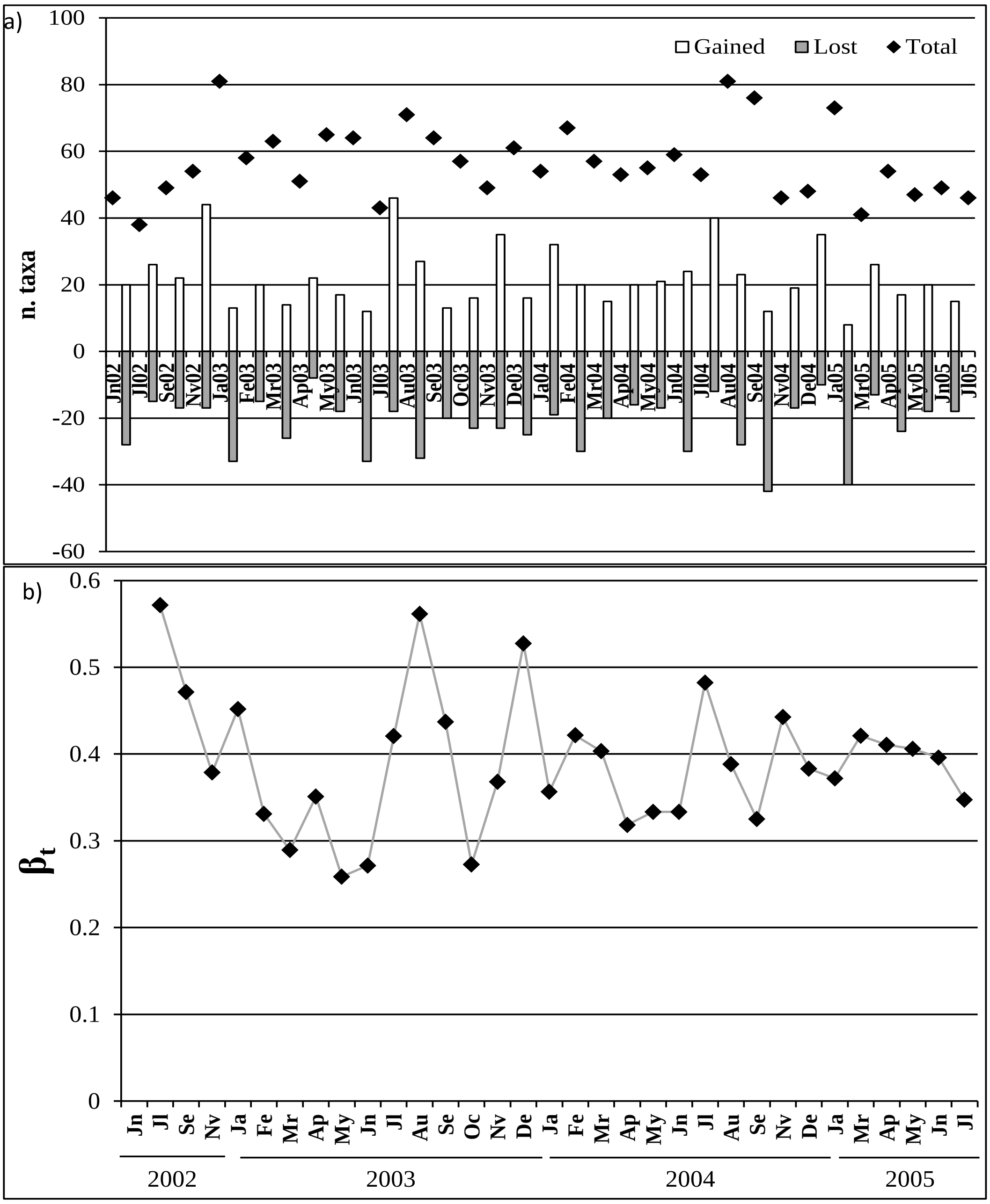

a)
100
80
60
40
20
0
-20
-40
-60
n. taxa
☐ Gained
▨ Lost
◆ Total
Jn02
Jl02
Se02
Nv02
Ja03
Fe03
Mr03
Ap03
My03
Jn03
Jl03
Au03
Se03
Oc03
Nv03
De03
Ja04
Fe04
Mr04
Ap04
My04
Jn04
Jl04
Au04
Se04
Nv04
De04
Ja05
Mr05
Ap05
My05
Jn05
Jl05
b)
0.6
0.5
0.4
0.3
0.2
0.1
0
β_t
Jn
Jl
Se
Nv
Ja
Fe
Mr
Ap
My
Jn
Jl
Au
Se
Oc
Nv
De
Ja
Fe
Mr
Ap
My
Jn
Jl
Au
Se
Nv
De
Ja
Mr
Ap
My
Jn
Jl
2002
2003
2004
2005

Figure 5

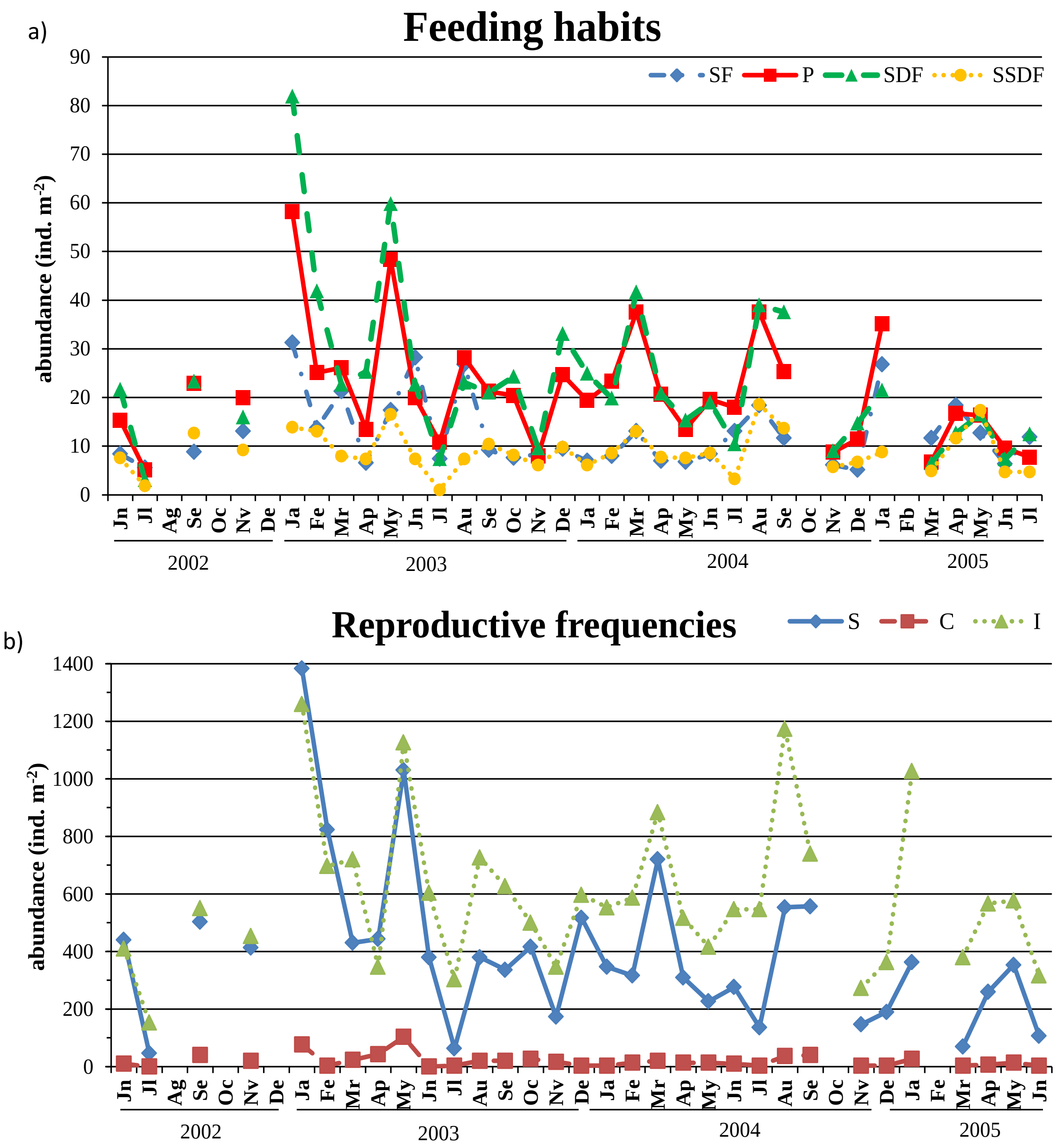

a)
Feeding habits
SF
P
SDF
SSDF
abundance (ind. m-2)
0
10
20
30
40
50
60
70
80
90
Jn Jl Ag Se Oc Nv De Ja Fe Mr Ap My Jn Jl Au Se Oc Nv De Ja Fe Mr Ap My Jn Jl Au Se Oc Nv De Ja Fb Mr Ap My Jn Jl
2002
2003
2004
2005
b)
Reproductive frequencies
S
C
I
abundance (ind. m-2)
0
200
400
600
800
1000
1200
1400
Jn Jl Ag Se Oc Nv De Ja Fe Mr Ap My Jn Jl Au Se Oc Nv De Ja Fe Mr Ap My Jn Jl Au Se Oc Nv De Ja Fe Mr Ap My Jn
2002
2003
2004
2005

Figure 6

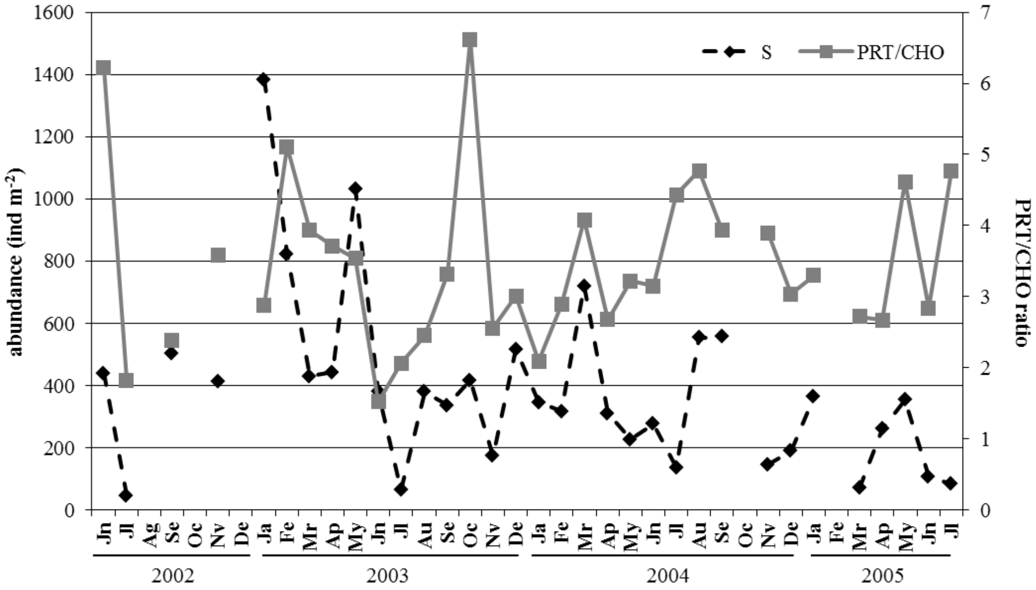
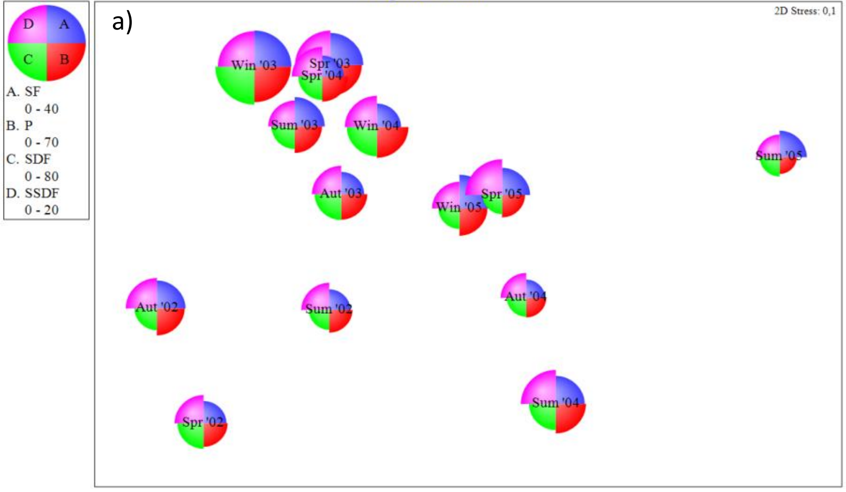

1600
1400
1200
1000
800
600
400
200
0
abundance (ind m-2)
7
6
5
4
3
2
1
0
PRT/CHO ratio
S
PRT/CHO
Jn
Jl
Ag
Se
Oc
Nv
De
Ja
Fe
Mr
Ap
My
Jn
Jl
Au
Se
Oc
Nv
De
Ja
Fe
Mr
Ap
My
Jn
Jl
Au
Se
Oc
Nv
De
Ja
Fe
Mr
Ap
My
Jn
Jl
2002
2003
2004
2005

Figure 7

Trophic habits



Reproductive frequencies

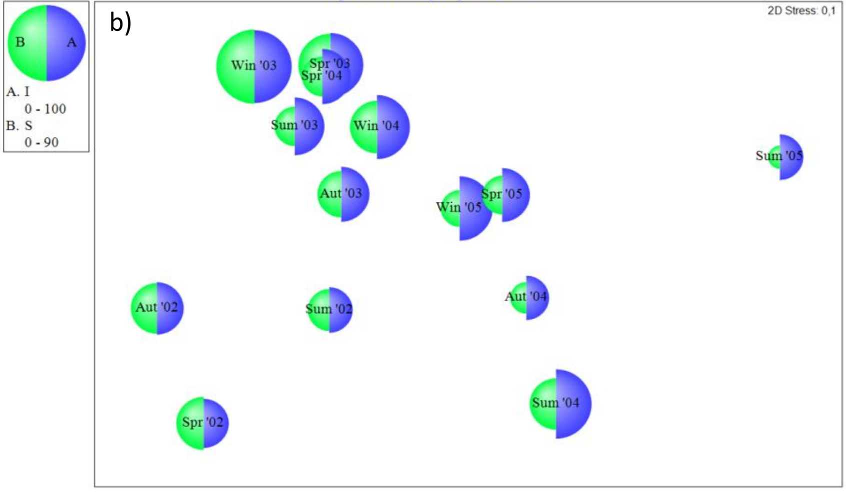


Figure 8

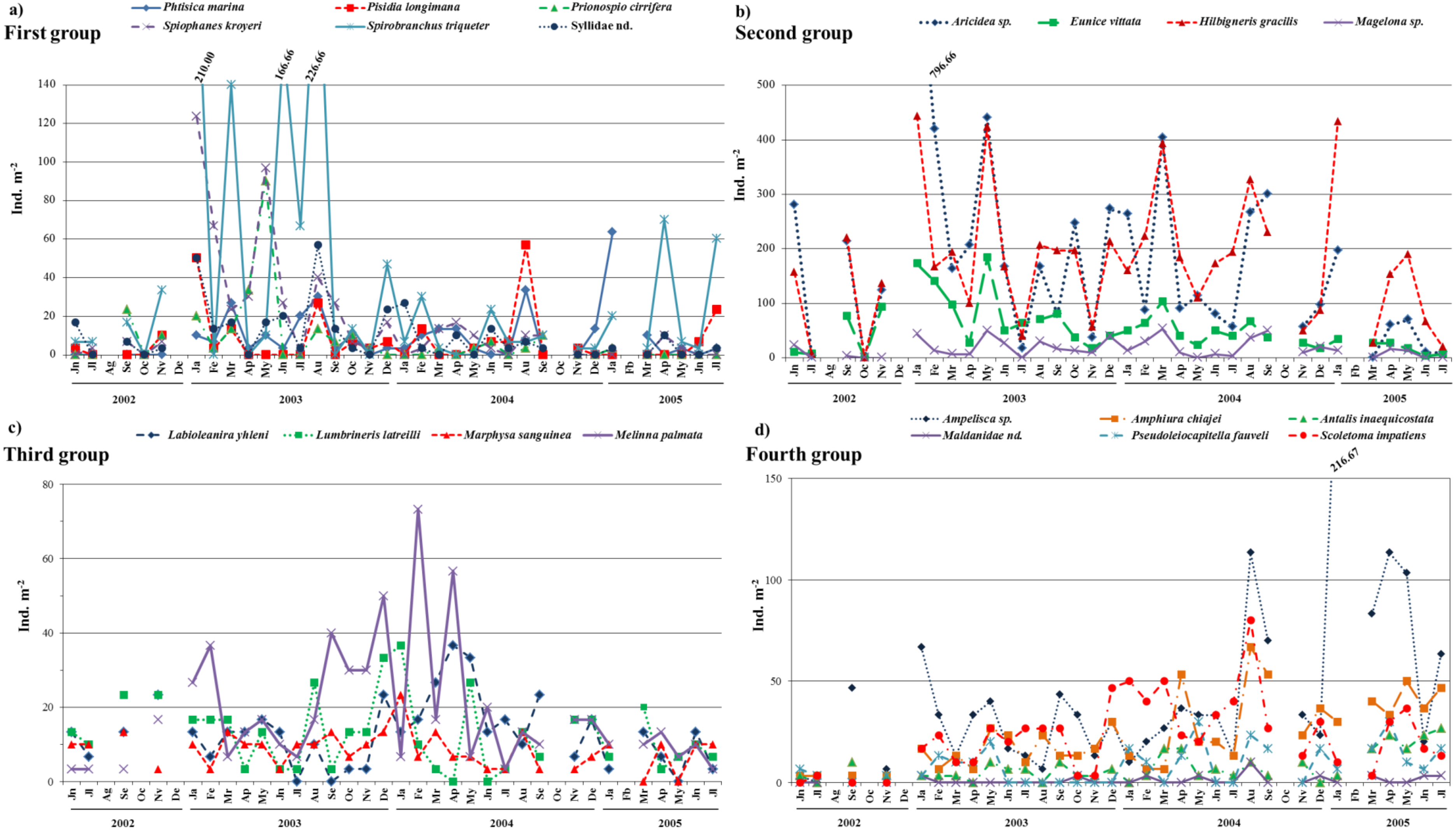
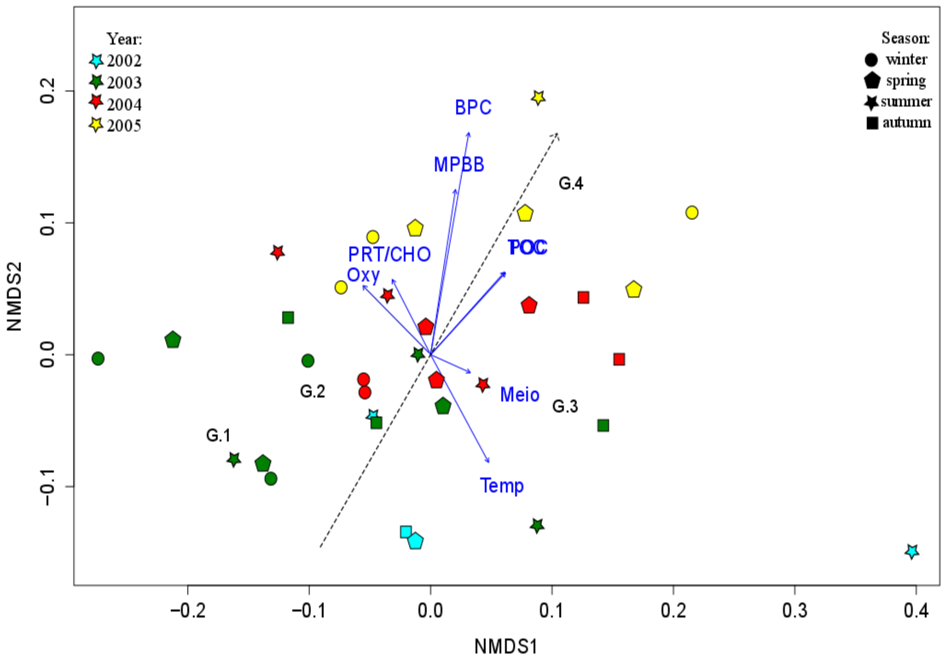

a)
First group
Phtisica marina
Pisidia longimana
Prionospio cirrifera
Spiophanes kroyeri
Spirobranchus triqueter
Syllidae nd.
210.00
166.66
226.66
Ind. m-2
140
120
100
80
60
40
20
0
Jn Jl Ag Se Oc Nv De Ja Fe Mr Ap My Jn Jl Au Se Oc Nv De Ja Fe Mr Ap My Jn Jl Au Se Oc Nv De Ja Fb Mr Ap My Jn Jl
2002
2003
2004
2005
b)
Second group
Aricidea sp.
Eunice vittata
Hilbigneris gracilis
Magelona sp.
796.66
Ind. m-2
500
400
300
200
100
0
2002
2003
2004
2005
c)
Third group
Labioleanira yhleni
Lumbrineris latreilli
Marphysa sanguinea
Melinna palmata
Ind. m-2
80
60
40
20
0
2002
2003
2004
2005
d)
Fourth group
Ampelisca sp.
Amphiura chiajei
Antalis inaequicostata
Maldanidae nd.
Pseudoleiocapitella fauveli
Scoletoma impatiens
216.67
Ind. m-2
150
100
50
0
2002
2003
2004
2005

Figure 9


Year:
2002
2003
2004
2005
Season:
winter
spring
summer
autumn
BPC
MPBB
PRT/CHO
Oxy
POC
TOC
Meio
Temp
G.1
G.2
G.3
G.4
NMDS1
NMDS2
−0.2
−0.1
0.0
0.1
0.2
0.3
0.4
−0.1
0.0
0.1
0.2

Paper II

TROPHIC SUBSIDY FROM THE PO RIVER DELTA AFFECT THE NEARBY COASTAL MACROBENTHIC COMMUNITY

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Manuscript

Author contribution statement:

Design and methods: LB, **FN**, FF

Data collection: LB,**FN**, FF

Data analyses: LB, **FN**, FF, RA,FR, DB

Manuscript preparation: LB, **FN**, FF,RA, FR, NM, DB

Project leader: LB

Abstract

Estuaries are highly productive ecosystems that can export organic matter to the coastal seas. However this process is seldom investigated taking into account the entire adjacent ecosystem (*e.g.* river distributaries and deltaic area) and hydrological setting (*i.e.* river discharge). Therefore we investigated the proportions of various primary producers contributing to plume of suspended particulate organic matter (SPOM) pool and the extent to which subsidies from rivers may influence macrobenthic community trophic structure and biomass. In December 2014 sediments and macrofauna samples were collected in the prodelta region (9 stations), at increasing distance from the main Po River mouth (*i.e.* Po di Pila). Potential estuarine food sources (*end members*) were collected within the Po River delta in three stations representing as much as possible the variability of the whole area (*i.e.* main river bank, branches and saltmarshes).

By mixing model the river SPOM and fringing vegetation were the principal riverine food sources for the macrofaunal community, highlighting the wide variation of organic load from the river distributaries of this delta. The main mouth exports organic matter from terrigenous and riverine phytoplankton, whereas the other river distributaries were important for seagrass input on the coastal trophic web. The community were dominant by surface deposit feeders in the station nearby the river mouth (the polychaete *Heteromastus filiformis*). The great majority of suspension feeders (above all the polychaete *Owenia fusiformis*) which correspond to the highest biomass were observed at the station slightly distant from the main organic loads and characterized by high idrodinamism. Subsurface deposit feeders gradually increased toward the deepest and clayey stations.

Overall, the macrofaunal community from the biomass measurement seem to be more structured due to the freshly organic matter observed at Po di Pila rather than the deepest stations, related to the high values of pheopigments/chlorophyll-a ratio. These findings highlighted the importance of integrative ecosystem-based management that maintains the connectivity of estuarine and coastal ecosystem.

Keywords: Food webs, Macrofauna, Stable isotopes, Food subsidy, River delta,

Introduction

Estuaries are considered among the most productive habitats due to the high *in situ* primary production, and inputs of organic matter transported from rivers (Dias et al. 2016). Due to tide water movements these environments typically exchange materials with outside receiving ecosystems, generating among the largest cross-boundary fluxes globally (Schlünz et al. 1999). These large quantities of terrestrial organic materials fuel disproportionately high rates of transformations in coastal ecosystems (estimate around 90% of modern carbon burial), impacting global bio-geochemical cycles (McKee et al. 2004), enhancing biological production and fisheries yields (Gaston et al. 2006). Organic matter and nutrients transfer across terrestrial and marine ecosystem boundaries and their incorporation by macrofauna in receiving ecosystems can influence communities' dynamics and feeding strategies and exert a relevant positive impact on the ecosystems functioning and services of adjacent marine coastal areas (Hocking et al. 2011; Savage et al. 2012).

The benthic habitats are important ecological components of the coastal zone. Moreover, the effect of rivers plumes in supporting macrozoobenthic feeding typologies in the adjacent coastal areas and the influence of riverine trophic subsidy on coastal macrofaunal abundance, biomass and diversity and trophic interactions remain poorly studied, limited to few estuaries or not inclusive of complex transitional areas like delta- prodelta systems. Prodelta areas are the primary interface between terrestrial and ocean environments and play a key role in linking the terrestrial and marine cycles of bioactive elements such as organic carbon. Indeed, it is thought that around 45% of the organic matter (OM) burial in marine environments occurs in deltaic regions (Aller 1998 and reference therein).

The Po River prodelta area receives a significant amount of OM and sediments from one of the largest European rivers. The Po River characterized by a mean daily discharge of 1500 m^3 s^{-1}, and periodically by floods (>5000 m^3 s^{-1})

represents the primary source of freshwater and nutrients entering the northern Adriatic Sea (Degobbis & Gilmartin 1990). The influence of this river can be observed in all the coastal ecosystem components especially because of the high land-derived input of allochtonous nutrients and the relevant transport of particulate inorganic suspended matter (Boldrin et al. 2005). The high nutrient concentrations discharged result in a highly impacted and eutrophic marine coastal area (Justic et al. 1995) which can lead to pathological condition of sediment hypoxia and consequently to the reduction or disappearance of sensitive organisms (Vollenweider et al. 1992). Changes of macrofaunal communities (abundances, diversity and trophic habits) in response to riverine inputs have been deeply investigated in coastal areas nearest to Po River delta (Ambrogi 1990; Simonini et al. 2004; Occhipinti-Ambrogi 2002; Occhipinti-Ambrogi et al. 2005). The macrobenthic community in this area, displays a high capacity to thrive and adapt to the peculiar environmental conditions of the area; it is characterized by a high abundance of a few dominant species (the bivalve *Corbula gibba* and the crustacean *Ampelisca diadema*), which are occasionally subjected to demographic blooms, and by a low dencity of many other species (Occhipinti-Ambrogi et al. 2005; Massamba N'Siala et al. 2008).

The condition and growth of macrofaunal organisms is dependent upon the quantity and quality of the food resources they assimilate over time. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) can provide time-integrated information about the source of food assimilated by macroinvertebrates and about trophic community structure and dynamics (Layman et al. 2012). If estuarine and marine food resources have their own distinct isotopic signatures the spatial subsidy of estuarine primary production and its contribution to secondary production can be assessed using stable isotopes in combination with mixing models (Philips et al. 2014). Moreover biomass-weighted isotope signatures can be used to assess the relative importance of different organic matter sources that contribute to animal's diet. Further, the study of trophic web over space, taking changes in species biomass provides opportunity to investigate energy pathways based on the structure and dynamics of species "feeding relationship" (Grall et al. 2006; Quillien et al. 2016). The potential of matching stable isotope analysis and mixing models with quali-quantitative studies of macrobenthic communities has been recently addressed as an effective strategy to assess and disentangle the effects of multiple environmental impacts in coastal marine ecosystems (Bongiorni et al. 2016).

In this study we attempt to estimate whether the Po River runoff, in particular after a period of major river floods, influences the food subsidies for the macrofaunal community in the adjacent prodelta area. More precisely, the aim of the present study was to answer the following questions 1) Are the macrofaunal isotopic composition and feeding habits of the prodelta area affected by riverine inputs? 2) Which is the contribution of terrestrial and marine origin food sources to the prodelta benthic consumer's diet? 3) At which trophic level the Po River subsidy influences macrofaunal biomass?

The isotopic composition of benthic infauna was investigated in the prodelta area at increasing distance from the main river distributaries, following the principal river front. The contribution of different terrestrial *vs* marine primary food sources to benthic consumers was evaluated using an isotopic mixing model.

Material and Methods

Study area and sampling

The Po River, with a drainage basin of 71000 km^2 and a length of 673 km, is the most important river in Italy and one of the largest in Europe. The Po supplies over 50% of the fresh water to the northern Adriatic (Mediterranean Sea) basin via a large delta (Degobbis et al. 1986). This river is characterized by an annual mean freshwater discharge of 1525 m^3 s^{-1} (recorded at Pontelagoscuro gauging station) and by two annual floods associated with rainfall in autumn and snowmelt in spring (Boldrin et al. 2005). The large Po delta includes five distributary mouths, from north to south: Maestra, Pila, Tolle, Gnocca and Goro (Fig. 1). Pila, the main distributary channel, accounts for 60% of the total freshwater discharged in the delta whereas Maestra, Tolle, Gnocca and Goro supply the remaining 3%, 12%, 16%, and 8%, respectively. During normal flow conditions, fine-grained sediments in the Po River undergo rapid deposition close to its mouths. Subsequently, the sedimentary plume is principally transported southward along the shelf due to the predominant cyclonic current Western Adriatic Coastal Current and wind-induced resuspension events promoted by the north-easterly Bora winds. Infrequently, southerly Scirocco wind events lead the river flow northward (Fain et al. 2007 and references therein).

Sampling was carried out in the Po prodelta area during a rapid-response survey (15th-19th December 2014). The sampling period followed the two most relevant river floods occurred during the whole 2014 (maximum discharge rates registered at the Pontelagoscuro gauging station on 19th November and 5th December, were ca. 8501 and 5930 $m^3 s^{-1}$ respectively (www.arpae.it). The river front was monitored in real time by satellite images and thus nine sampling stations located along the river plume were chosen (Fig 1). Bottom depth of sampling stations varied from 8 to 14 m, with the exception of stations C19, C22 and C23 in which depth ranged between 18 and 27 m.

Sample collection

In each sampling site, Suspended Particulate Organic Matter (SPOM) was collected using Niskin bottles at ca. 1 m below surface and 1 m above sea-bottom. At each station, sediments samples were collected using a van Veen grab (0.1 m^2) form sediment organic matter (SOM) and macrofaunal communities. Overall six replicated grabs were collected in each station and sediment samples were sieved through 1 mm mesh to retain the macrofaunal organisms.

Macrofauna obtained from three grabs were immediately fixed with formaldehyde solution (4 % v/v final concentration in seawater) for community structure analyses (abundance, biomass and species composition) while the other three grabs were used for stable isotopes analysis (SIA) of macrofaunal organisms.

Additional surface sediment samples were collected and immediately frozen for the determination of physical and chemical characteristics of the sediments: grain-size distribution, Total Organic Carbon (TOC), Total Nitrogen (TN), chlorophyll-*a* and phaeopigments.

Sample processing

Water sub-samples (300-1000 ml) for SPOM stable isotope analysis were suspensioned on pre-combusted (450°C, 4h) GFF suspensions (Whatman) and immediately frozen. Replicate suspensions were oven dried at 60 °C for 48 h before SIA. Sediments samples for SOM were sieved at 2 µm, oven dried (60°C, 48 hrs) and homogenized with a mortar and pestle before SIA.

For each station, aliquots (10-15 g) of homogenised sediment were collected for grain-size analyses and processed as described by Franzo et al. (2016). The analyses were performed using a Malvern Multisizer 2000S. Data are expressed as percentage of sand, silt and clay. For TOC analysis, triplicate subsamples of homogenised sediment (<250 µm) were weighed directly in a capsule (5x9 mm), treated with increasing concentrations of HCl (0.1N and 1N) to remove carbonates (Nieuwenhuize et al. 1994) and determined according to the methods of Pella and Colombo (1973) and Sharp (1974). TOC and TN values were used to calculate the C/N molar ratio as a proxy for the organic matter content and terrigenous input from the river. Pigments were extracted overnight (4°C, 90% acetone) from 0.7-0.9 g of wet sediment and analysed spectrofluorometrically following the procedures described by Lorenzen and Jeffrey (1980). In this study the pheopigments and chlorophyll-*a* ratio (PHEO/CHLA) was assessed to indicate the fraction of metabolically active auto-trophic component (Dell'Anno et al 2002).

Potential riverine food sources

Potential estuarine food sources (*end members*) were collected within the Po River delta in three stations: Pila (along the main distributary channel), Porto Barricata (at the mouth of Tolle distributary) and Gorino (inside Goro lagoon, Fig.1). Stations were chosen to represent as much as possible the variability of the whole area (*i.e.* main river banks, branches and saltmarshes). The collected material included bulk sediments, zooplankton, phytoplankton, SPOM, terrigenous detritus, microphytobenthos, seagrasses and riparian vegetations (C3 and C4 plants).

Bulk sediments were collected using a small van Veen (0.4 m^2) and treated as explained above. Zooplankton samples were collected by horizontal trawls of a WP2 net (200 µm mesh size). Organisms were sorted under a stereomicroscope, rinsed with distilled water and oven dried at 60ºC for 24-48 hours. Phytoplankton samples were collected by vertical deployment of a phytoplankton net (20 µm mesh size) and then filtered through a 200 µm Nitex mesh in order to discard big debris. Samples were mixed, decanted and filtered onto 25 mm precombusted GFF filters and oven-dried at 60ºC for 24-48 hours before SIA. However due to the low phytoplankton abundance and the predominance of riverine detritus in phytoplankton samples we were not able to analyse phytoplankton's stable isotopes. Therefore SPOM (including a mix of phytoplankton and detritus) was considered as *end member*. Samples for microphytobenthos were collected in the Po River delta using sediment cores (dark PVC, three cores *per* site) and kept in the dark. Sediment cores were upright covered with filer paper and exposed to light for ca. 10 hours to allow algal migration through filters. However, due to the low microphytobenthos biomass typical of the late autumn-winter period, and its further decrease likely occurring during river plume due to sediment burial (Cibic et al. 2012), did not allow us to including this source in the model. Seagrasses, plants and detritus samples were carefully cleaned from epiphytic organisms, rinsed in distilled water and oven dried for further analyses. *End members* of the Po delta were analysed separately for the three sampling sites but were pooled to obtain representative isotopic values of resources from the whole delta area.

Macrofauna samples processing

Samples for macrofauna were sorted using a stereomicroscope (Zeiss Discovery V.12, 8-100X magnifications) and all animals were counted and identified to the lowest possible taxonomical level. For identification, the taxonomical keys listed in Morri et al. (2004) were used. Biomass of each taxon was measured by ash-free dry weight after combustion at 450°C for 24h. Abundance and biomass were converted to units per m^2.

The feeding habits as functional traits for the analysed invertebrates were assigned in order to understand the predominant trophic strategies related to the available food sources in the sampled area. Six different feeding habits were assigned to all individuals: suspension feeders (SF), surface deposit feeders (SDF), subsurface deposit feeders (SSDF), predators (P) and omnivores (OMN), using the following criteria: morphology of the feeding apparatus, feeding mode, nature and origin of the food. Traits for each taxon were derived from literature sources (*i.e.* Desrosiers et al. 2000 and Jumars et al. 2015) and databases (*i.e.* MarLIN 2006 and Polytraits Team 2016).

Stable isotopes measurements

Stable isotopes analyses were carried out on the whole body of macrofaunal organisms. Since we were interested in the average stable isotope composition of each species and thereafter trophic guilds, individuals of each species were combined to reduce the effect of intra specific variability. Moreover to account for ontogenetic variations in diet for each taxon only organisms of similar size were analysed. Invertebrates' $\delta^{13}C$ were not corrected for lipid content because the shifts in $\delta^{13}C$ associated with lipid removal can be very variable and taxon-specific (Logan et al. 2008; Mateo et al. 2008).

Macrofauna specimens were oven dried at 60°C for 48-72 h prior to bulk SIA. Overall, prior to analyses, samples containing traces of carbonates (*e.g.* POM suspensions, seagrass and some invertebrate organisms) were divided into two subsamples, one of which was pre-treated with dilute (1 N) HCl to remove carbonates and the other was not acidified and was used for $\delta^{15}N$ analyses (Mateo et al. 2008). Bulk and acidified samples were weighed and placed into tin capsules to measure $\delta^{13}C$ and $\delta^{15}N$ and % of C and N in one run. The analyses were carried out on an Isotope Ratio Mass Spectrometer (Delta Plus, Thermo Scientific) coupled with an Elemental Analyzer Trace GQ (Thermo Fisher). The isotopic composition was denoted in delta (δ) notation, *i.e.* differences, between isotopic ratios in the samples and in international standard VPDB (Vienna Pee Dee Belemnite) for $\delta^{13}C$ and AIR for $\delta^{15}N$ and expressed in ‰ (Coplen 2011) using the formula: $\delta^{13}C$ or $\delta^{15}N$ (‰) = $(R_{sample}/R_{standard}) - 1) \times 10^3$; where $R={}^{13}C:{}^{12}C$ or ${}^{15}N:{}^{14}N$. The isotopic values were calculated against working in-house standards, which were themselves calibrated against international reference materials: L-glutamic acid USGS 40 (IAEA International Atomic Energy Agency, Vienna, Austria), fuel oil NBS-22 (IAEA) and sugar IAEA-CH-6 for $\delta^{13}C$, L-glutamic acid USGS 40 and potassium nitrate IAEA-NO3 for $\delta^{15}N$. The C% and N% were measured using as reference material atropine (Carlo Erba, Milano) with certified percentage of C and N. Uncertainty of determinations was 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$.

Data analyses

Based of sediment grain-size compositions which reflected a river depositional gradient, and distance from the main Pila distributary, three main groups of sampling stations could be distinguished: the northern stations (C1, C3 and C8), represented by stations close to the coastline and exclusively influenced by sediment deposition of the main Pila distributary mouth, the central group of near-coast stations (C10, C12, C16) located at increasing distance from Pila and influenced by secondary river distributary (Tolle), and the southern group of stations (C19, C20 and C23) located at increasing distance from the coastline and from Pila.

Biochemical and isotopic data of SOM were compared between stations' groups by mean of One- way ANOVA. All analyses were carried out using R software v 2.13.1. Prior to all analyses, data were checked for normality and homoscedasticy using Shapiro-Wilk's and Levene's test. When significant differences were observed, post hoc SNK tests was also performed.

Multivariate multiple regression analyses were performed to identify environmental drivers of macrofauna community composition in the investigated stations. The analysis was performed using the Distance-linear modelling (DistLM) analysis on macrofauna abundance matrix. Data were square root transformed and Bray-Curtis similarity was applied. Environmental variables were included as proxies of sediment texture (% of sand) and organic matter quality (PHEO/CHLA). Prior to the analysis, data were normalized. We used, as selection procedure, the option "All specified" and R^2 as the selection criterion. A distance-based redundancy analysis (dbRDA) plot was prepared using all the tested variables. Differences in macrofaunal species and stable isotope composition among the three groups of satiations were carry out with one-way PERMANOVA analysis (permutational multivariate analysis of variance). The two matrices (species abundance matrix and macrofaunal stable isotope one) were modified as follows: square root and log (X+1) transformation were used and Bray-Curtis similarity and Euclidean distance dissimilarity were applied, respectively. PERMANOVA test, DistLM and dbRDA analyses were performed using PRIMER 7 (PRIMER-E Ltd., Plymouth,UK).

In order to estimate the contribution to SOM of macroalgae, SPOM, terrigenous detritus and C3/C4 plants, the Bayesian mixing model SIAR (Stable Isotope Analysis in R, SIA TEF R v 4.2, Parnell et al. 2010) was applied. This model produces a range of feasible solutions given the available sources, while taking into account uncertainty and variation both in consumer and trophic enrichment factors. It also provide error terms that gives information on the variability that cannot be explained based on diet alone (residual error) (Parnell et al. 2010). Data for the mixing model consisted in carbon and nitrogen isotopic ratios of single sample and mean ± standard deviation of organic sources. For each model, discriminatory power was retained by selecting the best sources and by aggregating sources with similar isotopic values (Table S1, Phillips et al. 2014). In this study due to similar isotopic values, estuarine and prodelta SOM sources were pooled, as well as *Ulva* spp. and *Gracilaria* spp. macroalgae. Terrigenous sources such as the common reed (*Phragmites australis,* C3) and detritus (fragments of wood plants and leaves) display similar isotopic signatures and were distinct from C4 plant′ signals. Both fragments of riparian vegetation and wood-derived detritus were observed drifting in several stations inside the delta, therefore potentially transported offshore by currents, and were included in the model.

Marine *end members*′ values (phytoplankton, SPOM and zooplankton) were obtained from offshore North-West Adriatic water (Berto pers. comm., Table S1) and partially overlapped. Thus in the model only marine phytoplankton was included as representative of the main marine *end member*. Before running SIAR models, all δ^{13}C and δ^{15}N data were plotted to check the inclusion of samples in the convex polygon formed by the selected sources (Phillips et al 2014).

Bayesian stable-isotope mixing model was also used to estimate the contribution of terrigenous /riverine SPOM, marine SPOM, macroalgae, fringing vegetation/C3 to consumers′ diet. The contribution was estimated for each macrofaunal feeding habits. Models consider each station, organic pools and macrofauna feeding habits separately. Trophic enrichment factors (fractionation values) of + 2.5‰ ± 2.5 for δ^{15}N and 0.47‰ ± 1.23 for δ13C were used following Zaden and Rasmussen (2001).

Finally, in order to determine the influence of allochthonous food sources along the river plume on patterns of biomass (i.e. to identify changing pathways of trophic transfer) we weighted the isotope signature of every species funded in each sampling station and superimposed these to biomass (individual ash-free dry weight x abundance). This analysis produced a stable isotopes biomass bubbles plots where the area of circles reflected the biomass of each species. Further feeding habits information were added to the graph.

RESULTS

Environmental data and isotopic composition of basal sources

In the Po River prodelta, sediments were mainly represented by fine sediment fractions, as the percentage of silt, clay, and sand showed average of 68 ± 3, 28 ± 4, and 4 ± 3%, respectively. The sandy fraction decreased from north to south (11% and 1% St. C3 and St. C12, respectively) and from St. C16 to the more offshore and deepest stations (6% and 1% at St. C16 and C23, respectively), while an opposite pattern was observed for the percentage of clay. The TN content in sediments differed between stations' groups ($p<0.01$) and the SNK test showed higher values in the central (C8, C10, C12) and southern (C19, C22, C23) groups of sampling stations than in the northern ones (C1, C3 and C8, $p< 0.01$). Conversely, TOC values did not significant vary among groups (ANOVA, $p = 0.07$). The C/N ratio of sediments ranged between 8.62 and 10.59 (at C19 and C3, respectively), and value > 10 (10.17) occurred at the northern stations C1-C8 and at station C16 (in front of Scardovari lagoon). Chlorophyll-*a* content in sediments differed among groups (ANOVA, $p< 0.001$) and the SNK test showed higher values in the northern and central stations than in the southern group of stations. Phaeopigment values were higher in central and southern stations than in the northern ones (ANOVA; $p<0.05$, SNK test). The PHEO/CHLA of sediments ranged between 29.42 and 8.07 (at St. C23 and St. C3, respectively).

The $\delta^{13}C$ signature of SOM in the investigated area ranged between -22.42‰ and -28.87‰ at St. C23 and St. C16, respectively and its average value (-25.47 ± 1.71‰) was similar to bottom SPOM (Fig. 2). Prodelta $\delta^{15}N_{SOM}$ ranged between 1.75 and 3.74‰ at St. C1 and St. C23, respectively. And its average value

(2.82 ± 0.68‰) was less ^{15}N depleted than bottom SPOM (1.60 ± 1.55‰), and more similar to surface $\delta^{15}N_{SPOM}$.

Marine, riverine and terrigenous *end members* were distinct in term of isotopic compositions (Fig. 2). Po River $\delta^{13}C_{SPOM}$ was more ^{13}C-depleted than the prodelta SPOM, while $\delta^{13}C_{SOM}$ values were quite conserved in the river delta and in the adjacent prodelta. The δ^{15}N signatures of both SPOM and SOM of the Po River delta were higher than those of the prodelta area (Fig. 2). In order to run the mixing models, and avoid redundancy, whenever stable isotopic values of *end members* overlapped, data were pooled. Stable isotope data of SOM collected in the delta and prodelta area were pooled as well as values of fringing vegetation (mainly *Phragmites* spp., C3) and wood detritus, which were thereafter named as terrigenous detritus/C3 plants (Fig. 2).

The mixing model (SIAR), run to evaluate the mean contribution of terrigenous, riverine and marine organic sources to SOM isotopic composition, indicated that terrigenous detritus/C3 plants, was the dominant fraction, contributing between 22 and 60% (at St. C23 and at St. C1 and St. C16, respectively; Table S1). The riverine SPOM reached its highest contribution in stations St. C3, St. C10 and St. C12 (range 33-34%). The contribution of marine SPOM slightly decreased from north to south and from coast to more offshore stations (maximum contribution 21%, at St. C23). The highest seagrass contribution was observed at St. C16 (23%).

Macrobenthic community structure

Macrofaunal abundance in the Po River prodelta area ranged between 83 ± 75 ind. m^{-2} at St. C23 and 5333 ± 3409 ind. m^{-2} at St. C8. The lowest biomass value was measured at St. C8 (9.99 ± 6.70 g m^{-2}) while the highest at St. C8 (0.38 ± 0.50 g m^{-2}). Biomass decreased from the northern prodelta stations to the southern ones. Polychaetes were by far the most dominant group (60% of the total abundance), followed by molluscs (38%), crustaceans (7%), echinoderms (2%) and other minor groups (anthozoans, sipunculids and nemertines, >1%).

SF (58%) dominated the macrofauna communities and was followed by SDF (33%), SSDF (6%) and P (<2%). OMN remained the less represented group

(<1%) in the whole community. In stations located nearby the main Po River mouth (St. C1 and St. C3) the community was dominated by SDF (67% and 70%, respectively) due to the high presence of *Chamelea gallina* (bivalve) and *Heteromastus filiformis* (polychaete), respectively. At increasing distance from Pila (St. C8, St.C10 and St. C12), SF numerically dominated (82%, 76 % and 55%, respectively) mainly due to the presence of the polychaete *Owenia fusiformis* (St. C8) and the bivalve *Corbula gibba* (St. C12). Instead, the deepest stations (St. C19, St. C22 and St. C23) were characterized by an increasing abundance of SSDF. The polychaete *Sternaspis scutata* was numerically dominant at these stations reaching, at St. C22, the 83% of the whole community.

DistLM analyses showed that the cumulative percentage of the total variation of the macrofaunal community composition explained by environmental variables was 70%. A dbRDA analysis was used for the graphical visualization of the DistLM results. The first axis (dbRDA1) captures 43% of the fitted and 30% of the total variation between the macrofauna community composition of stations, while the second (dbRDA2) captures 23% of the fitted and 16% of the total variation. The C/N ratio explained the highest variability in the macrofauna communities (28%; Table S2). The dbRDA plot showed that the sampling stations were split into three groups of stations, located at increasing distance from the main river mouth main Pila mouth, as corroborated by PRMANOVA main test performed on species composition (Pseudo-F=2.52; $p<0.01$) (Fig. 3).

Isotopic composition of macrobenthic organisms

Isotopic analyses of macrobenthic consumers in the Po River prodelta area were obtained from 56 invertebrate taxa (24 molluscs, 15 polychaetes, 10 echinoderms, 5 crustaceans and 2 anthozoa). Within each taxon, the body size of specimens analysed for stable isotopes were homogeneous. The average values of the stable isotopes signals in macrobenthic organisms were -18.96 ± 2.80‰ ($\delta^{13}C$) and 7.30 ± 2.12‰ ($\delta^{15}N$, Table S3). $\delta^{13}C$ values of invertebrate consumers ranged between -7.52‰ and -25.02‰ (in the Scaphopoda *Antaris vulgaris*, St. C10 and the bivalve *Spisula subtruncata,* St. C3, Table S3). Nitrogen isotopes values in consumers ranged between 12.56 ‰ (in the bivalve *Nucula nucleus*, St. C10) and 1.74 ‰ (in the amphipod *Gammarus* sp.). Both $\delta^{15}N$ and δ13C of macrobenthic consumers show a significant differences among three group of stations (C1-C8, C10-C16, and C19-C23) representing area at increasing distance from the main Pila distributary River mouth (PERMANOVA: Pseudo-F=1.2 and 1.3; $p < 0.05$ for $\delta^{15}N$ and $\delta^{13}C$, respectively).

Differences in $\delta^{13}C$ signature of consumers reflect the origin (marine, terrigenous or riverine) of potential food source. Among macrofauna organisms, benthic primary consumers showed the most ^{13}C dappled isotopic signature typical of allochthonous riverine/terrestrial organic sources. The lower $\delta^{13}C$ values were observed at St. C3 in front of the Pila River mouth. Suspension feeders (SF) displayed lower $\delta^{13}C$ value at St. C3 (-22.61 ± 3.49‰) and gradually increased moving farther away toward north and south (-21.27 ± 2.65‰ and -17.28 ± 1.15‰, at stations C1-C8 and C10-C16, respectively, Fig. 4). In the southernmost C19-23 stations the $\delta^{13}C$ signal was again ^{13}C depleted (-20.21 ± 2.70‰). Similarly the average $\delta^{13}C$ signature of surface deposit feeders was lower at St. C3 (-22. 00 ± 2.18‰) compared to the other stations, even if a less clear decreasing gradient was evident. No clear differences were observed for SSDF between stations.

Contributions of food sources to primary consumers and analysis of trophic pathways

Overall, Bayesian mixing models for primary consumers included a high level of uncertainty for the primary producers, as indicated by the large credibility intervals that often included 0 at their lowest limit (Parnell et al., 2008; Table S4). The model showed some differences in the relative contribution of the OM sources to the diet of primary consumers (Fig. 5). The dual-isotope plots of macrofauna species and their potential food sources (not shown) showed that most animals were included in the convex hull formed by the identified sources, once they were corrected for fractionation. However some values did not fit the hull and were excluded, as result data for St. C12 and St. C19 (SF and SDF, respectively) and St. C22 (SSDF) could not be included in the analysis. Output of the model indicate that fringing vegetation/C4 plants contributed more than 28% to the diet of suspension feeders in all stations (Fig. 5) and the highest contribution (46%) was observed at St. C16. Riverine SPOM was the second source contributing to the SF diet (33 and 13 % at St. C3 and St. C16, respectively). Contribution of riverine SPOM gradually decreased from C3 toward C16 while increasing at the southern and deepest stations (St. C22 and St. C23; Fig. 5).

A similar pattern for riverine SPOM was also visible for SDF, even if differences were less marked. Fringing vegetation/C4 was again the main source contributing to the SDF diet (20 and 36% at St. C3 and St. C16, respectively, Fig. 5). The different sources almost equally contributed to SSDF diet.

By weighting the mean isotope signature of every taxon in the food web (food sources, primary and secondary consumers) by their respective mean biomass per square metre, major trophic pathways were visually identified. Near the Pila mouth (St. C1 and St. C3) main trophic pathway was channelled to SDF SSDF and SF (in particular, the bivalve *Pharaonella astula*: 1.05 ± 1.44 g m^{-2}; the polychaete *H. filiformis* biomass: 0.85 ± 0.28 g m^{-2} and the polychaetes *O. fusiformis* biomass: 2.99 ± 2.35 g m^{-2}, respectively). At the St. C8 the major trophic pathways was observed for SF due to the highest dominance in terms of abundance and biomass of the polychaetes *O. fusiformis*. At increasing distance from the Pila mouth SSDF generally dominated the community due to the high biomass of the polychaete *S scutata* (maximum at St. C19: 0.84 ± 0.22 g m^{-2}) (Fig. 6).

DISCUSSION

Riverine resource subsidies to coastal ecosystem

Primary production in the deltaic area provided food subsidies, contributing to secondary production of the adjacent coastal benthic communities. The stable isotope ratios indicated an important subsidy (up to 32%) from delta primary food sources (SPOM) to SOM in coastal area. These riverine sources were important food subsidies to *C. gibba* (SF), *O fusiform* (SF) and *P. astula* (SDF) particularly near Pila mouth. Further, the isotopic signatures of the SF cnidarians (Anthozoa sp.) in southern station (St. C23) were similar to SF bivalves (*Barbatia barbatia*) in the northern one (St. C3) (see Fig. 5 and Table S3), suggesting that similar organic matter sources were utilized and assimilated by both species. Accordingly, our findings support the “outwelling hypothesis” proposed by Odum et al 1979, demonstrating that excess estuarine primary production is adverted to the adjacent coastal system where benthic communities can take advantage of the higher production. A gradient of decreasing contribution of riverine primary production to coastal invertebrates an increasing distance from the main river distributary mouth was observed. The latter was in consistent with our hypothesis and other studies. In according with Savage et al (2012) and Claudino et al (2015) in estuarine areas the riverine primary production is principally basal sources for coastal marine food web.

Our results evidenced other allochthonous sources such as, fringing vegetation/C3 plants that contributed for more than 30% to SF and SDF invertebrates, especially toward the southern stations. This high percentages was principally observed at the group of station (C10-C12) located adjacent to the coastline, nearby to the river distributary (Porto Tolle). Within this deltaic area, numerous lagoons (*e.g.* Scardovari) with saltmarsh environments were present. We inferred that during the river flood the distributaries (above all Po di Tolle) could have influenced the coastal macrofaunal trophic pathways in the same way of the principal distributary (Pila). As highlighted by Tesi et al. 2011 when the river experience large floods the relative proportion between the main and secondary distributary channels change.

In this deltaic area we observed two different contributions from the main distributary and the secondary one. From Pila mouth riverine-derived SPOM likely contained a mixture of estuarine plankton and resuspended microphytes that was advected to the open coast and provided a high quality food resource (confirmed by the high amount of chlorophyll-*a* and proteins recorded at St. C3) to the SF macrofaunal invertebrates. SF in fact, tend to feed at the sediment-water interface (van Oevelem et al. 2006). By contrast, from Po Tolle, which goes through saltmarsh environments prior to touch the open sea mouth (Tesi et al. 2008), could be exported larger quantities of seagrass detritus to the offshore benthic community. Seagrass is more refractory than plankton and microphytobenthos and must be degraded by bacteria before it being available to SF and SDF invertebrates (Caraco et al. 1998) (see Fig. 5). This was also supported by our isotope values of macrofaunal invertebrates, which showed substantial enrichment in ^{15}N of the detrital seagrass offshore. In particular this was more evident at St. C16, with the macrolagae contribution of SOM was very high (up to 60%). The enrichment in ^{15}N at the latter station was more promptly in some SDF echinoderms (*Amphiura filiformis*) and SF cnidarians (Anthozoa nd.) (see Fig. 6).

Community structure

Many studies highlighted the influence that the river flows have on macrofaunal community structure from experimental (Eggleston et. al 1999; Thrush et al. 2003) spatial (Thrush et al. 2004; Hermand et al. 2008) and temporal scales (Ambrogi et al. 1990; Occhipinti-Ambrogi 2000; Occhipinti-Ambrogi et al. 2005). At a large scale, physical environmental factors, mainly grain size distribution and depth, determine broad patterns of benthic organism distribution (Hermand et al. 2008; Occhipinti-Ambrogi et al 2005). Species composition significantly related to the grain size was observed from the norther stations (more sandy) to the southern ones (more clayey). In according to previous studies occurred in this area (Ambrogi et al. 1990; Massamba N'Siala et al. 2008) the stations nearby the main river mouth were characterized by the following dominant species: *C. gibba*, *H. filiformis, O fusiformis* and *P. astula*. The latter decreased toward the deepest stations were species alike small gain-size distribution.

At smaller scale, macro-invertebrate distribution is drove by other abiotic and biotic factors such as quantity and quality of food supply (Thrush et al. 2004). Our results are in according to Hermand et al. (2008); no significant change in measured TOC, due to inorganic inputs that dilute the whole organic matter fraction, were observed. However, there were significantly variations in origin and quality (C/N ratio) of the organic matter which may explain changes in macrofauna composition.

The three main groups of species distinguished by dbRDA (see Fig. 3), correspond to a successional dynamic in response to change in sediment conditions. At the main river mouth stations (St. C1 and St. C3) the community is subject to a high amount of terrestrial organic load and the numerically dominant taxa were SDF. The high density of *H. filiformis* was in consistent with other studies (Thrush et al. 2003; Hermand et al. 2008; Savage et al. 2012). The latter authors describe *H. filiformis* as deposit feeders, known to have an opportunistic behaviour. In fact, favourable environmental conditions which following an increase of food supply can rapidly stimulate the growth, reproduction rate and survivorship of individuals. The SDF are not bulk ingestors of surface sediments, but they are rather able to modify their behaviour and feed on freshly organic metter at a very small spatial scale (Cruz-Rivera and Hay 2000), reaching high densities with a patch distributions (Thrush et al. 2004). The same opportunistic behaviour was observed for the SF O. *fusiformis* and *C gibba*, which greater densities were observed at St. C8. These species, in spite of fresh supplies was observed with few individuals at St. C1 and St. C3. The latter were characterized by high organic deposition, and thus affecting suspension feeding organisms by clogging feeding structure, inferring with particle selection and require the use of energy to clear away unwanted particles (Ellis et al. 2002). In fact the highest values of SF were observed at the St. 8 where fresh organic matter supplies were equally present with high hydrodynamic condition which enables a moderate terrestrial organic matter deposition (see Fig. 4 and 6). This community sampled at stations C10-C16 were characterized by higher structural variability behaving as transition zone (ecotone) between the northern and the southern stations. In according with other studies the deltaic/estuarine areas are characterized by ecotone as boundary zones between rivers and the sea with high variability of river loads (Attrill & Rundle 2002; Occhipinti-Ambrogi 2002). In this study, we observed a well-balance distribution of the feeding habits analysed between the northern stations and southern deepest ones, this can be linked to the presence of an ecotone.

The deepest group of stations (C19-C23) were characterised by SSDF in particular the species: S. *scutata* and the echinoderm *O. digitata*. These species are common in estuarine/deltaic area as belonged typically to Mediterranean VTC (biocoenosis of terrigenous mud), above all *S. Scutata* (Hermand et al. 2008 and reference therein). These species are significantly related to PHEO/CHLA ratio and fine grain-size distribution. SSDF with dwelling behaviour are also able to export organic matter in the deepest layers of sediment. In according to other studies (*e.g.* Desrosiers et al. 2000), SSDF were observed at stations with low chlorophyll-*a* contents and with more refractory organic matter (Herman et al

2008). This SSDF have generally a delay answer to food supply (Jumars & Wheatcroft 1989). This is also highlighted by mixing model, in which SF and SDF responded promptly (in their isotope signals) to the variation of food sources (*end members*) from northern to southern stations, whereas SSDF did not show the same patter.

Biomass structure and trophic level

Crossing between trophic levels measured and the biomass structure of the community was made in Fig. 6. To our knowledge, such approach combining species biomass distribution within the benthic community with stable isotope data for a large number of species was used by few studies (Grall et al. 2006; Quillien et al. 2016). From our analysis, it appears that the majority of the macrobenthic biomass is trapped into SF (in particular *O. fusiformis* at St. C8) SDF, SSDF and P. This confirmed that sediment organic matter originating from the water column (riverine loads) is indeed the major food source for the benthic community in the whole area. However we observed a simplification of community structure from the northern stations toward southern ones. Although southern stations received inputs from the main river mouth the quality of organic matter is low (express as high PHEO/CHLA ratios) and this could lead to a decrease in organic matter incorporation and therefore low macrofaunal biomass (see Fig. 6).

Conclusions

Deltaic areas provide important resource subsidies and contribute to secondary production of macrofaunal community in open coast ecosystems. This study highlighted the principal contribution of the river SPOM and fringing vegetation from the main river mouth and its distributaries along river plume, respectively. An increase in biomass close to the tributary of the principal river was registered, as well as changes in the trophic composition along the river gradient. Further, the study highlighted a close link between grain-size and macrofaunal structure and trophic pathways in a variable and highly hydrodynamic system as this deltaic area.

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References

Aller, R. C. (1998). Mobile deltaic and continental shelf muds as suboxic, fluidized bed reactors. Marine Chemistry, 61(3), 143-155.

Ambrogi, R., Bedulli, D., & Zurijni, G. (1990). Spatial and temporal patterns in structure of macrobenthic assemblages. A three-year study in the northern Adriatic Sea in front of the Po River Delta. Marine Ecology, 11(1), 25-41.

Attrill, M. J., & Rundle, S. D. (2002). Ecotone or ecocline: ecological boundaries in estuaries. Estuarine, Coastal and Shelf Science, 55(6), 929-936.

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. Marine Geology, 222, 135-158.

Bongiorni, L., Fiorentino, F., Auriemma, R., Aubry, F. B., Camatti, E., Camin, F., ... & Grall, J. (2016). Food web of a confined and anthropogenically affected coastal basin (the Mar Piccolo of Taranto) revealed by carbon and nitrogen stable isotopes analyses. Environmental Science and Pollution Research, 23(13), 12725-12738.

Caraco, N. F., Lampman, G., Cole, J. J., Limburg, K. E., Pace, M. L., & Fischer, D. (1998). Microbial assimilation of DIN in a nitrogen rich estuary: implications for food quality and isotope studies. Marine Ecology Progress Series, 167, 59-71.

Cibic, T., Acquavita, A., Aleffi, F., Bettoso, N., Blasutto, O., De Vittor, C., ... & Tamberlich, F. (2008). Integrated approach to sediment pollution: A case study in the Gulf of Trieste. Marine pollution bulletin, 56(9), 1650-1657.

Cibic, T., Comici, C., Bussani, A., & Del Negro, P. (2012). Benthic diatom response to changing environmental conditions. Estuarine, Coastal and Shelf Science, 115, 158-169.

Claudino, M. C., Pessanha, A. L. M., Araújo, F. G., & Garcia, A. M. (2015). Trophic connectivity and basal food sources sustaining tropical aquatic consumers along a mangrove to ocean gradient. Estuarine, Coastal and Shelf Science, 167, 45-55.

Coplen, T. B. (2011). Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Communications in Mass Spectrometry, 25(17), 2538-2560.

Crema, R., Castelli, A., & Prevedelli, D. (1991). Long term eutrophication effects on macrofaunal communities in northern Adriatic Sea. Marine Pollution Bulletin, 22(10), 503-508.

Cruz-Rivera, E., & Hay, M. E. (2000). Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. Ecology, 81(1), 201-219.

Dias, E., Morais, P., Cotter, A. M., Antunes, C., & Hoffman, J. C. (2016). Estuarine consumers utilize marine, estuarine and terrestrial organic matter and provide connectivity among these food webs. Marine Ecology Progress Series, 554, 21-34.

Degobbis, D., & Gilmartin, M. (1990). Nitrogen, phosphorus, and biogenic silicon budgets for the northern Adriatic Sea. Oceanologica Acta, 13(1), 31-45.

Degobbis, D., Gilmartin, M., & Revelante, N. (1986). An annotated nitrogen budget calculation for the northern Adriatic Sea. Marine Chemistry, 20(2), 159-177.

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.

Desrosiers, G., Savenkoff, C., Olivier, M., Stora, G., Juniper, K., Caron, A., ... & Roy, S. (2000). Trophic structure of macrobenthos in the Gulf of St. Lawrence and on the Scotian Shelf. Deep Sea Research Part II: Topical Studies in Oceanography, 47(3), 663-697.

Eggleston, D. B., Elis, W. E., Etherington, L. L., Dahlgren, C. P., & Posey, M. H. (1999). Organism responses to habitat fragmentation and diversity: habitat colonization by estuarine macrofauna. Journal of Experimental Marine Biology and Ecology, 236(1), 107-132.

Ellis, J., Cummings, V., Hewitt, J., Thrush, S., & Norkko, A. (2002). Determining effects of suspended sediment on condition of a suspension feeding bivalve (Atrina zelandica): results of a survey, a laboratory experiment and a field transplant experiment. Journal of Experimental Marine Biology and Ecology, 267(2), 147-174.

Fain, A. M. V., Ogston, A. S., & Sternberg, R. W. (2007). Sediment transport event analysis on the western Adriatic continental shelf. Continental Shelf Research, 27(3), 431-451.

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.

Gaston, T. F., Schlacher, T. A., & Connolly, R. M. (2006). Flood discharges of a small river into open coastal waters: plume traits and material fate. Estuarine, Coastal and Shelf Science, 69(1), 4-9.

Grall, J., Le Loc'h, F., Guyonnet, B., & Riera, P. (2006). Community structure and food web based on stable isotopes (δ 15 N and δ 13 C) analysis of a North Eastern Atlantic maerl bed. Journal of experimental marine biology and ecology, 338(1), 1-15.

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). Estuarine, Coastal and Shelf Science, 79(3), 367-376.

Hocking, M. D., & Reynolds, J. D. (2011). Impacts of salmon on riparian plant diversity. Science, 331(6024), 1609-1612.

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.

Justić, D., Rabalais, N. N., Turner, R. E., & Dortch, Q. (1995). Changes in nutrient structure of river-dominated coastal waters: stoichiometric nutrient balance and its consequences. Estuarine, Coastal and Shelf Science, 40(3), 339-356.

Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., ... & Post, D. M. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biological Reviews, 87(3), 545-562.

Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. Journal of Animal Ecology, 77(4), 838-846.

Lorenzen, C. J. (1967). Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnology and oceanography, 12(2), 343-346.

MarLIN, 2006.BIOTIC - Biological Traits Information Catalogue. Available at: https//www.marlin.ac.uk/biotic.

Mateo, M. A., Serrano, O., Serrano, L., & Michener, R. H. (2008). Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. Oecologia, 157(1), 105-115.

McKee, K. L., Mendelssohn, I. A., & D Materne, M. (2004). Acute salt marsh dieback in the Mississippi River deltaic plain: a drought-induced phenomenon? Global Ecology and Biogeography, 13(1), 65-73.

Morri, C., Bellan-Santini, D., Giaccone, G., & Bianchi, C. N. (2004). Principles of bionomy: definition of assemblages and use of taxonomic descriptors (macrobenthos). Biol. Mar. Medit, 11(Suppl 1), 573-600.

Massamba N'Siala, G. M., Grandi, V., Iotti, M., Montanari, G., Prevedelli, D., & Simonini, R. (2008). Responses of a Northern Adriatic Ampelisca–Corbula community to seasonality and short-term hydrological changes in the Po river. Marine environmental research, 66(4), 466-476.

Occhipinti-Ambrogi, A. (2002). Susceptibility to invasion: Assessing scale and impact of alien biota in the Northern Adriatic. CIESM Workshop, Monographs, 20.

Occhipinti-Ambrogi, A., Savini, D., & 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(1), 317-328.

Odum, W. E., Fisher, J. S., & Pickral, J. C. (1979). Factors controlling the flux of particulate organic carbon from estuarine wetlands. In Ecological processes in coastal and marine systems (pp. 69-80). Springer US.

Parnell, A. C., Inger, R., Bearhop, S., & Jackson, A. L. (2010). Source partitioning using stable isotopes: coping with too much variation. PloS one, 5(3), e9672.

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., ... & Ward, E. J. (2014). Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology, 92(10), 823-835.

Polytraits Team (2016). Polytraits, A database on biological traits of polychaetes. Available at http//polytraits.lifewatchgreece.eu.

Quillien, N., Nordström, M. C., Schaal, G., Bonsdorff, E., & Grall, J. (2016). Opportunistic basal resource simplifies food web structure and functioning of a highly dynamic marine environment. Journal of Experimental Marine Biology and Ecology, 477, 92-102.

Rossi, F. (2003). Short-term response of deposit-feeders to an increase of the nutritive value of the sediment through seasons in an intertidal mudflat (Western Mediterranean, Italy). Journal of experimental marine biology and ecology, 290(1), 1-17.

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.

Schlünz, B., Schneider, R. R., Müller, P. J., Showers, W. J., & Wefer, G. (1999). Terrestrial organic carbon accumulation on the Amazon deep sea fan during the last glacial sea level low stand. Chemical Geology, 159(1), 263-281.

Simonini, R., Ansaloni, I., Pagliai, A. B., & 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: Journal du Conseil, 61(6), 871-881.

Tesi, T., Langone, L., Goni, M. A., Miserocchi, S., & Bertasi, F. (2008). Changes in the composition of organic matter from prodeltaic sediments after a large flood event (Po River, Italy). Geochimica et Cosmochimica Acta, 72(8), 2100-2114.

Tesi, T., Miserocchi, S., Goñi, M. A., Turchetto, M., Langone, L., De Lazzari, A., ... & 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. Frontiers in Ecology and the Environment, 2(6), 299-306.

Thrush, S. F., Hewitt, J. E., Norkko, A., Cummings, V. J., & Funnell, G. A. (2003). Macrobenthic recovery processes following catastrophic sedimentation on estuarine sandflats. Ecological Applications, 13(5), 1433-1455.

Van Oevelen, D., Soetaert, K., Middelburg, J. J., Herman, P. M., Moodley, L., Hamels, I., ... & Heip, C. H. (2006). Carbon flows through a benthic food web: Integrating biomass, isotope and tracer data. Journal of Marine Research, 64(3), 453-482.

Vollenweider, R. A., Rinaldi, A., & Montanari, G. (1992). Eutrophication, structure and dynamics of a marine coastal system: results of ten-year monitoring the Emilia-Romagna coast (Northwest Adriatic Sea). Science of the total environment, 63-106.

Jumars, P. A., & Wheatcroft, R. A. (1989). Responses of benthos to changing food quality and quantity, with a focus on deposit feeding and bioturbation. Productivity of the ocean: Present and past. Wiley, 235-253.

Zanden, M., & Rasmussen, J. B. (2001). Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. Limnology and oceanography, 46(8), 2061-2066.

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Figures captions

Figure 1. Location of the coastal sampling stations in the Po River prodelta area. The main Po delta distributaries are depicted.

Figure 2. Stable isotopes bi-plot for prodelta sediment organic matter (SOM, green tringles) in all analysed prodelta stations, and autoctonous and allochthonous *end members* (mean ± SE). Average prodelta surface and bottom SPOM are depicted with blue and red diamonds, respectively. *End members* are: marine and riverine_zoo (zooplankton), marine and riverine_ phyto (phytoplankton), macroalgae, fringing veg/C4 (mainly undetermined plants having a C4 patway), terr det/C3 (wood detritus and C3- plants, mainly *Phragmites* canebrakes).

Figure 3. dbRDA ordinations of the distLM model which describe the relationship between the macrobenthic community composition and the sediments environmental variables: Organic Carbon (Corg), Carbon and Nitrogen ratio (C/N), Pheaopigments Chlorophyll-a ratio (PHAEO/CHLA), sand and clay content (%).

Figure 4. Mean $\delta^{13}C$ values of primary consumers' trophic guilds (suspended feeders and surface deposit feeders) in the Po River prodelta stations.

Figure 5. Output of the mixing model (SIAR) showing the mean percentage of contribution of potential food sources to primary consumers (suspension feeders, surface deposit feeders and subsurface deposit feeders). Values (mean, and 95 % confidence intervals) for each source are shown in Table S3 in the supplementary material

Figure 6. Stable isotope bi-plots showing macrofaunal consumers' isotope signature weighted by biomass per square meter (filled circles). Macro faunal feeding habits are indicated with different colours and sours isotope signatures with different shapes. DMalgae=delta macroalgae; DSPOM=delta suspended organic matter; Dplants=fringing vegetations, MSPOM=marine suspended organic matter; MSOM= marine sediment organic matter. Each macrofaunla consumer species is denoted by a number: 1=*Abra alba*; 2=*Acrocnida brachiata*,

3=*Acteon Tornatilis*; 4=*Amphiura chiajei*; 5=*Amphiura filiformis*; 6=*Antalis vulgaris*; 7=Anthozoa; 8=*Aricidea* sp.; 9=*Atlantella distorta*; 10=*Barbatia barbata*; 11=*Brada villosa*; 12=*Chamelea gallina*; 13=*Corbula gibba*; 14= *Cylichna cylindracea*; 15=*Diopatra neapolitana*; 16=*Dosinia lupinus*; 17=Echinodermata; 18=*Eulima glabra*; 19=*Euspira nitida*; 20=*Glycera* sp.; 21=*Glycera unicornis*; 22=*Heteromastus filiformis*; 23=*Hiatella arctica*; 24=*Hilbigneris gracilis*; 25=*Labioleanira yhleni*; 26=*Lanice conchilega*; 27=*Leptopentacta elongate*; 28=*Leptopentacta tergestina*; 29=*Loripinus fragilis*; 30=*Maldane glebifex*; 31=*Musculus subpictus*; 32=*Necallianassa truncata*; 33=*Nephtys hystricis*; 34=Nereidae; 35=*Nereis lamellosa*; 36=*Nucula nucleus*; 37=*Oestergrenia digitata*; 38=*Ostrea edulis*; 39=*Owenia fusiformis*; 40=*Pharaonella astula*; 41=*Phascolosoma (Phascolosoma) strombus*; 42=*Pisidia longimana*; 43=*Pitar rudis*; 44=*Processa* sp.; 45=*Sabella pavonina*; 46=*Scoletoma impatiens*; 47=*Sipunculus (Sipunculus) nudus*; 48=*Spisula subtruncata*; 49=*Sternaspis scutata*; 50=*Tellimya ferruginosa*; 51=*Thyone fusus*; 52=*Tritia mutabilis*; 53=*Tritia reticulata*; 54=*Turritella comunis*; 55=*Upogebia tipica*; 56=*Virgularia mirabilis*.

Figure 1

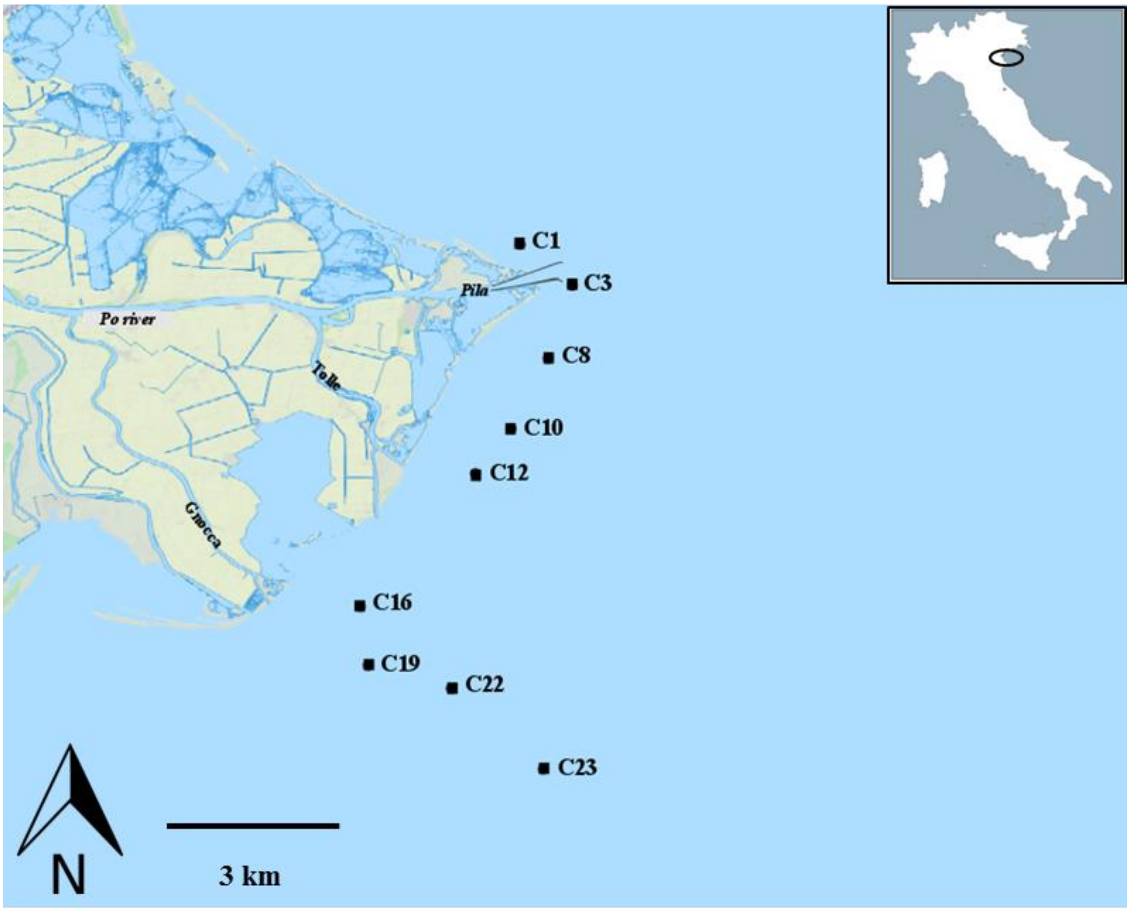
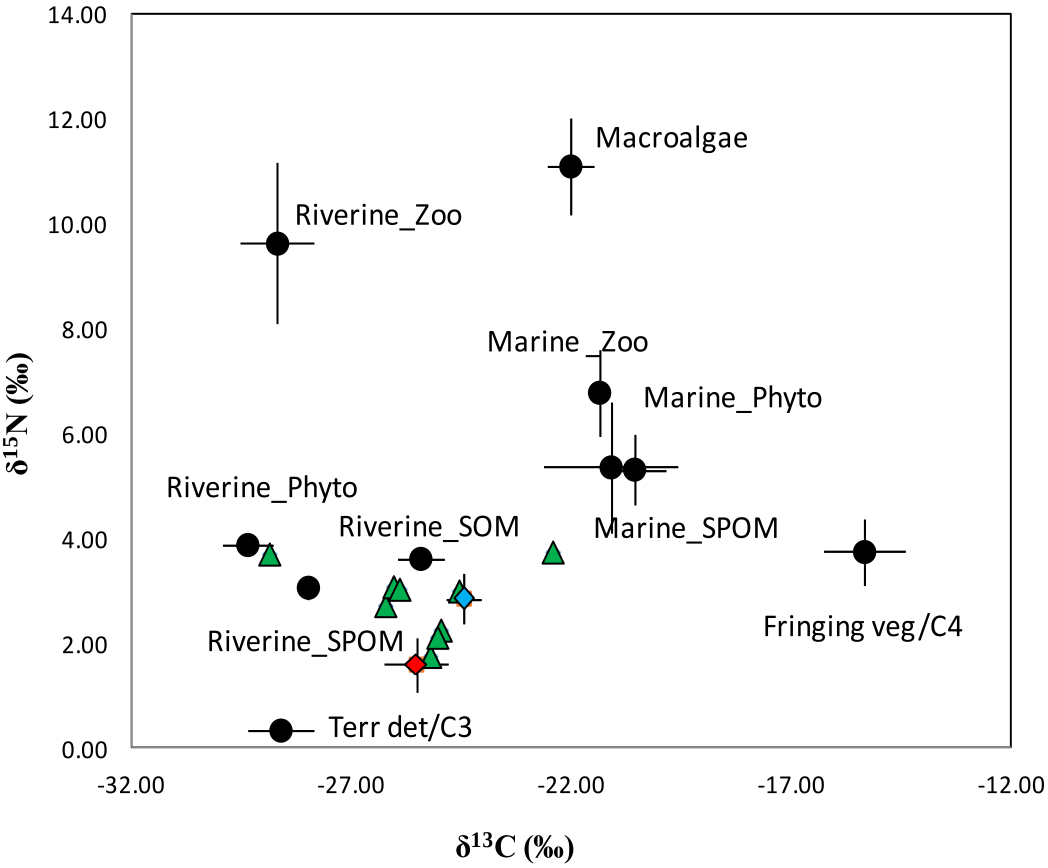

C1
C3
Pila
Po river
C8
Tolle
C10
C12
Gnocca
C16
C19
C22
C23
N
3 km

Figure 2



14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
δ15N (‰)
Macroalgae
Riverine_Zoo
Marine _Zoo
Marine_Phyto
Riverine_Phyto
Riverine_SOM
Marine_SPOM
Fringing veg/C4
Riverine_SPOM
Terr det/C3
-32.00
-27.00
-22.00
-17.00
-12.00
δ13C (‰)

Figure 3

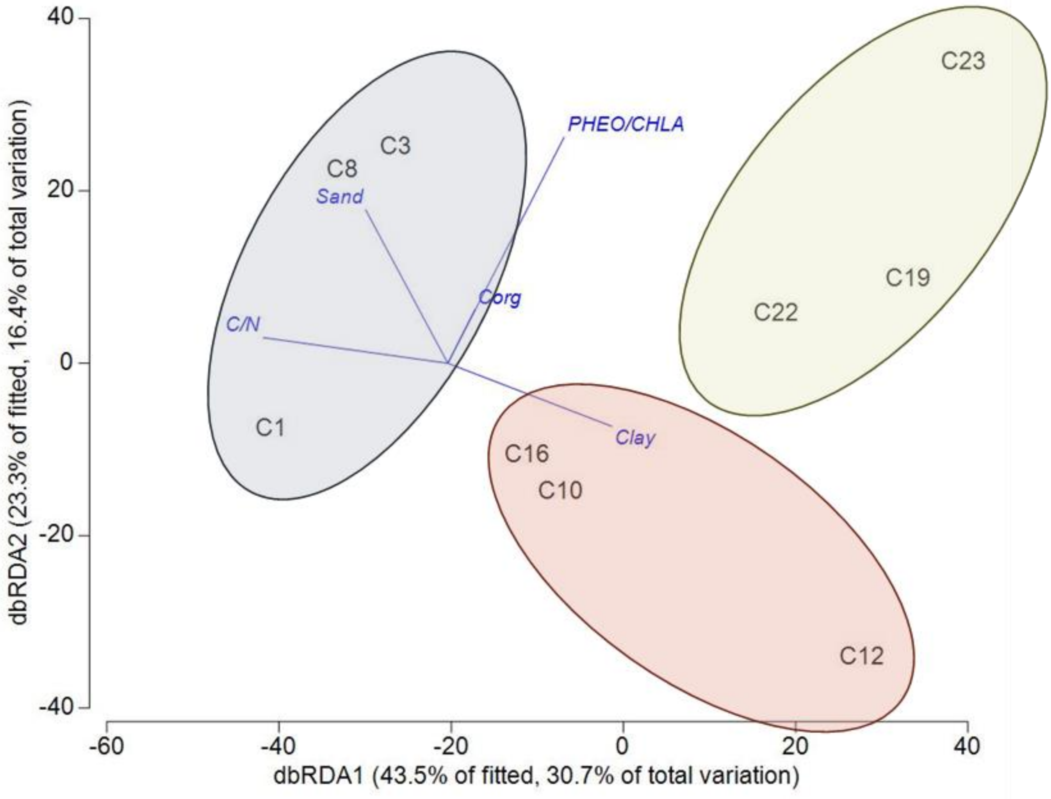

40
20
0
-20
-40
dbRDA2 (23.3% of fitted, 16.4% of total variation)
-60
-40
-20
0
20
40
dbRDA1 (43.5% of fitted, 30.7% of total variation)
C23
PHEO/CHLA
C3
C8
Sand
C19
Corg
C22
C/N
C1
Clay
C16
C10
C12

Figure 4

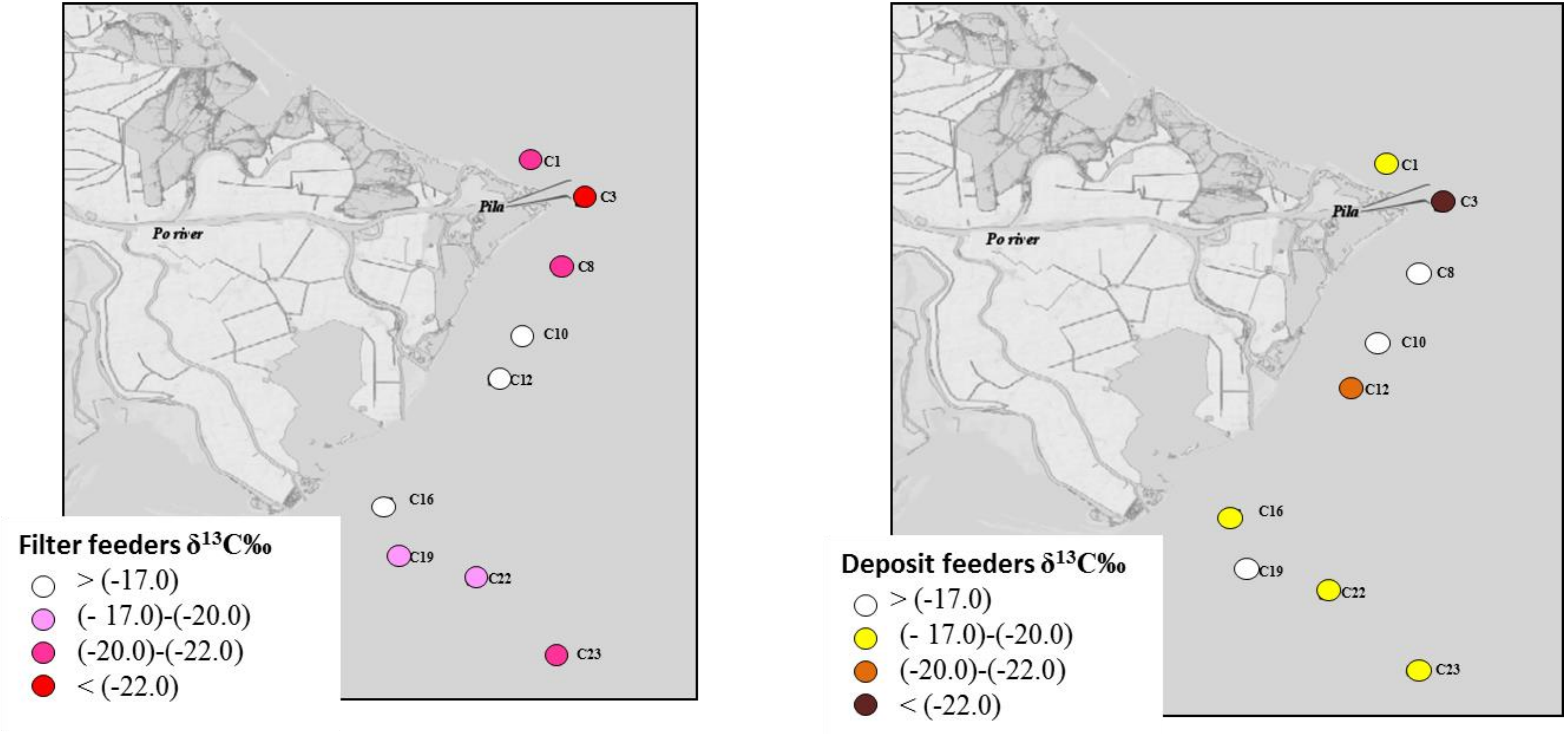

Po river
Pila
C1
C3
C8
C10
C12
C16
C19
C22
C23
Filter feeders δ13C‰
○ > (-17.0)
● (- 17.0)-(-20.0)
● (-20.0)-(-22.0)
● < (-22.0)
Po river
Pila
C1
C3
C8
C10
C12
C16
C19
C22
C23
Deposit feeders δ13C‰
○ > (-17.0)
● (- 17.0)-(-20.0)
● (-20.0)-(-22.0)
● < (-22.0)

Figure 5

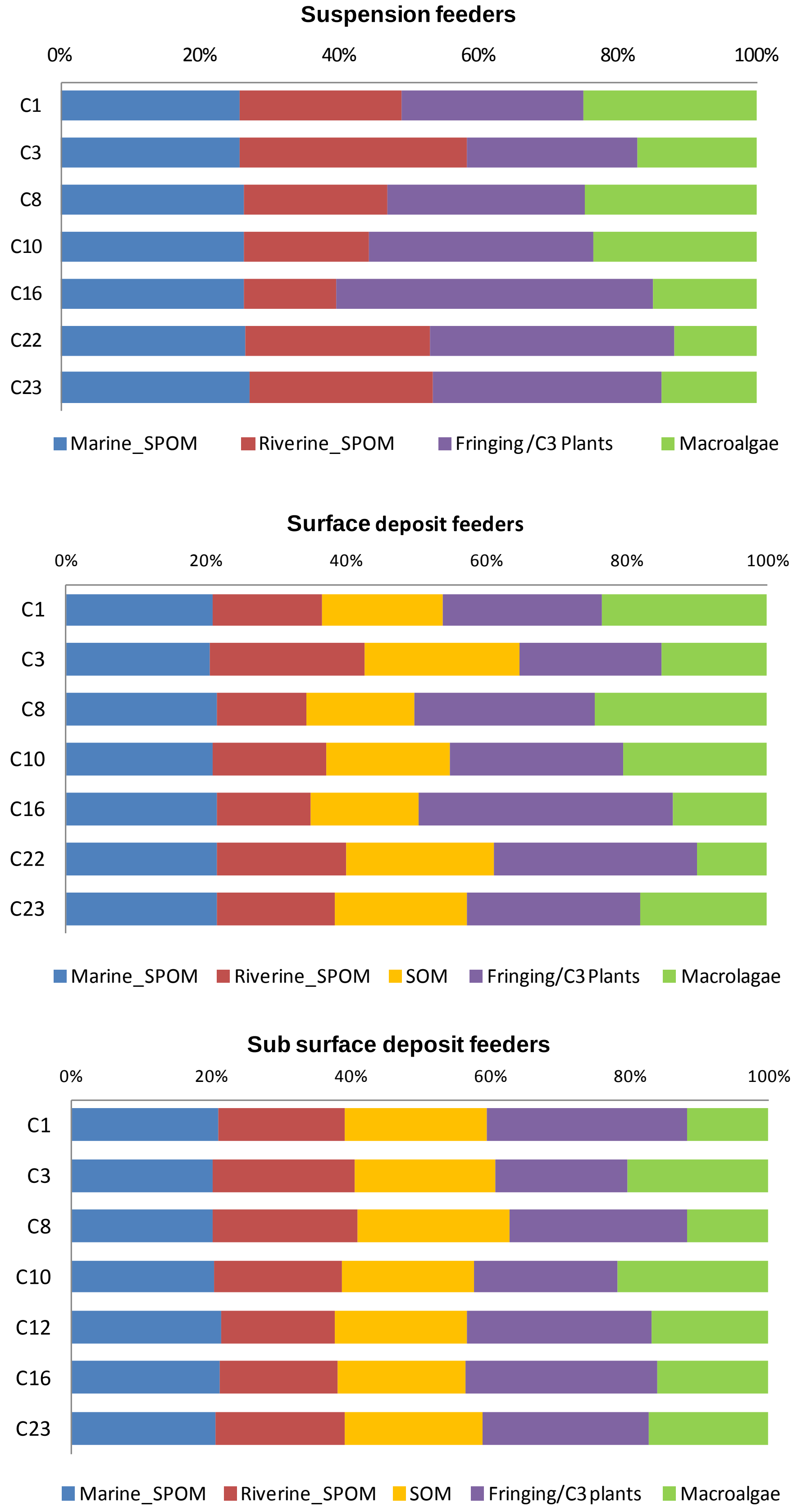
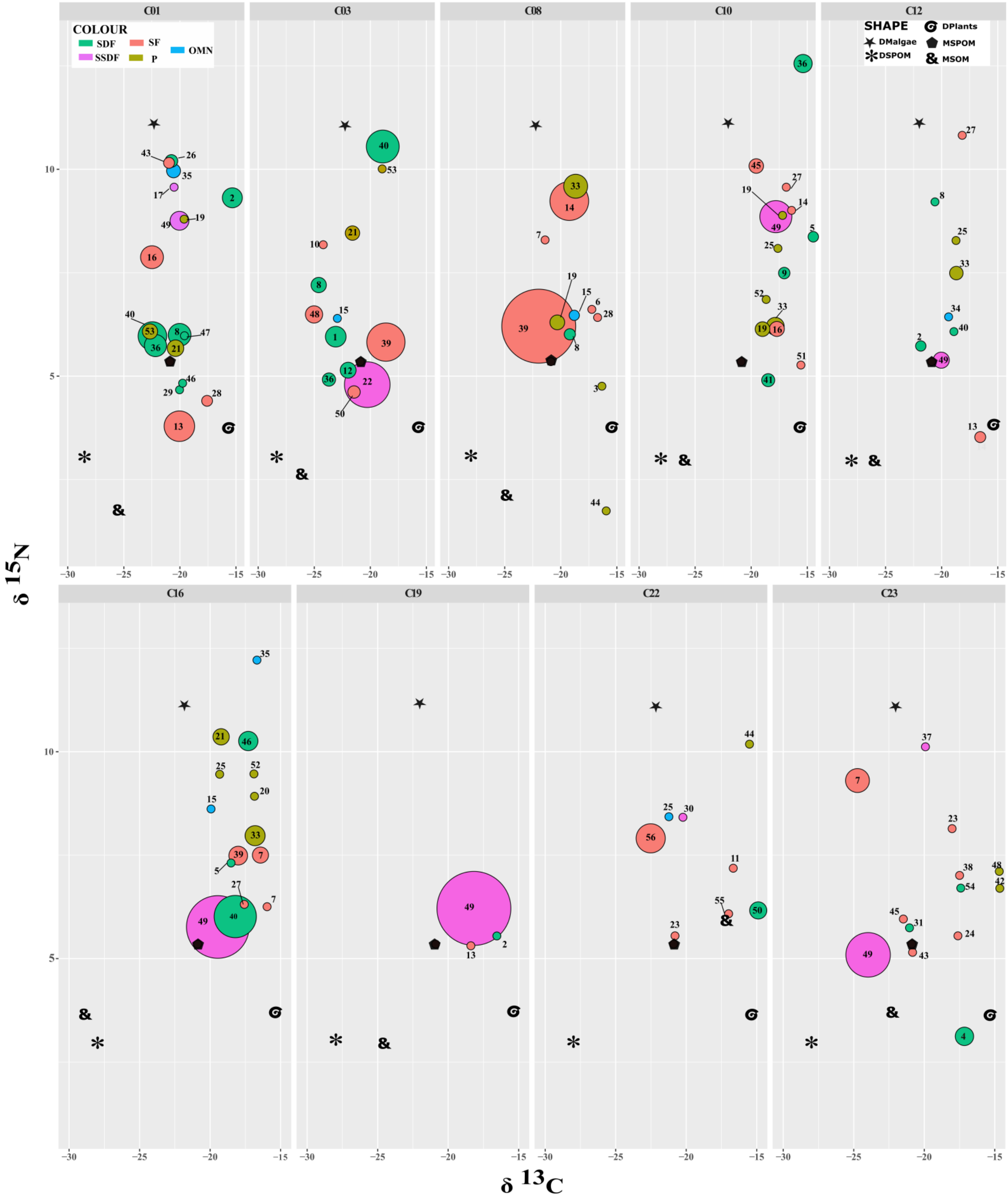

Suspension feeders
0%
20%
40%
60%
80%
100%
C1
C3
C8
C10
C16
C22
C23
Marine_SPOM
Riverine_SPOM
Fringing /C3 Plants
Macroalgae
Surface deposit feeders
0%
20%
40%
60%
80%
100%
C1
C3
C8
C10
C16
C22
C23
Marine_SPOM
Riverine_SPOM
SOM
Fringing/C3 Plants
Macrolagae
Sub surface deposit feeders
0%
20%
40%
60%
80%
100%
C1
C3
C8
C10
C12
C16
C23
Marine_SPOM
Riverine_SPOM
SOM
Fringing/C3 plants
Macroalgae

Figure 6


C01
C03
C08
C10
C12
C16
C19
C22
C23
COLOUR
SDF
SF
OMN
SSDF
P
SHAPE
DPlants
DMalgae
MSPOM
DSPOM
MSOM
δ 15N
δ 13C
10
5
−30
−25
−20
−15
43
26
35
17
2
49
19
16
40
53
8
47
36
21
46
29
28
13
40
53
21
10
8
48
15
1
39
12
36
22
50
33
14
7
39
19
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6
28
8
3
44
36
45
27
19
14
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5
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9
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33
19
16
51
41
27
8
25
33
34
40
2
49
13
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21
46
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15
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7
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49
43
4

Supplementary material

Table S1. Output of the mixing model (SIAR) showing the mean percentage of contribution of potential organic matter sources to the sediment organic matter (SOM) pool. Values (mean and 95 % confidence intervals) for each source.

Stations	Mean relative contribution (CI=95%) SOM				
	Marine_SPOM	Riverine_SPOM	Fringing veg/C4	Terrigenous det/C3	Macroalgae
C1	9 (0-24)	12 (0-29)	16 (2-28)	60 (44-75)	3 (0-7)
C3	12 (0-26)	33 (2-58)	7 (0-15)	41 (20-65)	7 (0-15)
C8	12 (0-31)	20 (0-40)	17 (2-29)	48 (29-66)	4 (0-9)
C10	13 (0-28)	34 (3-62)	7 (0-15)	37 (14-70)	9 (0-18)
C12	14 (0-30)	33 (3-60)	8 (0-16)	37 (15-60)	8 (0-17)
C16	3 (0-8)	12 (0-32)	1 (0-4)	60 (45-72)	23 (16-30)
C19	18 (0-37)	26 (1-47)	16 (1-28)	34 (16-54)	6 (0-14)
C22	11 (0-29)	18 (0-37)	17 (2-29)	51 (32-69)	3 (0-9)
C23	21 (0-40)	19 (0-38)	28 (14-42)	22 (4-39)	9 (0-19)

Table S2. Output of the DistLM analysis on macrofauna community composition in the Po River prodelta. Reported are the results of the Marginal Tests. SS: Sum of square. Prop: percentage of variance explained by each variable. P values having statistical significance are highlighted in bold. C/N= Carbon and Nitrogen ratio; PHAEO/CHLA = Pheaopigments Chlorophyll-*a* ratio; Corg=Organic Carbon.

MARGINAL TESTS

Variable	SS(trace)	Pseudo-F	P	Prop.
C/N	6794.6	2.77	**0.002**	0.28
PHAEO/CHLA	5548.1	2.11	**0.028**	0.23
Corg	2785.9	0.92	0.543	0.11
Clay	6156	2.42	0.006	0.25
Sand %	5060	1.87	0.053	0.21
res.df: 7				
Total SS(trace): 23967				

Table S3. Values of δ^{15}N, δ^{13}C and C:N (Carbon to Nitrogen ratio)of macrofauna specimens collected in the Po River Prodelta. Anfip (Amphipod); Ant (Anthozoa); Biv (Bivalve); Decap (Decapod); Ech (Echinoderm); Gast (Gastropod); Pol (Polychaete), Scaf (Scaphopoda); Sip (Siponculid); SF (Suspension Feeders); SDF (Surface Deposit Feeders); SSDF (Sub Surface Deposit Feeders); OMN (Omnivores); P (Predators).

Station	**Species**	**Trophic group**	**δ^{15}N**	**δ^{13}C**	**C:N**
C1	*Corbula gibba (Biv)*	SF	3.79	-20.05	7.45
	Dosinia lupinus (Biv)	SF	7.88	-22.49	5.98
	Leptopentacta tergestina (Ech)	SF	7.01	-18.47	4.76
	Pitar rudis (Biv)	SF	10.16	-20.97	5.33
	Acrocnida brachiata (Ech)	SDF	9.31	-15.33	5.30
	Aricidea sp. *(Pol)*	SDF	6.00	-20.03	6.42
	Lanice conchilega (Pol)	SDF	10.20	-20.76	5.98
	Loripinus fragilis (Biv)	SDF	4.67	-20.03	5.61
	Nucula nucleus (Biv)	SDF	5.74	-22.18	6.23
	Pharaonella astula (Biv)	SDF	5.97	-22.45	6.10
	Scoletoma impatiens.(Pol)	SDF	4.83	-19.76	5.98
	Sipunculus (Sipunculus) nudus (Sip)	SDF	5.98	-19.59	6.31
	Spisula subtruncata(Biv)	SDF	2.37	-11.46	16.35
	Sternaspis scutata (Pol)	SSDF	8.76	-20.04	5.76
	Echinodermata nd. *(Ech)*	SSDF	9.57	-20.52	5.01
	Nereis lamellosa (Pol)	OMN	9.96	-20.57	5.67
	Euspira nitida (Gast)	P	8.79	-19.63	5.75
	Glycera tessellata (Pol)	P	5.68	-20.39	5.65
	Tritia reticulate (Gast)	P	6.08	-22.66	5.45
C3	*Barbatia barbatia (Biv)*	SF	8.18	-24.20	6.15

	Owenia fusiformis (Pol)	SF	5.82	-18.61	4.97
	Spisula subtruncata (Biv)	SF	6.49	-25.02	6.26
	Abra alba (Biv)	SDF	5.95	-23.08	6.25
	Aricidea sp. *(Pol)*	SDF	7.20	-24.60	6.23
	Atlantella distorta (Biv)	SDF	4.62	-21.46	7.36
	Chamelea gallina (Biv)	SDF	4.79	-20.27	6.04
	Nucula sulcata (Biv)	SDF	4.92	-23.69	7.02
	Pharaonella astula (Biv)	SDF	10.55	-18.89	5.64
	Heteromastus filiformis nd. (Pol)	SSDF	4.79	-20.27	6.04
	Diopatra sp. *(Pol)*	OMN	6.40	-22.94	7.21
	Glycera unicornis (Pol)	P	8.46	-21.59	6.15
	Nassarius reticulatus (Gast)	P	10.01	-18.94	5.28
C8	*Anthozoa* nd. *(Ant)*	SF	8.29	-21.40	5.85
	Corbula gibba (Biv)	SF	9.23	-19.24	5.13
	Leptopentacta tergestina (Ech)	SF	9.59	-18.46	5.07
	Owenia fusiformis (Pol)	SF	6.21	-21.97	6.95
	Antalis vulgaris (Scaf)	SDF	6.51	-17.04	5.99
	Aricidea sp. *(Pol)*	SDF	6.01	-19.19	5.61
	Atlantella distorta (Biv)	SDF	4.82	-10.71	12.78
	Diopatra sp. (Pol)	OMN	6.47	-18.80	5.67
	Acteon tornatilis (Gast)	P	4.76	-16.33	6.86
	Euspira nitida (Gast)	P	6.30	-20.31	6.42
	Nephtys hystricis (Pol)	P	9.59	-18.67	6.24
	Processa sp. *(Decap)*	P	9.73	-18.49	5.55
	Gammaridae nd. *(Anfip)*	nd	1.74	-15.95	4.56
C10	*Cylichna cylindracea (Gast)*	SF	9.01	-16.40	5.34

	Dosinia lupinus (Biv)	SF	6.14	-17.71	14.28
	Leptopentacta elongata (Ech)	SF	9.57	-16.87	5.01
	Sabella pavonina (Pol)	SF	10.08	-19.55	5.12
	Thyone fusus (Ech)	SF	5.27	-15.57	4.97
	Amphiura filiformis (Ech)	SDF	8.20	-14.49	4.76
	Antalis vulgaris (Scaf)	SDF	9.35	-7.52	14.28
	Atlantella distorta (Biv)	SDF	7.49	-17.06	5.30
	Nucula nucleus (Biv)	SDF	12.56	-15.38	4.90
	Phascolion (Phascolion) strombus (Ech)	SDF	4.90	-18.49	6.59
	Sternaspis scutata (Pol)	SSDF	8.86	-17.80	5.54
	Eulima glabra (Gast)	P	8.89	-17.21	6.30
	Euspira nitida (Gast)	P	6.14	-18.99	5.80
	Leanira yhleni (Pol)	P	8.09	-17.62	5.32
	Nephtys hystricis (Pol)	P	6.20	-17.86	5.35
	Titria mutabilis (Gast)	P	6.85	-18.67	5.20
C12	*Corbula gibba (Biv)*	SF	3.53	-16.56	5.76
	Leptopentacta elongata (Ech)	SF	10.82	-18.17	4.35
	Acrocnida brachiata (Ech)	SDF	5.73	-21.86	17.23
	Aricidea sp. *(Pol)*	SDF	9.21	-20.60	5.52
	Pharaonella astula (Biv)	SDF	6.43	-19.38	6.00
	Sternaspis scutata (Pol)	SSDF	5.38	-20.04	4.57
	Nereidae nd. *(Pol)*	OMN	11.31	-18.66	5.34
	Leanira yhleni (Pol)	P	8.28	-18.72	4.65
	Nephtys hystricis (Pol)	P	7.49	-18.69	5.10
C16	*Anthozoa* nd. *(Ant)*	SF	7.50	-16.43	9.59
	Leptopentacta elongata (Ech)	SF	6.31	-17.58	4.50

	Owenia fusiformis (Pol)	SF	7.49	-18.01	6.23
	Amphiura filiformis (Ech)	SDF	7.31	-18.52	13.16
	Pharaonella astula (Biv)	SDF	6.02	-18.22	5.85
	Scoletoma sp. *(Pol)*	SDF	10.26	-17.29	5.42
	Sternaspis scutata (Pol)	SSDF	5.76	-19.46	5.23
	Diopatra sp. (Pol)	OMN	8.62	-19.94	5.38
	Nereis lamellosa (Pol)	OMN	12.22	-16.68	5.95
	Glycera sp. *(Pol)*	P	8.96	-19.40	6.00
	Glycera unicornis (Pol)	P	10.36	-19.22	5.38
	Hilbigneris gracilis (Pol)	P	9.46	-19.32	8.20
	Nephtys hystricis (Pol)	P	7.97	-16.81	5.12
	Titria mutabilis (Gast)	P	9.46	-16.89	5.66
C19	*Corbula gibba (Biv)*	SF	5.31	-18.39	5.47
	Acrocnida brachiata (Ech)	SDF	5.55	-16.55	12.32
	Sternaspis scutata (Pol)	SSDF	6.21	-18.17	5.38
C22	*Hiatella artica (Biv)*	SF	5.55	-21.73	6.13
	Upogebia tipica (Decap)	SF	6.08	-16.99	5.38
	Virgularia mirabilis (Ant)	SF	7.91	-22.52	6.75
	Brada villosa (Pol)	SDF	7.01	-17.49	4.86
	Tellimya ferruginosa (Biv)	SDF	6.16	-15.94	6.66
	Maldane glebifex (Pol)	SSDF	8.42	-20.23	5.32
	Leanira yhleni (Pol)	P	8.43	-21.23	4.88
	Processa sp. *(Decap)*	P	10.26	-15.93	4.74
C23	*Anthozoa* nd. *(Ant)*	SF	9.30	-24.74	5.21
	Hiatella artica (Biv)	SF	7.78	-17.76	4.78
	Modiolarca subpicta (Biv)	SF	5.96	-21.48	5.90

Ostrea edulis (Biv)	SF	5.55	-17.61	4.97
Pitar rudis (Biv)	SF	5.74	-21.05	5.66
Sabella pavonina (Pol)	SF	8.14	-18.03	5.31
Amphiura chiajei (Ech)	SDF	3.12	-17.15	6.88
Turritella communis (Gast)	SDF	5.74	-21.05	5.66
Sternaspis scutata (Pol)	SSDF	5.09	-23.98	5.14
Oestergrenia digitata (Ech)	SSDF	10.12	-19.92	4.74
Necallianassa truncata (Decap)	P	7.16	-14.85	6.69
Pisidia longimana (Decap)	P	7.02	-14.85	5.37

Paper III

BENTHIC ECOSYSTEM FUNCTIONING APPROACH AS A TOOL FOR SUSTAINABLE MANAGEMENT OF A PORT AREA IN THE NORTHERN ADRIATIC SEA

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Under second revision to Frontiers in Marine Science.

Author contribution statement:

Design and methods: TC

Data collection: **FN**

Data analyses: TC, AF, **FN**, RA

Manuscript preparation: TC, AF, **FN**, RA, PD

Project leader: TC, PD

Abstract

The present study is an example of "the ecosystem approach to management" that has been carried out to provide practical support to decision makers in managing the port area, thus evaluating whether or not to exclude a part of the bay from the Site of National Interest (SIN) of Trieste – a very large contaminated area in need of remediation. To assess how the benthic ecosystem functioning varies in the port macrosites subjected to diversified industrialization and anthropization, we evaluated the structural characteristics of the sediments, both heterotrophic and phototrophic communities together with the main processes of production, transformation and consumption of organic matter at seven stations and a reference one. The port-, shipbuilding- and iron foundry areas, characterised by high levels of contaminants, low macrozoobenthic diversity, major organic contents (up to 51.1 mgC g^{-1}) and higher numbers of hydrocarbon degrading bacteria (up to 5,464 MPN g_{dry}^{-1}) significantly differed ($R_{ANOSIM} = 0.463$, $p = 2.9\%$) from the other stations. In the contaminated areas, the oxygen consumption (-15.22±1.59 mgC m^{-2}) prevailed over the primary production and the trophic state was net heterotrophic. Therefore, according to our results, the environmental status of these macrosites justifies their inclusion in the SIN of Trieste.

In contrast, in the residential area/center bay where contamination levels were below the legal limits, the microalgal and macrobenthic communities were more biodiverse. Higher macrofaunal abundances (up to 753 ± 174.7 ind.m^{-2}), primary production rates (up to 58.60 ± 8.41 mgC m^{-2}) and exoenzymatic activities were estimated. nMDS and SIMPROF analyses performed on benthic communities significantly separated the most contaminated stations from the other ones. Overall, the environmental conditions in this part of the SIN were similar to the reference site and therefore not so severe as to justify their inclusion in the SIN.

Our work is one among the first study cases, where such an ecosystem approach has been applied in a port area to provide practical support to decision makers in the spatial planning of the harbour zone. Although our results derive from one single harbor, and are therefore case-specific, they may have broader implications and offer insights to scientists and stakeholders alike.

Keywords: benthic ecosystem functioning, microphytobenthos, meiofauna, macrozoobenthos, benthic primary production, prokaryotic heterotrophic production, extracellular enzymatic activities, trophic state.

Introduction

The increasing pressure on the marine realm calls for a well-planned approach of managing use of marine space. Fisheries and aquaculture, gas and oil industry, shipping, tourism and on the other hand, the urging need for marine conservation, all compete for the same valuable space. All these activities influence the structure and functioning of marine ecosystems and the use of coastal zones, calling for a robust approach of future spatial planning. Marine areas have been traditionally managed on a case-by-case, sector-by-sector basis, ignoring the interdependent nature of ecosystem components (Katsanevakis et al., 2011). Although informative, the assessment of the environmental status of a given area based solely on the commonly used parameters such as contaminants in the sediments and abundance/composition of macrofauna, could lead to an insufficient understanding of the whole ecosystem functioning, overlooking other environmental aspects that should be considered when addressing proper corrective actions. There is, therefore, an urgent need for a fundamental shift in the way we manage our coast towards the development of a holistic approach that considers all the main components of the ecosystem functioning and its integration into management.

The study of the ecosystem functioning is a complex issue which implies the simultaneous investigation of both structural and functional parameters and their consequent integration in order to depict an overall view of the C flow and cycling through the system. An ecosystem may be defined as an open dynamic system, composed of “subunits” that are interlinked by a complex web of “connections”. In benthic ecosystems, the soft bottoms are inhabited by several communities of organisms arbitrarily classified according to their body size in: pico-, microphyto-, meio- and macrobenthos. From an ecological point of view, organisms could be grouped into three main categories: producers (autotrophs), consumers (heterotrophs) and decomposers (prokaryotes) that are interlinked with each other by a dense web of connections. Primary producers represent a food source for herbivores which graze on algae and are, in turn, food for carnivores along what is commonly considered as the classical grazing benthic food web. However, both meio- and macrofaunal detritivores do not graze exclusively on microalgae but ingest large amounts of sediments obtaining energy from the organic matter present in them. Therefore the grazing food web is refined with the integration of the detrital food web, *i.e.* the flow of energy from the (non-living) detrital organic matter towards the higher trophic levels such as meio- and macrobenthos. The biotic and abiotic components of the system are further interlinked by processes of organic matter production (primary production), its transformation (mineralisation) and energy dissipation (respiration). The system is extremely flexible and responds to physical, chemical and biological factors of both natural and anthropogenic origin. Modifications of the ecosystem structure and C flow give, therefore, indications about the presence of a stressor.

The idea behind "the ecosystem approach to management" is that the management of human activities is based on the limits within which ecosystem structure, functioning, productivity and biological diversity can be maintained. The concept has been developed and incorporated into a number of international agreements over the past 10-15 years (Ottersen et al., 2011). There are still very few examples of its actual practise, mainly due to the difficulties met in coupling environmental safety and sustainable use of resources with stakeholder's needs and expectations. There are still major science and knowledge gaps in applying the ecosystem approach to management, related to our limited understanding of the dynamics and resilience of ecosystems, the cumulative impacts of human uses on the marine environment and the effectiveness of management and governance systems (Katsanevakis et al., 2011 and references therein). Moreover, the details outlined in the scientific literature are often only loosely incorporated into management plans and actions (Ottersen et al., 2011).

Port terminals and harbor areas play important roles in the economy worldwide through the transport and storage of traded goods. Since they are often installed in highly important ecological areas, such as bays, they have several negative environmental impacts on the coastal zone, such as pollution due to the discharge of contaminants, *e.g.* wastewater, petroleum and its derivatives. Port activities are often associated with aquatic pollution and the spreading of contaminants along different compartments, such as water, sediments and biota. Special attention must be given to sediments, which frequently present higher concentrations of contaminants compared to the water column, and may constitute not only a sink but also a secondary source of contaminants to the water column and biota (Buruaem et al., 2012). Benthic organisms, in particular, due to their limited mobility, are exposed to the accumulated contaminants and respond to stress conditions both at the individual and community level through the selection of taxa, elimination of the sensitive ones and abundance changes (Solis-Weiss et al., 2004). Many studies report changes in abundance or diversity of micro- or macrobenthic communities in chronically polluted marine environments. Other fundamental aspects, such as how pollution affects respiration rates, primary production (Sundbäck et al., 2004; Forster et al., 2006; Rubino et al., 2016), prokaryotic heterotrophic production or other microbial processes (Manini et al., 2004; Pusceddu et al., 2014; Sweetman et al., 2014; Franzo et al. 2016a) have been seldom analysed in chemically polluted environments. Studies on benthic ecosystem functioning based on actual estimates of the biological processes integrated with the qualitative and quantitative composition of communities at different trophic levels are even rarer (Schaffner et al., 2008; Cibic et al., 2012b; Franzo et al. 2016b).

The port of Trieste (northern Adriatic Sea) is located within a Site of National Interest (SIN). These sites are defined as very large contaminated areas, classified as the most dangerous by the Italian State and in need of remediation of soil, surface water and groundwater. SIN are identified in relation to the characteristics of the site, the quantity and hazard of pollutants, the importance of the impact on the surrounding environments, in terms of health and ecology, as well as damage to the cultural and environmental heritage. The SIN of Trieste was bounded by Decree of the Italian Ministry of the Environment in 2003 and covers a marine-coastal area of about 12,000,000 m^2 within the eastern part of the Gulf of

Trieste. It is divided in 5 macrosites on the basis of the main activities located there and their consequent anthropogenic pressures: 1) a port area, 2) a shipbuilding area, 3) an iron foundry area with a steel plant, 4) a petroleum area where handling, storage and processing of petroleum products take place and 5) a residential area/centre bay, the largest among the 5 areas. The Port Authority of Trieste, which is the managing body of the SIN, adopted a strategy based on the integrated ecosystem approach in order to carry out the new Environmental Characterisation Plan of the SIN as required by the most recent Italian law. Therefore, to verify whether it was possible to exclude some specific areas (the strip of sea off Muggia and part of the center of the bay) from the SIN, an assessment of the benthic ecosystem functioning in the harbor area was requested. In this study, the following questions were addressed: 1) How do the benthic ecosystem functioning vary in the investigated macrosites of the port subjected to diversified industrialization and anthropization? 2) Do the environmental status of all the macrosites justify their inclusion in the Site of National Interest?

Material and methods

Study site

The Gulf of Trieste, located at the north-western end of the Adriatic Sea, is a shallow embayment of about 500 km^2 with a coastline of about 100 km. It is almost completely surrounded by land, its southwest limit is the line connecting Punta Tagliamento in Italy with Punta Salvore in Croatia. It is isolated from the rest of the Adriatic by a sill from Grado to the Salvore peninsula (Ogorelec et al., 1991); 10% of its area is < 10 m and maximum depth is about 25 m. Average salinities range from 33 to 38 psu at the surface and from 36 to 38.5 psu at the bottom. Annual temperatures fluctuate from 8°C to ≥ 24°C at the surface and from 8°C to ≥ 20°C at the bottom. Tidal amplitude is about 1.5 m, which is the highest in the Mediterranean Sea (Cardin and Celio, 1997). Water enters the Gulf from the southeast and circulation at the surface is predominantly from southeast to northwest. Sedimentation is controlled mainly by river input rather than by marine currents (Brambati and Catani, 1988). Winds and water column stratification are the major factors influencing the characteristics of the composition, evolution and persistence of marine life in the Gulf of Trieste.

Within the Gulf of Trieste, the Bay of Muggia is a shallow embayment (8-20 m) about 7 km long and 4 km wide and oriented NW- SE (Ghirardelli and Pignatti, 1968). Sedimentation is controlled by the low hydrodynamism (Solis-Weiss et al., 2004 and references therein) and fluvial inputs: two streams enter the Bay, Rosandra and Ospo that may discharge large amounts of fine sediments containing chemical fertilisers (www.porto.trieste.it). The Bay of Muggia houses the port and industrial area of Trieste and its morphology makes it prone to the accumulation of contaminants, since it is sheltered from currents and it is characterised by an elongated shape and low hydrodynamism.

The development of the port dates back to the early 1900s with the construction of three external dams (1904-1909), the creation of large industrial structures in the area Gaslini as well as the industrial complex for the manufacture of iron and steel. In the following decades other industrial structures were built, such as the industrial channel (completed in the 50s), the navigation channel (1966) as well as the construction of the pipeline terminal Trieste - Monaco of Bavaria (SIOT) (1967) (Solis-Weiss et al., 2004 and references therein), the most important pipeline that serves central Europe (about $36 \cdot 10^6$ tons of crude oil discharged in 2001) (www.porto.trieste.it) and finally, the expansion of the commercial docks.

Since 2003, the SIN of Trieste has been intensely monitored to assess the chemical quality status of the sediments and the water column, as required under the current legislation. In the latest Environmental Characterisation Plan, carried out in 2013, contaminants in the surface sediments were evaluated by the Port Authority of Trieste in more than 40 sampling stations. Among those we chose the ones closest to our 7 sampling stations to have an indication of the concentrations of the major contaminants (Table 1).

Sampling

In June 2013, seven stations were sampled within the SIN of the Port of Trieste (see Figure 1 for description of the macrosites). St. C1, situated within the Marine

Reserve of Miramare (not shown in Figure 1), approximately 5.4 nm (about 10 km) far from the port, was chosen as the reference station (45° 42.050′ N, 13° 42.600′ E). At each station, 6 virtually undisturbed sediment cores were collected by an automatic KC Haps bottom corer (KC-Denmark) using polycarbonate sample tubes (12.7 cm i.d. with a sample area of 127 cm^2); 1 sediment core was used for meiofauna sampling, 1 for oxygen microprofiling, while from the 4 remaining cores the uppermost layer (0-1 cm) was sampled and homogenised. Macrozoobenthos was sampled in three replicates per station using a stainless steel van Veen grab (sampling area 0.1 m^2). Macroalgae were absent at all sampling stations. Bottom water samples were collected using a 2-L horizontal Niskin bottle. At the moment of sampling, the Photosynthetically Available Radiation (PAR) was recorded *in situ* by a Profiling Natural Fluorometer PNF-300 (Bioshperical instruments Inc., San Diego, CA, USA). PAR at the bottom was expressed as the percentage of measured irradiance with respect to surface irradiance (%PAR). Bottom sea temperature, dissolved oxygen and salinity were measured by a CTD probe model Sea-Bird Electronics 19plus SEACAT profiler (Sea-Bird Electronics, Inc., Bellevue, Washington, USA).

Granulometry, total organic carbon (TOC) and pigments

For each station, aliquots (10-15 g) of homogenised sediment were collected for grain-size analyses and processed as described by Franzo et al. (2016b). The analyses were performed using a Malvern Multisizer 2000S. Data are expressed as percentage of sand, silt and clay. For TOC analyses, triplicate subsamples of homogenised sediment (< 250 µm) were weighed directly in a capsule (5x9 mm), treated with increasing concentrations of HCl (0.1N and 1N) to remove carbonates (Nieuwenhuize et al., 1994) and determined according to the methods of Pella and Colombo (1973) and Sharp (1974). Pigments were extracted overnight (4°C, 90% acetone) from 0.7-0.9 g of wet sediment and analysed spectrofluorometrically following the procedures described by Lorenzen and Jeffrey (1980).

Biopolymeric carbon (BPC)

Subsamples of homogenised sediment were freeze-dried and processed for the determination of carbohydrates, lipids and proteins. Colloidal and EDTA extractable carbohydrates (CHO) were analysed following the method described by Blasutto et al. (2005). Lipids were analysed following the method proposed by Bligh and Dryer (1959) and modified for sediments. Proteins were extracted in NaOH (0.5M) for 4 h and determined according to Hartree (1972). All analyses were carried out in four replicates. Carbohydrate, lipid and protein concentrations were converted to carbon equivalents (Fichez, 1991). The sum of carbohydrates, lipids and proteins was referred to as Biopolymeric Carbon (BPC).

Enumeration of alkane- and petroleum-degrading bacterial groups

The standard five-tubes most-probable-number (MPN) method (Alexander, 1965) was adopted to estimate the abundances of some specific bacterial groups. Subsamples of homogenised sediment (~5 g_{wet}) were placed in sterile tubes with 45 mL of pre-filtered bottom water (0.2 µm filters), stirred, sonicated and centrifuged at 956 x *g* for 1 minute to remove sediment particles. Supernatant and 5 serial 10-fold diluted inoculums were added to the wells containing specific medium (10% v/v) and incubated at 20°C. Alkane- and petroleum-degrading bacteria were enumerated by adding n-Hexadecane (C16) (3.4% v/v) and unleaded petrol (3.4% v/v) to BH medium (Bushnell and Haas, 1941). Hydrocarbon degradation was detected after 15 days by adding a solution of 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium (INT, 0.6 g L^{-1} final concentration) and considering as positive reaction the red-violet dye turning (Johnsen et al., 2002).

Microphytobenthos (MPB)

For the microphytobenthic analyses, aliquots of 2 cm^3 of homogenised sediment were withdrawn using a syringe and directly fixed with 10 mL of formaldehyde (4% final concentration) buffered solution $CaMg(CO_3)_2$ in pre-filtered bottom seawater (0.2 µm filters). After manual stirring, 20-µL aliquots of the sediment suspension were drawn off from the slurries and placed into a 2.5 mL counting chamber. Only cells containing pigments and not empty frustules were counted under a Leitz inverted light microscope (Leica Microsystems AG, Wetzlar, Germany) using a ×32 objective (×320 final magnification). Samples were counted in three replicates following the protocol described by Cibic et al. (2007a, 2012a). The qualitative identification was carried out using floras cited therein. The microalgal taxonomy was based on Round et al. (1992).

Meiofauna

From a virtually undisturbed sediment core, three replicates were gently taken using cut-off plastic syringes (2.7 cm i.d., length 11.4 cm) and immediately frozen at −20 °C (Higgins and Thiel, 1988). The top 10 cm of the sediment core was subsampled and preserved in buffered 4% formaldehyde solution using prefiltered seawater and stained with Rose Bengal (0.5 g L^{-1}). Sediment samples were sieved through 1000 and 38 μm mesh net and the extraction of organisms (from the sediment retained on the 38 μm sieve) was performed by three times centrifugation (1932 x *g*, 10 min) with Ludox HS-40 (density 1.15-1.18 g cm^{-3}) as described by Danovaro et al. (2004). All meiobenthic organisms were counted and taxonomically classified to the main groups according to Higgins and Thiel (1988) under a stereomicroscope (Olympus SZX12; final magnification of 40 or 80X). The abundance was expressed as individuals per 10 cm^2.

Macrofauna

Macrofauna was sampled with a van Veen grab; three replicates per station were taken, sieved through a 1000 μm sieve and immediately frozen at –20°C. After defrosting and sorting, animals were counted and identified to the lowest possible taxonomic level using a stereomicroscope (Olympus SZX12) at 7-40X magnification (Rees et al., 1990). For the identification of organisms the taxonomic keys listed in Morri et al. (2004) were used. The abundance was expressed as individuals m^{-2}. In order to investigate the functional structure of the macrozoobenthic community in relation with the other benthic communities (*i.e.*

microphytobenthos and meiofauna), the macrofaunal feeding habits were considered. Six different feeding habits were assigned to all individuals: surface deposit feeders (SDF), subsurface deposit feeders (SSDF), suspension feeders (SF), predators (P), omnivores (OMN) and grazers (G) using the following criteria: morphology of the feeding apparatus, feeding mode, nature and origin of the food. Traits for each taxon were derived from Roth and Whilson (1998) and Jumars et al. (2015). Taxonomic resolution was kept at the species level whenever possible but adjusted to genus or family when the information on traits were only available on a higher taxonomic level.

Gross primary production (GPP), oxygen consumption, and benthic trophic state

GPP was estimated in the laboratory from ^{14}C-incubation of slurries (Cibic et al., 2008). At each station, ten cm^3 of homogenised surface sediment was sucked up with a syringe, resuspended in 190 mL of overlying filtered seawater (0.2 µm filter) and inoculated with 20 µCi (0.74 MBq) of $NaH^{14}CO_3$ (DHI, Denmark) (Steemann-Nielsen, 1952). After stirring, the slurry was transferred into 9 mL glass vials and incubated in a thermostatic chamber at *in situ* temperature under a gradient of light intensities (20-50-100-200-300 µmol photons m^{-2} s^{-1}). After 45 minutes carbon incorporation was stopped by adding 200 µL of HCl 5N (final HCl concentration 0.11N) (Cibic and Virgilio, 2010). Subsequently, samples were treated as described in detail by Cibic et al. (2008). At each station, the highest GPP value obtained from the light gradient was used. O_2 consumption rates were obtained from oxygen microprofiling carried out on intact sediment cores in the darkness. For technical reasons, at St. 4 oxygen consumption rates are not available. Steady-state O_2 microprofiles were measured using Clark-type O_2 microelectrodes with a guard cathode (Revsbech, 1989) having external tip diameter <100 µm, stirring sensitivity <2 %, and a 90% response time <8 s. The sensor current was measured using a Unisense PA2000 picoammeter; data was recorded with the Unisense Profix software version 3.10 (Unisense, Aarhus, Denmark). A step size of 100 µm was used. For the interpretation of the measured O_2 concentration profiles the software PROFILE version 1.0 (Berg et al., 1998) was used. Areal rates of oxygen respiration were calculated as described by Cibic et al. (2007b).

The oxygen data were converted to mg C m^{-2} h^{-1} to allow an estimation of the trophic status (autotrophy/heterotrophy) of the investigated sites. Gross primary production (GPP) represents the sum of net primary production (NPP) and community respiration (CR). The ^{14}C technique measures something between GPP and NPP, depending on the incubation time: shorter incubation times are closer to GPP whereas incubation times ≥ 6 h are closer to NPP (Gazeau et al., 2004). In our study, the incubation time was about 45 minutes, therefore a GPP rate was measured. Subtracting CR, assessed as the oxygen consumption in the dark, we attempted to estimate NPP.

Degradative extracellular bacterial activities

Extracellular enzymatic activities were assayed using fluorogenic substrate analogues (Hoppe, 1993) derived from 7-amino-4-methyl-coumarin (AMC) and 4-methyl-umbelliferone (MUF). Protease activity (leucine aminopeptidase activity – AMA) was assayed as the hydrolysis rate of leucine-AMC while β-glucosidase (β-GLU), lipase (LIP) and chitinase (CHIT) were assayed using MUF-β-D-glucoside and MUF-oleate and MUF- β-D-glucosamide (Sigma-Aldrich), respectively. Enzyme activities were expressed in terms of the rate of MUF or AMC production. Sediment slurries were prepared by adding 6 mL of 0.2 µm-filtered bottom water to 0.5 g of wet sediment. After evaluation of the saturating concentrations, hydrolysis rates were measured by incubating slurries with (final concentrations) 800-µM leucine-AMC, 400-µM MUF-β-D-glucoside, MUF-oleate and 200-µM MUF- β-D-glucosamide for 1 h in the dark at *in situ* temperature. Before spectrofluorometric measurement each sample was centrifuged 2 min at 3000 rpm. Fluorescence increase due to MUF and AMC hydrolysed from the model substrates was measured using a Jasco FP 6500 spectrofluorometer (MUF = 365-nm excitation and 455-nm emission; AMC = 380-nm excitation and 440-nm emission). Standard solutions of MUF and AMC were used to produce calibration curves with 0.2 µm-filtered bottom water. Triplicate blanks without fluorogenic substrate were used to determine the natural fluorescence increase in the samples not attributable to the tested enzymes. Hydrolytic activities were converted into C mobilisation using the conversion factor 72 for glucose and leucine and 216 for oleic acid.

Prokaryotic carbon production (PCP)

PCP in sediment samples was carried out by the method of van Duyl and Kop (1994), as detailed by Manini et al. (2004). Each sediment sample (0.2 mL of 1:1 vol/vol slurry) was added to 6 μCi of ^{3}H-leucine and incubated in the dark for 1 h at *in situ* temperature. After incubation, radiotracer incorporation was stopped by adding 80% ethanol (1.7 mL). After two washes of the samples with ethanol (80%) by mixing, centrifuging and decanting the supernatant, the sediment was transferred with ethanol (80%) onto a polycarbonate filter (0.2 μm mesh size). Subsequently, the filters were washed twice with 5% trichloroacetic acid. Samples were heated in 2M NaOH for 2 h in a water bath at 100°C. One mL of supernatant was transferred to scintillation vials and 10 mL of Hionic Fluor scintillation fluid was added. For each sample, three replicates and two ethanol-treated blanks were analysed. Activity in the samples was determined by a β-counter (TRI-CARB 2900 TR Liquid Scintillation Analyser).

Statistical analyses

Descriptive statistics was performed on microphytobenthos, meiofauna and macrofauna in order to calculate the Relative Abundance (RA) of the main taxa. Univariate diversity analysis was applied to the abundances of both benthic diatoms and macrofaunal taxa considering richness (d, Margalef, 1986), equitability (J', Pielou, 1966), diversity (H', Shannon and Weaver, 1949) and dominance (λ, Simpson, 1949).

Prior to analysis, the microphytobenthic and macrozoobenthic abundance data from the eight stations (seven from the port area and the reference one) were square-root transformed to reduce the influence of dominant species. The transformed data were then analysed using cluster analysis (performed with the complete linkage clustering algorithm) in conjunction with the similarity profiles (SIMPROF) routine, performed on the same Bray-Curtis similarity matrices

(Clarke et al., 2014). This analysis was applied to highlight possible significant differences ($p < 0.05$) in microphytobenthic and macrofaunal communities among sites. The groups of stations significantly gathered by the SIMPROF test were overlaid on the non-metric multidimensional scaling (nMDS) ordination plots. These tests were not applied to meiofauna because the taxonomic level of identification was too low (large taxonomic groups).

Principal Component Analysis (PCA) was carried out on environmental data of the port stations in order to visualise the trends of the main structural parameters (sand fraction, TOC, BPC and pigments) and those contaminants that were above the legal limits (As, Hg, Pb, Cu, Va, Zn, PAHs and PCBs, see Table 1). The biotic components (MPB, meio- and macrofauna, extracellular enzymatic activities, PCP, GPP, petroleum and n-hexadecane degrading bacteria) were projected on the factor plane as additional variables without contributing to the results of the analysis. This can provide an insight into the possible influence of the environmental variables upon each biotic variable.

To test for the variability of abiotic variables (the same used to construct the PCA matrix) among stations, the analysis of similarity (ANOSIM) test was applied. The ANOSIM statistic R is based on the difference of mean ranks between groups and within groups. The statistical significance of observed R is assessed by permuting the grouping vector to obtain the empirical distribution of R under null-model. ANOSIM tests a priori-defined groups (in our case stations at which contaminants were above or below the legal limits) against random groups in ordinate space. A zero ($R=0$) indicates that there is no difference among groups, while a one ($R=1$) indicates that all the samples within groups are more similar to one another than any samples from different groups (Clarke et al., 2014).

All univariate and multivariate analyses were performed using PRIMER 7 (PRIMER-E Ltd., Plymouth, UK) software.

The main chemical and biological structural data were checked for significant differences between the three contaminated stations (St. 1, 2 and 3) and the other ones, by applying the Mann-Whitney test (STATISTICA 7).

Results

Physico-chemical data

Temperature ranged between 17.29 °C at the deepest St.7 and 23.01 °C at the shallowest St. 6. At the latter station, the bottom layer was supersaturated in O_2 whereas at the other stations O_2 ranged from 80.6 to 90.5%. The lowest salinity was registered at St. 6 while it did not vary much among the other stations (range: 37.07-37.23 psu). The lowest PAR at the bottom was measured at St. 2 due to cloudy weather conditions, which also occurred during sampling at St. 6. However at this latter site, PAR at the bottom showed an intermediate value due to the limited depth (Table 2).

According to Shepard's (1954) classification, the sediment was clayey silt at all stations except for St. 6 where it was sandy silt. The percentage of sand at St. 6 was in fact > 40% while it ranged between 7.0% (St. 1) and 17.1% (St. 3) at the other sites (Table 3). Mean TOC content reached 51.1 ± 2.2 mg g^{-1} at St. 3, thrice the value recorded at the reference station, which was likely due to the steel plant activity. At St. 2 and 3 much higher values of BPC were detected compared to the other investigated sites. The lipid content represented the main fraction of the labile organic matter pool at St. 2, 3, 4 and C1, while at St. 1, 6 and 7 the protein content dominated, varying from 29.8 to 46.3% of the total BPC. EDTA-extractable carbohydrates did not exceed 22.9% of BPC and their content was higher at St. 6 and 7, while the contribution of colloidal carbohydrates was negligible (Table 3). The highest Chl *a* value was measured at St. 4 (43.4 ± 3.3 µg g^{-1}) while major phaeopigment contents were observed at St. 2 and 3 compared to the other sites. TOC, BPC and phaeopigment contents were significantly higher at St. 1, 2 and 3 compared to the other sites (Mann-Withney test, $z = 2.12$, $p<0.05$).

Benthic communities

Petroleum degrading bacteria were isolated from the surface sediments of St. 3, 1, 2 and 7 (up to 23 MPN g^{-1}) whereas they were not observed at the other stations. Similarly, the presence of bacteria able to degrade n-Hexadecane was higher at St. 3 (5,464 MPN g^{-1}), St. 2 and 1, *i.e.* stations more contaminated by hydrocarbons. Lower values were obtained offshore of Muggia and in the centre of the bay even if they were one order of magnitude higher than those at the reference site (Table 4). Both bacterial groups were significantly higher at St. 1, 2 and 3 compared to the other sites (Mann-Withney test, z = 2.12, p<0.05).

Total microphytobenthic abundances were high, exceeding 200,000 cells cm^{-3}, and comparable at St. 1, 2, 3, 4 and C1 (Table 4), whereas the highest density was observed at St. 5. At St. 7 the microalgal density was approximately half of that obtained at the first four stations, probably because of the greater depth (19.5 m) and lower irradiance at the bottom to support a thriving microbial phototrophic community. The lower microphytobenthic abundance at St. 6 was likely due to the different grain-size composition, *i.e.* the higher percentage of sand but also the high hydrodynamism that thwart the attachment of diatom cells to the sea bottom. The qualitative analysis of the microphytobenthos revealed that the microalgal community was dominated by diatoms (>99% in all the investigated sites). Slightly higher percentages of resting cells (spores and cysts) were observed at St. 2 and 3 compared to the other stations, suggesting adverse or stress conditions in these sites. Among diatoms, *Nitzschia* was the most abundant genus, with a relative abundance (RA) varying from 26.6% at St. 7 to 44.1% at St. 3, followed by *Navicula* (11.5% < RA < 27.9%), at all stations except for St. 5 where *Gyrosigma* prevailed. *Cylindrotheca* was observed with a higher RA at the deeper stations (St. 7, C1 and 1), where a lower hydrodynamism probably allowed a better development of this tychopelagic (loosely associated to the bottom) taxon (Table 5). *Nitzschia* cfr. *commutata* was the dominant species at St. 2, 3 and 4, with an RA of 22.6%, 14.2% and 11.0%, respectively. In contrast, in the reference site and in the centre of the bay its percentage did not exceed 1.5%. The univariate diversity indices applied to the diatom community revealed that among the eight sites the highest richness and diversity were observed at St. 5 (d = 3.24; H′ = 3.03), whereas the lowest diversity (H′ = 2.51) as well as the major dominance were obtained at St. 2 (λ = 2.63) likely due to the prevalence of *Nitzschia* cfr. *commutata* in this site. At St. 7 the diatom community resulted more evenly distributed (J′ = 0.84) compared to the other stations.

The nMDS analysis based on the microphytobenthic species highlighted clear spatial differences among stations (Figure 2). Three significant groups of samples were identified using the SIMPROF test: those stations where the level of contamination was above the legal limits (St.1, St. 2 and St.3) were gathered in the same group. The other stations were split into two different groups (St. 4, St. 5 and St. 6 and St. 7) whereas St. C1 was completely separated from the port stations.

Meiofaunal abundances ranged between 324.5 ± 31.7 ind. 10 cm^{-2} and 2,167.9 ± 549.0 ind. 10 cm^{-2} at St. 7 and St. 3, respectively (Table 4). A total of 15 taxa were detected: 9 belonged to permanent meiofauna (Nematoda; Copepoda Harpacticoida and their naupliar stages; Kinorhyncha; Ostracoda; Tardigrada; Turbellaria; Gatrotricha; Acarina) and 6 to temporary meiofauna (*i.e.* juvenile macrofauna: Polychaeta, Bivalvia, Gastropoda, Amphipoda, Decapoda and Tanaidacea). Nematoda represented the dominant group at all the studied stations. Their Relative Abundance (RA), in fact, reached more than 60.2% (St. 1) of the whole community, with a maximum of 95.1% at St. 7. Copepoda Harpacticoida, together with their naupliar stages, represented the second most abundant taxon, with RA ranging from 4.1% and 36.9% at St. 7 and St. 1, respectively (Table 5). The other observed taxa showed very low RA (<1%) at all sites.

Macrofaunal abundances ranged from 130.0 ± 62.5 ind. m^{-2} at St. 2 to 753.3 ± 174.7 ind. m^{-2} at St. 6 (Table 4). The qualitative analysis revealed that this community was dominated by Polychaeta (>40% in all the investigated sites), except for St. 2 and St. 3, where Mollusca reached 46.1 % and 66.4% of the total abundance, respectively. Overall, Crustacea were less abundant, although with slightly higher numbers at St. 2, 6 and 7 (25.6%, 30.6% and 21.5%, respectively). Echinodermata represented more than 10% of the whole community only at St. 7 (15.4%) (Table 5). The univariate diversity indices applied to macrofauna revealed that among the eight sites the highest richness and diversity were observed at St. 4 (d= 10.78 and H’= 5.10), whereas the lowest diversity (H’= 3.22) as well as a lower value of evenness were obtained at St. 3 (J= 0.66) likely due to the prevalence of *Corbula gibba* (45%). The macrofaunal community was characterised by suspension feeders (SF, 37.2%), followed by surface deposit feeders (SDF, 23.7%), predators (P, 15.5%) and subsurface deposit feeders (SSDF, 14.0%). Omnivores and grazers were the less represented, 3% of the total community. Overall, the community feeding habits were well expressed at each sampling site. Suspension feeders were observed in high numbers particularly at St.6 and St. 3 (306.6 and 283.6 ind m^{-2}, respectively). On the contrary, deposit feeders (both SDF and SSDF) reached a high RA (>60%) at St. 1, St.7 and St. C1, mainly on the account of SDF at St. C1 and St. 7 (83.3 and 73.3 ind. m^{-2}, respectively) and to SSDF at St. 1 (63.3 ind. m^{-2}). The RA of predators was >12% at all the investigated sites, except for St.7 where only 16.6 ind. m^{-2} were observed (Figure 3).

The nMDS analysis based on the macrozoobenthic species also revealed spatial differences among stations, though less pronounced than that based on the microphytobenthos (Figure 4). The SIMPROF analysis gathered St.2 and St.3 in the same group, while the other contaminated St. 1 was grouped with St. 7. St.4 and St.5 showed a high level of macrofaunal similarity whereas the reference site was separated from the other stations.

Gross primary production (GPP), oxygen consumption, and benthic trophic state

The highest GPP rates were reached at the given light intensity of 300 μE m^{-2} s^{-1} at all stations except for the shallowest St. 6, where the maximum was estimated at 200 μE m^{-2} s^{-1}. At the deepest St. 7 (19.5 m), the GPP rate did not exceed 3.23 ± 0.57 mg C m^{-2} h^{-1} whereas the absolute maximum was estimated at the shallowest St.6 (58.60 ± 8.41 mg C m^{-2} h^{-1}). The maximum oxygen consumption, converted to C equivalents, was observed at St. 3 (-15.22 ± 1.59 mg C m^{-2}), followed by that obtained at St. 2 (-11.90 ± 2.15 mg C m^{-2}) whereas at the other stations more modest rates were obtained and were comparable to the oxygen consumption calculated at the reference site (-5.66 ± 0.77 mg C m^{-2}) (Figure 5). Offshore Muggia, the benthic system resulted net autotrophic, with an estimated value of 3.99 mg C m^{-2} h^{-1} at St. 5 while at St. 6 it was highly autotrophic (52.81 mg C m^{-2} h^{-1}). At the reference site, the estimated Net Production was barely positive (0.52 mg C m^{-2} h^{-1}) whereas at all the other stations the benthic system was net heterotrophic (Figure 5).

Prokaryotic carbon production (PCP) and extracellular enzymatic activities

Higher PCP values were measured at St. 1, 2, 3 and 4, with a mean rate of 0.98 ± 0.02 µg C cm^{-3} h^{-1}. The stations within the residential area/centre of the bay were characterised by slightly lower PCP rates that ranged between 0.46 ± 0.06 µg C cm^{-3} h^{-1} (St. 7) and 0.77 ± 0.11 µg C cm^{-3} h^{-1} at St. 6 (Figure 6). Degradation rates of the polysaccharides, lipids, chitin and proteins were measured in the SIN and at the reference site (Figure 7). β-glucosidase rates did not varied substantially at the eight stations and values ranged from 6.67 ± 0.22 nmol cm^{-3} h^{-1} (St. C1) to 12.09 ± 0.44 nmol cm^{-3} h^{-1} (St. 3). Lipolytic activities were low and quite homogeneous at all stations. Only at St. 5 sensibly higher rates were measured (14.31 ± 1.05 nmol cm^{-3} h^{-1}). Chitinase showed a mean value of 7.3 ± 0.4 nmol cm^{-3} h^{-1} and did not vary among stations, higher hydrolytic rates were observed at St. 7 (14.51 ± 0.50 nmol cm^{-3} h^{-1}). Protease activities showed the highest hydrolysis rates in the study area. The maximum value was measured at St. 4 (320.69 ± 9.31 nmol cm^{-3} h^{-1}), while the minimum at St. 6 (160.15 ± 17.22 nmol cm^{-3} h^{-1}). The other stations showed an average protease activity of 222.2 ± 16.7 nmol cm^{-3} h^{-1}.

Multivariate statistical analysis

Since the nMDS analysis, based on both benthic communities, and the SIMPROF test revealed that the reference station significantly differed from the port stations, the further multivariate analysis was performed on the port stations only, to better highlight the differences among the stations located within the port area. To integrate all the considered variables in a comprehensive manner, a Principal Component Analysis (PCA) was carried out (Figure 8).

On the PCA plot, stations were separated into two groups: St. 1, 2 e 3 were placed on the right side of the plot, far from the other stations. The analysis of similarity further confirmed that the group gathering the contaminated stations (St. 1, 2 e 3) was significantly different from that gathering all the other stations (R_{ANOSIM} = 0.463, p = 2.9%). The position of the abiotic variables used for the PCA gives an overall indication on the ecosystem state. TOC, BPC and phaeopigments in the proximity of St. 1, 2 and 3 indicate that these areas of the SIN are more subjected to a high organic load than the remaining zones of the SIN. Sediments with high organic content represent, in turn, favourable conditions for the accumulation of heavy metals and organic contaminants, as also evidenced by the PCA. The position of the additional biotic variables used in the PCA gives supplementary information that allow to integrate the abiotic components of the system with the biological ones. This leads to a more comprehensive interpretation of the benthic ecosystem functioning of the port area. The position of both petroleum and n-Hexadecane degrading bacteria close to St. 1, 2 and 3 confirms the presence of a specialised microbial community inhabiting those sediments. The position of microphytobenthos nearby the same stations indicates the presence of an active microalgal community in the contaminated sites, which seems to partly support higher organisms inhabiting these sediments. On the left side of the plot, St. 4, 5 and 7 were gathered together, suggesting similar environmental conditions in these sites. Although St. 4, 5 and 7 are included in the SIN of Trieste, the PCA highlights that in the residential area/centre of the bay the benthic processes of primary production and exoenzymatic degradation of the organic matter were particularly active. As already revealed by the structural and functional parameters, the environmental features of St. 6 (different grain-size and minor depth) rendered this site diverse from the others and supported an abundant macrofaunal community. Indeed, also in the PCA St. 6 was isolated from the other stations.

Discussion

Benthic trophic web in the port area

In the benthic trophic web the primary producers (microphytobenthos), consumers (meio- and macrofauna) and decomposers (bacteria) are interlinked with each other by a dense web of connections. On soft bottoms of shallow areas, where macroalgae are lacking, benthic microalgae represent the main photoautotrophs of the system. The high abundance of benthic diatoms in the port area and in the three contaminated sites suggests that the latter are not negatively affected by high concentrations of heavy metals and hydrocarbons.

Benthic diatom mats are rich in extracellular polymeric substances (EPS) which may function as a protective barrier against toxic compounds, as well as enhance the uptake of favorable ones. This feature could decrease the vulnerability of microbial mats to toxic compounds (Sundbäck et al., 2007 and reference therein). Overall, the microphytobenthic abundance in the port area was of the same order of magnitude as that previously estimated in a site in the middle of the Gulf of Trieste (Cibic et al., 2012a) but higher than that reported at the coastal St. C1 of the gulf (Franzo et al., 2016b). Although the total microphytobenthic abundances did not vary much among stations, the community structure in the investigated sites was quite diverse, as confirmed by the nMDS and SIMPROF analysis that separated the contaminated St. 1, 2 and 3 from the others. Especially at St. 2 and 3 some diatom species that are tolerant to pollution were observed. For instance, *Nitzschia* cfr. *commutata* was the most abundant species both in the shipbuilding and the iron foundry area, with relative abundances (RA) of 25.9 and 14.4%, respectively. In contrast, this diatom was observed in very low RA at St. 7, while it was completely absent at St. C1. *N. commutata* has similar ecological features to *Nitzschia tryblionella*; the latter was previously described as a nutrient-loving diatom that thrives under high organic matter conditions (Agatz et al., 1999) such as those present at St. 2 and 3. However, even at these two more impacted sites of the port of Trieste the microalgal community seemed to be affected by contamination to a much lesser extent than the one thriving in the surface sediments of the Mar Piccolo of Taranto (Ionian Sea, Italy), where only few diatom specimens were encountered in the most contaminated site (Rubino et al., 2016). The authors reported that only those diatoms that thrive just above the surface sediments, *i.e. Paralia sulcata* and *Bacillaria paxillifera,* rather than within the sediments, survived the extremely high concentrations of PCBs and heavy metals whereas diatoms with other life strategies likely died. Similarly, the paucity of benthic diatoms compared to their planktonic counterparts was recently highlighted by Potapova et al. (2016) in lagoonal sediments contaminated by heavy metals and PAHs.

From a trophic point of view, the microalgal proliferation in the Gulf of Trieste is known to support the meiofaunal community. Particularly in summer, meiofauna showed to respond to pulsed inputs of fresh organic matter, which are ascribable to the higher abundances of benthic diatoms, representing an important food source for these heterotrophs by increasing the sediment nutritional value (Franzo et al., 2016b). In the SIN of Trieste, meiofaunal numbers showed the same order of magnitude reported in the virtually pristine St. C1 over a 2-year study (Franzo et al., 2016b). When comparing similar sampling periods (*i.e.* June), abundances resulted even more alike, suggesting that sediment contamination, especially at St. 2 and 3, did not seem to severely affect the meiofaunal numbers. The community, dominated by nematodes and copepods as in most coastal areas of the Adriatic Sea (Balsamo et al., 2010), reflected the composition already reported in the Gulf of Trieste (Cibic et al., 2009; Franzo et al., 2016b). Compared to the stations inside the port area, at St. C1 the highest number of meiofaunal groups (n =10) was reported, suggesting the presence of a more structured community at the reference station. The taxonomic level obtained in this study may not be sufficient to properly detect possible changes induced by the different degree of contamination within the SIN. Accounting for more than 60% of the whole community, nematodes were the dominant meiofaunal group at all stations. These organisms are known to survive to severe contamination. Some taxa, as *Theristus*, *Terschellingia* and *Desmodora*, were reported to tolerate extremely high concentrations of heavy metals, PAHs and PCBs. Nematodes were reported, in fact, as almost the only representatives of meiofauna in the sediments of the Mar Piccolo of Taranto, where the levels of Hg and PCBs were higher than those of the SIN (Franzo et al., 2016a; Cibic et al., 2016). The resistance of nematodes to such inhospitable environments allows meiofauna to keep its ecological role within the trophic web, *i.e.* to convey C and energy from the detritus to the higher trophic levels. Although the meiofaunal community in the SIN was likely not well structured, our results indicate, to some extent, the presence of an active community at the middle level of the trophic web and responsible for the transfer of C to macrofauna.

The macrofaunal development in the port area was influenced by bathymetry and sediment grain-size. Indeed, among the physical variables, the grain-size is considered one of the most important factors influencing the colonisation of sediments by benthic organisms at different trophic levels from microphytobenthos (Round et al., 1992), to meiofauna (Balsamo et al., 2010) and macrofauna (Solis-Weiss et al., 2004). Within the port area, St. 6 differed from the other stations primarily for its reduced depth (8.5 m) and a higher percentage of sand (about 40%) that favoured the macrofaunal development. Besides these physical features, also the different levels of contamination among stations clearly affected the species distributional patterns, as revealed by the nMDS and SIMPROF analysis that separated the most contaminated St. 2 and St. 3 from the other stations. Indeed, moving from the external boundaries of the SIN towards St. 2 and St. 3 (*i.e.* stations closer to the main sources of pollution), the macrozoobenthic community responded to the increasing stress conditions with lower biodiversity and the presence of stress-tolerant species. Among them, the polychaetes *Marphysa sanguinea, Lumbrineris latreilli* and the bivalves *Corbula gibba, Abra alba* and *Atlantella distorta* deserve to be mentioned (Simboura and Zenetos, 2002). These results are in accordance with previous studies carried out in the industrial area of the port of Trieste (Ghirardelli et al., 1973; Solis-Weiss et al., 2004). In contrast, the sandier site (St. 6) and the shallower ones (St. 4 and St. 5) were characterised by the highest abundances and diversity. According to Ergen et al. (2007), the high biodiversity observed at St.4 in the petrol area could be related to the spatial complexity of sediments, resulting in additional ecological niches. Indeed, due to the occurrence of pebbles in this site, both muddy-bottom species (the polychaete *Vermiliopsis striaticeps*) and typically hard-substrate species (*e.g*. the bivalve *Hiatella arctica* and the polychaete *Serpula vermicularis*) were found. Further, at St. C1 and at St. 6 two species indicators of environmental stability were observed: the echinoidea *Echinocardium cordatum* and *Ova canaliferus* (Clarke et al., 2014).

High numbers of deposit feeders were observed particularly at St.1, St.2 and St.7 that could be related to the high microphytobenthic densities at these stations. According to Checon et al. (2016) diatoms are an important component of benthic food webs due to their high assimilation and low gut residence. Benthic diatoms, in particular, are the most palatable food for deposit feeder polychaetes. In the guts of most polychaetes the authors observed, among others, diatom specimens belonging to *Navicula* and *Nitzschia* that were the dominant genera also in our study, particularly at St. 2 and 3. The amount of lipids (*e.g*. triglycerides and long fatty acids chains) contained inside the microalgal cells renders them very palatable to deposit feeder invertebrates (Goedkoop et al., 2011). This indicates the importance of local benthic primary production in the trophic web at these stations. Among predators, high abundances of *Hilbigneris gracilis* and *Eunice vittata* were observed at St. 4 and St. 6, in correspondence with high meiofaunal densities. Predators generally prey on small invertebrates and a potential top-down control on meiofauna is well known (Van Colen et al., 2015). The polychaetes observed at these two stations belong to the families Lumbrineridae and Eunicidae, respectively that have paired mandibles and complex sets of maxillae in a strongly muscular and eversible pharynx and are able crawlers and borrowers in muddy sediments (Jumars et al., 2015). These features render them very effective predators on meiofauna. The low numbers of predators observed at the most contaminated sites of the port, *i.e.* St. 2 and St. 3, are in contrast with the findings of Franzo et al. (2016a). The authors reported high numbers of predators at the severely polluted site in the Mar Piccolo of Taranto, where mobile organisms (as predators) likely survived due to their ability to avoid hotspots of contaminants through their active movements (Ward et al., 2013). Overall, despite the presence of macrofaunal stress-tolerant species (*e.g. A. alba, C. gibba,* and *L. latreilli*), the environmental contamination within the SIN seems not so severe to affect the macrofaunal feeding structure.

As representatives of decomposers, in this study we focused on the bacterial community capable of exploiting specific C sources, *i.e.* n-Hexadecane and petroleum. The presence of bacteria capable of degrading n-Hexadecane and petroleum, especially at St. 2 and 3, suggests that contaminated sediments harbor consortia of bacteria that are highly specialised in exploiting non-readily-available sources of C as hydrocarbons. These bacteria were detected also off Muggia (St. 5 and 6), although in lower numbers, indicating that the ability of the bacterial community to degrade hydrocarbons was widespread in the entire area. Our findings are consistent with Cibic et al. (2012b), who reported the presence of a specialised bacterial community inhabiting sediments in a severely impacted channel, but with remarkably higher abundances of both petroleum and n-Hexadecane degrading bacteria. The difference in abundance could be due to the higher concentrations of contaminants in the channel and different environmental contexts (brackish conditions in the channel *vs* a shallow marine environment in the SIN). Overall, the benthic ecosystem seems to adapt to pollution, with the development of stress resistant communities able to occupy new ecological niches. The freshly produced C is conveyed, via bacterial biomass, to higher trophic levels, establishing highly specialised food webs in these contaminated sites.

Benthic processes and ecosystem functioning

Ecosystem functioning is a general concept that refers, in essence, to the overall performance of ecosystems. Describing or measuring ecosystem functioning is difficult: as it encompasses a number of phenomena, the overall functioning of an ecosystem is complex and involves many factors related to the chemical, physical and biological components of the system. No one individual parameter can be used to describe the functioning of entire ecosystems, so consideration of multiple variables may be the most appropriate way to shed light on the concept (Bremner, 2008 and references therein). In the literature, studies on benthic ecosystem functioning that consider both the main autotrophic and heterotrophic pathways are still very rare (Cibic et al., 2012b; Cibic et al., 2016; Franzo et al., 2016b). Most of them, in fact, investigate the autotrophic side of the ecosystem functioning or its heterotrophic counterpart. In the present study, the synoptic investigation of both pathways allowed to elucidate their reciprocal interactions and how their combination resulted in the observed ecosystem functioning.

Ecosystem functioning encompasses several processes as organic matter production, decomposition, consumption and C transfer to higher trophic levels. Regarding the process of phototrophic C fixation, the main driver of the productivity of the microalgal community inhabiting surface sediments is the light availability at the bottom. In fact, GPP rates were lower at the deeper stations (St. 7, 1, C1) and higher at the shallowest sites. Indeed, at St. 6 the microalgal community was extremely active, as also indicated by the oxygen oversaturation at the bottom layer (107.1%). Overall, GPP rates did not seem to be affected by contamination levels since they were comparable to those estimated at the reference site. This further indicates that benthic microalgae were able to adapt to stress induced by the high levels of chemical compounds accumulated in these sediments. In contrast, Rubino et al. (2016) estimated extremely low primary production rates (close to zero) in a shallow area of the Ionian Sea severely contaminated by PCBs and heavy metals. The authors infer that the synergistic effect of the contaminants was such as to interfere with the proper functioning of certain benthic processes, *i.e.* the microalgal photosynthetic capability. Interestingly, in another contaminated area located in a riverine-lagoonal system, Cibic et al. (2012b) reported GPP values that were almost three times higher than those found in our port area. In the latter study, the high contamination levels seemed to strongly affect the meiofaunal and macrofaunal numbers rather than the microalgal density and due to the consequent low grazing pressure a very abundant and highly productive microalgal community was obtained.

The trophic state of the system varied noticeably according to the site: it was net autotrophic offshore Muggia (St. 5 and 6) and net heterotrophic in the other sites, where the oxygen consumption prevailed over the primary production. Again, the trophic state was primarily influenced by depth, since positive values were observed only at the shallower stations (depth< 11.5 m) where the microalgal assemblages were photosynthetically active. In the other sites of the SIN, the values ranged between -2.76 mg C m^{-2} h^{-1} at St.1 and -5.37 mg C m^{-2} h^{-1} at St. 3. Especially in the iron foundry area the high oxygen uptake was probably due to the degradation of the accumulated synthetic organic substances. However, the minimum (as absolute number) observed in the iron foundry area was still twice lower than that previously reported for a riverine-lagoonal system that was strongly heterotrophic (-12.00 mg C m^{-2} h^{-1}) (Cibic et al., 2012b), in which the major oxygen demand was due to degradation of the high organic load. We further compared our values to those reported in the severely contaminated Mar Piccolo of Taranto, where the trophic state of the benthic system was net heterotrophic in all sites and seasons (April and June), with values ranging from -3.62 mg Cm^{-2} h^{-1} to -8.17 mg C m^{-2} h^{-1} (Rubino et al., 2016). However, it is likely that in the Bay of Muggia the residence time of the water masses is much lower compared to the semi-confined Mar Piccolo, with a direct influence on dilution rates of contaminants as well as on the overall trophic state of the system.

Prokaryotic C conversion efficiency is the ratio between the Prokaryotic C Production (PCP) and the amount of C degraded enzymatically, expressed as a percentage (Danovaro and Pusceddu 2007). Since it combines in a direct way two key aspects of the benthic ecosystem functioning (*i.e.* the organic matter re-cycling and its incorporation into new living biomass at the lower level of the trophic web), it can be considered as a proxy of ecosystem functioning, at least for these pivotal aspects. In the SIN of Trieste, higher efficiencies were calculated at St. 1, 2 and 3, with a maximum of 5.96% in the port area, while the sites within the residential/centre of the bay and St. C1 showed efficiencies <4%. St. 6 (5.76%) represented an exception due to its limited depth and coarser sediments (Figure 9). Our results are comparable with the findings of a study carried out in a riverine-lagoonal system severely contaminated by PAHs and Hg (Cibic et al., 2012b). The authors reported a more efficient microbial community at the most contaminated site, the Banduzzi channel, with a prokaryotic C conversion efficiency of 6.21%. Our results from the SIN of Trieste confirm that contaminated sediments can harbour a very active microbial community. Such organisms can be autotrophic, as indicated by high abundances of microalgae, and heterotrophic, as pointed out by the presence of specialised consortia of bacteria that are able to use and tolerate toxic compounds. The fresh organic material derived by the microalgal proliferation fuels prokaryotic processes of mineralisation and C incorporation and represents a food source for meio- and macrofauna. In another site of the Gulf of Trieste, a shift of the benthic ecosystem from a "source system" during summer to a "detritus sink system" during winter was documented (Franzo et al., 2016b). The variable proliferation of microalgae was identified as one of the main drivers responsible for such change. Our results, carried out in early summer, confirmed the pivotal role of these organisms in the ecosystem functioning of the SIN. The active microbial community observed even at the most contaminated sites increases the nutritional value of the sediments masking, consequently, the potential negative effects of contamination on meio- and macrofauna. Thanks to the presence of tolerant taxa as nematodes, the system showed to be adapted to the anthropogenic stressors that insist on the area. There is a flow of C and energy both within a consolidated microbial loop and, although not well structured, even towards the higher trophic levels. This was confirmed by the well balanced structure of macrofaunal feeding habits.

Conclusions

The present study is an example of "the ecosystem approach to management" that has been carried out to provide practical support to decision makers in managing the port area, thus evaluating whether or not to exclude a part of the bay from the SIN of Trieste. To assess how the benthic ecosystem functioning varies in the investigated macrosites of the port subjected to diversified industrialization and anthropization, we evaluated the structural characteristics of the sediments, both heterotrophic and phototrophic communities together with the main processes responsible for the C flow through the system. The applied multivariate statistical analysis significantly separated St. 1 (port area), St. 2 (shipbuilding area) and St. 3 (iron foundry area) from the other four stations of the SIN. The first three stations were characterised by high levels of contaminants, low macrozoobenthic diversity, major TOC and BPC contents. Overall, at these stations, the oxygen consumption prevailed over the primary production and the trophic state was net heterotrophic. Therefore, according to our results, the environmental status of these macrosites justifies their inclusion in the SIN of Trieste. Yet, the higher numbers of hydrocarbon degrading bacteria and major Prokaryotic C conversion efficiency suggest that the sediments at these three stations are colonised by an active and specialised microbial community capable of exploiting other sources of C, such as different hydrocarbons that have accumulated in these sites. Moreover, despite the presence of macrofaunal stress-tolerant species (*e.g. Abra alba*, *Corbula gibba* and *Lumbrineris latreilli*), the overall environmental contamination in these three macrosites seems not so severe to affect either the macrofaunal feeding structure or the meiofaunal and microphytobenthos total numbers. In contrast, at St. 5, 6 and 7 (residential area/center bay), where the contamination levels were below the legal limits, both the microalgal and macrobenthic communities were more biodiverse and higher macrofaunal abundances were observed. Also the primary production and the tested exoenzymatic activities were higher at these stations.

Overall, the environmental conditions in this part of the SIN were similar to the reference site and therefore not so severe as to justify their inclusion in the SIN. Further samplings are needed along a finer sampling grid, in order to confirm these first results.

In this study, to assess the benthic ecosystem functioning, a holistic approach was followed by integrating the ecosystem structure, its functioning and biological diversity at several trophic levels. Our work is one among the first study cases, where such an ecosystem approach has been applied in a port area to provide practical support to decision makers in the spatial planning of the harbour zone. Although our results derive from one single harbor, and therefore are necessarily case-specific, depending upon the particular features of the port (*e.g.* depth, grain-size, productive activities, level of contamination, etc.), they may have broader implications and offer insights to scientists and stakeholders alike. Indeed, this novel approach could be implemented into management techniques for the zoning of harbors, and more broadly, contaminated areas, an environmental issue of widespread importance.

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References

Agatz, M., Asmus, R., Deventer, B. (1999). Structural changes in the benthic diatom community along a eutrophication gradient on a tidal flat. *Helgol. Mar. Res.* 53, 92-101.

Alexander, M. (1965). "Most-Probable-Number Method for Microbial Populations", in *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, ed. A. G. Norman (American Society of Agronomy, Soil Science Society of America) 1467-1472.

Balsamo, M., Albertelli, G., Ceccherelli, V. U., Coccioni, R., Colangelo, M. A., Curini-Galletti, et al. (2010). Meiofauna of the Adriatic Sea: present knowledge and future perspectives. *Chem. Ecol.* 26, 45-63.

Berg, P., Risgaard-Petersen, N., Rysgaard, S. (1998). Interpretation of measured concentration profiles in sediment pore water. *Limnol. Oceanogr.* 43, 1500-1510.

Blasutto, O., Cibic, T., De Vittor, C., Fonda Umani, S. (2005). Microphytobenthic Primary Production and Sedimentary Carbohydrates Along Salinity Gradients in the Lagoons of Grado and Marano (Northern Adriatic Sea). *Hydrobiologia* 550, 47-55.

Bligh, E. G., and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911-917.

Brambati, A., and Catani, G. (1988). Le coste e i fondali del Golfo di Trieste dall'Isonzo a Punta Sottile: aspetti geologici, geomorfologici, sedimentologici e geotecnici. *Hydrores Information* 5, 13-28.

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

Buruaem, L. M., Hortellani, M. A., Sarkis, J. E., Costa-Lotufo, L. V., Abessa, D. M. S. (2012). Contamination of port zone sediments by metals from Large Marine Ecosystems of Brazil. *Mar. Pollut. Bull.* 64, 479-488.

Bushnell, L., and Haas, H. (1941). The utilization of certain hydrocarbons by microorganisms. *J. Bacteriol.* 41, 653.

Cardin, V., and Celio, M. (1997). Cluster analysis as a statistical method for identification of the water bodies present in the Gulf of Trieste (Northern Adriatic Sea). *Bollettino di Geofisica Teorica ed Applicata* 38, 119-135.

Checon, E. E., Pardo, E. V., Zacagnini Amaral, A. C. (2016). Breadth and composition of polychaete diets and the importance of diatoms to species and trophic guilds. *Helgol. Mar. Res.* 70(1), 19.

Cibic, T., Blasutto, O., Bettoso, N. (2009). Microalgal-meiofaunal interactions in a sublittoral site of the Gulf of Trieste (northern Adriatic Sea, Italy): A three-year study. *J. Exp. Mar. Biol. Ecol.* 370, 144-154.

Cibic, T., Blasutto, O., Burba, N., Fonda Umani, S. (2008). Microphytobenthic primary production as ^{14}C uptake in sublittoral sediments of the Gulf of Trieste (northern Adriatic Sea): Methodological aspects and data analyses. *Estuar. Coast. Shelf S.* 77, 113-122.

Cibic, T., Blasutto, O., Fonda Umani, S. (2007a). Biodiversity of settled material in a sediment trap in the Gulf of Trieste (northern Adriatic Sea). *Hydrobiologia* 580, 57-75.

Cibic, T., Blasutto, O., Hancke, K., Johnsen, G. (2007b). Microphytobenthic species composition, pigment concentration, and primary production in sublittoral sediments of the Trondheimsfjord. *J. Phycol.* 43, 1126-1137.

Cibic, T., Bongiorni, L., Borfecchia, F., Di Leo, A., Franzo, A., Giandomenico, S., et al. (2016) Ecosystem functioning approach applied to a large contaminated coastal site: the study case of the Mar Piccolo of Taranto (Ionian Sea). *Environ. Sci. Pollut. Res.* 23, 12739-12754 doi: 10.1007/s11356-015-4997-2

Cibic, T., Comici, C., Bussani, A., Del Negro, P. (2012a). Benthic diatom response to changing environmental conditions. *Estuar. Coast. Shelf S.* 115, 158-169.

Cibic, T., Franzo, A., Celussi, M., Fabbro, C., Del Negro, P. (2012b). Benthic ecosystem functioning in hydrocarbon and heavy-metal contaminated sediments of an Adriatic lagoon. *Mar. Ecol. Prog. Ser.* 458, 69-87.

Cibic, T., and Virgilio, D. (2010). Different fixatives and chloridric acid concentrations in microphytobenthic primary production estimates using radiolabeled carbon: their use and misuse. *Limnol. Oceanogr.-Meth.* 8, 453-461.

Clarke, K. R., Gorley, R. N., Somerfield, P. J., Warwick, R. M. (2014). *Change in marine communities: An Approach to Statistical Analysis and Interpretation*. Plymouth: PRIMER-E Ltd.

Danovaro, R., Gambi, C., Mirto, S., Sandulli, R., Cecchierelli, V. (2004). "Meiofauna", in *Mediterranean marine benthos: a manual of methods for its samplings and study*, eds M. C. Gambi and M Dappiano (Biol. Mar. Medit. 11), 55-97.

Danovaro, R., and Pusceddu, A. (2007). Biodiversity and ecosystem functioning in coastal lagoons: Does microbial diversity play any role? *Estuar. Coast. Shelf S.* 75, 4-12.

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 and D. George. (Scientia Marina 70S3), 197-207.

Fichez, R. (1991). Composition and fate of organic-matter in submarine cave sediments-implications for the biogeochemical cycle of organic-carbon. *Oceanol. Acta* 14, 369-377.

Forster, R. M., Créach, V., Sabbe, K., Vyverman, W., Stal, L. J. (2006). Biodiversity-ecosystem function relationship in microphytobenthic diatoms of the Westerschelde estuary. *Mar. Ecol. Prog. Ser.* 311, 191-201.

Franzo, A., Auriemma, R., Nasi, F., Vojvoda, J., Pallavicini, A., Cibic T., et al. (2016a) Benthic ecosystem functioning in the severely contaminated Mar Piccolo of Taranto (Ionian Sea, Italy): focus on heterotrophic pathways. *Environ. Sci. Pollut. Res.* 23, 12645-12661 doi: 10.1007/s11356-015-5339-0

Franzo, A., Cibic, T., Del Negro, P. (2016b). Integrated approach for the assessment of the benthic ecosystem functioning at a coastal site in the northern Adriatic Sea. *Cont. Shelf Res.* 121, 35-47 doi: 10.1016/j.csr.2015.12.005

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.

Ghirardelli, E., Orel, G., Giaccone, G. (1973). L'inquinamento del Golfo di Trieste. *Atti Mus. Civ. Stor. Nat.* 28, 431-450.

Ghirardelli, E., and Pignatti, S. (1968). Conséquences de la pollution sur le peuplements du 'Vallone de Muggia' pre`s de Trieste. *Rev. Intern. Oceanogr. Med.* 10, 111-122.

Goedkoop, W., Sonesten, L., Ahlgren, G., Boberg, M. (2011). Fatty acids in profundal benthic invertebrates and their major food resources in Lake Erken, Sweden: seasonal variation and trophic indications. *Canadian Journal of Fisheries and Aquatic Sciences*, 57, 2267-2279.

Hartree, E. F. (1972). Determination of protein: A modification of the lowry method that gives a linear photometric response. *Anal. Biochem.* 48, 422-427.

Higgins, R. P., and Thiel, H. (1988). *Introduction to the Study of Meiofauna*. London: Smithsonian Institution Press.

Hoppe, H.-G. (1993). "Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria", in *Current Methods in Aquatic Microbial Ecology*, ed P. F. Kemp, B. F. Sherr, E. B. Sherr, J. J. Cole (Boca Raton, FL: CRC Press), 423-431.

Johnsen, A. R., Bendixen, K., Karlson, U. (2002). Detection of microbial growth on polycyclic aromatic hydrocarbons in microtiter plates by using the respiration indicator WST-1. *Appl. Environ. Microbiol.* 68, 2683-2689.

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

Katsanevakis, S., Stelzenmüller, V., South, A., Sørensen, T. K., Jones, P. J. S., Kerr, S., et al. (2011). Ecosystem-based marine spatial management: Review of concepts, policies, tools, and critical issues. *Ocean Coast. Manage.* 54, 807-820.

Lorenzen, C., and Jeffrey, S. (1980). Determination of chlorophyll in seawater. *Unesco Tech. Pap. Mar. Sci* 35, 1-20.

Manini, E., Luna, G. M., Danovaro, R. (2004). Benthic bacterial response to variable estuarine water inputs. *FEMS Microbiol. Ecol.* 50, 185-194.

Margalef, R. (1986). *Ecología*. Barcelona: Editorial Omega SA.

Morri, C., Bellan-Santini, D., Giaccone, G., Bianchi, C. (2004). Principles of bionomy: Definition of assemblages and use of taxonomic descriptors (macrobenthos). *Biol. Mar. Medit.* 11, 573-600.

Nieuwenhuize, J., Maas, Y. E. M., Middelburg, J. J. (1994). Rapid analysis of organic carbon and nitrogen in particulate materials. *Mar. Chem.* 45, 217-224.

Ogorelec, B., Mišič, M., Faganeli, J. (1991). Marine geology of the Gulf of Trieste (northern Adriatic): Sedimentological aspects. *Mar. Geol.* 99, 79-92.

Ottersen, G., Olsen, E., van der Meeren, G. I., Dommasnes, A., Loeng, H. (2011). The Norwegian plan for integrated ecosystem-based management of the marine environment in the Norwegian Sea. *Mar. Policy* 35, 389-398.

Pella, E., and Colombo, B. (1973). Study of carbon, hydrogen and nitrogen determination by combustion-gas chromatography. *Microchimica Acta* 61, 697-719.

Pielou, E. (1966). Shannon's formula as a measure of specific diversity: its use and misuse. *Am. Nat.* 118, 463-465.

Potapova, M., Desianti, N., Enache, M. (2016). Potential effects of sediment contaminants on diatom assemblages in coastal lagoons of New Jersey and New York States. *Mar. Pollut. Bull.* 107, 453-458

Pusceddu, A., Gambi, C., Corinaldesi, C., Scopa, M., Danovaro, R. (2014). Relationships between meiofaunal biodiversity and prokaryotic heterotrophic production in different tropical habitats and oceanic regions. Plos ONE http://dx.doi.org/10.1371/journal.pone.0091056

Rees, H. L., Moore, D. C., Pearson, T. H., Elliot, M., Service, M., Pomfret, J., et al. (1990). Procedures for the Monitoring of Marine Benthic Communities at UK Sewage Sludge Disposal Sites. Department of Agriculture and Fisheries for Scotland. Scottish Fisheries Information Pamphlet No. 18. pp. 79.

Revsbech, N. P. (1989). An oxygen microsensor with a guard cathode. *Limnol. Oceanogr.* 34, 474-478.

Roth, S., and Wilson, J. G. (1998). Functional analysis by trophic guilds of macrobenthic community structure in Dublin Bay, Ireland. *J. Exp. Mar. Biol. Ecol.* 222, 195-217.

Round, F. E., Crawford, R. M., Mann, D. G. (1992). *The Diatoms*. Cambridge: Cambridge University Press.

Rubino, F., Cibic, T., Belmonte, M., Rogelja, M. (2016). Microbenthic community structure and trophic status of sediments in the Mar Piccolo of Taranto (Mediterranean, Ionian Sea). *Environ. Sci. Pollut. Res.* doi 10.1007/s11356-015-5526-z

Schaffner, L.C., Anderson, I. C., Stanhope, J. W., Gillett, D. J., Metcalfe, W. J., Brylawski, A. M. Z. (2008). An Integrated Approach to Understand Relationships Between Shallow Water Benthic Community Structure and Ecosystem Function. Department of Defense Strategic Environmental Research and Development Program SERDP Project SI-1335.

Shannon, C. E., and Weaver, W. (1949). *The mathematical theory of communication*. Urbana, IL: University of Illinois Press IL.

Sharp, J. H. (1974). Improved analysis for particulate organic carbon and nitrogen from seawater. *Limnol. Oceanogr.* 19, 984-989.

Shepard, F. P. (1954). Nomenclature based on sand-silt-clay ratios. *J. Sediment. Res.* 24, 151-158.

Simboura, N., and Zenetos, A. (2002). Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic Index.*Medit. Mar. Sci.* 3/2, 77-111.

Simpson, E. H. (1949). Measurement of diversity. *Nature* 163, 688

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). *Sci. Total Environ.* 328, 247-263.

Steemann-Nielsen, E. (1952). The use of radioactive carbon (^{14}C) for measuring organic production in the sea. *Journal of Conservation International Exploration Meridian* 16, 117-140.

Sundbäck, K., Linares, F., Larson, F., Wulff, A. (2004). Benthic nitrogen fluxes along a depth.

gradient in a microtidal fjord: The role of denitrification and microphytobenthos. *Limnol. Oceanogr.* 49, 1095–1107.

Sundbäck, K., Petersen, D. G., Dahllöf, I., Larson, F. (2007). Combined nutrient-toxicant effects on a shallow-water marine sediment system: sensitivity and resilience of ecosystem functions. *Mar. Ecol. Prog. Ser.* 330, 13-30.

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.

Van Colen, C., Thrush, S. F., Parkes, S., Harris, R., Woodin, S. A., et al. (2015). Bottom–up and top–down mechanisms indirectly mediate interactions between benthic biotic ecosystem components. *J. Sea Res.* 98, 42–48.

van Duyl, F. C., and Kop, A. J. (1994). Bacterial production in North Sea sediments: clues to seasonal and spatial variations. *Mar. Biol.* 120, 323-337.

Ward, D. J., Simpson, S. L., Dianne, F. J. (2013). Slow avoidance response to contaminated sediments elicits sublethal toxicity to benthic invertebrates. *Environ. Sci. Technol.* 47, 5947-5953.

http.www.porto.trieste.it. Website of the Port Authority of Trieste. Visited October 2016.

Table 1: Contaminants in sediments within the Site of National Interest of Trieste. PCBs = Polychlorobiphenyls; PAHs = Polycyclic Aromatic Hydrocarbons. Concentrations of heavy metals and hydrocarbons are expressed in mg kg^{-1}; PAHs and PCBs in μg kg^{-1} (sediment dry mass). Concentrations marked in bold exceed the legal limits (column A, Table 1, Annex 5 of the Italian Legislative Decree 152/06; the thresholds marked with an asterisk (*) were specifically fixed for the marine part of the SIN of Trieste in the Environmental Characterisation Plan required by the Italian Ministry of Environment). Contaminant data, obtained from surveys carried out in 2013, were provided by the Port Authority of Trieste.

	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7	threshold
As	18.1	**23.8**	15.5	15.8	10.4	12.1	16.1	20
Cd	0.25	0.25	0.25	0.25	0.25	0.25	0.25	2
total Cr	64.1	67.9	62.6	60.2	69.1	60.5	64.7	150
Hg	**3.40**	**4.37**	0.52	0.14	0.10	0.58	0.37	1
Ni	52.3	52.2	52.2	51.5	60.1	52.1	56.4	120
Pb	57.1	**388.0**	73.9	4.0	8.7	11.0	33.1	100
Cu	36.8	**112.0**	28.7	16.6	24.4	22.2	22.5	80*
Va	**101.0**	**105.0**	**91.5**	**90.0**	65.8	83.0	**93.5**	90
Zn	137	**770**	**205**	57.0	56.0	67.0	78.6	150
total hydrocarbons (13>C<40)	**147**	**1,050**	**215**	38.0	33.5	39.8	44.5	50
total PAHs	**4,870**	**19,000**	**14,950**	46	207	802	1,424	4,000*
total PCBs	74.7	**907**	50.8	1.0	0.5	3.3	3.9	190*

Table 2: Physical data measured in the water column at the eight stations. %PAR = percentage of measured irradiance at the bottom with respect to surface irradiance.

Site	depth	temperature	O_2%	salinity	bottom PAR	surface PAR	%PAR
	(m)	(°C)		psu	μmol photons m^{-2} s^{-1}		
St.1	18.5	17.30	80.7	37.23	23.68	1,539.36	1.54
St.2	15.0	17.47	83.7	37.20	14.16	589.40	2.40
St.3	13.0	17.84	88.5	37.14	36.69	1,678.52	2.19
St.4	10.5	18.07	89.0	37.07	61.58	2,358.84	2.61
St.5	11.5	17.86	90.5	37.14	115.99	1,840.87	6.30
St.6	8.5	23.01	107.0	35.03	57.25	467.43	12.25
St.7	19.5	17.29	80.6	37.22	20.85	1,087.11	1.92
St. C1	17.0	17.33	89.0	37.21	37.52	1,253.32	2.99

Table 3: Sand, expressed in %, and structural chemical data measured in the eight sites. TOC = Total Organic Carbon; CHO$_{EDTA}$ = EDTA-extractable carbohydrates; CHO$_{H20}$ =Colloidal carbohydrates extracted in water; BPC = Biopolymeric C; phaeo = phaeopigments. Values are averages of three replicates, SD = standard deviation.

Site	**sand**	**TOC**	SD	**proteins**	SD	**lipids**	SD	**CHO-$_{EDTA}$**	SD	**CHO-$_{H2O}$**	SD	**BPC**	**Chl *a***	SD	**phaeo**	SD
	%	mg C g^{-1}		µg C g^{-1}		µg C g^{-1}		µg C g^{-1}		µg C g^{-1}		µg C g^{-1}	µg g^{-1}		µg g^{-1}	
St.1	7.0	28.6	± 1.2	2,130.1	± 39.0	1,921.8	± 0.9	418.8	± 13.1	133.8	± 4.1	4,604.5	26.4	± 1.6	41.5	± 0.5
St.2	14.2	40.8	± 1.9	2,331.3	± 61.0	3,671.7	± 20.8	531.2	± 25.4	116.0	± 5.4	6,650.0	25.8	± 1.1	52.2	± 2.2
St.3	17.1	51.1	± 2.2	2,458.4	± 94.0	2,866.3	± 48.5	554.5	± 7.4	136.7	± 8.2	6,015.9	29.4	±1.2	48.1	± 3.0
St.4	11.3	22.3	± 1.8	1,162.1	± 50.0	2,052.6	± 10.2	530.3	± 42.3	160.5	± 0.9	3,905.5	43.4	± 3.3	35.5	± 1.2
St.5	10.6	18.9	± 0.2	1,242.9	± 17.0	2,073.5	± 6.5	484.2	± 14.6	156.4	± 3.8	3,956.9	22.6	± 0.9	28.1	± 1.1
St.6	40.2	8.6	± 0.5	676.4	± 13.0	573.0	± 20.8	385.2	± 12.9	67.8	± 2.4	1,702.4	6.3	± 0.1	10.4	± 0.2
St.7	8.8	19.9	± 0.7	914.5	± 14.0	812.4	± 37.5	539.3	± 15.6	93.7	± 1.4	2,359.9	8.8	± 0.3	28.9	± 0.9
St. C1	9.0	14.4	± 0.3	1,153.3	± 17.0	1,529.3	± 48.4	453.7	± 18.6	152.5	± 5.2	3,288.8	16.3	± 0.3	31.3	± 1.6

Table 4: Biological structural parameters measured at the investigated stations. MPB = Microphytobenthos; MPN = Most Probable Number. Except for functional bacterial groups, values are averages of three replicates, SD = standard deviation. NA = Not Available

Site	**Petroleum**	**n-Hexadecane**	**MPB**	SD	**meiofauna**	SD	**macrofauna**	SD
	degrading bacteria	degrading bacteria						
	MPN g $_{dry}^{-1}$	MPN g $_{dry}^{-1}$	cell cm^{-3}		ind. 10 cm^{-2}		ind. m^{-2}	
St. 1	16	2,423	230,800	±30,924	654.0	±467.2	153.3	±37.9
St. 2	15	4,333	227,600	±13,232	1,579.8	±787.8	130.0	±62.5
St. 3	23	5,464	225,400	±8,190	2,161.9	±549.0	466.7	±330.1
St. 4	0	1,423	212,000	±25,486	1,242.5	±402.6	706.7	±188.8
St. 5	0	1,910	265,400	±18,003	1,262.7	±322.7	383.3	±56.9
St. 6	0	1,800	123,200	±3,995	1,780.4	±407.3	753.3	±174.7
St. 7	5	928	108,600	±19,827	324.5	±31.7	216.7	±5.1
St. C1	0	34	226,600	±24,857	1,416.0	±474.6	290.0	NA

Table 5: Relative abundance, expressed as percentage, of the major groups constituting the three benthic communities and the ten major benthic diatom genera in the eight sites. Microphytobenthos were analysed in the surface layer while meiofauna from the top 10 cm.

Microphytobenthos	**St. 1**	**St. 2**	**St. 3**	**St. 4**	**St. 5**	**St. 6**	**St. 7**	**St. C1**
Nitzschia	41.0	44.0	44.2	43.8	40.2	29.1	26.6	27.6
Navicula	11.9	22.2	21.3	11.5	12.5	27.9	20.7	16.7
Gyrosigma	3.7	2.1	3.9	11.3	14.9	4.7	7.8	14.4
Cylindrotheca	8.3	5.7	3.9	5.8	3.0	4.7	10.2	15.3
Paralia	7.2	1.0	0.9	1.6	3.7	5.2	6.3	3.5
Caloneis	3.0	2.7	2.6	2.0	1.0	0.0	0.0	1.6
Rhopalodia	1.2	0.2	2.6	1.0	1.8	2.0	1.7	1.9
Amphora	1.6	1.8	1.4	1.0	0.7	2.0	2.2	1.4
Entomoneis	1.1	2.1	1.3	2.3	0.8	2.6	0.9	0.5
Bacillaria	1.1	0.6	1.1	2.7	3.4	0.0	1.5	0.0
Other diatom genera	20.1	17.7	16.9	17.3	17.9	21.7	22.3	17.1
Meiofauna	**St. 1**	**St. 2**	**St. 3**	**St. 4**	**St. 5**	**St. 6**	**St. 7**	**St. C1**
Copepoda	17.9	9.6	4.5	9.4	5.2	4.2	3.1	10.2
Nauplii	19.0	14.3	2.7	10.0	6.0	2.5	1.0	10.9
Nematoda	60.2	75.6	92.5	80.2	87.8	92.2	95.1	77.2
Polychaeta	0.4	0.1	0.1	0.2	0.3	0.1	0.5	0.3
Kinorhyncha	0.1	0.0	0.1	0.0	0.6	0.8	0.0	0.7
Ostracoda	0.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Others	1.5	0.3	0.1	0.2	0.1	0.2	0.3	0.6
Macrofauna	**St. 1**	**St. 2**	**St. 3**	**St. 4**	**St. 5**	**St. 6**	**St. 7**	**St. C1**
Polychaeta	59.7	25.6	18.6	48.6	46.1	49.1	46.2	58.6
Mollusca	14.0	46.2	66.4	37.7	38.3	26.5	15.4	24.1
Crustacea	5.3	25.6	13.6	11.3	8.7	16.8	21.5	13.8
Echinodermata	1.8	2.6	1.4	1.4	1.7	4.4	15.4	3.5
Others	0.0	0.0	0.0	0.9	5.2	3.1	1.5	0.0

Figure legend

Figure 1. Location of the seven sampling stations within the five macrosites of the Site of National Interest (SIN) of Trieste.

Figure 2. non-metric multidimensional scaling (nMDS) analysis based on microphytobenthic abundance data from the eight stations (seven from the port area and the reference one). On the nMDS ordination plot, groups of stations significantly gathered by the SIMPROF test are superimposed. Red triangles: stations where contaminants are above the legal limits, green inverted triangles: stations where contaminants are below the legal limits.

Figure 3. Relative abundance, expressed as percentage, of the macrofaunal feeding habits in the eight sites. SDF=surface-deposit feeders; SSDF=subsurface-deposit feeders; SF=suspension feeders; P=predators; O=omnivores and G=grazers.

Figure 4. non-metric multidimensional scaling (nMDS) analysis based on macrozoobenthic abundance data from the eight stations (seven from the port area and the reference one). On the nMDS ordination plot, groups of stations significantly gathered by the SIMPROF test are superimposed. Red triangles: stations where contaminants are above the legal limits, green triangles: stations where contaminants are below the legal limits.

Figure 5. Rates of Gross Production (from ^{14}C data), Consumption estimated in the darkness and at *in situ* temperature (converted from O_2 consumption data) and Net Production (estimated) at the seven stations in the port area and in the reference site. NA = data not available.

Figure 6. Prokaryotic C production (PCP) estimated at the seven stations within the Site of National Interest and in the reference site (St. C1).

Figure 7. Extracellular enzymatic activities estimated at the seven stations within the Site of National Interest and in the reference site: a) Leucine aminopeptidase; b) ß-glucosidase; c) Lipase; d) Chitinase. For Leucine aminopeptidase a different scale on the y-axis is used.

Figure 8. Principal Component Analysis (PCA) ordination diagram based on the main structural parameters (sand fraction, TOC, BPC and phaeopigments) and contaminants (As, Hg, Pb, Cu, Va, Zn, PAHs and PCBs) of the port stations (upper panel). TOC = Total Organic C; BPC = Biopolymeric C; Phaeo = phaeopigments. The biotic components (MPB, meio- and macrofauna, extracellular enzymatic activities, PCP, GPP, petroleum and n-hexadecane degrading bacteria) were projected on the factor plane as additional variables without contributing to the results of the analysis (lower panel). MPB = microphytobenthos; Meio = meiofauna; Macro = macrofauna; Chit = Chitinase; Lip = lipase; AMA = protease; β-glu = β-glucosidase; PCP = Prokaryotic C Production GPP = Gross Primary production; Petr Bac = Petroleum degrading bacteria; n-Hex Bac = n-Hexadecane degrading bacteria. Red triangles: stations where contaminants are above the legal limits, green triangles: stations where contaminants are below the legal limits.

Figure 9. Relationship between Biopolymeric C and organic C conversion efficiency determined as the ratio of Prokaryotic C Production and the amount of C degraded enzymatically and expressed as a percentage (as proposed by Danovaro and Pusceddu (2007)). The Prokaryotic C conversion efficiency combines in a direct way two key aspects of the benthic ecosystem functioning *i.e.* the organic matter re-cycling and its incorporation into new living biomass at the lower level of the trophic web and can be therefore considered as a proxy of ecosystem functioning.

Figure 1

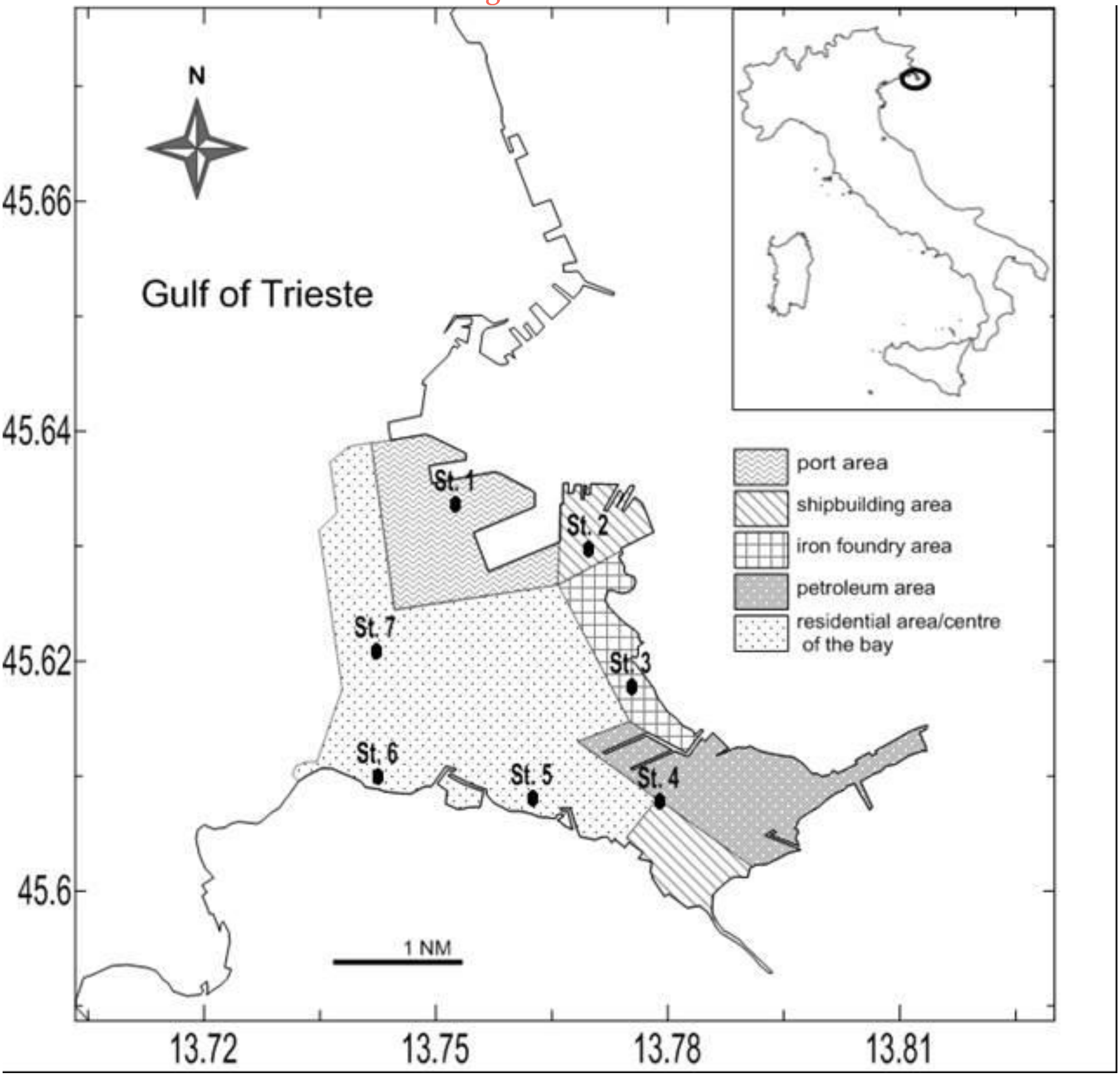

N
Gulf of Trieste
45.66
45.64
45.62
45.6
13.72
13.75
13.78
13.81
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
1 NM
port area
shipbuilding area
iron foundry area
petroleum area
residential area/centre of the bay

Figure 2

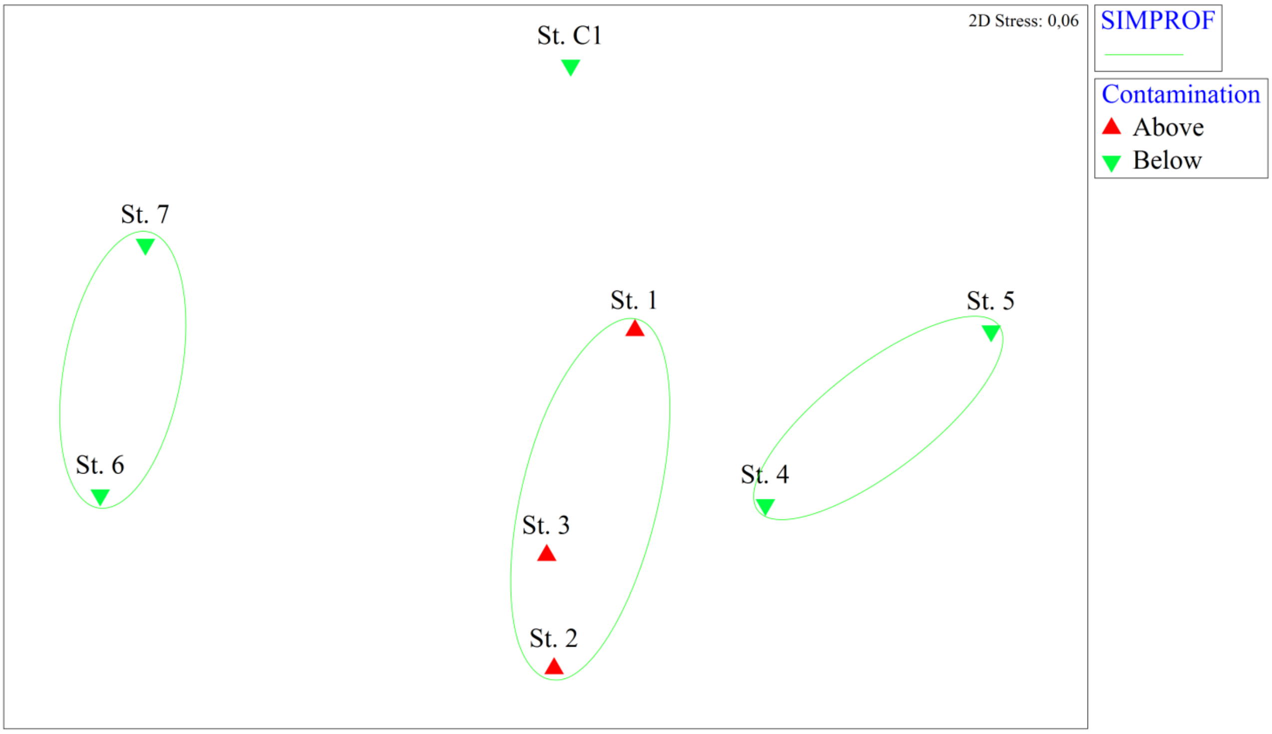

2D Stress: 0,06
St. C1
St. 7
St. 6
St. 1
St. 3
St. 2
St. 5
St. 4
SIMPROF
Contamination
▲ Above
▼ Below

Figure 3

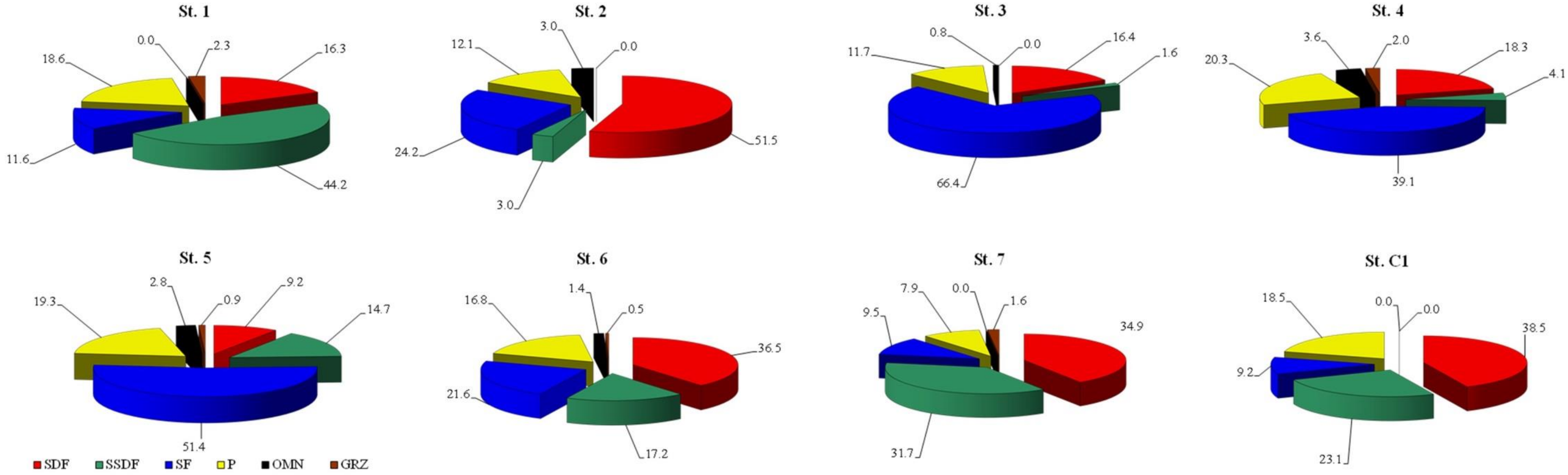

St. 1
16.3
44.2
11.6
18.6
0.0
2.3
St. 2
51.5
3.0
24.2
12.1
3.0
0.0
St. 3
16.4
1.6
66.4
11.7
0.8
0.0
St. 4
18.3
4.1
39.1
20.3
3.6
2.0
St. 5
9.2
14.7
51.4
19.3
2.8
0.9
St. 6
36.5
17.2
21.6
16.8
1.4
0.5
St. 7
34.9
31.7
9.5
7.9
0.0
1.6
St. C1
38.5
23.1
9.2
18.5
0.0
0.0
SDF
SSDF
SF
P
OMN
GRZ

Figure 4

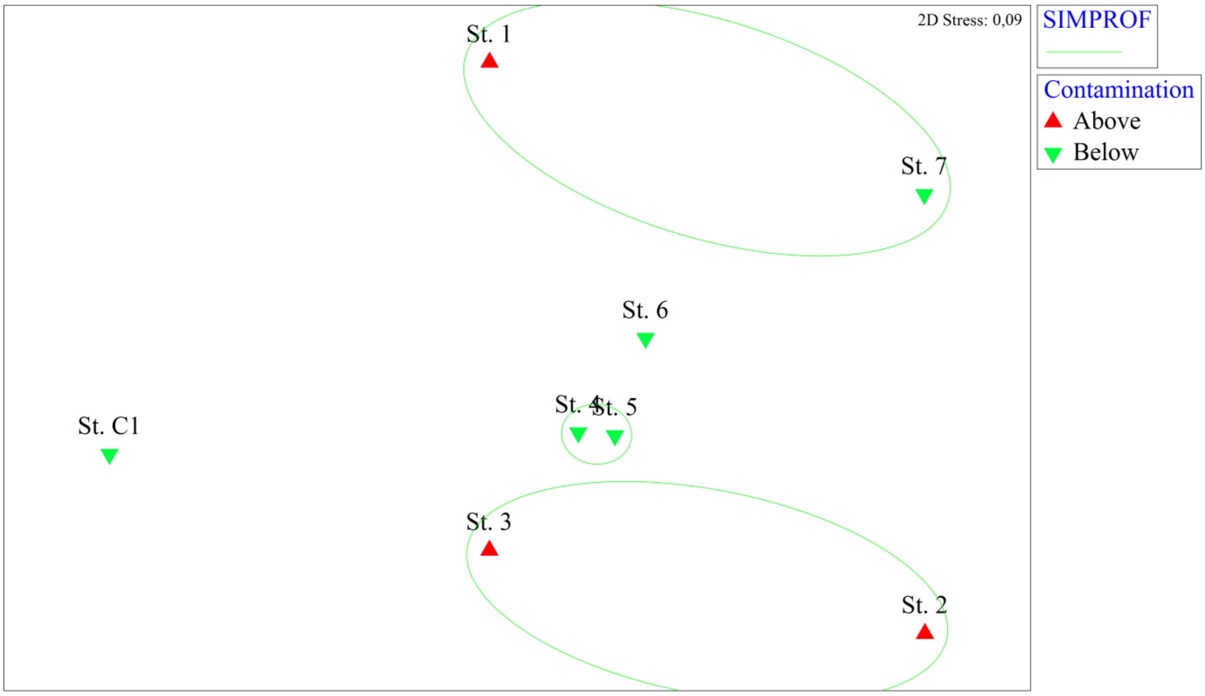

2D Stress: 0,09
St. 1
St. 7
St. 6
St. 4
St. 5
St. C1
St. 3
St. 2
SIMPROF
Contamination
Above
Below

Figure 5

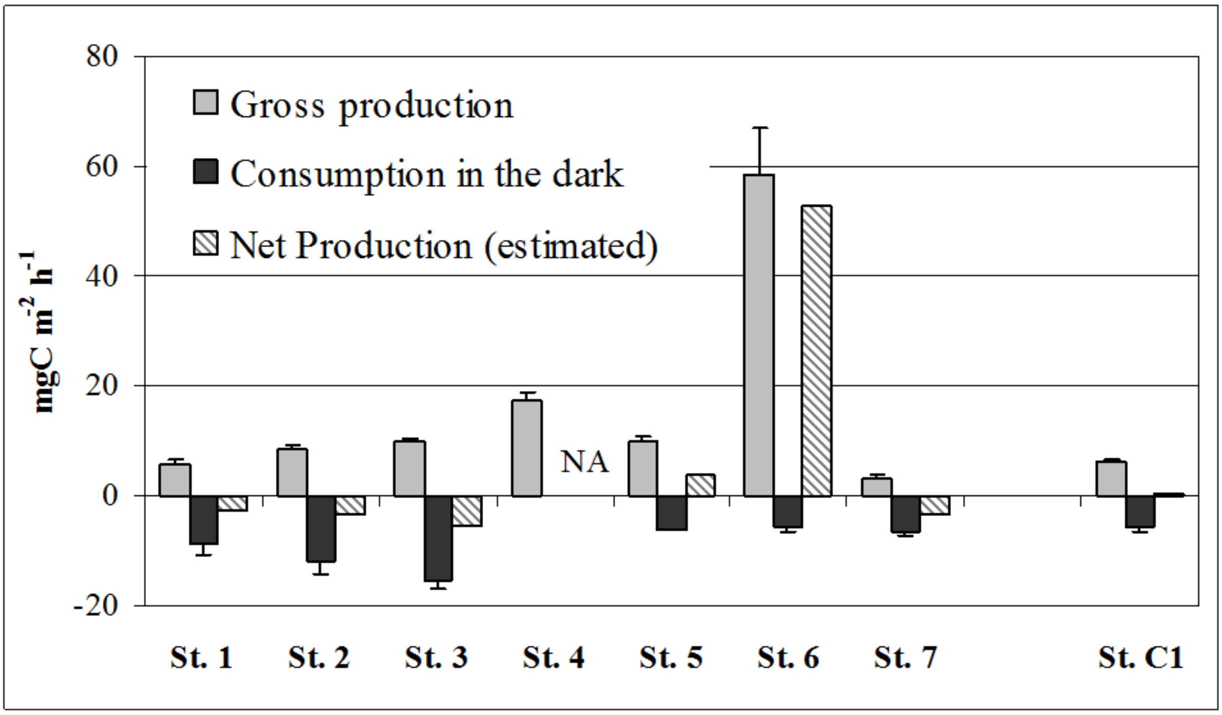

80
60
40
20
0
-20
mgC m^{-2} h^{-1}
Gross production
Consumption in the dark
Net Production (estimated)
NA
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
St. C1

Figure 6

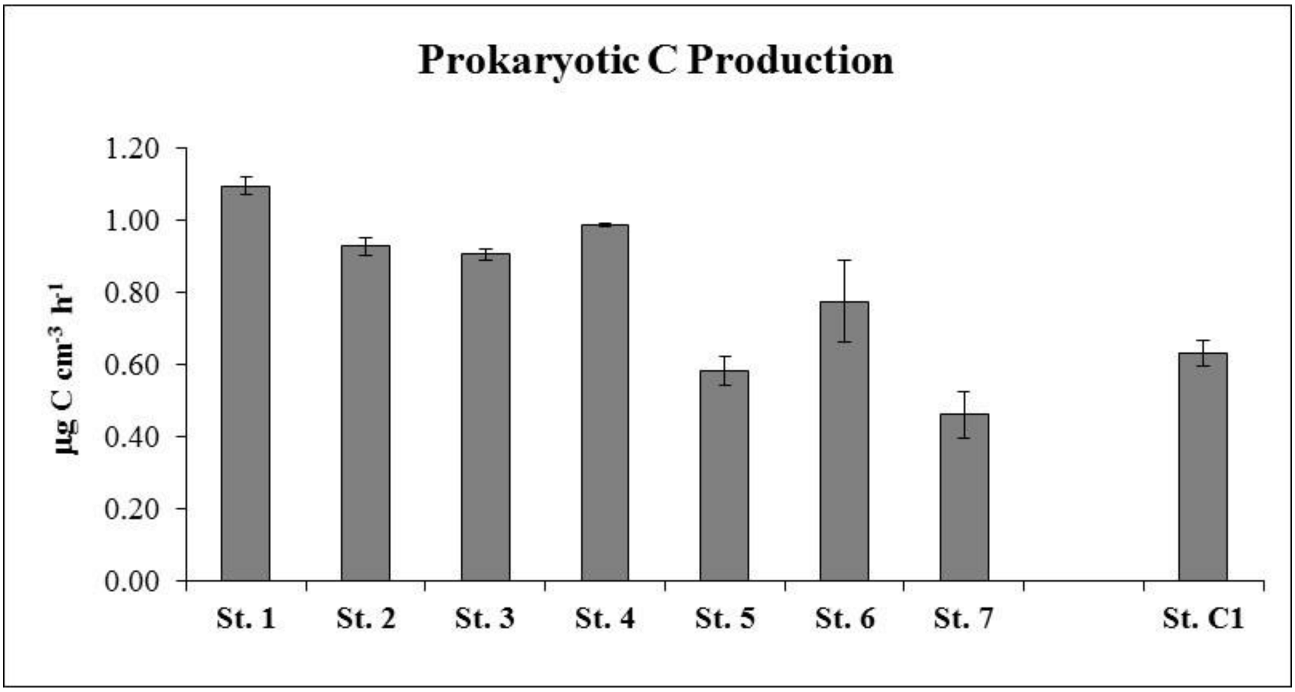

Prokaryotic C Production
µg C cm-3 h-1
1.20
1.00
0.80
0.60
0.40
0.20
0.00
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
St. C1

Figure 7

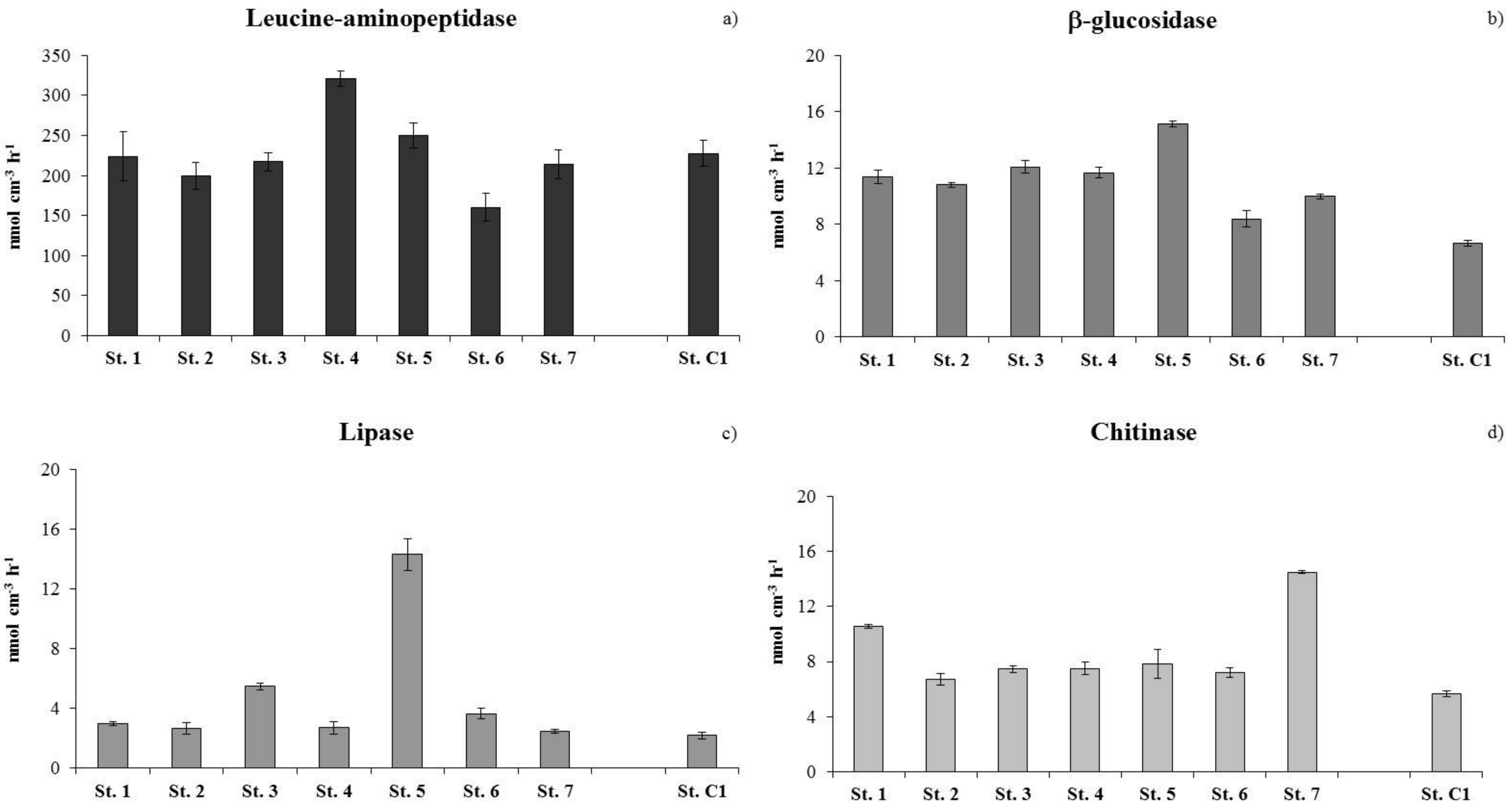

Leucine-aminopeptidase
a)
nmol cm^{-3} h^{-1}
0
50
100
150
200
250
300
350
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
St. C1
β-glucosidase
b)
nmol cm^{-3} h^{-1}
0
4
8
12
16
20
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
St. C1
Lipase
c)
nmol cm^{-3} h^{-1}
0
4
8
12
16
20
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
St. C1
Chitinase
d)
nmol cm^{-3} h^{-1}
0
4
8
12
16
20
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
St. C1

Figure 8

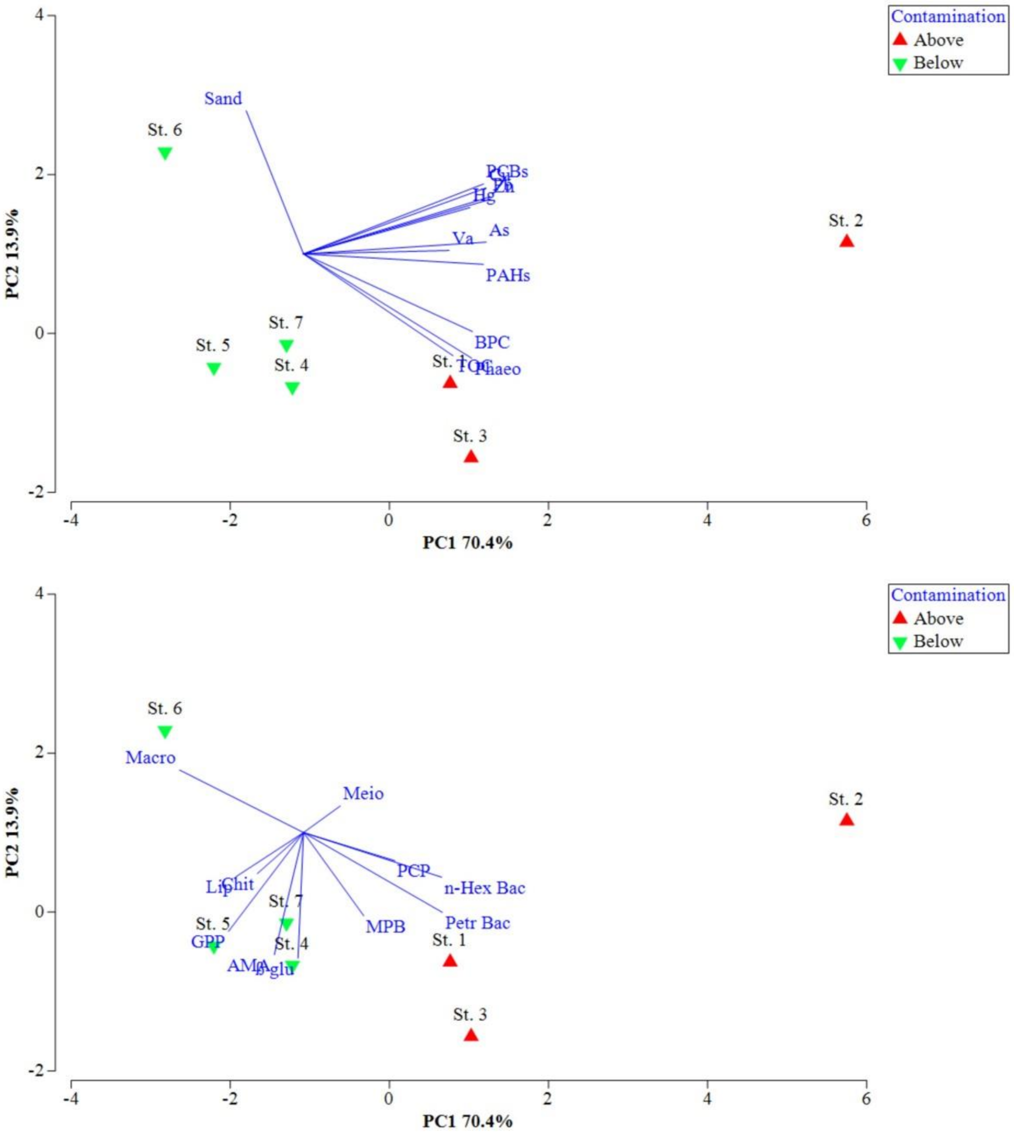
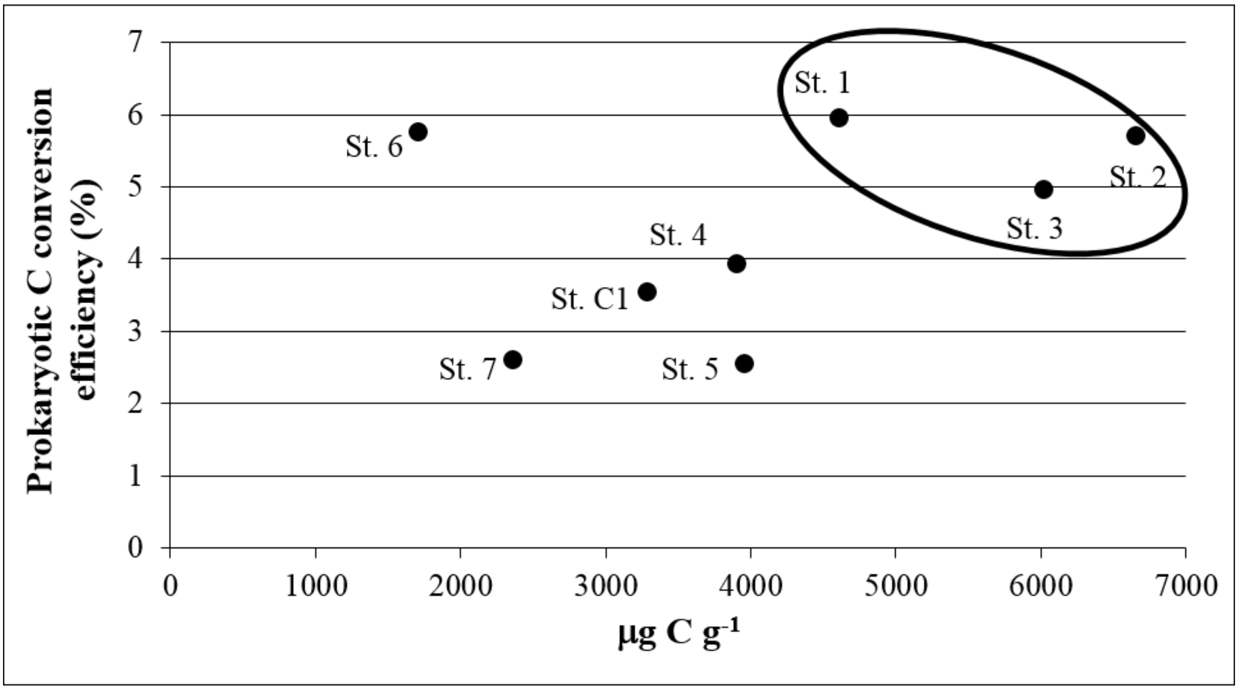

Contamination
Above
Below
Sand
PCBs
Pb
Zn
Hg
Va
As
PAHs
BPC
TOC
Phaeo
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
PC1 70.4%
PC2 13.9%
-4
-2
0
2
4
6
Contamination
Above
Below
Macro
Meio
PCP
n-Hex Bac
Petr Bac
MPB
Lip
Chit
GPP
AMA
βglu
St. 1
St. 2
St. 3
St. 4
St. 5
St. 6
St. 7
PC1 70.4%
PC2 13.9%

Figure 9


Prokaryotic C conversion efficiency (%)
7
6
5
4
3
2
1
0
St. 1
St. 6
St. 2
St. 3
St. 4
St. C1
St. 7
St. 5
0
1000
2000
3000
4000
5000
6000
7000
μg C g^{-1}

Paper IV

BENTHIC ECOSYSTEM FUNCTIONING IN THE SEVERELY CONTAMINATED MAR PICCOLO OF TARANTO (IONIAN SEA, ITALY): FOCUS ON HETEROTROPHIC PATHWAYS

Annalisa Franzo, Rocco Auriemma, **Federica Nasi,** Jana Vojvoda, Alberto Pallavicini, Tamara Cibic, Paola Del Negro *(2016). Environmental Science and Pollution Research, 23(13), 12645-12661.*

Author contribution statement:

Design and methods: AF, RA, **FN**, JV, TC, PDN

Data collection: AF, TC

Data analyses: AF, **FN**, RA, AP, JV

Manuscript preparation: AF, RA, **FN,** JV, AP, TC, PDN

Project leader: PDN, TC

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Benthic ecosystem functioning in the severely contaminated Mar Piccolo of Taranto (Ionian Sea, Italy): focus on heterotrophic pathways

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Abstract The benthic ecosystem functioning is a rarely applied holistic approach that integrates the main chemical and biological features of the benthic domain with the key processes responsible for the flux of energy and C through the system. For the first time, such conceptual model, with an emphasis on the heterotrophic pathways, has been applied to the sediments at four stations within one of the most polluted coastal areas in Italy: the Mar Piccolo of Taranto. The functioning of the benthic ecosystem was different according to the investigated site. Nearby the military arsenal, i.e., the main source of organic contaminants and heavy metals, the system seemed inhibited at all the investigated structural and functional levels. Slow microbial processes of C reworking together with very limited densities of benthic fauna suggested a modest transfer of C both into a solid microbial loop and to the higher trophic levels. On the other hand, the ingression of marine water through the "Navigabile" channel seemed to stimulate the organic matter degradation and, consequently, the proliferation of meiofauna and macrofauna. In the innermost part of the basin, the system functioning, to some extent, is less impacted by contaminants and more influenced by mussel farms. The organic matter produced by these bivalves fueled faster C reworking by benthic prokaryotes and enhanced the proliferation of filter feeders.

Keywords Sediments · Mar Piccolo of Taranto · Semi-enclosed basin · Organic enrichment · Contamination · Ecosystem functioning · Benthic communities · Heterotrophic pathways

Introduction

The United Nations Convention on the Law of the Sea defined pollution as "the introduction by man, directly or indirectly, of substances or energy into the marine environment, including estuaries, which results or is likely to result in such deleterious effects as harm to living resources and marine life, hazards to human health, hindrance to marine activities, including fishing and other legitimate uses of the sea, impairment of quality for use of the sea water and reduction of amenities". Contaminants of major concern include persistent organic pollutants, nutrients, oil, heavy metals, pathogens, etc. Categorization of pollution only facilitated discussion: most contaminants are interrelated and jeopardize the environment and organisms regardless of the source of contamination (Islam and Tanaka 2004). Coastal areas deserve proper attention regarding diffuse and synergistic forms of pollution. Disposal into waterways is, in fact, a very ancient practice of dealing with wastes and the open waterways have been used by mankind for dumping all kinds of waste. Furthermore, the industrial areas are generally highly populated or the industries are usually established near highly populated areas. Therefore, the pollution load from industrial resources is generally coupled with domestic and sewage wastes. Among coastal areas, the effects of diffuse and synergistic forms of pollution are even more emphasized in enclosed basins, where

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the water circulation is restricted and, therefore, the dilution effect of contaminants is limited.

The Mar Piccolo of Taranto (Ionian Sea, southern Italy) appears to comprise all these features since this semi-enclosed basin collects the wastes from the densely populated urban center of Taranto and from the long-lasting industrial activities settled in the area. Since the 1960s, the city of Taranto and its coastline have been subjected, in fact, to the industrialization process that has caused profound environmental changes. The industrial zone is characterized mainly by the presence of the largest steelworks in Europe and navy arsenal in Italy, a major oil refinery, shipbuilding, and other industrial activities that are responsible for severe environmental contamination, mainly due to heavy metals, asbestos, polycyclic aromatic hydrocarbons (PAHs), organic solvents, polychlorinated biphenyls (PCBs), and dioxins. Previous surveys focused on the evaluation of organic and inorganic pollutants have shown so high levels of contamination as to consider the Mar Piccolo of Taranto one of the most polluted areas in Italy (Cardellicchio et al. 2007; Spada et al. 2012). This basin is included, in fact, in one of the largest Sites of National Interest (SIN) that has been declared as such by the Italian State according to the Law n. 426/ 1998. SIN are identified in relation to the characteristics of the site, the quantity and hazard of pollutants, the importance of the impact on the surrounding environments, in terms of health and ecology, as well as injury to the cultural and environmental heritage. The basin is even the most important area of mussel farming in Italy with an annual production of bivalves of about 40, 000 tons per year (Caroppo et al. 2012). This economic activity ensures noticeable incomes to the local population, but it could be endangered by the high contamination levels that affect the Mar Piccolo.

In a severely polluted environment such as the Mar Piccolo of Taranto, sediments deserve special attention. Due to the interactions with the water column, in fact, the benthic domain may be considered as the repository of the overlying water and consequently may act as a biogeochemical record of the past. Sediments represent a sink of contaminants that occur in concentrations up to orders of magnitude higher than in the overlying water (Cardellicchio et al. 2007). Benthic organisms, remaining in place, react to these high contamination levels without any escape possibility. The resulting community, in terms of species composition, replacements, eliminations, diversity, or abundance changes can give a proper idea of the recent history of the environmental events affecting the area (Solis-Weiss et al. 2001). In the Mar Piccolo, the chemical characterization of sediments, with an emphasis on contaminants, is well documented (Cardellicchio et al. 2007; Spada et al. 2012), and benthic communities have been studied focusing more on their macroscopic fraction (Matarrese et al. 2004; Prato and Biandolino 2005). On the contrary, the benthic ecosystem functioning, i.e., a holistic approach that integrates the main chemical and biological features of the system together with the main processes responsible for the flux of energy and C, has not been investigated yet.

The benthic ecosystem is able to adapt to anthropogenic pressure, to some extent, by trying to minimize its impact through the development of stress-resistant communities that occupy new ecological niches. A highly specialized food web, which allows the benthic ecosystem to reach a state of equilibrium, can thus be established even in a heavily contaminated site (Cibic et al. 2012). The primary step of organic matter breakdown is mediated by extracellular enzymatic activities that render organic macromolecules available for bacterial uptake. Sediment organic matter can be channeled to higher trophic levels both directly (e.g., through deposit-feeding meiofauna and macrofauna) and/or indirectly, i.e., through bacterial uptake, Heterotrophic C Production (HCP), and subsequent consumption by bacterial grazers such as protozoa and bacterivorous meiofauna (Manini et al. 2003).

Due to its particular features, the Mar Piccolo of Taranto can be considered as a unique macrocosm where the application of a holistic approach could help to properly address future remediation actions that are urgently required in the area. Thus, we applied the benthic ecosystem functioning approach at four sites that should represent different forms/ degrees of pollution. More precisely, we focused mainly on the key heterotrophic pathways and investigated in particular (1) how the main benthic heterotrophic communities (prokaryotes, meiofauna, and macrofauna) respond to severe forms of pollution in terms of abundance and composition, and (2) to what extent does the ability of the system in transferring organic detritus to the higher trophic levels change through microbial degradation activities and the subsequent incorporation of mobilized C into prokaryotic biomass.

Materials and methods

Study site and sampling

The study area is an inner, semi-enclosed basin, with lagoonal features and divided into two inlets: the first is close to the navy arsenal and the steelworks plant, while the second is mainly influenced by mussel farms. The Mar Piccolo is characterized by scarce hydrodynamism and low water exchange since it is connected with the Mar Grande by just two narrow channels in the first inlet: the “Navigabile” and the “Porta Napoli” channels (Fig. 1). The main hydrological and chemical-physical features of the study area have been exhaustively described by Cardellicchio et al. (2015, this issue).

In June 2013 and April 2014, sampling was carried out at four sites selected as representatives of different environmental issues and anthropogenic impacts (Fig. 1). In the first inlet, St. 1E (40° 29′ 01 N, 17° 14′ 46 E) and St. 1I (40° 28′ 46 N,

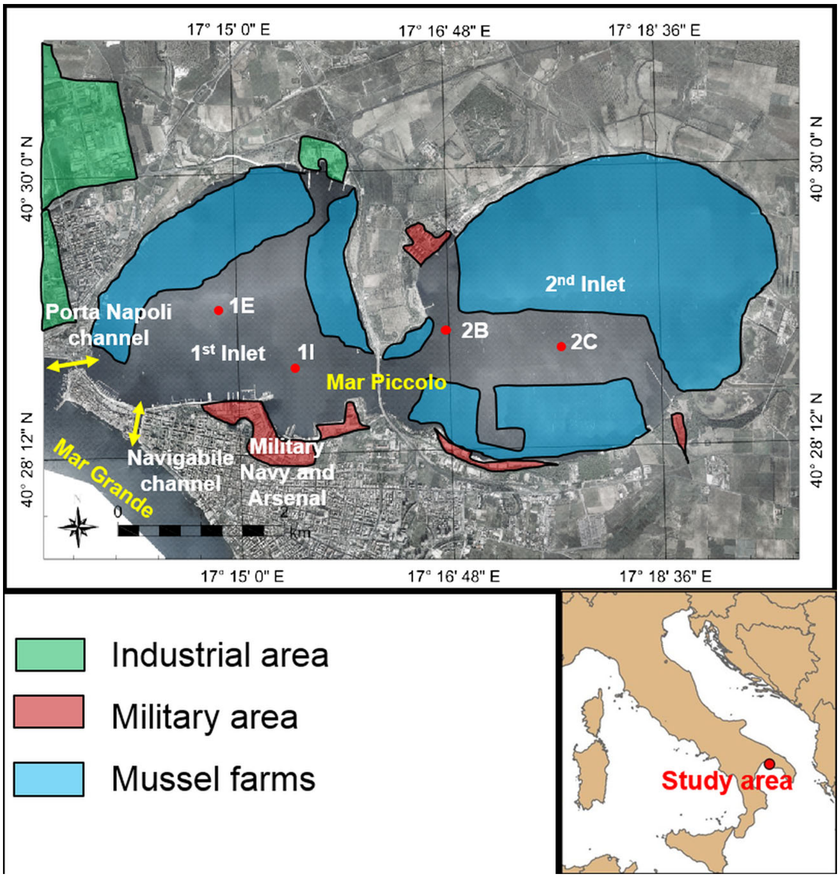

Fig. 1 Location of the four sampling stations in the Mar Piccolo of Taranto (*red circles*). The main anthropogenic pressures that exist along its coast are highlighted: the industrial area (*green*), the military area (*red*), and the mussel farms (*blue*). "Porta Napoli" and "Navigabile" channels are indicated by *yellow arrows*

17° 15′ 38 E) should summarize the environmental features of the area in front of the "Navigabile" channel and the one nearby the military navy arsenal, respectively. In the second inlet, St. 2B (40° 28′ 57 N, 17° 16′ 42 E) and St. 2C (40° 28′ 57 N, 17° 17′ 41 E) were selected as representatives of the most enclosed part of the Mar Piccolo. Such stations were chosen following an increasing distance from the main sources of industrial pollution (located in the first inlet) and, at the same time, a more pronounced influence of mussel farms.

At each station, scuba divers collected five virtually undisturbed sediment cores using polycarbonate sample tubes (12.7 cm i.d. with a sample area of 127 cm^2). Scuba divers avoided macroalgae when conspicuous as at St. 2B and 2C in order to maintain the same sampling conditions in all sites. One sediment core was partially extruded on board and the oxic sediment layer (0–1 cm ca) was subsampled and processed for the incubation in situ of the Heterotrophic C Production (HPC). The other cores were brought to the laboratory; one was dedicated to meiofauna subsampling while the others were partially extruded and the collected oxic sediment layer (0–1 cm ca) of each core was homogenized for the analyses of the main chemical (i.e., total and organic C, total N and biopolymeric C) and microbial parameters (i.e., prokaryotic abundances and exoenzymatic activities). The same homogenized oxic sediment was also subsampled for the determination of heavy metals and PCBs as detailed in Cibic et al. (2015, this issue).

During each sampling, seawater temperature, dissolved oxygen, and salinity at the bottom were measured using a Seabird 19 Plus Seacat probe.

Total nitrogen (TN), total Carbon (TC), and total organic carbon (TOC)

For chemical element analyses, sediments were freeze-dried and ground using a mortar and pestle, and >250 μm fraction was separated from the rest of the sample. Triplicate of subsamples (~8–12 mg) were directly weighed in capsules (5× 9 mm) on a microultrabalance Mettler Toledo model XP6 (accuracy of 0.1 μg). Tin and silver capsules were used for TC/TN and TOC analyses, respectively. The contents of TN, TC, and TOC were measured using an elemental analyzer CHNO-S Costech model ECS 4010. Before TOC determination, obtained following the methods of Pella and Colombo (1973) and Sharp (1974), subsamples were treated with increasing concentrations of HCl (0.1 and 1 N) to remove the carbonate (Nieuwenhuize et al. 1994).

Biopolymeric carbon (BPC)

Subsamples of homogenized sediment were freeze-dried and processed for the determination of carbohydrates, lipids, and proteins. Colloidal and EDTA extractable carbohydrates (CHO) were analyzed following the method described by Blasutto et al. (2005). Lipids were analyzed following the method proposed by Bligh and Dyer (1959) and modified for sediments. Proteins were extracted in NaOH (0.5 M) for 4 h and determined according to Hartree (1972). All analyses were carried out in four replicates. Carbohydrate, lipid, and protein concentrations were converted to carbon equivalents (Fichez 1991). The sum of carbohydrates, lipids, and proteins carbon was referred to as biopolymeric carbon (BPC).

Prokaryotic abundance

Three aliquots of sediment (0.5 g_{wet}) were withdrawn from each sediment sample, transferred into sterile test tubes, and fixed with 3 mL of pre-filtered (0.2-μm pore size) and buffered formaldehyde solution (3 %*v*/*v* final concentration in autoclaved seawater). The sediment slurry was kept at +4 °C for 24 h, then washed twice with 3 mL of 1× phosphate-buffered saline (PBS) (pH=7.2) by centrifuging at 500×*g* to remove the supernatant, and stored in 1× PBS-ethanol (1:1) at −20 °C (Ravenschlag et al. 2000). For the subsequent sample processing, a modified protocol by Lunau et al. (2005) was followed: 100 μL of sediment slurry was diluted with 900 μL sterilized Milli-Q water and 100 μL methanol. The diluted samples were placed in a water bath at 35 °C for 15 min, sonicated in ice (three times for 1 min with two 30-s in-between breaks), diluted again with sterile Milli-Q water to a final concentration of 1:110, and filtered on black Nuclepore polycarbonate 0.2-μm-pore-size filters. Filters were mounted on microscope slides, stained with a SYBR Green I-mounting medium Mowiol solution (1:15), and counted by epifluorescence microscopy at ×1000 magnification (LEICA DM2500). A minimum of 300 cells were counted for each filter in at least 20 randomly selected fields under a blue filter set (BP 450–490 nm, BA 515 nm).

Benthic bacterial community structure

Bacterial community structure was assessed in the homogenized oxic sediment only in April 2014. Bacterial community DNA was extracted from 0.5 g_{wet} of sediment using PowerSoil DNA extraction kit (MoBIO) with a slightly modified protocol. To facilitate the cell lysis, 0.5 g of muffled glass beads (0.1 mm) was added before the bead-beating step, which was then repeated four times for 2.5 min followed by 10 min of incubation at 70 °C. DNA was eluted in 50 μL of preheated (70 °C) elution buffer. Bacterial 16S rRNA amplicon library was generated by two PCR amplifications. The first PCR was performed using universal bacterial primers F8 (5′-AGA GTT TGA TCC TGG CTC AG-3′) and U534R (5′-ATT ACC GCG GCT GCT GGC-3′) in a reaction containing 1× KAPA HiFi HotStart ReadyMix, BSA (3 mM), 0.5 μM of each primer, 1× EvaGreen, up to 10 μL of molecular-grade water, and 1 μL of extracted DNA. Amplification was carried out in a qPCR cycler with an initial denaturation step at 95 °C (3 min), followed by 23 cycles of denaturation at 98 °C (20 s), primer annealing at 59 °C (15 s), and extension at 72 °C (15 s). Cycling was completed by a final extension at 72 °C for 1 min, followed by melting curve analysis obtained by increasing the temperature from 65 to 95 °C. PCR products were diluted based on Ct values to obtain the equimolar concentrations for all of the samples and used as template to amplify the 16S rRNA hypervariable region V3. Amplification was performed in 25 μL reaction containing 1× KAPA HiFi HotStart ReadyMix, 3 mM BSA, additional 0.5 mM $MgCl_2$, 1× EvaGreen fluorescent dye, and 0.4 μM of both forward primer (B338F-5′-ACT CCT ACG GGA GGC AGC AG-3′) fused with P1-adapter sequence, and reverse primer (U534R-5′-ATT ACC GCG GCT GCT GGC-3′) fused with A-Adaptor plus key sequence. Nested PCR was performed under the same cycling conditions as the first PCR with an exception of annealing temperature of 57 °C and 14 amplification cycles. Amplicons were gel-purified using HiYield™ Gel/PCR DNA Fragments Extraction Kit (RBC Bioscience). Amplicon library concentration was estimated with Qubit® dsDNA BR Assay Kit (Life Technologies), while the size and purity were examined by on-chip electrophoresis in 2100 BioAnalizer instrument using DNA 1000 kit (Agilent). Using the concentration data and the average amplicon length (261 bp), the molarity of amplicon library was calculated, assuming that an average base pair has a molecular weight of 650 g mol^{-1}. All the libraries were pooled in equimolar amount and the pool diluted to the concentration of 25 pM just before sequencing. Sequencing of the amplicon libraries was carried out on the Ion Torrent Personal Genome Machine (Life Technologies) system using the Ion Sequencing 200 kit (Life Technologies) on a Ion 316 chip v2 (Life Technologies).

For sequence analysis, reads were processed for demultiplexing, adapters, and quality trimming using the CLC genomics workbench 7.5 (CLCbio). Reads from each sample were analyzed using several software packages. Richness and diversity metrics, including the number of observed OTUs, Chao1 estimator, Shannon index, and inverse Simpson's index, were calculated using the Mothur-based analysis embedded in the Clove software pipeline (http://www.ncbi.nlm.nih.gov/pubmed/21878105). Taxonomic assignments were performed with MG-RAST (http://www.biomedcentral.com/1471-2105/9/386) versus the non-redundant database M5RNA. The on-line software METAGENassist was used to perform statistical differential OTUs representation and to link taxonomic assignments to metabolic features (http://nar.oxfordjournals.org/content/40/W1/W88).

Meiofauna

From a virtually undisturbed sediment core, three replicates were gently taken using cut-off plastic syringes (2.7 cm i.d., length 11.4 cm) and immediately frozen at −20 °C (Higgins and Thiel 1988). The top 10 cm of the sediment core was considered; subsamples were preserved in buffered 4 % formaldehyde solution using prefiltered seawater and stained with Rose Bengal (0.5 g L^{-1}). Sediment samples were sieved through 1000 and 38 μm mesh net and the extraction of organisms (from the sediment retained on the 38 μm sieve) was performed by three times centrifugation (1932×*g*, 10 min) with Ludox HS-40 (density 1.15–1.18 g cm^{-3}) as described by Danovaro et al. (2004). All meiobenthic organisms were counted and taxonomically classified to the main groups according to Higgins and Thiel (1988) under a stereomicroscope (Olympus SZX12; final magnification of ×40 or ×80). The abundance was expressed as individuals per 10 cm^2.

Macrofauna

Macrobenthos were sampled with a van Veen grab sampling area of 0.06 m^2; three replicates per station were taken, sieved through a 1000 μm sieve, and immediately fixed with formaldehyde solution (4 %*v*/*v* final concentration in seawater). After sorting, all animals were counted and identified to the lowest possible taxonomical level using a stereomicroscope (Zeiss Discovery V.12) at ×8 to ×100 magnification (Rees et al. 1990). For the identification of organisms, the taxonomical keys listed in Morri et al. (2004) were used. The abundance was expressed as individuals per square meter. Individuals were grouped in five trophic guilds according to several criteria as the feeding apparatus morphology, the feeding mode, and the food source (Desrosiers et al. 2000). Such groups were surface deposit feeders (SDF), subsurface deposit feeders (SSDF), filter feeders (FF), carnivores (CRN), and grazers (GRZ).

Degradative activities

Extracellular enzymatic activities were assayed using fluorogenic substrate analogues (Hoppe 1993) derived from 7-amino-4-methyl-coumarin (AMC) and 4-methylumbelliferone (MUF). Protease activity (leucine aminopeptidase activity-AMA) was assayed as the hydrolysis rate of leucine-AMC while β-glucosidase (β-GLU), lipase (LIP), and chitinase (CHIT) were assayed using MUF-β-D-glucoside and MUF-oleate and MUF-β-D-glucosamide (Sigma-Aldrich), respectively. Enzyme activities were expressed in terms of the rate of MUF or AMC production.

Sediment slurries were prepared by adding 6 mL of 0.2-μm-filtered bottom water to 0.5 g of wet sediment. After evaluation of the saturating concentrations, hydrolysis rates were measured by incubating slurries with (final concentrations) 800 μM leucine-AMC, 400 μM MUF-β-D-glucoside, MUF-oleate, and 200 μM MUF-β-D-glucosamide for 1 h in the dark at in situ temperature. Before spectrofluorometric measurement, each sample was centrifuged for 2 min at 3000 rpm. Fluorescence increase due to MUF and AMC hydrolyzed from the model substrates was measured using a Jasco FP 6500 spectrofluorometer (MUF=365-nm excitation and 455-nm emission; AMC=380-nm excitation and 440-nm emission). Standard solutions of MUF and AMC were used to produce calibration curves with 0.2-μm-filtered bottom water. Triplicate blanks without fluorogenic substrate were used to determine the natural fluorescence increase in the samples not attributable to the tested enzymes. Hydrolytic activities were converted into C mobilization using the conversion factor 72 for glucose and leucine and 216 for oleic acid.

Heterotrophic Carbon Production (HCP)

HCP was measured following the method of ^{3}H-leucine uptake for sediment samples (van Duyl and Kop 1994, as detailed by Manini et al. 2004). Each sediment sample (0.2 mL of 1:1 *v*/*v* slurry) was added to 6 μCi of ^{3}H-leucine (Perkin Elmer, specific activity=69) and incubated in the dark for 1 h at experimental temperature. After incubation, radiotracer incorporation was stopped by adding ethanol 80 % (1.7 mL). After two washes of the samples with ethanol (80 %) by mixing, centrifuging, and supernatant removal, the sediment was transferred with ethanol (80 %) onto a polycarbonate filter (0.2 μm mesh size). Subsequently, the filters were washed twice with 5 % trichloroacetic acid. Samples were heated in NaOH 2 M for 2 h in a water bath at 100 °C, cooled on ice, and centrifuged at 425×*g* for 3 min. One milliliter of supernatant was transferred to scintillation vials and 10 mL of Hionic Fluor® (Perkin Elmer) scintillation fluid was added. Activity in the samples was determined by a β-counter (Packard Tri-Carb 2900TR). For each sample, three replicates and two ethanol-treated blanks were analyzed.

Statistical analyses

Descriptive statistics was performed on meiofauna and macrofauna in order to calculate the relative abundance (RA) of the main taxa.

Univariate diversity analysis, cluster analysis, and similarity percentage analysis (SIMPER) were performed on macrofauna using PRIMER software v.5 (PRIMER-E Ltd., Plymouth, UK). The applied univariate diversity indices were number of taxa (S), richness (d; Margalef 1986), equitability (J′, Pielou 1966), and diversity (H′log2; Shannon and Weaver 1949).

To test for the variability of the main variables as a whole among stations, the analysis of similarity (ANOSIM) was applied using PRIMER software v.5 (PRIMER-E Ltd., Plymouth, UK). ANOSIM tests a priori-defined groups (corresponding to the stations) against random groups in ordinate space. R_{ANOSIM} statistic values are a relative measure of separation of the a priori-defined groups. A zero (0) indicates that there is no difference among groups, while a one (1) indicates that all the samples within groups are more similar to one another than any samples from different groups.

Principal component analysis (PCA) was carried out on environmental data in order to visualize the trends of the main abiotic variables. TOC, proteins, lipids, and total carbohydrates were included in the analysis together with total PCBs and total Hg (data presented in Cibic et al. 2015, this issue). The biotic components (benthic prokaryotes, meiofauna and macrofauna abundances, extracellular enzymatic activities, HCP) were projected on the factor plane as additional variables without contributing to the results of the analysis. This can provide an insight into the possible influence of the environmental variables upon each benthic group (STATISTICA 7).

Multidimensional scaling analysis (MDS) was performed on the abundances of macrofaunal trophic guilds. Square root transformation of the data matrix and Bray-Curtis similarity were applied using PRIMER software v.5 (PRIMER-E Ltd., Plymouth, UK).

Results

Chemical characterization of sediments

The results of the main chemical parameters (TN, TC, TOC, and BPC) are reported in Table 1. During both campaigns, the highest values of TN were measured at St. 2B (~4 mg C g^{-1}) while minima at St. 1E (<2.5 mg C g^{-1}). Regarding TC, higher contents characterized the first inlet (~80 mg C g^{-1}) compared to the second one, especially St. 2C (≤60 mg C g^{-1}). Almost half of the TC load was constituted by TOC, with maxima at St. 1I and minima at St. 2C during both campaigns. In June 2013 and April 2014, protein contents of St. 2C were slightly higher than lipids while the latter represented the dominant BPC fraction in the first inlet. The site in front of the navy arsenal (St. 1I) showed, in fact, elevated lipid contents with values equal to 5256.3±189.1 μg C g_{dry}^{-1} and 3157.3±74.3 μg C g_{dry}^{-1} in June 2013 and April 2014, respectively. Carbohydrates constituted the minor fraction of the BPC pool, with values at least one order of magnitude lower than proteins and lipids. A clear difference between the two inlets was not observed for the EDTA-extractable carbohydrates whereas the colloidal carbohydrates were very low in the first inlet during June 2013.

Benthic prokaryotes and bacterial community structure

Although benthic prokaryotic numbers did not vary either among stations or during the two campaigns (Table 2), some differences were observed in the prokaryotic community structure in April 2014.

Out of 430,976 obtained reads, 396,591 were left after the removal of the low quality sequences (limit p_{err}=0.5) and/or with a length <150 bp. A total of 355,513 reads were recognized as bacterial 16S rRNA gene sequences, and out of this, approximately 56 % (197,692 reads) could be affiliated to a specific bacterial class. Based on 97 % similarity, 6537, 9761, 11,283, and 8963 OTUs were observed in samples from St. 1E, St. 1I, St. 2B, and St. 2C, respectively. According to the Shannon diversity index (H′), the most diverse communities were those from St. 1I and St. 2B (H′=7.3 and 7.2, respectively), while at St. 1E and St. 2C diversity was slightly lower (H′=6.5 and 6.6, respectively). Taxonomic classification of the reads revealed that the most abundant bacterial groups were *Gammaproteobacteria*, *Deltaproteobacteria*, *Bacteroidetes*, and *Alphaproteobacteria* (Fig. 2). On a family level, *Flavobacteriaceae* (*Bacteroidetes*), *Rhodobacteraceae* (*Alphaproteobacteria*), and *Desulfobulbaceae* (*Deltaproteobacteria*) were most frequently detected at St. 1E. *Desulfobacteraceae* (*Deltaproteobacteria*) were more abundant than *Flavobacteriaceae* at St. 1I and St. 2B, while all four families were equally abundant in the middle of the second inlet (St. 2C).

The taxon-to-phenotype mapping (done by METAGENassist) did not show clear differences among the four stations in the metabolic potential (Fig. 3). The most common trait identified at all four stations (around 20 % of population) was the oxidation of ammonia, followed by the dehalogenation ability, sulfate reduction and oxidation, nitrite reduction, and N_2 fixation.

Benthic communities at higher trophic levels

The absolute abundances of meiofaunal and macrofaunal organisms are summarized in Table 2.

In June 2013, meiofaunal numbers ranged from 321.5±223.3 ind. 10 cm^{-2} to 1303.9±721.9 ind. 10 cm^{-2} at St. 1I and St. 1E, respectively. During the second campaign, St. 1I showed very low densities (91.3±58.7 ind. 10 cm^{-2}) while the highest value was reported at St. 1E (695.0±393.6 ind. 10 cm^{-2}), comparable to the abundance observed at St. 2C (636.9±218.9 ind. 10 cm^{-2}) (Table 2).

Meiofauna were dominated by Nematoda during both campaigns and at all stations, with relative abundances (RA) >75 %. Copepoda and their naupliar stages were the second

Table 1 The main structural chemical data measured in the four sites

	St. 1E	St. 1I	St. 2B	St. 2C
TN (mg N g^{-1})	2.2 ± 0.0	3.0 ± 0.1	3.9 ± 0.1	3.7 ± 0.1
	2.5 ± 0.0	2.7 ± 0.0	4.0 ± 0.0	2.8 ± 0.0
TC (mg C g^{-1})	79.8 ± 0.9	81.1 ± 0.5	69.6 ± 0.6	60.4 ± 0.3
	76.9 ± 0.4	83.7 ± 1.3	64.4 ± 0.1	55.3 ± 0.5
TOC (mg C g^{-1})	35.6 ± 0.6	42.9 ± 1.5	38.1 ± 0.2	30.2 ± 0.5
	33.2 ± 2.1	38.1 ± 1.5	32.2 ± 1.6	22.3 ± 0.3
C-PRT (µgC g^{-1})	693.7 ± 12.0	1943.6 ± 30.0	1522.9 ± 8.6	2145.4 ± 69.3
	1200.3 ± 29.9	1963.8 ± 45.6	2420.7 ± 69.7	1591.5 ± 22.0
C-LIP (µgC g^{-1})	2497.0 ± 58.7	5256.3 ± 189.1	2580.3 ± 68.8	1896.1 ± 86.5
	2498.4 ± 97.4	3157.3 ± 74.3	1685.9 ± 90.5	1068.0 ± 75.3
C-CHO$_{EDTA}$ (µgC g^{-1})	197.3 ± 10.0	292.8 ± 12.8	321.3 ± 16.5	269.7 ± 12.7
	132.8 ± 6.1	187.5 ± 6.4	154.2 ± 5.4	117.6 ± 4.0
C-CHO$_{H2O}$ (µgC g^{-1})	13.1 ± 1.2	16.7 ± 0.5	109.8 ± 7.0	154.6 ± 1.3
	96.5 ± 2.6	90.8 ± 1.8	136.5 ± 3.5	93.2 ± 0.9
BPC (µgCg^{-1})	3401.1	7509.4	4534.3	4465.7
	3928.0	5399.4	4397.2	2870.4

Values are averages of three replicates (±standard deviations). White and gray backgrounds outline data of June 2013 and April 2014, respectively

TN total nitrogen, *TC* total carbon, *TOC* total organic carbon, *C-PRT* proteins, *C-LIP* lipids, C-CHO_{EDTA} EDTA-extractable carbohydrates, *C*-CHO_{H20} colloidal carbohydrates extracted in water, *BPC* biopolymeric C

most abundant taxon with higher RA at St. 2C both in June 2013 and in April 2014. Kinorhyncha were observed only in sediments of St. 1E and in very low percentages (Table 3). Overall, in April 2014, a higher number of taxa was observed than during the first campaign.

In June 2013, total macrofaunal abundances varied from 605.0±543.1 ind. m^{-2} to 1916.0±341.9 ind. m^{-2} at St. 1I and St. 1E, respectively. In April 2014, values were lower than those observed during the previous campaign and the minimum, equal to 145.0±7.1 ind. m^{-2}, was recorded at St. 1I, while the highest density was at St. 2C (1050.0±14.1 ind. m^{-2}) (Table 2). Overall, in June 2013, the community was dominated by polychaetes (41 %) and molluscs (40 %), followed by crustaceans, which represented ~13 % of the total abundance. The remaining 6 % included taxa with a sporadic occurrence as anthozoans, sipunculids, and nemertines. The dominance of polychaetes was due to the high abundance of this taxon at St. 1E (RA=56.2 %), while at

Table 2 Mean abundance of the three benthic communities analyzed at the four stations. Values are averages of three replicates (± standard deviations)

	Benthic prokaryotes	Meiofauna	Macrofauna
	*10^9 cells g$_{dry}$ $^{-1}$	ind. 10 cm^{-2}	ind. m^{-2}
St. 1E	2.3 ± 0.0	1303.9 ± 721.9	1916.0 ± 341.9
	2.5 ± 0.2	695.0 ± 393.6	755.0 ± 106.1
St. 1I	1.9 ± 0.0	321.5 ± 223.3	605.0 ± 543.1
	2.0 ± 0.1	91.3 ± 58.7	145.0 ± 7.1
St. 2B	2.5 ± 0.1	713.8 ± 154.0	683.3 ± 294.9
	1.8 ± 0.0	437.7 ± 185.9	255.0 ± 21.2
St. 2C	1.8 ± 0.3	589.3 ± 108.0	977.0 ± 209.2
	1.9 ± 0.1	636.9 ± 218.9	1050.0 ± 14.1

White and gray backgrounds outline data of June 2013 and April 2014, respectively

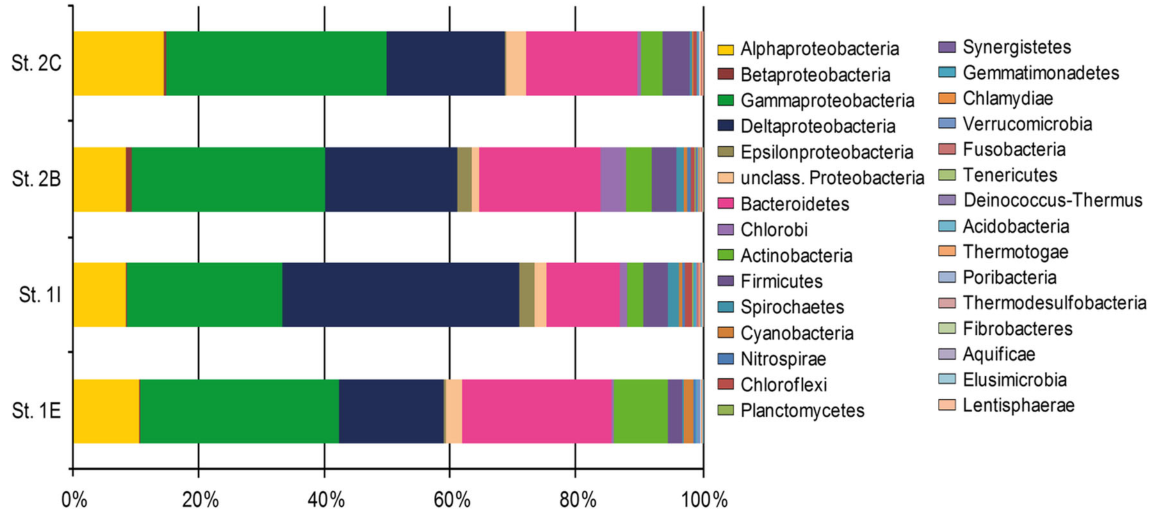

Fig. 2 Relative abundance and affiliation (class level) of the reads from each sampling station in April 2014

the other stations, molluscs represented the most abundant group of organisms (Table 3). Crustaceans accounted for only 4.3 % of the whole community at St. 1E and their RA increased considerably at the other three sites. Echinoderms were observed only in the first inlet and with low percentages. Similarly, in April 2014, macrofauna were constituted mainly by molluscs (41 %), polychaetes (38 %), and crustaceans (13 %), while the remaining 8 % comprised anthozoans, sipunculids, and nemertines. The dominance of molluscs was ascribable to their high numbers at St. 2C, where these organisms, mainly represented by bivalves, accounted for >50 % of the whole community. RA of polychaetes decreased from St. 1E to St. 2C while higher RA of crustaceans were reported at St. 1I and 2B. Similarly to the first campaign, echinoderms were observed in low RA and mainly at the station close to the channel, i.e., St. 1E (RA=10.6 %).

Focusing on the qualitative composition of macrofauna, sediments of St. 1E harbored typically marine species during both campaigns, i.e., *Papillicardium papillosum* and *Pitar rudis* among bivalves and *Amphiura chiajei* and *Ophiotrix quinquemaculata* among echinoderms. The other stations were characterized by organisms that inhabit paralic environments as *Abra segmentum*, *Cerastoderma glaucum* (bivalves), *Gammarus aequicauda*, and *Gammarus insensibilis* (amphipods). At St. 1I and 2B, taxa typically associated to the presence of macroalgae were observed: *Gammarus* sp. and *Leuchotoe* sp. among amphipods and *Musculus subpictus* and *Modiolula phaseolina* among bivalves.

Applying the diversity indices to macrofauna, H′ values were higher in June 2013 than in April 2014 (Table 4). During the first campaign, St. 2B resulted the most diverse station with H′ equal to 4.41 whereas the lowest diversity

Fig. 3 Relative abundances of specific metabolic traits obtained with taxon-to-phenotype mapping (METAGENassist)

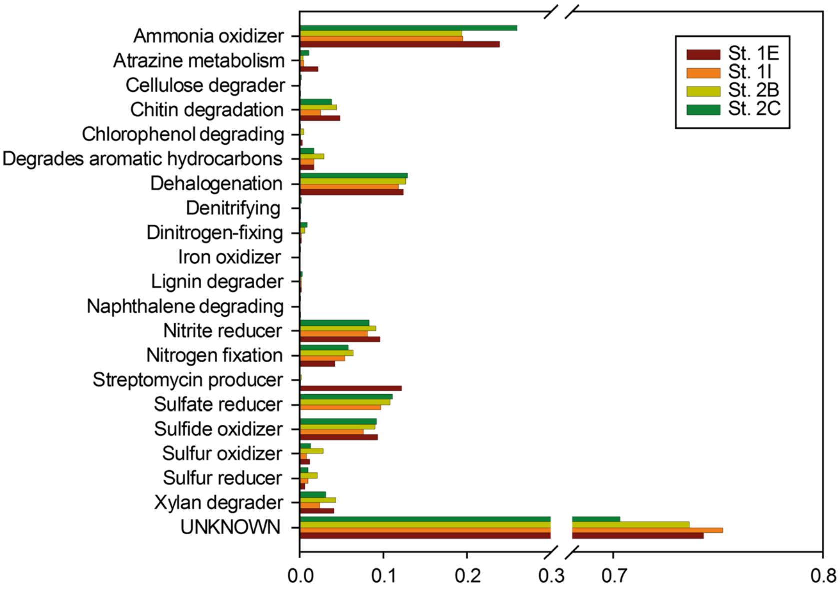

Table 3 Relative abundance (%) of major groups constituting meiofauna and macrofauna at the four stations

Meiofauna	St. 1E	St. 1I	St. 2B	St. 2C
Nematoda	94.0	93.1	93.4	90.3
	92.0	86.7	79.9	75.6
Copepoda	2.1	4.1	5.0	3.0
	3.1	4.2	5.6	6.6
Nauplii	1.7	1.9	1.5	6.8
	3.3	6.7	11.4	17.2
Kinorhyncha	1.8	0.0	0.0	0.0
	1.1	0.0	0.0	0.0
Others	0.3	0.9	0.1	0.0
	0.5	2.4	3.2	0.6
Macrofauna				
Polychaeta	56.2	27.5	31.7	24.4
	72.8	51.7	35.3	13.3
Mollusca	29.3	43.1	48.8	54.5
	7.3	10.3	23.5	73.8
Crustacea	4.3	26.6	16.3	18.8
	1.3	37.9	39.2	12.4
Echinodermata	3.8	0.9	0.0	0.0
	10.6	0.0	0.0	0.0
Others	6.4	1.8	3.3	2.3
	7.9	0.0	2.0	0.5

Meiofauna were analyzed in the top 10 cm. White and gray backgrounds outline data of June 2013 and April 2014, respectively

characterized St. 2C (H′=3.72). The highest number of taxa (S) and richness (d) were calculated at St. 1E. Although this site was characterized also by the second highest H′ value, the minimum of equitability (J′) was due to the dominance of *Notomastus formianus* (polychaete) and *Abra alba* (mollusc). In April 2014, the maxima of both d and H′ were calculated at St. 1E, whereas the lowest values characterized St. 2C. The dominance of *Arcuatula senhousia* at this site contributed also to the minimum of equitability (J′=0.43).

Table 4 Univariate diversity indices applied to macrofauna at the four stations

	S	d	J'	H'($\log_2$)
St. 1E	57	9.66	0.75	4.39
	24	4.61	0.87	3.98
St. 1I	26	5.33	0.88	4.12
	12	3.30	0.89	3.20
St. 2B	35	7.10	0.86	4.41
	12	2.83	0.89	3.20
St. 2C	24	4.47	0.81	3.72
	16	2.82	0.43	1.71

White and gray backgrounds outline data of June 2013 and April 2014, respectively

S number of taxa, *d* richness, *J′* equitability, *H′(log2)* diversity

While grazers were absent at St. 1E, macrofauna were dominated by deposit feeders that represented >69 % of the whole community during both periods. The highest contribution was attributable to surface deposit feeders (738.8 ind. m^{-2}) and subsurface deposit feeders (305.0 ind. m^{-2}) in June 2013 and April 2014, respectively. The other sampling stations were characterized by the presence of all trophic guilds during both campaigns, and some differences were observed. At St. 1I, macrofauna were dominated by grazers (the isopod *Paracerceis sculpta* and the bivalve *Haminoea navicula*) and carnivores (the polychaetes *Lumbrineris latreilli* and *Eunice vittata*), which together accounted for 55.2 % (June 2013) and 62.1 % (April 2014) of the whole community. At St. 2C, filter feeders were well represented, especially during April 2014, when this group accounted for 79.4 % of the total abundance due to the high density of

A. senhousia (bivalve). St. 2B showed an intermediate situation compared with the other sites and a clear dominance of a trophic association over the others was not highlighted.

Functional parameters

The rates of β-glucosidase, lipase, and chitinase were low (<4 nmol cm^{-3} h^{-1}) both in June 2013 and April 2014, and the variability among stations did not allow to highlight any pattern within the study area. The highest enzymatic activities were directed to the degradation of proteins. The minima were observed at St. 1I during both campaigns (18.63±1.75 nmol cm^{-3} h^{-1} and 11.71±0.82 nmol cm^{-3} h^{-1}) while the maxima were reached at St. 2C (56.50±1.42 nmol cm^{-3} h^{-1}) and St. 1E (126.19±1.62 nmol cm^{-3} h^{-1}) in June 2013 and April 2014, respectively. Alkaline phosphatase showed intermediate rates, with lower values in the first inlet and slightly higher values in the second one, both in June 2013 and April 2014 (Fig. 4).

In June 2013, HCP rates, ranging from 0.99±0.05 μg C $g_{dry}{}^{-1}$ to 2.53±0.38 μg C $g_{dry}{}^{-1}$, were higher than during April 2014, when data varied between 0.17±0.01 μg C $g_{dry}{}^{-1}$ and 0.59±0.05 μg C $g_{dry}{}^{-1}$. During both campaigns, sediments of St. 1I were characterized by the HCP minima, while the maxima were observed at St. 2B (June 2013) and St. 2C (April 2014), although the latter value was almost equal to the rate estimated at St. 2B (Fig. 4f).

PCA

The ordination plot of the considered functional and structural parameters accounted for 78.36 % of the total variance. Two principal components (factors) were identified (eigenvalue >1) which together explained 78.36 % of the total variance, whereas the first and the second factors explained 45.22 and 33.14 % of the total variance, respectively (Fig. 5). Total lipids, total PCBs, and TOC were the predominant elements of the first factor, while the major contributors of the second one were total proteins, total carbohydrates, and TN (Fig. 5a). All the sampling stations were plotted on the PCA factor-plane 1×2, which grouped the stations according to their environmental conditions (Fig. 5b). During both periods, the two inlets of the Mar Piccolo of Taranto were well separated with St. 1E and St. 1I in the left half of the plot, and St. 2B and St. 2C in the right one. Focusing on the first inlet, St. 1E and St. 1I were clearly separated. The position of the latter was influenced mainly by lipid load, total Hg, and total PCBs.

Discussion

In the present study, the benthic ecosystem functioning of the Mar Piccolo of Taranto was investigated at four stations that could be considered, to some extent, as representatives of different environmental issues and anthropogenic impact. Furthermore, the survey was carried out in early summer and spring conditions, i.e., during June 2013 and April

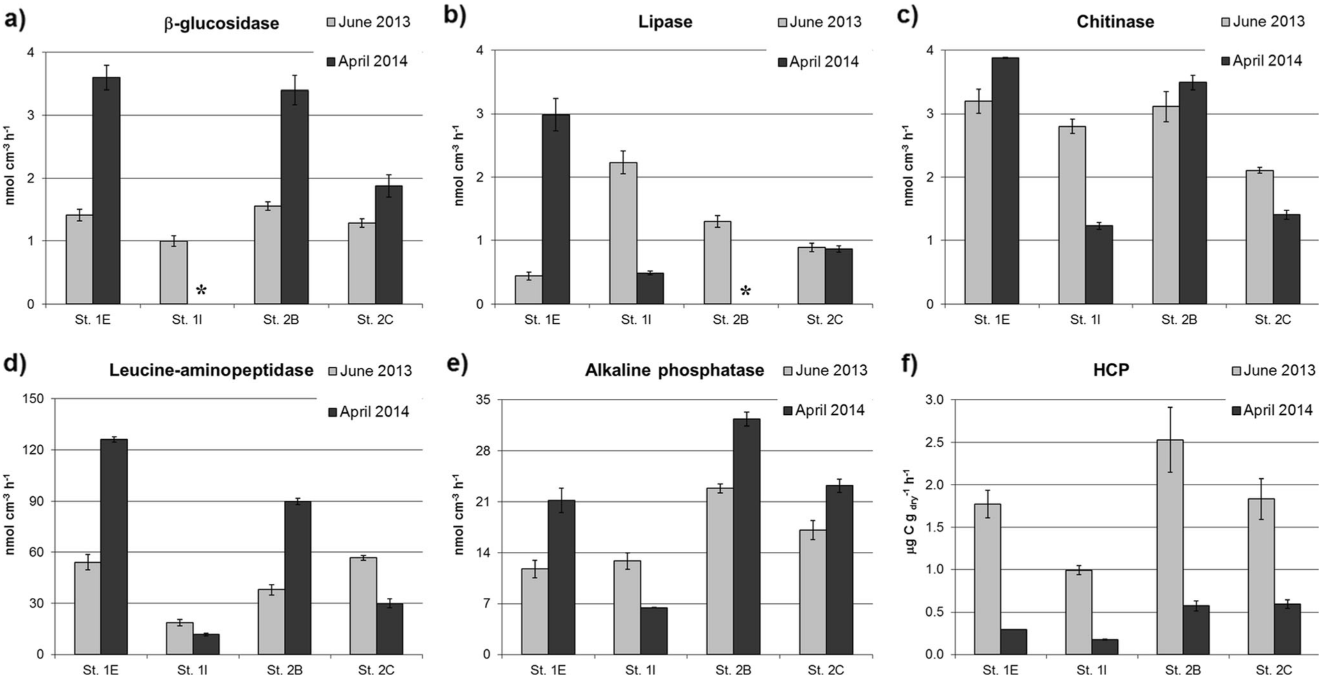

Fig. 4 Extracellular enzymatic activities and Heterotrophic C Production (HCP) at the four stations in June 2013 and in April 2014. *Asterisks* indicate that data are not available because below the detection limit of the instrument

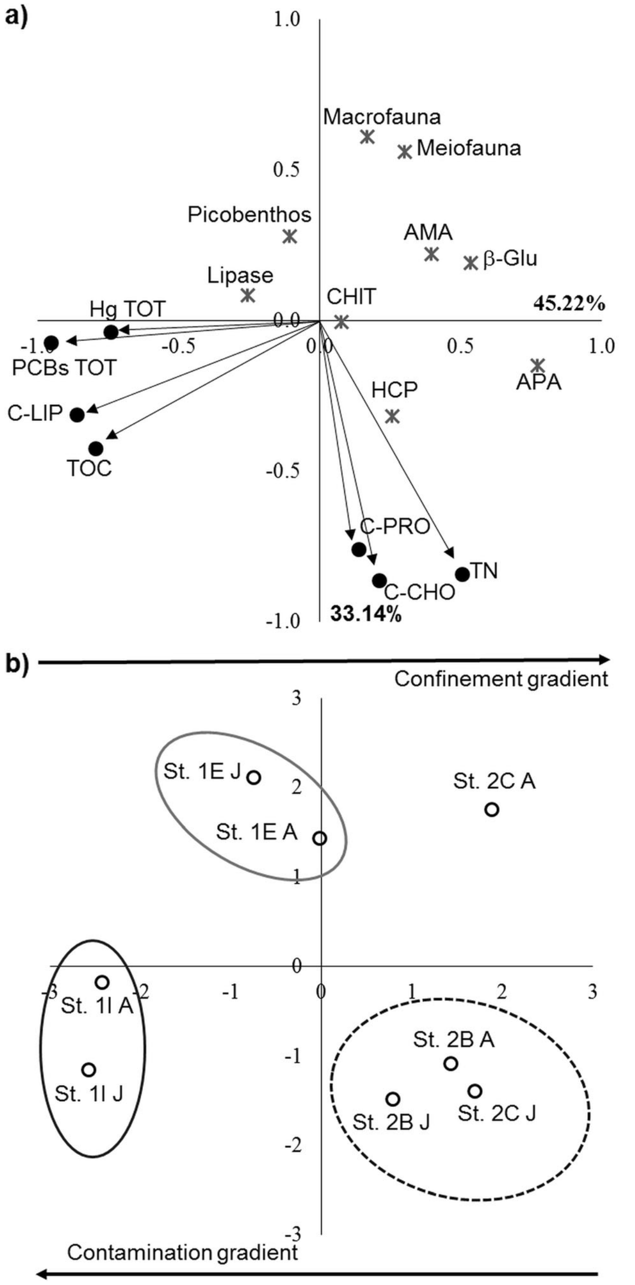

Fig. 5 PCA ordination diagram of the selected variables (**a**). Scatter diagram plotting factors 1 and 2 of sampling stations (**b**). *C-LIP* total lipids; *C-PROT* total proteins; *C-CHO* total carbohydrates; *TN* total nitrogen; *TOC* total organic carbon; *Hg TOT* total Hg; *PCBs TOT* total PCBs; *AMA* protease; *APA* alkaline phosphatase; *CHIT* chitinase; *β-Glu* β-glucosidase; *HCP* Heterotrophic C Production; *J* June 2013; *A* April 2014

2014, respectively. Focusing on the temporal variability, the ANOSIM test was performed using the two periods as discriminating factors in order to investigate the role exerted by seasonality in amplifying or reducing the differences among stations. The output highlighted that, for the selected variables (chemical structural parameters, total Hg, total PCBs, abundances of the main benthic communities, and the investigated functional parameters), the stations of June 2013 were not statistically different from those of April 2014. Thus, we infer that for the main benthic parameters, the seasonal variation was not strong enough to hide the intrinsic and more profound differences among stations. On the other hand, performing the ANOSIM test on the same parameters using the stations as the discriminating factor, the output showed that the four sampling sites were statistically different among each other, corresponding, therefore, to different environmental situations at the bottom ($R_{\text{ANOSIM}}=0.521$, $p<0.05$). These findings suggest that the spatial variability among stations exceeded the temporal one, revealing the presence of different environmental contexts that were maintained over time.

Chemical characterization of sediments and synergistic effects of contaminants

Our results pointed out that the sediments of all the studied sites contained an elevated load of both TC and TOC. However, some differences among stations were detectable. The sampled site in front of the navy arsenal showed the maxima of TC and TOC during both periods while the station located in the middle of the second inlet displayed the minima. The high TC content observed in the first inlet could be ascribable to the industrial activities that exist along its coast, e.g., the introduction of coal, which could be associated to the steelworks plant. On the contrary, the increasing distance from these sources of C could explain its lower values in the second inlet.

Focusing on the organic fraction, a pattern similar to that observed for TC was highlighted, i.e., higher TOC content at St. 1I and a gradual decrease towards the second inlet. Our findings revealed a considerable organic enrichment that tends to remain in sediments over time. During both campaigns, TOC data were, in fact, just slightly higher compared to those previously reported in the same basin (Calace et al. 2005) and fell within the range observed in another severely polluted area characterized by brackish water and lagoonal features, i.e., the Banduzzi-Aussa river system (Cibic et al. 2012). Although the peculiarities of these two environments (different depth, salinity, etc.) limit any further comparison, data confirm the high organic enrichment as a common feature for environments subjected to a diffuse and multiple degree of pollution. In the Mar Piccolo of Taranto, besides the impact of several anthropogenic activities that exist in the area (e.g., mussel farming, industrial plants, etc.), the low hydrodynamism in this semi-enclosed basin likely contributes to the accumulation of organic matter.

The discharge of municipal and industrial wastes is generally associated with the introduction of a wide array of contaminants that are tightly linked to the sedimentary organic matrix. Among them, PAHs and PCBs are widely recognized as the major environmental issue in sediments of the Mar

Piccolo of Taranto (Storelli and Marcotrigiano 2000; Cardellicchio et al. 2007). Such contaminants show a great resistance to degradation and, then, they tend to accumulate in sediments for decades. Their toxicity affects benthic organisms at several trophic levels by reducing their abundances or altering the communities' structures. Focusing on PCBs, they occur in high or warning concentrations as reported in previous surveys (Cardellicchio et al. 2007) and confirmed by Cibic et al. (2015, this issue) in the uppermost sediment layer (0–1 cm). These hydrophobic contaminants enter the coastal marine areas through primary and secondary sources (e.g., effluent outfalls, river discharges, urban runoff, etc.) and tend to settle on the seabed where they are absorbed by fine mineral particles or organic matter forming sedimentary contaminant sinks (Kapsimalis et al. 2014). In the present study, the highest values of PCBs (1159.7 and 1067.6 ng g_{dry}^{-1}, in June 2013 and April 2014, respectively, from Cibic et al. 2015, this issue) co-occurred with the maxima of TOC, i.e., at St. 1I, indicating a high level of pollution nearby the navy arsenal. At this station, the maxima of lipids were also observed, confirming the strong association of PCBs with this biopolymeric C fraction. Sediments of the first inlet, in fact, were characterized by a lipid content of >70 and >58 % over the total BPC in June 2013 in April 2014, respectively, and at St. 1I this fraction reached extremely high values during June 2013 (5256.3 ±189.1 μg C g^{-1}). On the other hand, sediments of the second inlet showed PCB concentrations about one order of magnitude lower than those reported at St. 1I, indicating that this area is less contaminated by such contaminants.

Both BPC and TN contents measured at St. 2B and St. 2C revealed a situation of substantial organic enrichment likely ascribable to mussel farming that is widely spread over the second inlet. The biodeposits (feces and pseudofeces) continuously produced by suspended bivalves settle to the bottom increasing the organic load as reported by Franzo et al. (2014) in a marine costal site. In the Mar Piccolo of Taranto, the long line mussel farms, although present in both inlets, are more widespread in the second one (i.e., ~66 % of the whole second inlet's coverage against ~26 % of the first one; Caroppo et al. 2012), where the low hydrodynamism facilitates the accumulation of such biodeposits in the seabed.

Benthic communities

The ecotoxicological evaluation of sediments of the Mar Piccolo of Taranto, based on the international Sediment Quality Guidelines (SQGs) and mSQGq indices, was applied to organic contaminants (PAHs and PCBs) and heavy metals by Cardellicchio et al. (2007) and Cardellicchio et al. (2009), respectively. Overall, their findings demonstrated that sediments of the Mar Piccolo of Taranto, particularly those in the first inlet, show medium-high or high ecological risk for marine organisms, especially for benthic species. Organic contaminants, and in particular PCBs that are strictly associated to the sedimentary organic matrix, could directly reduce deposit feeders' numbers by killing them. An indirect effect could be represented by the alteration of the inter-species relationships with the disappearance of sensitive taxa and the dominance of the most tolerant ones. We infer that the high PCB toxicity could be responsible for the low densities of meiofauna and macrofauna observed at St. 1I, where the concentration of such contaminants was extremely high. These low abundances could be reflected in a lower top-down pressure leading to the accumulation of OM, and in particular of lipids. This could partially explain the extremely high lipid contents in sediments nearby the navy arsenal.

Focusing on heavy metals, a clear distinction between the two inlets was observed, with high levels at St. 1I in accordance with previous studies in which the navy arsenal has been identified as one of the most important sources of these contaminants (Cardellicchio et al. 2009; Spada et al. 2012). Spada et al. (2012) reported that total Hg (THg) could be even 110 times higher than the background levels. However, THg in sediments is not the most important chemical form to consider, as the different Hg chemical species have also different bioavailabilities, thereby affecting their biogeochemical behavior and toxicity to organisms (Covelli et al. 2009). Oxygen concentrations in sediments modify the Hg speciation and consequently its bioavailability. In the Mar Piccolo of Taranto, although Hg is present mainly in its elemental form, a noticeable fraction of it may be "mercuric sulfide" (HgS), due to the co-occurrence of strong anoxic conditions and the high availability of sulfides (Caroppo and Cardellicchio 1995). Both these forms are insoluble and potentially less available for methylation (Covelli et al. 2009). However, bioturbation and particularly resuspension events, which often occur in the Mar Piccolo due to its shallow depth, could be responsible for the oxidation of HgS, rendering a fraction of this form eventually available for methylation. In the present study, the low abundances of meiofauna and macrofauna observed at St. 1I could be ascribable not only to PCBs but partially also to Hg contamination. Although data on Hg speciation are not available, oxygen microprofiles did not indicate a severe oxygen depletion (Rubino et al. 2015, this issue), suggesting that a fraction of HgS could be oxidized posing the risk for its consequent methylation. Bioaccumulation and biomagnification phenomena already documented in the whole area of the Mar Piccolo (Spada et al. 2012) and particularly in front of the navy arsenal corroborate the hypothesis that the benthic trophic chain is affected by Hg contamination. However, the evaluation of this aspect and its ecological implications need great caution and deserve further investigations.

To the best of our knowledge, very few data are available on meiofauna inhabiting sediments of the Mar Piccolo of Taranto. However, our findings are in accordance with

Sandulli et al. (2004) who reported the presence of a not well structured meiofaunal community characterized by low abundances. In our study, the higher densities observed at St. 1E could be ascribable to the ingression of marine water through the channel and to several associated effects as inputs of fresh organic matter and enhanced oxygenation of sediments. In the second inlet, gradually increasing meiofaunal numbers were observed both in June 2013 and April 2014, along a decreasing gradient of contamination. Although the determination at the level of the main meiofaunal groups limits further considerations about the structure and biodiversity of this community, our findings suggest that meiofauna were more structured at St. 1E due to the occurrence of several groups even if with very low RA. In accordance with our results, Sandulli et al. (2004) reported a community strongly dominated by Nematoda. Although this feature is common for both polluted and unpolluted environments, the authors observed the presence of nematode genera well adapted to strongly contaminated sediments as *Theristus*, *Terschellingia*, and *Desmodora*. Being the most diverse taxon of meiofauna, this well-represented group of organisms has a high ecological valence and therefore likely includes opportunistic taxa, i.e., able to tolerate such inhospitable environments. Moreover, Sandulli et al. (2004) observed higher meiofaunal numbers in sediments colonized by *Caulerpa racemosa*, and related the presence of such macroalgae to an increased microenvironmental complexity, which could offer new exploitable surfaces for meiofauna colonization. In our study, the presence of *Caulerpa* spp. and *Chaetomorpha linum* was observed in the second inlet during both campaigns (Cibic et al. 2015, this issue). These algae could alter the sedimentary environment by increasing the habitat complexity not only for meiofauna but also for macrofauna. The occurrence of new ecological niches could contribute, therefore, to the settlement of benthic communities different from those observed in the first inlet.

The geomorphological features of the Mar Piccolo of Taranto seem to play a pivotal role on macrofaunal abundance and community structure within the area. This semi-enclosed basin shows the peculiarities of coastal transitional environments, with decreasing salinity, limited depth, and slow water renewal rates going from the “Navigabile” channel towards the second inlet. The presence of a confinement gradient, in fact, was already reported by Alabiso et al. (2006). In similar environmental contexts, the increased distance from seawater inputs is associated to the gradual reduction of marine species, which are replaced by taxa adapted to brackish conditions (Perthuisot and Guelorget 1983). Along a confinement gradient, macrofauna tend to show large seasonal variations, low benthic diversity, and strong dominance of few paralic species (Arias and Drake 1994). In the present study, macrofauna seem to follow such general patterns. The station nearby the “Navigabile” channel (St. 1E) showed a well-developed macrobenthic community as supported by the high values of richness and biodiversity. During both sampling campaigns, marine species were observed mainly at this site, such as the echinoderms *Amphiura chiajei* and *Ophiothrix fragilis*, the bivalves *Pitar rudis* and *Papillicardium papillosum*, and the polychaete *Notomastus formianus* (Pérès 1967). These findings suggest that the proximity to the channel mouth, and the ingression of marine water through it, may influence the environmental characteristics of the sediments favoring marine environmental features. On the other hand, the inner part of the study area, i.e., the second inlet, displayed species typical of the paralic environment (the bivalves *Abra segmentum* and *Cerastoderman glaucum*; the amphipods *Gammarus aequicauda*, *G. insensibilis*, and *Microdeutopus anomalus*, Perthuisot and Guelorget 1983), reflecting the increased degree of confinement. Moreover, the paralic species *Arcuatula senhousia* and *Paracerceis sculpta* were only found at these stations.

Even considering the natural variability of the community due to the confinement gradient, different responses of macrofauna to anthropogenic stressors were detected. Focusing on the trophic guilds, the dominance of infaunal organisms and deposit feeders at St. 1E suggests, to some extent, the co-occurrence of oxygenated sediments and less severe levels of contamination. This hypothesis is corroborated by the lower load of contaminants reported at this site when compared to St. 1I and also by the oxygen microprofiles which revealed a slightly deeper oxygen penetration at that station, up to 3 mm in June 2013 (Rubino et al. 2015, this issue). On the other hand, the community nearby the navy arsenal was dominated by more mobile invertebrates as carnivores and grazers. This finding suggests that deposit feeders could be killed by the ingestion of toxic sediments due to high contaminants contents as those observed at St. 1I. Even if Hg is likely present in its insoluble forms, the digestive acids of such organisms could transform less bioavailable Hg compounds in more active ionic counterparts. Furthermore, in such inhospitable environment, mobile organisms could have more probability to survive because they are able to avoid hotspots of contaminants through their active movements (Ward et al. 2013). In the second inlet, the presence of mussel farms could enhance the proliferation of filter feeders, which obtain food via filtration of the organic matter settled from the suspended ropes. Since this pattern was more accentuated at St. 2C than at St. 2B, and the latter showed intermediate characteristics between St. 1I and St. 2C, we infer that the three stations are located along a decreasing gradient of chemical (heavy metals, PCBs, etc.) contamination. Increasing the distance from the main contamination source (that, overall, is represented by the first inlet), the community seems to adapt to an impacted environmental context in which the main stressor is the mussel farm activity. Moreover, as already mentioned for meiofauna, the presence of macroalgae could exert an influence on macrofaunal composition and structure. During April 2014, the elevated abundances of *A. senhousia* (760 ind. m^{-2}) at St. 2C could derive from the presence of the nitrophilic algae observed in our samples. The association between this bivalve and nitrophilic macroalgae has been already reported by Matarrese et al. (2004). Vegetation represents, in fact, a suitable substratum for the attachment of this species by byssal threads. The variability observed in its abundance between June 2013 and April 2014 could be explained by the intrinsic characteristics of this bivalve. It is well known, in fact, that *A. senhousia* tends to reach very high densities (150,000 ind. m^{-2}; Crooks 2001) under favorable environmental conditions.

The MDS plot performed on trophic guilds shows a clear separation of the marine St. 1E from all the other sites, and the latter seem to follow a confinement gradient. The location of the most polluted stations (St. 1I) at the bottom right of the plot suggests that the sediments in front of the navy arsenal harbor a different macrofaunal assemblage. The reciprocal positions of the other sites (especially those of the second inlet) seem to follow a decreasing contamination gradient (Fig. 6).

Benthic ecosystem functioning in the Mar Piccolo of Taranto

Ecosystem functioning involves several processes which can be summarized as production, consumption, and transfer to higher trophic levels, organic matter decomposition, and nutrient regeneration (Danovaro and Pusceddu 2007). In this study, we investigated two aspects of the heterotrophic pathways that are crucial for the transfer of detritus to the higher trophic levels and, therefore, for the ecosystem functioning performance: the organic matter decomposition rates (i.e., extracellular enzymatic activities) and the incorporation of such mobilized C into new prokaryotic biomass (i.e., HPC).

In the four studied stations, all the tested hydrolytic rates were extremely low even compared with other polluted environments characterized by high sedimentary organic matter (Manini et al. 2003; Cibic et al. 2012). Since both TOC and BPC data indicated an elevated organic content at all sites, the limited degradation rates suggest that just a small fraction of the organic pool was enzymatically degradable and, therefore, available for heterotrophic metabolism. We calculated the turnover rates of the main macromolecules as the ratio of enzymatically mobilized carbohydrates, lipids, and proteins to the corresponding labile organic fraction. Values are expressed as days required for completing the turnover of each molecules' group. St. 1I was characterized by the slowest turnover rates of both proteins (24.1 and 32.9 days in June 2013 and April 2014, respectively) and carbohydrates (28.3 and 33.6 days in June 2013 and April 2014, respectively) whereas the other stations showed values comparable with those reported for Lesina and Goro lagoons (Manini et al. 2003) and slightly lower than those calculated in the polluted Banduzzi-Aussa river system (Cibic et al. 2012). Lipid turnover was slow at all stations and particularly in the first inlet, where more than 400 days were potentially required for the degradation of the whole lipid pool (St. 1E in June 2013 and St. 1I in April 2014).

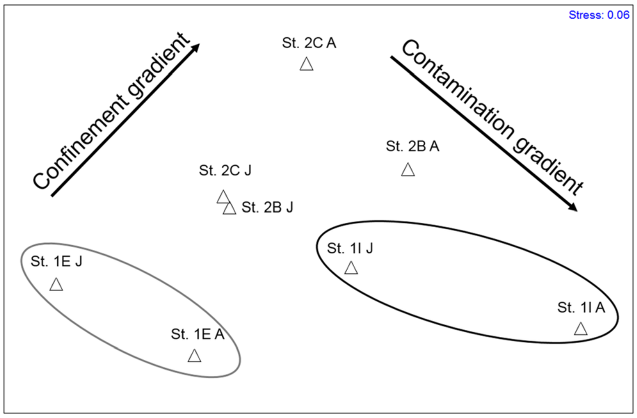

Fig. 6 MDS output performed on the abundances of main macrofauna trophic guilds. *J* June 2013; *A* April 2014

The refractory nature of sedimentary OM could be attributable to processes such as sorption or complexation, which may partially sequester potentially degradable substrates and, then, impede their mineralization. Mayer et al. (2004) demonstrated that the network of pores that allow the access of enzymes to organic matter within the aggregates might include "throats" of small mesopore size. Thus, some pores could be too small to allow enzyme diffusion to parcels of organic matter held in larger pore spaces. Furthermore, the diffusional hindrance caused by some combination of pore size and tortuosity will slow access of digestive agents to organic substrate, and return of solubilized food, that makes it physiologically unprofitable for an organism to make the attempt. In addition, Zimmerman et al. (2004) confirmed such "mesopore protection" mechanism for sequestration and preservation of sedimentary organic matter, extending the concept also to organic contaminants. Sediments of the Mar Piccolo of Taranto are an extremely complex environment where an elevated organic load co-occurs with several other compounds, such as organic contaminants and heavy metals. Unfortunately, we are not able to investigate the fraction of mesopores over the total range of pores in our sediment aggregates. However, the high complexity of the sedimentary matrix should not exclude the occurrence of adsorption/complexation processes, which alter the function of enzymes and/or their access to the substrates, resulting, therefore, in partially impeded carbon mineralization.

From an "ecosystem" point of view, the benthic system of the Mar Piccolo of Taranto showed different functioning scenarios. Nearby the navy arsenal, the high amounts of several pollutants strictly associated to the OM pool (and in particular to lipids) could increase its refractory nature, limiting therefore the degradation of the organic detritus. Furthermore, the lowest HCP rates observed at St. 1I during both campaigns suggest a limited transfer of mobilized C to the higher trophic levels. From a top-down perspective, the scarce presence of meiofaunal and macrofaunal organisms at this station could reflect limited inputs of "fresh" organic matter (e.g., exudates, feces, etc.), which could be readily available to fuel the degradation activities. Moreover, it is important to take into account that a fraction of the observed enzymatic activities should be attributable to benthic microalgae (Misic and Covazzi Harriague 2009; Cibic et al. 2012). At St. 1I, Rubino et al. (2015, this issue) reported surprisingly modest abundances and activities associated to such organisms. These findings could contribute to explain the low degradation rates observed nearby the navy arsenal.

Although the degradation rates remained rather slow at all stations, the heterotrophic microbial community seems more active at the other investigated sites if compared to St. 1I. The ingression of marine water through the "Navigabile" channel (St. 1E) and the higher abundances of both benthic microalgae (Rubino et al. 2015, this issue) and meiofauna/macrofauna could in fact contribute to the faster prokaryotic processes. This hypothesis is corroborated by the higher HCP rates observed, especially during June 2013. On the other hand, the low HCP rates measured during the second campaign may reflect the winter conditions in which slower microbial metabolisms associated to the lower bottom temperature lead to a less active microbial community. Our findings are consistent with Cibic et al. (2012) who reported comparable HCP rates in a severely polluted environment during winter.

Focusing on the main actors responsible for the investigated mineralization processes, i.e., benthic bacteria, our data on their potential metabolic traits (Fig. 4) did not clearly reveal that specific pathways prevailed over the others according to the different stations. However, the lower microbial activities observed at St. 1I suggest that the more unfavorable environmental conditions nearby the navy arsenal could exert an inhibition effect on bacteria. Heavy metals, observed in higher amounts at this station, have documented toxic effects on bacterial activity and were already reported by Sun et al. (2013) as the main factor able to shape bacterial community composition, structure, and diversity in sediments of estuaries subjected to multiple contamination. Moreover, the large percentage of "unknown" metabolic traits makes the interpretation of results particularly arduous since the real metabolic potential of the community is likely hidden. Much of our knowledge on pollutant tolerant strains in literature comes from studies that involve laboratory isolation from environmental samples. However, moving from environment into controlled laboratory settings will favor certain bacterial types. The high abundance of unclassified groups in this and other studies (Sun et al. 2013) suggests that current knowledge on contaminant tolerant species has been biased by the difficulties faced in mimicking environmental settings in laboratory contaminant exposures. Our findings reiterate how little is known about bacterial communities inhabiting coastal sediments, their diversity, and associated functions.

Finally, we cannot exclude that some methodological limitations could be partly responsible for the observed low degradative activities. In the present study, the hydrolytic rates were estimated using small substrate proxies, typically a monomer (an amino acid or a monosaccharide analogue) linked to a fluorophore. Such method measures only the activities of exo-acting enzymes, overlooking completely the action of endo-acting enzymes that cleave a polymer midchain. In addition to such considerations, the MUF and AMC fluorophores have excitation and emission maxima, which overlap with natural DOC in marine environments, so measurements can be problematic, particularly in high-DOC-content pore waters (Arnosti 2011). In the Mar Piccolo of Taranto, De Vittor et al. (2015, this issue) reported elevated pore water DOC concentrations especially at St. 1I, in correspondence to our lowest degradative activities. These findings corroborate the hypothesis of an antagonistic effect exerted by DOC fluorescence on that of the fluorophores used for measuring the exoenzymatic rates. Keeping in mind the drawbacks described above, we cannot exclude, therefore, that the measured degradative rates could represent an underestimation of the real mineralization processes ongoing in the area.

Conclusions

Our main findings suggest that the benthic ecosystem functioning could be quite different between the two inlets. Although a noticeable organic enrichment existed at all stations, the two inlets differed in the intrinsic characteristics of such sedimentary organic matter. The sites located in the first inlet showed an extremely elevated organic content strongly associated with high amounts of contaminants, rendering the sediments more toxic and limiting the role of OM as a food source for the analyzed benthic communities. The stations of the second inlet were characterized by less contaminated sediments with organic matter that derived mainly from mussel farming and that tended to accumulate due to the low renewal of bottom waters. St. 1I displayed the highest concentrations of both organic matter and contaminants (PCBs and heavy metals) due to the presence of one of the main sources of pollution, i.e., the military arsenal. The benthic system functioning seemed affected at all the investigated structural and functional levels. Both meiofauna and macrofauna showed low abundances, while likely inhibited microbial processes led to limited transfer of C either into a solid microbial loop or to the higher trophic levels. On the other hand, at St. 1E, the ingression of seawater through the "Navigabile" channel and the increased distance from the navy arsenal likely enhanced the overall benthic ecosystem functioning as indicated by the faster microbial reworking of the organic matter and by the higher densities of meiofauna and macrofauna. St. 2B and 2C were located along a decreasing gradient of contamination (from St. 1I) and at the same time along an increasing gradient of confinement (from St. 1E). The brackish conditions in the inner part of the second inlet enhanced the presence of communities different from those observed at St. 1E, and the main distance from the navy arsenal contributed to a less impacted benthic ecosystem. Its functioning seemed mainly driven by the mussel farms. The fresh organic matter produced by bivalves partially fueled microbial processes and the mobilized C was transferred to the higher trophic levels mainly represented by paralic filter feeders.

Our results demonstrated that the evaluation of the benthic ecosystem functioning could allow to detect different environmental conditions that would be likely neglected using more traditional approaches as the sole chemical characterization of sediments. However, we warmly recommend further investigations, especially on a wider sampling grid and by deepening the study of the bacterial community structure, prior to any corrective action on the area. This latter aspect deserves to be developed extensively in order to understand the potential ability of specific prokaryotes in degrading persistent contaminants. The main findings by Matturro et al. (2015, this issue), in fact, suggest that the autochthonous microbial community (sampled at St. 1I) of the Mar Piccolo of Taranto showed to be potentially efficient in sustaining the biodegradation of PCBs. Although the study was performed in controlled anaerobic microcosms, which unlikely simulate the environmental conditions, these results provide a promising starting point for addressing corrective plans.

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References

Alabiso G, Giacomini M, Milillo M, Ricci P (2006) Chemical-physical conditions in the Taranto Sea system from 2002 to 2004. Bio Mar Mediter 13(1):1055–1058

Arias AM, Drake P (1994) Structure and production of benthic macroinvertebrate community in a shallow lagoon in the Bay of Cadiz. Mar Ecol Prog 115:151–167

Arnosti C (2011) Microbial extracellular enzymes and the marine carbon cycle. Annu Rev Mar Sci 3:401–425

Blasutto O, Cibic T, De Vittor C, Fonda Umani S (2005) Microphytobenthic primary production and sedimentary carbohydrates along salinity gradients in the lagoon of Grado and Marano (Northern Adriatic Sea). Hydrobiologia 550:47–55

Bligh EG, Dyer W (1959) A rapid method for total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Calace N, Ciardullo S, Petronio BM, Pietrantonio M, Abbondanzi F, Campisi T, Cardellicchio N (2005) Influence of chemical parameters (heavy metals, organic matter, sulphur and nitrogen) on toxicity of sediments from the Mar Piccolo (Taranto, Ionian Sea, Italy). Microchem J 79:243–248

Cardellicchio N, Buccolieri A, Giandomenico S, Lopez L, Pizzulli F, Spada L (2007) Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, Southern Italy). Mar Pollut Bull 55(10–12):451–458

Cardellicchio N, Buccolieri A, Di Leo AD, Librando V, Minniti Z, Spada L (2009) Methodological approach for metal pollution evaluation in sediments collected from the Taranto Gulf. Toxicol Environ Chem 91(7):1273–1290

Cardellicchio N, Annicchiarico C, Di Leo A, Giandomenico S, Spada L (2015) The Mar Piccolo of Taranto: an interesting marine ecosystem for the environmental problems studies. Environ Sci Pollut Res. doi: 10.1007/s11356-015-4924-6

Caroppo C, Cardellicchio N (1995) Preliminary study on phytoplankton communities of Mar Piccolo in Taranto (Jonian Sea). Oebalia 21: 61–76

Caroppo C, Giordano L, Palmieri N, Bellio G, Paride Bisci A, Portacci G, Sclafani P, Sawyer Hopkins T (2012) Progress toward sustainable mussel aquaculture in Mar Piccolo, Italy. Ecol Soc 17(3):10

Cibic T, Franzo A, Celussi M, Fabbro C, Del Negro P (2012) Benthic ecosystem functioning in hydrocarbon and heavy-metal contaminated sediments of an Adriatic lagoon. Mar Ecol Prog Ser 458:69–87

Cibic T, Bongiorni L, Borfecchia F, Di Leo A, Franzo A, Giandomenico S, Karuza A, Micheli C, Rogelja M, Del Negro P (2015) Ecosystem functioning nearby the largest steelworks in Europe and the main Italian naval base: the study case of the Mar Piccolo of Taranto. Environ Sci Pollut Res. doi:10.1007/s11356-015-4997-2

Covelli S, Acquavita A, Piani R, Predonzani S, De Vittor C (2009) Recent contamination of mercury in an estuarine environment (Marano lagoon, northern Adriatic, Italy). Estuar Coast Shelf Sci 82:273–284

Crooks JA (2001) Assessing invader roles within changing ecosystems: historical and experimental perspectives on an exotic mussel in an urbanized lagoon. Biol Invasions 3:23–36

Danovaro R, Pusceddu A (2007) Biodiversity and ecosystem functioning in coastal lagoons: does microbial diversity play any role? Estuar Coast Shelf Sci 75:4–12

Danovaro R, Gambi C, Mirto S, Sandulli R, Cecchierelli VU, Meiofauna (2004) Mediterranean marine benthos: a manual of methods for its samplings and study. Biol Mar Mediter 11(Suppl 1):55–97

De Vittor C, Relitti F, Kralj M, Covelli S, Emili A (2015) Oxygen, carbon and nutrient exchanges at the sediment-water interface in the Mar Piccolo of Taranto (Ionian Sea, southern Italy). Environ Sci Pollut Res DOI. doi:10.1007/s11356-015-4999-0

Desrosiers G, Savenkoff C, Olivier M, Stora G, Juniper K, Caron A et al (2000) Trophic structure of macrobenthos in Gulf of St. Lawrence and on the Scotian Shelf. Deep Sea Res Part II 47:663–697

Fichez R (1991) Composition and fate of organic matter in submarine cave sediments; implications for the geochemical cycle of organic carbon. Oceanol Acta 14:369–377

Franzo A, Cibic T, Del Negro P, Solidoro C (2014) Microphytobenthic response to mussel farm biodeposition in coastal sediments of the northern Adriatic Sea. Mar Pollut Bull 79:379–388

Hartree EF (1972) Determination of proteins: a modification of the Lowry method that give a linear photometric response. Anal Biochem 48: 422–427

Higgins RP, Thiel H (1988) Introduction to the study of meiofauna. Smithsonian Institution Press, London

Hoppe HG (1993) Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Current methods in aquatic microbial ecology. CRC Press, Boca Raton, pp 423–431

Islam Md 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. Mar Pollut Bull 48:624–649

Kapsimalis V, Panagiotopoulos IP, Talagani P, Hatzianestis I, Kaberi H, Rousakis G, Kanellopoulos TD, Hatiris GA (2014) Organic contamination of surface sediments in the metropolitan coastal zone of Athens, Greece: sources, degree, and ecological risk. Mar Pollut Bull 80(1–2):312–324

Law n. 426 (1998) Nuovi interventi in campo ambientale, Gazzetta Ufficiale della Repubblica Italiana del 14 dicembre 1998., p 291, **Serie generale**

Lunau M, Lemke A, Walther K, Martens-Habbena W, Simon M (2005) An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. Environ Microbiol 7(7):961–968

Manini E, Fiordelmondo C, Gambi C, Pusceddu A, Danovaro R (2003) Benthic microbial loop functioning in coastal lagoons: a comparative approach. Oceanol Acta 26:27–38

Manini E, Luna GM, Danovaro R (2004) Benthic bacterial response to variable estuarine water inputs. FEMS Microbiol Ecol 50:185–194

Margalef R (1986) Ecologia. Omega, Barcelona

Matarrese A, Mastrototaro F, D'onghia G, Maiorano P, Tursi A (2004) Mapping of the benthic communities in the Taranto seas using side-scan sonar and an underwater video camera. Chem Ecol 20(5):377–386

Matturro B, Ubaldi C, Grenni P, Barra Caracciolo A, Rossetti S (2015) Polychlorinated biphenyl (PCB) anaerobic degradation in marine sediments: microcosm study and role of autochthonous microbial communities. Environ Sci Pollut Res DOI. doi:10.1007/s11356-015-4960-2

Mayer LM, Schick LL, Hardy KR, Wagai R, McCarthy J (2004) Organic matter in small mesopores in sediments and soils. Geochim Cosmochim Ac 68:3863–3872

Misic C, Covazzi Harriague A (2009) Organic matter characterisation and turnover in the sediment and seawater of a tourist harbour. Mar Environ Res 68:227–235

Morri C, Bellan-Santini D, Giaccone G, Bianchi C (2004) Principles of bionomy: definition of assemblages and use of taxonomic descriptors (macrobenthos. Biol Mar Mediter 11(Suppl 1):573–600

Nieuwenhuize J, Maas YEM, Middelburg JJ (1994) Rapid analysis of organic carbon and nitrogen in particulate materials. Mar Chem 45: 217–224

Pella E, Colombo B (1973) Study of carbon, hydrogen and nitrogen determination by combustion-gas chromatography. Mikrochim Acta 5:697–719

Pérès JM (1967) The Mediterranean benthos. Oceanogr Mar Biol Annu Rev 5:449–533

Perthuisot JP, Guelorget O (1983) Le confinement, parametre essentiel de la dynamique biologique du domaine paralique. Sci Géol Bull 36(4): 239–248

Pielou EC (1966) Shannon's formula as a measure of species diversity: its use and misuse. Am Nat 118:463–465

Prato E, Biandolino F (2005) Amphipod biodiversity of shallow water in the Taranto seas (north-western Ionian Sea). J Mar Biol Ass 85:333–338

Ravenschlag K, Sahm K, Knoblauch C, Jørgensen BB, Amann R (2000) Community structure, cellular rRNA content and activity of sulfate-reducing bacteria in marine arctic sediments. Appl Environ Microbiol 66(8):3592–3602

Rees HL, Moore DC, Pearson TH, Elliott M, Service M, Pomfret J, Johnson D (1990) Procedures for the monitoring of marine benthic communities at UK sewage sludge disposal sites. Scottish Fisheries Information Pamphlet, 18. Department of Agriculture and Fisheries, Scotland

Rubino F, Cibic T, Belmonte M, Rogelja M, Rubino F, Cibic T, Belmonte M, Rogelja M (2015) Microbenthic community structure and trophic status of sediments in the Mar Piccolo of Taranto (Mediterranean, Ionian Sea). Environ Sci Pollut Res, this issue

Sandulli R, Carriglio D, Deastis S, Marzano A, Gallo D'Addabbo M, Gerardi D, De Zio GS (2004) Meiobenthic biodiversity in areas of the Gulf of Taranto (Italy) exposed to high environmental impact. Chem Ecol 20(Suppl 1):379–386

Shannon CE, Weaver W (1949) The mathematical theory of communication. Illinois Press, Urbana

Sharp JH (1974) Improved analysis for "particulate" organic carbon and nitrogen from seawater. Limnol Oceanogr 19:984–989

Solis-Weiss V, Rossin P, Aleffi F, Bettoso N, Orel G, Vrišerv B (2001) Gulf of Trieste: sensitivity areas using benthos GIS techniques. In: Özhan E (ed) Proceedings of the Fifth International Conference on the Mediterranean Coastal Environment. Middle East Technical University, Ankara, pp 1567–1578

Spada L, Annichiarico C, Cardellicchio N, Giandomenico S, Di Leo A (2012) Mercury and methylmercury concentrations in Mediterranean seafood and surface sediments, intake evaluation and risk for consumers. Int J Hyg Envir Heal 215(3):418–426

Storelli MM, Marcotrigiano GO (2000) Polycyclic aromatic hydrocarbons distributions in sediments from the Mar Piccolo, Ionian Sea, Italy. Bull Environ Contam Toxicol 65:537–544

Sun MY, Dafforn KA, Johnston EL, Brown MV (2013) Core sediment bacteria drive community response to anthropogenic contamination over multiple environmental gradients. Environ Microbiol 15(9): 2517–2531

van Duyl FC, Kop AJ (1994) Bacterial production in North Sea sediments: clues to seasonal and spatial variations. Mar Biol 120:323–337

Ward DJ, Simpson SL, Dianne FJ (2013) Slow avoidance response to contaminated sediments elicits sublethal toxicity to benthic invertebrates. Environ Sci Technol 47:5947–5953

Zimmerman AR, Chorover J, Goyne KW, Brantley SL (2004) Protection of mesopore-adsorbed organic matter from enzymatic degradation. Environ Sci Technol 38:4542–4548

Websites

http://www.ncbi.nlm.nih.gov/pubmed/21878105
http://www.biomedcentral.com/1471-2105/9/386
http://nar.oxfordjournals.org/content/40/W1/W88

Paper V

FOOD WEB OF A CONFINED AND ANTHROPOGENICALLY AFFECTED COASTAL BASIN (THE MAR PICCOLO OF TARANTO) REVEALED BY CARBON AND NITROGEN STABLE ISOTOPES ANALYSES

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Data analyses: LB, FF, RA, FBA, EC, FC, **FN**, MP, LZ

Manuscript preparation: LB, FF, RA, FBA, EC, FC, **FN**, MP, LZ, JG

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INTEGRATED ENVIRONMENTAL CHARACTERIZATION OF THE CONTAMINATED MARINE COASTAL AREA OF TARANTO, IONIAN SEA (SOUTHERN ITALY) - THE RITMARE PROJECT

Food web of a confined and anthropogenically affected coastal basin (the Mar Piccolo of Taranto) revealed by carbon and nitrogen stable isotopes analyses

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Abstract Carbon and nitrogen stable isotope analysis was used to examine the food web of the Mar Piccolo of Taranto, a coastal basin experiencing several anthropogenic impacts. Main food sources (algal detritus, seaweeds, particulate organic matter (POM) and sediment organic matter (SOM)) and benthic and pelagic consumers were collected during two contrasting seasons (June and April), at four sites distributed over two inlets, and characterized by different level of confinements, anthropogenic inputs and the presence of mussels farming. $\delta^{13}C$ values of organic sources revealed an important contribution of POM to both planktonic and benthic pathways, as well as the influence of terrigenous inputs within both inlets, probably due to high seasonal land runoff. Although $\delta^{13}C$ of both sources and consumers varied little between sampling sites and dates, $\delta^{15}N$ spatial variability was higher and clearly reflected the organic enrichment in the second inlet as well as the uptake of anthropogenically derived material by benthic consumers. On the other hand, within the first inlet, the isotopic composition of consumers did not change in response to chemical contamination. However, the impact of polluted sediments near the Navy Arsenal in the first inlet was detectable at the level of the macrobenthic trophic structure, showing high dominance of motile, upper level consumers capable to face transient conditions and the reduction of the more resident deposit feeders. We therefore underline the great potential of matching stable isotope analysis with quantitative studies of community structure to assess the effects of multiple anthropogenic stressors.

Keywords Food webs · Stable isotopes · Macrofauna · Zooplankton · Feeding guilds · Anthropogenic pressures

Introduction

Estuarine and coastal ecosystems are among the most diverse and productive natural systems on earth and provide a large variety of goods and services for human well-being (Costanza et al. 1997). Over the past decades a wide range of human activities have threatened these environments and the many benefits they provide (Lotze et al. 2006; Halpern et al. 2008). Threats include habitat and biodiversity loss, fishery decline, and an overall decrease in the whole system functioning and quality of life. It is now recognized that successful management actions need effective tools to assess those interacting impacts and to acknowledge the complexity of ecosystem functioning per se, rather than simplifying and scaling down the system into its components (Parrott 2010). These issues have been recognized as major gaps to the application of the principle of "the ecosystem approach to management" (EAM,

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Katsanevakis et al. 2011 and literature therein). In this context, the study of food webs offers the possibility to integrate the effects of multiple human pressures on natural ecosystems, as internal food web mechanisms can buffer or even reverse the effects exerted at the population or species scale (Gray et al. 2014; Strong and Frank 2010).

Food webs describe the exchange of organic matter between organisms of an ecosystem, and energy flow from basal sources to top predators (Krumins et al. 2013) and stable isotopes analysis (SIA) has been commonly used in diverse ecosystems to study trophic pathways (Fry 2006; Layman et al. 2012). This analysis is based on the fractionation of stable isotopes in animal tissue due to metabolic processes such as assimilation, excretion and respiration and thus can provide a time-integrated measure of trophic position and sources of energy. The preferential assimilation of the lighter form of stable N isotope between the consumer and its food source constitutes a useful tool to determine relative trophic position of consumers along coexisting food chains within an ecosystem (Peterson and Fry 1987; Post 2002; Vander Zanden and Rasmussen 2001). The trophic position identifies how much above the primary producer's level the organism in question feeds on average. This concept is a schematic but useful abstraction to clarify and organize the understanding of energy transfers in ecosystems and to overcome bias when comparing structure and functioning of different ecosystems.

Compared to stable nitrogen, the enrichment of stable carbon per trophic level is negligible; therefore, the ratio of ^{13}C to ^{12}C can be used to find the carbon flow to consumers and diet composition especially in cases when the diet sources have relatively large differences in δ^{13}C values (De Niro and Epstein 1978; Post 2002). This is particularly useful in coastal waters which receive a complex mix of organic sources of both terrestrial and marine origin. Moreover, when potential food sources (terrestrial plants, marine detritus, phytoplankton, etc.) are identified by their isotopic signatures, the fate of the nutrients in marine ecosystems and their relative contribute to consumers' diet can be inferred by using mixing models (Layman et al. 2012).

In recent years, natural variations in the isotopic composition of marine organisms have been also used to detect and quantify the impact of anthropogenic activities, such as fish farming and sewage outfall (Costanzo et al. 2001; Dolenec et al. 2006; Vizzini et al. 2005; Vizzini and Mazzola 2006 and literature therein). Lately, the SIA approach has emerged as a useful tool to support conservation, restoration and management, and the potentials of SIA to provide food web scale indicators for estimating cumulative anthropogenic stressors have been recently addressed (Mancinelli and Vizzini 2015 and literature therein).

The Mar Piccolo is a transitional basin partially connected to the Gulf of Taranto (Mar Grande, Ionian Sea) and represents an example of coastal marine ecosystem whose biological balances have been modified by increasing anthropic development (iron and steel factory, petroleum refinery and shipyard, discharge of municipal waste water flow, and mussel culture activities). For these intense anthropogenic impacts, Taranto City has been identified as an "Area of High Environmental Risk" (Cardellicchio et al. 2007).

In order to fill the gap related to the lack of information on the food web structure of this area, we mainly address two questions: (i) Is there a spatial variability in the isotopic composition of organic sources and consumers of this coastal basin? (ii) Is it possible to link the isotopic composition to anthropogenic impacts and identify the effect of these on the food webs? To answer those questions SIA was applied to main organic matter sources and benthic and pelagic consumers, a Bayesian Stable Isotope Mixing Model was used to quantify the proportional contribution of each basal resource to primary consumers diets.

Materials and methods

Study area

Main features of the Mar Piccolo have been exhaustively described by Cardellicchio et al. (this issue). Briefly, the Mar Piccolo is a coastal, inner sheltered sea of 20.72 km^2 located north of the town of Taranto. The basin is structured in two shelves: the first inlet and the second inlet which reach maximum depths of 13 and 9 m, respectively (Fig. 1). The two inlets are characterized by different levels of confinement: The first inlet is directly linked with the Gulf of Taranto (Ionian Sea) through two channels, while the second inlet is internal and linked only with the first basin. On the base of its whole hydrographic characteristics, the Mar Piccolo can be compared to an estuarine ecosystem (Umgiesser et al. 2007 and literature therein). Water circulation and exchange are restricted and mainly driven by tide, whereas the wind seems to have a marginal role (Umgiesser et al. 2007). Due to low hydrodynamics, water stratification occurs mainly in the dry summer season, and salinity is influenced by the input of freshwater coming from small tributary rivers and freshwater submarine springs *citri*.

In the last years, the Mar Piccolo has experienced urban expansion and intensive agriculture which have caused increased nutrient and organic-matter levels (Cardellicchio et al. 2007, This issue). Major anthropogenic impacts include industrial pollution (port and arsenal navy, fishing port and shipbuilding activity in the first inlet; military fuel dock in the second inlet) and widespread mussel-breeding activity. Lately, mussels are reared only in inlet 2 (ca. 20, 000 t year^{-1}), and only juvenile collection is allowed in inlet 1 (ca.5000 t year^{-1}, Pugnetti et al. 2013). In addition, the basin gets considerable amount of sewage coming from the northern area of the city of Taranto and from eight nearby towns.

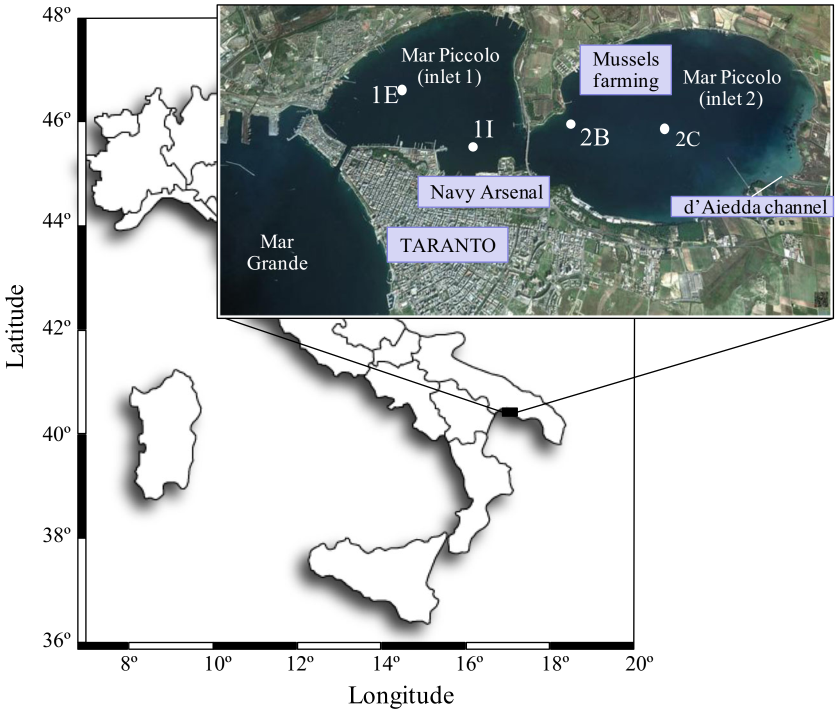

Fig. 1 Location of sampling sites in the Mar Piccolo

Currently, seven sewage pipes (three in the first and four in the second inlet) are operating. Bottom sediments are sandy near the shore and covered by reduced mud in the central zone of both inlets. Sedimentation is mainly influenced by land runoff, presence of *citri*, small streams, and urban and industrial sewage.

Sample collection

Samples were collected during June 2013 and April 2014 at four sites representative of different levels of confinement and anthropogenic impacts (Fig. 1). Stations 1E and 1I are located in the first inlet. St. 1E is close to the connection with the open sea and therefore can be classified as the less confined site. St. 1I is located at ca.0.6 nautical miles from the Navy base and is characterized by sediments contaminated by PCBs and heavy metals (Cardellicchio et al. 2007; Cibic et al. this issue, Fig. 1). Stations 2B and 2C are located in the second inlet, both in proximity to the commercial mussel farming area (Fig. 1). Station 2B is in the north-western side of the inlet, and station 2C is in the innermost part in correspondence of a long-term monitoring site.

Macrofauna was sampled in each station using a van Veen grab (0.06 m^2). Three grab replicates were gently sieved (1 mm) to collect invertebrates. Additional macrofaunal organisms were collected by scuba divers directly from the bottom, on buoy ropes and on the mussel-rearing lines. Mesozooplankton was collected by repeated horizontal hauls (towing speed and time 1–1.5 kn, for 20–30 min) of a WP2 net (0.57 m Ø, 200 μm mesh), across the first and second inlet. In addition, fishes caught in the Mar Piccolo basin during May 2012 were also analysed for stable isotopes. Since fish specimens were collected a year before this study, SI data were kept as indicative of the isotopic signatures of high trophic positions but were not included in the statistical analyses.

At each sampling station, bulk sediment samples were collected by scuba divers with polycarbonate cores (n=3, 12.7-cm diameter). The upper 3-cm core layer was frozen for isotopic analyses. Water samples for suspended particulate organic matter analyses were collected using Niskin bottles at ca. 1 m above sandy-bottom. Seaweeds and algal detritus were collected either by scuba divers or trapped by the van Veen grab. Algae samples were carefully scraped to remove epiphytes, rinsed in distilled water and freeze dried.

Sample processing

Benthic invertebrates were starved over-night in 0.2-μm filtered seawater before being frozen (−20 °C) and freeze-dried. For the identification of organisms, the taxonomical keys listed in Morri et al. (2004) were used. Individuals were assigned to the following trophic guilds: suspension feeders (SF), surface deposit feeders (SDF), subsurface deposit feeders (SSDF), grazers (GRZ), omnivores (OMN) and carnivores (CRN) according to literature (Desrosiers et al. 2000 and reference therein). Mesozooplankton was starved for 60 min in containers filled with 0.2-μm filtered seawater to allow for gut evacuation. Afterwards animals were concentrated on 200-μm Nitex mesh and immediately blast frozen to −20 °C. Successively, pools were sorted into the most representative taxa under a stereomicroscope. Between 100 and 500, individuals of main zooplankton taxa were picked, pooled, cleaned from detritus and dried at 60 °C for 24–48 h prior to bulk SIA.

SIA was carried out on dorsal muscles of fish (Yokoyama et al. 2005), muscle or mantle of molluscs, and flesh of crustaceans, after an accurate removal and dissection of their shells and cuticles. For smaller invertebrates and zooplankton taxa, the analysis was conducted on the whole body. In order to rule out possible changes due to onthogenic shift, within each taxon, only organisms of similar size were used for isotopic analyses. Water samples (ca. 1.5–2 L) were filtered on precombusted (450 °C, 4 h) GF-F, filters, 25-mm diameter, until clogging. Filters with suspended POM were kept frozen and then oven dried (60 °C for 48 h). The upper layer of each sediment core was rinsed with distilled water and dried in oven (60 °C for 48 h). Sediments were sieved onto 2-mm sieve to remove big debris. All samples (sediments, primary producers and animals) were homogenized with clean mortar and pestle until obtaining a fine powder.

Prior to the analyses samples containing traces of carbonates (e.g. POM filters, SOM, seaweeds, zooplanktonic taxa, crustaceans, anthozoa) were divided into two subsamples, one of which was pre-treated with dilute (1 N) HCl to remove carbonates and the other not receiving acid treatment to avoid effects of acidification on δ^{15}N (Mateo et al. 2008).

Stable isotope measurements

Bulk and acidified samples were weighed and placed into tin capsules to measure δ^{13}C and δ^{15}N and % of C and N in one run. The analyses were carried out using a Delta Plus XP isotope ratio mass spectrometer (ThermoFinnigan) equipped with a Flash EA 1112 elemental analyser (ThermoFinnigan).The isotopic composition was denoted in delta (δ) notation, i.e. differences, between isotopic ratios in the samples and in international standard VPDB (Vienna Pee Dee Belemnite) for δ^{13}C and AIR for δ^{15}N and expressed in ‰ (Coplen 2011). The isotopic values were calculated against working in-house standards, which were themselves calibrated against international reference materials: L-glutamic acid USGS 40 (IAEA-International Atomic Energy Agency, Vienna, Austria), fuel oil NBS-22 (IAEA) and sugar IAEA-CH-6 for δ^{13}C, L-glutamic acid USGS 40 and potassium nitrate IAEA-NO3 for δ^{15}N. The C% and N% were measured using as reference material atropine (Carlo Erba, Milano) with certified percentage of C and N. Uncertainty of determinations was 0.3‰ for δ^{13}C and δ^{15}N.

Values of δ^{13}C of invertebrates and fish samples (having C/N >3.5) were corrected according to Logan et al. (2008) for the effect of individual and species-specific lipid content by applying a mathematical model which uses the carbon-to-nitrogen (C/N) ratio. For zooplanktonic species, lipids corrections were made according to D'Ambra et al. (2013). To estimate the trophic position (λ) of consumers, the following equation was used:

$$\lambda = \left(\left(\delta^{15}\text{Nconsumer} - \delta^{15}\text{Nbase}\right)/\Delta\delta^{15}N\right) + 2,$$

where δ^{15}Nbase is the nitrogen isotopic signature of a representative baseline. Due to the low temporal variance in their isotopic signatures, long-lived primary consumers (λ=2) have been suggested to provide an appropriate baseline to quantify consumers' trophic positions (Post 2002). In this study, we used as baseline the average δ^{15}N value the long-lived and commonly occurring filter feeder *Corbula gibba*, which reflected the average filter feeders bivalve values. As best estimate of consumer's fractionation, a mean isotopic N shift ($\Delta\delta^{15}$N) of 2.30±0.28‰ was considered (McCutchan et al. 2003).

Statistical analyses

Potential food sources (SOM and POM) were compared between sampling dates and stations by mean of one- and two-way ANOVA. Macroalgae samples were found and collected only in the second inlet, and no statistical comparison among stations was performed. All analyses were carried out using R software v 3.1.2 (R Core Team 2014). Differences in the stable isotopes ratios of macrobenthic consumers between sampling dates and stations were tested by a two-way crossed (two and four fixed levels for each factors), univariate distance-based permutational multivariate analysis of variance (PERMANOVA, McArdle and Anderson 2001). This analysis is based on Euclidean distances of untransformed data, and it was carried out using 4999 random permutations of the appropriate units under a reduced model using PRIMER v.6 and the add-on PERMANOVA+ module (Anderson et al. 2008). Prior to analyses, data were checked for normality and homoscedasticy using Shapiro-Wilk's and Levene's test, and no transformation was necessary. When significant differences were observed, post hoc Tukey's HSD and Pair-wise comparison tests were also performed.

A mixing model based on a Bayesian approach Stable Isotope Analysis in R (SIAR) v 4.2 developed by Parnell et al. (2010) was used to determine the likely contribution of each of the potential food sources to the diet of primary consumers. Data for the mixing model consisted in carbon and nitrogen isotopic ratios per species belonging to different feeding guilds of primary consumers (suspension feeders, deposit feeders, sub-surface deposit feeder and grazers) and mean± standard deviation (SD) of carbon and nitrogen isotopic ratios per source. In order to reduce potential pitfalls due to reduction in estimated contribution of unlikely food sources, SOM pool was not included in the analyses (Vafeiadou et al. 2013). According to McCutchan et al. (2003), mean±SD trophic enrichment factors used in the model were 2.30±0.28‰ for Δ^{15}N and 0.3±0.14‰ for Δ^{13}C of consumers analysed as whole and 1.3±0.30‰ for consumers analysed as muscle.

Results

Stable isotopic ratios of organic matter sources

Average values (±SD) of δ^{13}C and δ^{15}N of potential food sources for consumers in the whole basin and in first and second inlet are shown in Fig. 2 and Table 1, respectively. In the first inlet, we were not able to collect fresh algal materials as macroalgae covered less than 15 % (Cibic et al. this issue), and only algal detritus was analysed. Particulate organic matter (POM) δ^{13}C was on average −24.12±1.14‰ and ranged between −26.49‰ (St. 2C, April) and −21.75‰ (St. 1I, June, Figs. 2 and 3, Table 1). Values of δ^{13}C POM were higher in June than in April and at St. 1I compared to the other stations (Two-way ANOVA, $F_{1,16}$=39.40, p<0.001; Tukey's HSD test, p<0.01, and $F_{3,16}$=11.81, p<0.001; Tukey's HSD, p<0.01, respectively). Depleted δ^{13}C signature was observed to occur during April in particular at St. 2C (inlet 2, average −25.92‰, Fig. 3). POM was more ^{13}C-depleted than sedimentary organic matter (SOM) and macroalgae (Table 1, Fig. 2, t test p<0.05). SOM δ^{13}C (average −22.01±1.01) ranged between −22.98‰ (St. 1I, April) and −20.09‰ (St. 2B, April), and values were constant during sampling months and between stations (one-way ANOVAs, $F_{1,6}$=0.01, p=0.9 and $F_{1,6}$=1.03, p=0.35, respectively). Macroalgae δ^{13}C values (average −16.95±2.46‰) ranged between −22.75‰ (red algae, *Rhodophyceae*) and −13.64‰ (*Ulva* sp.), and algal detritus showed intermediate δ^{13}C signature (−18. 39‰, Fig. 2, Table 1).

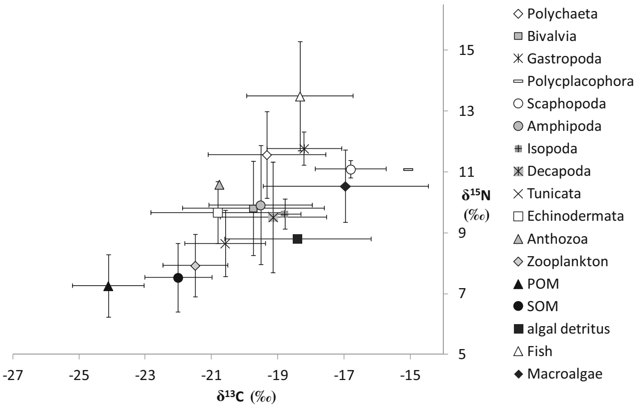

Fig. 2 Stable isotope (δ^{13}C and δ^{15}N) bi-plots of consumers and basal resources in the whole Mar Piccolo basin. *POM* particulate organic matter, *SOM* sedimentary organic matter. Values are reported as SI average of single taxa and food sources for the whole sampling area and periods. *Bars* represent standard deviation

δ^{15}N POM (average 7.26±1.05‰) ranged between 4.92‰ (St. 1E, June) and 9.81‰ (St. 2B, June). Values did not change between months (two-way ANOVA, $F_{1,16}$=1.03, p=0.32) but were higher in St. 2B than in to all others and in St. 2C compared to St.1E (two-way ANOVA, $F_{3,16}$=21.85, p<0.001, Tukey's HSD, p<0.01 and 0.5, respectively). δ^{15}N of SOM (average 7.54±1.12‰) varied from 6.17‰ (St. 1E, June) to 9.00‰ (St. 2B, June, Table 1, Fig. 3). Values were homogeneous between the two sampling months (one-way ANOVA, $F_{1,6}$=0.12, p=0.74) and higher in stations 2B and 2C compared to St. 1E and St. 1I (one-way ANOVA, $F_{1,6}$=13.6, p<0.05, Fig. 3). δ^{15}N signature of POM and SOM was lower compared to signals of algal detritus and fresh macroalgae (average 10.47±1.21‰, Table 1, Fig. 2). Seaweeds δ^{15}N ranged between 9.01‰ (*Gracilaria gracilis*) and 12.46‰ (*Ulva rotundata*). δ^{15}N value of senescent algal detritus was on average 8.82‰ (Table 1, Fig. 2).

Macrobenthos and mesozooplankton communities' compositions and distribution

Macrobenthos and mesozooplankton communities in the Mar Piccolo have been extensively described by Franzo et al. (this issue) and Karuza et al. (this issue). Briefly, macrofauna abundance ranged between 605±543 and 1916±342 ind m^{-2} and was generally higher in June compare to April. Higher values were reached at St. 1E (June) and St. 2C (April, Franzo et al. this issue). In both sampling months, the community was dominated by polychaetes (38–41 %, mainly at St. 1E) and molluscs (40–41 %), followed by crustaceans (13 %) and other taxa (mainly anthozoans, sipunculids and nemertines, 6–8 %). Station 1E appeared as the most diverse (high diversity and richness) and presents a typical marine community dominated by polychaetes (*Notomastus formianus*), and echinoderms (mainly *Amphiura chiajei* and *Ophiothrix quinquemaculata*). In the other three stations, polychetaes abundances was lower, and the molluscs were the most abundant group followed by crustaceans (Franzo et al. this issue). In inlet 2 (St. 2C) bivalves accounted for more than half of the whole community. Inlet 2 harboured species typical of paralic environments (the bivalves *Abra segmentum*, *Cerastoderma glaucum* and *Arcuatula senhousia*, the amphipods *Gammarus aequicauda* and *G. insensibilis*, and the isopod *Paracerceis sculpta*).

The four sites differ in terms of feeding guilds as St.1E was numerically dominated by deposit feeders, while in St. 1I, the community was dominated by grazers (GRZ, especially the isopod *Paracerceis sculpta* and the bivalve *Haminoea navicula*) and by carnivores (CRN, the polychaetes *Lumbrineris latreilli* and *Eunice vittata*). In the second inlet, suspension feeders (SF) numerically dominated St. 2C (79 %, mainly the bivalve *Arcuatula senhousia* during April), while St. 2B did not show a clear dominance (Franzo et al. this

Table 1 Mean (±SD) values of δ^{15}N, δ^{13}C (‰) and Carbon to Nitrogen ratio of primary sources collected in inlet 1 and 2 of the Mar Piccolo

June 2013	Inlet 1				Inlet 2			
	n	δ^{15}N (‰)	δ^{13}C (‰)	C/N	*n*	δ^{15}N (‰)	δ^{13}C (‰)	C/N
POM	6	6.72±1.00	−23.51±1.10	7.15±0.64	6	8.02±1.34	−23.40±0.40	6.68±0.47
SOM	2	6.59±0.60	−22.60±0.38	13.79±2.20	2	8.80±0.29	−21.52±0.89	10.00±0.48
Algal detritus	2	8.82±0.02	−18.39±2.22	13.19±0.50	–	–	–	–
Caulerpa prolifera	–	–	–	–	3	9.61±1.38	−15.36±0.89	9.50
Chaetomorpha linum	–	–	–	–	3	11.67±0.55	−18.25±1.49	26.64±0.17
Cladophora prolifera	–	–	–	–	2	10.35±1.08	−14.80±0.14	12.28
Ulva sp.	–	–	–	–	1	10.39	−13.64	10.33
April 2014								
POM	6	6.50±0.45	−24.04±0.76	7.12±0.09	6	7.82±0.36	−25.52±0.62	6.77±0.49
SOM	2	6.52±0.01	−22.77±0.29	15.96±1.65	2	8.27±0.52	−21.15±1.51	11.89±1.12
Caulerpa prolifera	–	–	–	–	1	9.59	−15.33	14.76
Cladophora prolifera	–	–	–	–	1	9.24	−17.43	12.54
Caulacantus ustulatus	–	–	–	–	1	11.72	−19.34	8.95
Gracilaria gracilis	–	–	–	–	1	9.01	−18.68	9.95
Gracilaria bursa pastoris	–	–	–	–	1	11.05	−18.69	9.34
Ulva rutundea	–	–	–	–	1	12.46	−14.96	11.53
Rhodophyta und.	–	–	–	–	1	9.32	−22.75	16.59

Isotopic analysis of SOM was made on a homogenized pool obtained by mixing three replicated sediment cores from each sampling station

POM particulate organic matter, *SOM* sedimentary organic matter

issue). Similar results were reflected by biomasses (Fig. 4). At St 1E and 2C filter and deposit feeders contribute up to 87 % of the total biomass (St. 1E, April), while at St. 1I, CRN reached 50 and 70 % in June and April, respectively. At this site, both SDF and SSDF contribution to total biomass was comparatively lower (0.15 and 0.05 g m^{-2} in June and April, respectively corresponding to ca. 9 %) than the other sites (16–52 %, Fig. 4).

Mesozooplankton community in both sampling months and inlets was characterized by low species and taxa diversity (Karuza et al. this issue). Copepods reached up to 50 % of the total abundances and were represented by *Acartia* sp. (about 30 and 25 % of the total abundances in both basins in June and April, respectively) and *Centropages* spp. (about 7 % in June at both basin and 2 % in April, only in the first basin). The rest of the assemblage was prevalently characterized by meroplanktonic forms such as larvae of decapods (about 10 and 24 % of the total abundances in both basins in June and April, respectively), gastropods and ascidians in both months

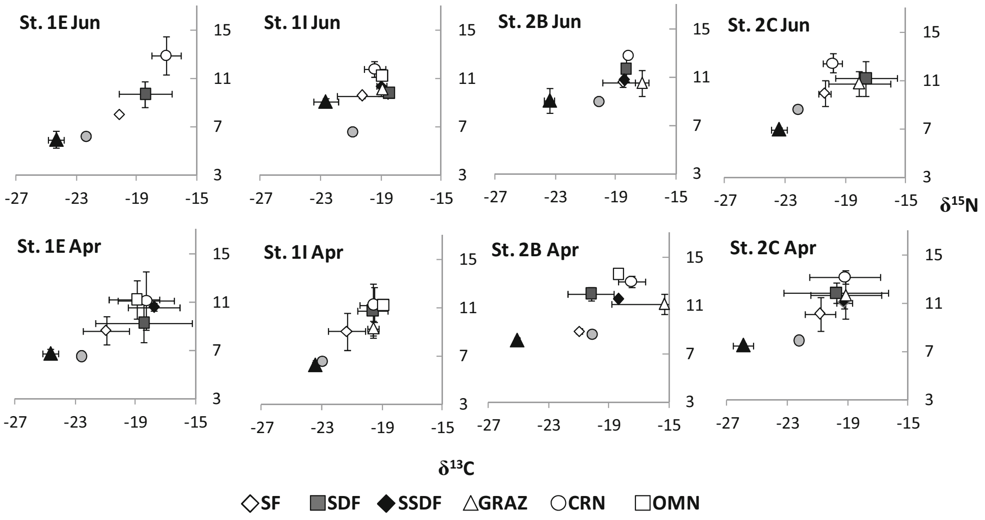

Fig. 3 Stable isotope (δ^{13}C and δ^{15}N) bi-plots of macrobenthic community feeding guilds in four stations of the Mar Piccolo and two sampling dates. *SF* suspension feeders, *SDF* surface deposit feeders, *SSDF* sub-surface deposit feeders, *GRAZ* grazers, *CRN* carnivores, *OMN* omnivores, *POM* particulate organic matter, *SOM* sedimentary organic matter. Values are SI average of each feeding guilds. *Bars* represent standard deviation

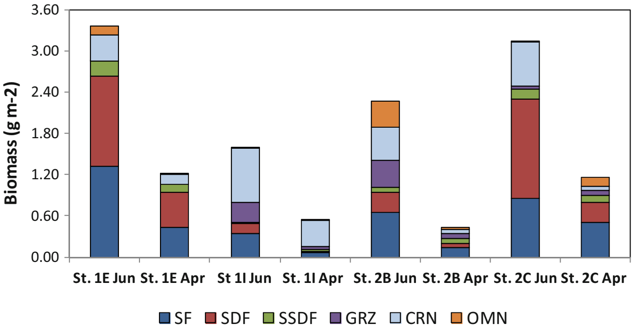

Fig. 4 Biomass of macrobenthos community feeding guilds. *SF* suspension feeders, *SDF* surface deposit feeders, *SSDF* subsurface deposit feeders, *GRAZ* grazers, *CRN* carnivores, *OMN* omnivores

(Karuza et al. this issue). These latter two taxa could not be used for SI analyses because did not reach a sufficient biomass.

Isotopic composition of consumers

Isotopic analyses in the Mar Piccolo were obtained from a total of 66 benthic invertebrates' taxa, 3 mesozooplankton taxa and 5 fish taxa. Within each taxon, body size of specimens analysed for stable isotopes was similar. Benthic invertebrates consumers were represented as follows: molluscs (27), polychetaes (21), crustaceans (11), echinoderms (4), tunicates (2) and anthozoans (1), and reflected the dominance of the macrobenthic communities. When excluding the lowest isotopic values (−30.43 and −1.30‰, δ^{13}C and δ^{15}N, respectively) found in *Loripes lucinalis* (St. 2C, in June), the mean values of SI of macrobenthos in the whole Mar Piccolo were −19.35±2.36‰ (δ^{13}C) and 10.49±2.01 (δ^{15}N, Fig. 2). δ^{13}C values ranged between −26.26 and −10.18‰ (in the polychete *Anphitrite* sp., St. 2C, April and in the isopod, *Paracerceis sculpta*, St. 2B, June, respectively, Table 2) and varied among sampling dates and stations (PERMANOVA, $p<0.05$ and $p<0.01$, respectively, Table 3). δ^{13}C values of benthic consumers were lower in April than in June (Table 3) and higher at St. 1E than at St. 1I and at St. 2B than at St. 2C and 1I (Table 3).

δ^{15}N values in benthic consumers ranged between 5.70‰ (in the bivalve *Mytilus galloprovincialis*, St. 1I, April) and 14.2‰ (in the gastropod *Diodora graeca*, St. 2C, April, Table 2). No differences were observed between sampling months, while differences were observed among stations (PERMANOVA $p<0.05$, Table 3). Post hoc test revealed that δ^{15}N values of consumers in St. 2B and 2C (second inlet) were higher than in St. 1E and St. 1I (first inlet, Table 3). Comparison between suspension feeders (SF), surface deposit feeders (SDF) and subsurface deposit feeders (SSDF) showed that SF exhibited a more depleted C signal (−20.99±1.97‰) compared to SDF (−18.93±2.23‰, Kruskal-Wallis=9.91, *df*=2, $p<0.01$, Fig. 3).

Mesozooplankton values of δ^{13}C (average −23.33±0.80 ‰) varied between −20.03‰ (Decapods larvae, Inlet 2, June) and −23‰ (in the copepod *Centropages* spp., Inlet 2, June, Table 2). Higher δ^{13}C values occurred in June compared to April (*t* test, $t=3.34$, $df=4$, $p<0.05$), but no differences were observed between inlets. δ^{15}N zooplankton was on average 7.93±1.03‰ (Fig. 2) and ranged between 5.55 and 8.93‰ in *Centropages* spp., inlet 2 in June and in *Acartia* sp., inlet 1, June, respectively (Table 2). No differences were observed between sampling months and inlets (*t* test, ns).

Mean fish SI signatures were −18.33±1.61 and 14.63±1.69‰ (for δ^{13}C and δ^{15}N, respectively, Table 2). δ^{13}C in fish ranged from −20.23±1.56 to −15.69‰ (in *Sarpa salpa* and *Mugil cephalus*, respectively). δ^{15}N ranged between 11.70 and 17.05‰ (*Mugil cephalus* and *Dichentrarchus labrax*, respectively, Fig. 2, Table 2).

Values of δ^{15}N placed consumers at four different trophic positions (Table 2). Polychaetes, molluscs and echinorderms were classified as primary and secondary consumers ($\lambda=2$ and 3). Exceptions were the polychaete *Sabella spallanzani* which showed a slightly low λ value (1.6) and the bivalve *Mythilus galloprovincialis*, *Corbula gibba* and *Flexopecten glaber* which showed lower λ values in the first inlet ($\lambda=0.6$–1.1) compared to the second inlet. Crustaceans occupied mainly the second trophic position with few species showing λ values<2 (mainly mesozooplanktonic specimens, Table 2). Tunicates generally displayed λ values below 2 (range 0.8–1.9). Fish were δ^{15}N-classified at the third (*Gobius niger*, *Boops boops* and *Sarpa salpa*) and at the fourth position (*Mugil cephalus* and *Dichentrarchus labrax*, respectively, Table 2). The λ values of SF and SDF were variable and in SF ranged between 0.6 (*Mythilus galloprovincialis*) and 3.3 (*Crassostrea gigas*, at St. 1I and St. 2C, April, respectively). In the surface deposit feeders, λ values ranged between 0.8 (*Gammarus* sp.) and 3.1 (Terebellidae und., Table 2). SSDF and grazers occupied position 2, and despite few exceptions, omnivores and carnivores occupied from positions 2 to 3 and from 3 to 4, respectively.

Contributions of food sources to primary consumers

Bayesian mixing models showed some differences in the relative contribution of the OM sources to the diet of primary consumers (Fig. 5). POM mainly contributed to SF diet (range between 36 and 61.5 %). The lower contribution of POM was observed in St. 2B (22 and 62 % expressed as lower and higher 95th confidence intervals, CI). At this station, the contribution of algal detritus and macroalgae (especially *Caulerpa* and *Gracilaria*) to the diet became important (46.6 %, Fig. 5). Contribution of POM to the diet of SDF was comparatively lower compared to SF (range from 24 to

Table 2 Mean values (±SD) of δ^{15}N and δ^{13}C and trophic position (λ) of macrofauna, zooplankton and fish specimens collected in inlets 1 and 2 of the Mar Piccolo

Species/feeding guild	Inlet 1				Inlet 2			
	n	δ^{15}N (‰)	δ^{13}C (‰)	*λ*	*n*	δ^{15}N (‰)	δ^{13}C (‰)	*λ*
Suspension feeders								
Sabella spallanzanii (Pol)	3	8.85±0.48	−21.26±0.91	1.6	1	8.74	−20.96	1.6
Striarca lactea (Biv)	2	9.50±0.28	−19.14±0.12	1.9	–	–	–	–
Arcuatula senhousia (Biv)	1	8.90	−23.13	1.6	2	10.45±0.49	−18.85±2.60	2.3
Ascidia mentula (Tun)	1	8.00	−20.15	1.2	2	9.45±0.64	−21.02±0.18	1.9
Ascidiella aspersa (Tun)	1	7.30	−20.86	0.9	4	9.08±0.96	−19.86±1.58	1.7
Corbula gibba (Biv)	1	7.80	−19.35	1.1	2	10.65±0.35	−19.38±0.80	2.4
Flexopecten glaber (Biv)	2	7.85±0.64	−20.74±0.54	1.0	2	10.50±0.71	−20.70±0.70	2.3
Mimachlamys varia (Biv)	1	9.70	−20.44	2.0	–	–	–	–
Bispira volutacornis (Pol)	2	10.34±0.17	−20.53±0.00	2.3	–	–	–	–
Cardites antiquatus (Biv)	2	9.60±0.85	−20.92±1.39	1.7	–	–	–	–
Loripes lucinalis (Biv)	–	–	–	–	1	−1.30	−30.43	nd
Parvicardium exiguum (Biv)	–	–	–	–	1	10.60	−20.27	2.4
Ostrea edulis (Biv)	1	9.40	−22.15	1.8	1	9.30	−19.07	1.8
Mytilus galloprovincialis (Biv)	2	6.55±1.20	−22.61±0.57	0.6	1	10.30	−19.61	2.2
Styela plicata (Tun)	1	6.90	−22.23	0.8	1	9.20	−21.23	1.8
Antedon mediterranea (Ech)	3	9.35±0.21	−22.20±0.53	1.9	–	–	–	–
Ophiotrix sp. (Ech)	2	10.00±1.84	−20.02±3.11	2.1	–	–	–	–
Anthozoa und.	1	10.60	−20.77	2.4	–	–	–	–
Monia patelliformis (Biv)	–	–	–	–	1	11.50	−22.51	2.8
Modiolus barbatus (Biv)	–	–	–	–	1	8.70	−22.55	1.5
Crassostrea gigas (Biv)	–	–	–	–	1	12.80	−20.36	3.3
Decapods larvae	2	8.20±0.30	−21.04±1.26	1.3	2	8.70±0.31	−20.70±0.95	1.5
Surface deposit feeders								
Abra alba (Biv)	1	9.40	−18.35	1.8	3	11.37±1.07	−18.31±0.45	2.7
Nucula nucleus (Biv)	1	9.80	−18.59	2.0	–	–	–	–
Tellina distorta (Biv)	1	9.20	−16.89	1.8	–	–	–	–
Antalis inaequicostata (Scaph)	2	11.10±0.28	−16.80±1.06	2.6	–	–	–	–
Amphiura chiajei (Ech)	2	9.70±1.13	−19.52±1.95	2.0	–	–	–	–
Terebellidae und. (Pol)	1	10.81	−20.47	2.5	3	12.35±0.41	−18.11±0.05	3.1
Pista sp. (Pol)	–	–	–	–	3	11.73±0.90	−19.08±0.69	2.7
Abra prismatica (Biv)	–	–	–	–	2	11.45±0.21	−14.57±0.81	2.7
Lysianassa pilicornis (Amph)	1	11.20	−19.90	2.6	2	11.70±0.57	−19.68±1.65	2.8
Gammarella fucicola (Amph)	–	–	–	–	1	8.70	−18.92	1.5
Gammarus sp. (Amph)	2	7.00±0.85	−17.43±1.24	0.8	–	–	–	–
Opheliidae und. (Pol)		11.50	−20.89	2.8	–	–	–	–
Spionidae und. (Pol)	2	10.34±3.07	−21.25±2.52	2.3	–	–	–	–
Polynoidae und. (Pol)	1	12.25	−18.67	3.1	–	–	–	–
Amphitrite sp. (Pol)	–	–	–	–	1	12.30	−26.26	3.1
Leucothoe serraticarpa (Amph)	–	–	–	–	1	11.60	−21.30	2.8
Amphipholis squamata (Ech)	–	–	–	–	1	12.70	−20.47	3.3
Subsurface deposit feeders								
Capitellidae und. (Pol)	2	9.85±0.07	−19.11±0.31	2.0	–	–	–	–
Orbinidae und. (Pol)	2	10.95±0.50	−18.29±0.57	2.5	2	11.59±0.11	−18.49±0.23	2.7
Maldane glebifex (Pol)	2	10.36±0.08	−16.52±0.05	2.3	–	–	–	–
Cirratulidae und. (Pol)	1	11.01	−20.13	2.5	–	–	–	–

Table 2 (continued)

Species/feeding guild	Inlet 1				Inlet 2			
	n	δ^{15}N (‰)	δ^{13}C (‰)	*λ*	*n*	δ^{15}N (‰)	δ^{13}C (‰)	*λ*
Notomastus latericeus (Pol)	–	–	–	–	1	10.80	−18.45	2.5
Notomastus formianus (Pol)	–	–	–	–	2	10.77±0.04	−19.55±0.05	2.4
Grazers								
Haminoea hydatis (Gas)	–	–	–	–	4	11.18±0.68	−17.51±1.36	2.6
Chiton (Rhyssoplax) olivaceus (Polypl)	–	–	–	–	1	11.10	−15.06	2.6
Tricolia pullus pullus (Gas)	–	–	–	–	3	11.00±0.17	−15.72±3.64	2.5
Paracerceis sculpta (Isop)	–	–	–	–	4	9.63±0.50	−18.54±0.60	1.9
Diodora graeca (Gas)	–	–	–	–	1	14.20	−19.96	3.9
Gibbula albida (Gas)	–	–	–	–	1	12.00	−15.76	3.0
Gibbula umbilicalis (Gas)	1	10.10	−18.90	2.1	–	–	–	–
Elasmopus rapax (Amph)	1	9.70	−19.30	2.0	1	11.70	−21.75	2.8
Elasmopus affinis (Amph)	1	8.90	−19.83	1.6	–	–	–	–
Sarpa salpa (Fish)	2	13.05±0.07	−20.23±1.56	3.4	–	–	–	–
Omnivores								
Marphisa bellii (Pol)	2	12.46±0.44	−19.27±1.14	3.2	1	13.61	−18.38	3.7
Marphisa fallax (Pol)	1	10.73	−20.20	2.4	–	–	–	–
Melinna palmata (Pol)	1	9.23	−16.97	1.8	–	–	–	–
Centropages spp. (Cop)	2	7.19±0.51	−22.23±0.07	0.9	1	5.55	−23.00	0.2
Acartia spp. (Cop)	2	8.21±1.02	−20.80±0.29	1.3	2	8.57±0.20	−21.93±0.71	1.5
Carnivores								
Lumbrineris sp. (Pol)	4	11.97±0.37	−19.07±1.56	3.0	1	13.00	−20.32	3.4
Brachynotus gemmellaroi (Decap)	1	8.30	−17.07	1.4				
Pisidia longimana (Decap)	2	9.80±2.40	−20.19±1.19	2.0	1	12.80	−20.84	3.3
Paguristes eremita (Decap)	2	11.50±0.28	−19.02±1.25	2.8	–	–	–	–
Inachus sp. (Decap)	1	8.20	−21.23	1.3	–	–	–	–
Euthria cornea (Gas)	1	12.40	−16.77	3.1	–	–	–	–
Hexaplex trunculus (Gas)	–	–	–	–	1	13.40	−17.72	3.6
Nassarius cuvierii (Gas)	–	–	–	–	1	12.60	−16.71	3.2
Eunice vittata (Pol)	4	12.00±0.55	−19.20±0.25	3.0	3	12.42±0.47	−18.73±0.64	3.2
Glycera sp. (Pol)	3	13.10±1.65	−17.43±1.42	3.5	2	13.43±0.18	−17.21±0.45	3.6
Gobius niger (Fish)	1	13.70	−16.28	3.7	–	–	–	–
Dicentrarchus labrax (Fish)	1	16.40	−16.26	4.9	1	17.70	−16.05	5.5
Boops boops (Fish)	3	12.43±1.01	−17.47±0.40	3.2	–	–	–	–
Mugil cefalus (Fish)	3	14.37±2.33	−17.72±0.93	4.0	7	15.59±0.57	−19.33±0.81	4.5

For small invertebrates, *n* corresponds to replicate numbers of homogeneous pool of individuals

Pol Polychaeta, *Biv* Bivalvia, *Gas* Gastropoda, *Polypl* Polyplacophora, *Tun* Tunicata, *Ech* Echinodermata, *Scaph* Scaphopoda, *Amph* Ampipoda, *Isop* Isopoda, *Cop* Copepoda, *Dec* Decapoda, *nd* not determined, *und* undetermined

36 %). Detritus provided a higher contribution (15–16.8 %) to the total diet and together with macroalgae reached 64.2–76.3 %. Similarly, SSDF and GRZ relied on a higher contribution of algal OM (detritus and fresh algae) and a lower contribution of POM. Probably due to the narrow range of variability of the δ^{13}C macroalgae signature, the analysis could not discriminate different contributions to consumers' diets (Fig. 5).

Discussion

Nature and origin of OM sources in the Mar Piccolo

In coastal marine ecosystems, food webs are fuelled by pools of suspended and sediment particulate matter which are typically composed by a complex mix of phytoplankton, local benthic primary producers and terrestrial matter of riverine

Table 3 PERMANOVA tests for changes in δ^{15}N and δ^{13}C of macrobenthic consumers

Test	Source	*df*	SS	MS	Pseudo-*F*	*P* (perm)	Outputs of pair-wise tests
δ^{15}C	Months (mo)	1	20.218	20.218	5.4806	0.0222	June>April,
	Stations (st)	3	41.233	13.744	3.7258	0.016	St 1E>St 1I; St 2B>St 1I and St2C
	Moxst	3	3.6274	1.2091	0.32777	0.804	
	Res	122	450.05	3.6889			
	Total	129	530.49				
δ^{15}N	Months (mo)	1	0.38916	0.38916	0.14201	0.6924	
	Stations (st)	3	26.085	8.6949	3.1729	0.0244	St 2B>St 1I and 1E; St 2C>St 1I and 1E
	Moxst	3	6.4753	2.1584	0.78763	0.5008	
	Res	122	334.33	2.7404			
	Total	129	386.04				

origin (Cresson et al. 2012). Moreover, in these systems, high primary production is often associated to a wide diversity of benthic primary producers (macroalgae, salt marsh and seagrass beds, Bode et al. 2006). In such complex systems, the SIA is a powerful help when trying to resolve the nature and origin of organic pools and their contribution to higher trophic levels.

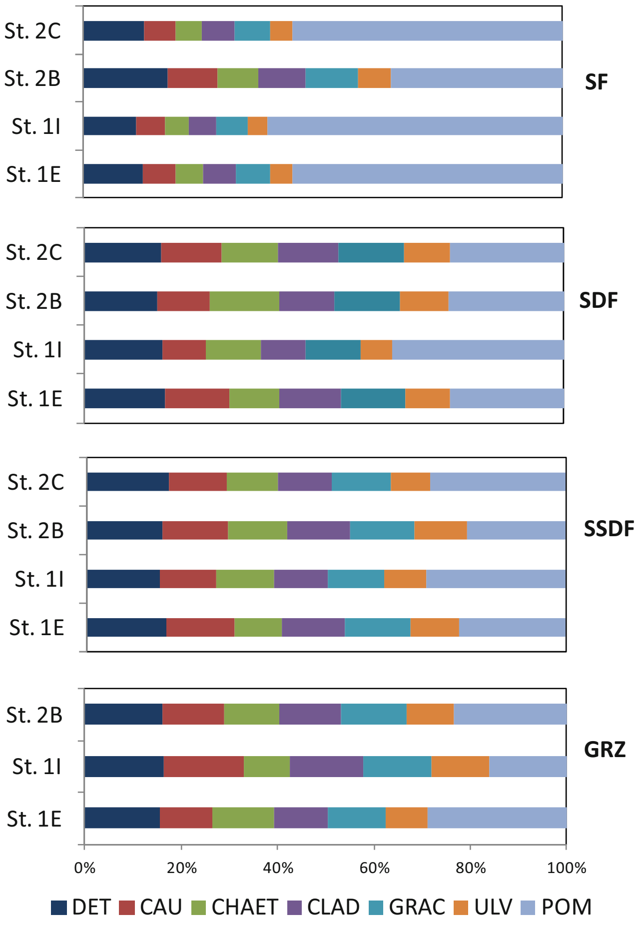

Fig. 5 Outputs of the Bayesian Mixing Model SIAR showing the average contribution of potential sources to primary consumers' feeding guilds in each sampling station. *SF* suspension feeders, *SDF* surface deposit feeders, *SSDF* subsurface deposit feeders, *GRAZ* grazers. Sources are the following: *DET* algal detritus, *CAU Caulerpa*, *CHAET Chaetomorpha*, *GRAC Gracilaria*, *CLAD Cladophora*, *ULV Ulva*, *POM* particulate organic matter

Mean δ^{13}C (−23.90‰) of particulate organic matter (POM) in the Mar Piccolo during both sampling months was lower when compared to phytoplankton values registered in temperate coastal waters (−20 and −22‰, Fry and Wainright 1991; Harmelin-Vivien et al. 2008) and in range or lower than in Mediterranean lagoons (−25 to −20.77‰ reported by Berto et al. 2013; Carlier et al. 2007, −16.12‰ reported by Vizzini and Mazzola 2003). δ^{13}C POM signature was significantly lower during April and in particular in stations located in the second inlet (Table 1).

Differences in δ^{13}C of suspended particles can depend on different cells size and phytoplankton taxa composition. In the Mediterranean Sea, Fry and Wainright (1991), and Rau et al. (1990) found that small cells (nano and pico)-phytoplankton were ^{13}C depleted (−25.4 and −22.5‰, respectively) compared to big diatoms and dinoflagellates. However, this hypothesis was not supported by results obtained in the Mar Piccolo by Karuza et al. (this issue) who reported in the same inlet and season a net predominance of bigger microphytoplankton cells (ca. 50 %). Low values of marine $\delta^{13}C_{POM}$ (below −24‰) are also typically considered to reflect substantial contribution of terrestrial OM (Harmelin-Vivien et al. 2008). Therefore, the low ^{13}C values detected in the Mar Piccolo could reflect either the seasonal intrusion of freshwater carrying terrestrial detritus from *citri* (estimate discharge of Le Kopre *citro* in the second inlet, 0.1–1.2 m^{3} s^{-1}) or most likely the high land runoff often detected at the end of the rainy winter period (Cardellicchio et al. this issue; Umgiesser et al. 2007). The predominant contribution of riverine/terrestrial POM in the Mar Piccolo was confirmed by the output of the SIAR model applied in Cibic et al. (this issue) to evaluate the contribution of different primary sources to the main organic pools. An important contribution to POM was also due to phytoplanktonic component. This is corroborated by high chlorophyll concentrations and POC/PN ratios (between 6 and 7) observed in June and coherent with average C/N ratios of phytoplankton cells (Table 1, Savoye et al. 2003).

Compared to POM, SOM exhibited higher δ^{13}C values. As observed by Cresson et al. (2012) in the bay of Marseille, such differences might rule out from major influence of ^{13}C enriched macroalgae or seagrasses detritus in sediments. This result is congruent with the high C/N ratio of SOM (≥10, Table 1) and by the mixing model which pointed to an important contribution of macroalgae (average 19.5 %) to this pool (Cibic et al. this issue). Although no SI data were available for the microphytobenthic fraction, the low benthic primary production and biomass detected in the Mar Piccolo basin (Cibic et al. this issue; Rubino et al. this issue) seems to exclude a main contribution of this component to the sediment pool. δ^{13}C of SOM tended to be spatially homogeneous at the whole basin scale, probably as results of intense tidal resuspension and mixing.

Conversely, N signature was more distinctive between the two inlets. δ^{15}N values around 9‰ in POM and SOM pools were observed in the second inlet especially in June. Similar or higher δ^{15}N values have been generally reported to reflect high biological metabolism or N input due to anthropogenic activities (Owens 1985; Vander Zanden and Rasmussen 2001). This hypothesis was supported by the high primary and secondary production and microbial metabolic activities measured at St. 2B and 2C during the same sampling period (Cibic et al., Franzo et al., Karuza et al. this issue). However, the vicinity of the mussel rearing area and the discharge of waste water through the D'Aiedda channel could have also contributed to the high δ^{15}N of POM and SOM detected in the second inlet. Indeed, sewage sludge and aquaculture waste introduce nutrients and organic matter which are typically ^{15}N enriched compared to naturally occurring dissolved inorganic nitrogen; both sources are uptaken by primary producers, and the signal is transferred through the food web (Vermuelen et al. 2011).

Trophic structures and C food web pathways of the Mar Piccolo

The study of the stable isotopic composition of organic sources and consumers and the Bayesian mixing model on primary consumers confirms the importance of POM in fuelling the food web. In the Mar Piccolo, high planktonic production appeared to supplement both planktonic and benthic pathways (also refer to Cibic et al. this issue). Generally, the δ^{13}C signature well discriminate consumers' diets (Grall et al. 2006). Despite certain variability, outputs of the mixing model were consistent with a diet of suspension feeders (SF) mainly dependent on POM. Differently, the δ^{13}C of deposit feeders (average −18.93 and −18.61‰, for SDF and SSDF, respectively) and the output of the mixing model were mainly in agreement in pointing to fresh algae and detritus as the most important ultimate C sources for these consumers. Despite that macroalgal coverage was significantly higher in the second inlet (up to 30–40 % at St. 2C and 90–100 % at St. 2B, Cibic et al. this issue), no significant difference was observed in their contribution to consumers between inlets 1 and 2. These results suggest that probably due to tide mixing between the two inlets, carbon inputs associated to benthic algae extend beyond inlet 2 influencing the whole basin. Compared to the average isotopic composition of feeding guilds, some species exhibited different signatures. The SF bivalve *Arcuatula senhousia*, and *Corbula gibba* and the tunicate *Ascidiella aspersa* in St 2B (Table 2) had a lower δ^{13}C which could suggest opportunistic shifts in their feeding strategy. While the low δ^{13}C signature of *Arcuatula senhousia* during April was mainly pointing to POM as primary source, the isotopic signal in June was less depleted. These could have been the results either of a shift toward benthic resources or the effect of resuspension events which would bring organic matter of algal origin in the water column. In St. 2C, the deposit feeders *Abra prismatica* exhibited higher δ^{13}C signature (−14.5‰) compared to other sites, which were consistent with a major influence of macroalgae such as *Caulerpa* and Ulvales. It is interesting to note that at this site, the lower ^{13}C/^{12}C signature of the congeneric *Abra alba* (−18.31‰) could suggest different feeding preference and thus the occurrence of resource partitioning between these two species. The plasticity of *A. alba* to feed on deposit as well as on suspended material (Holtmann et al. 1996) could explain these differences in δ^{13}C.

The SIA results also discriminated alternative feeding strategies in the Mar Piccolo. The bivalve *Loripes lucinalis* represented an outlier in the isotopic range of consumers in the Mar Piccolo as its SI values (−1.30 and −30.43‰ for δ^{15}N and δ^{13}C, respectively) were very low. Similar values have been reported for the bivalves belonging to the family Lucinidae living in estuarine seagrasses and mangrove habitats (Vafeiadou et al. 2013; Bouillon et al. 2008) and in the Sales-Leucate lagoon (Carlier et al. 2007). *L. lucinalis* is a suspension filter-feeder but can also exploit metabolites released by the endosymbiontic chemotrophic sulphur-oxidizing bacteria located in the animal gills. Those metabolites which are ^{13}C depleted as a result of bacterial C fixation (Herry et al. 1989; Spiro et al. 1986) would explain the low C stable isotope value found.

Effect of anthropogenic disturbance on the Mar Piccolo food web

Contrarily to what was expected, stable isotopes of consumers in the Mar Piccolo did not show a clear-cut seasonal and spatial variation; therefore, we could not link change in the SI signals to the effect of consumers' ontogenesis or to anthropogenic impacts. The only clear difference was the higher $\delta^{15}N$ signatures exhibited by consumers in the second inlet. For example, individuals of *Mytilus galloprovincialis* collected in the second inlet (St 2C) showed higher $\delta^{15}N$ (10.3‰) compared to those collected in the first inlet. A similar variation of the isotopic composition of the biota was found by Vizzini and Mazzola (2004) in proximity to land based fish farm. Vermuelen et al. (2011) found that while macroalgae exhibited equivocal signals, mussels provided the best time-integrated picture of the graduated exposure to anthropogenic impacts. In particular, bivalve owing to their large filtering capacity accumulates matter suspended in the water column and provides a long-term integrated signal of their food sources (Lassauque et al. 2010). In the Mar Piccolo, several indicators have pointed to a gradient of eutrophication from the first inlet toward the second inlet (Alabiso et al. 2005). $\delta^{15}N$ values of the biota in the second inlet suggested the persistence of eutrophic conditions mainly triggered by the release of N-rich waste from mussel farming facilities which occupies an extensive portion of the second inlet.

Although isotopic analysis was not able to detect a true response to anthropogenic stressor in inlet 1, clear-cut difference was observed in terms of benthic community composition (Franzo et al. this issue). At St.1E, the one close to the entrance of the basin and characterized by low contamination and high sediment oxygen penetration (Cibic et al. this issue), deposit feeders dominated the macrobenthic community (Fig. 4). Conversely at St. 1I, located in front of the navy arsenal, and more contaminated by PCB and heavy metals, filters feeders and mobile invertebrates (carnivores and grazers) biomass were more abundant, while surface and subsurface deposit feeders were dramatically reduced compared to the other sampling stations (Fig. 4). Reduction in trophic complexity in organically enriched and chemically contaminated sediments, in which the benthic assemblages were dominated by opportunistic species, has been observed by several authors (Gaston et al. 1998; Rakocinski et al. 2000). The structure of the assemblage of intermediate consumers that link basal OM sources and higher predators can be very relevant for food web processes and ecosystem functioning. For instance, disappearance of specific intermediate consumers may result in a simplification of the food web that compromises the efficiency of energy transfers ('energetic bottlenecks', *sensu* Iles and Rasmussen 2005 and literature therein). Therefore, we can infer that the simplification of the food web structure in the more contaminated site of the Mar Piccolo (see Figs. 3 and 4) could suggest a localized reduction in the efficiency of energy transfers towards higher trophic levels.

Conclusions

In this study, we analysed for the first time the food web structure of the Mar Piccolo and the influence of different organic sources to primary consumers using stable isotopes. This analysis reflected the nature of primary resources and its importance for consumers. In particular, $\delta^{13}C$ analysis of primary resources well distinguishes the influence of freshwater and/or terrigenous inputs. Moreover, SI pointed to POM as the major sources contributing to both benthic and pelagic pathways, while direct consumption of benthic resources was marginal and mainly related to feeding mode and site specific. Results of this study confirm that the analysis of stable isotopes (especially of $\delta^{15}N$ in sources and macrobenthic consumers) is a useful indicator of organic enrichment. Data were consistent with a gradient of eutrophication from the first to the second inlet probably due to the high sewage and/or the mussel aquaculture waste discharge. However, since both these sources of pollution trigger an increase of $\delta^{15}N$ signal, it was not possible to resolve the effect of one source over the other. A deeper investigation on $\delta^{15}N$ signatures of singles organic sources coupled with detailed measurements of nutrients concentration may help to unravel this issue. In the Mar Piccolo, due to the high level of urbanization and antrophogenic pressure, impacts are multiple thus making evaluation of single source very difficult. In this study, stable isotope of consumers could not discriminate the high chemical contamination of sediment near the navy arsenal. However, this effect was reflected at level of macrobenthic trophic structure, with the dominance at this site of more opportunistic species capable to exploit more transient condition. We therefore underpin the great potentials of matching stable isotopes analyses with studies of the community structures to better evaluate and disentangle the effects of multiple environmental impacts.

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References

Alabiso G, Giacomini M, Milillo M, Ricci P (2005) The Mar Piccolo of Taranto: 8 years of chemical-physical measurements. Biol Mar Med 12(1):369–373

Anderson M, Gorley R, Clarke K (2008) PERMANOVA+ for PRIMER: a guide to software and statistical methods Primer-e. Plymouth, UK

Berto D, Rampazzo F, Noventa S, Cacciatore F, Gabellini M, Aubry FB, Girolimetto A, Brusà RB (2013) Stable carbon and nitrogen isotope ratios as tools to evaluate the nature of particulate organic matter in the Venice lagoon. Estuar Coast Shelf Sci 135:66–76

Bode A, Alvarez-Ossorio MT, Varela M (2006) Phytoplankton and macrophyte contributions to littoral food webs in the Galician upwelling estimated from stable isotopes. Mar Ecol Prog Ser 318:89–102

Bouillon S, Borges AV, Castañeda-Moya E, Diele K, Dittmar T, Duke NC, Kristensen E, Lee SY, Marchand C, Middelburg JJ, Rivera-Monroy VH, Smith III TJ, Twilley RR (2008) Mangrove production and carbon sinks: a revision of global budget estimates. Global Biogeochemical Cycles 22. doi:10.1029/2007GB003052

Cardellicchio N, Buccolieri A, Giandomenico S, Lopez L, Pizzulli F, Spada L (2007) Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, Southern Italy). Mar Pollut Bull 55(10–12):451–458

Cardellicchio N, Annicchiarico C, Di Leo A, Giandomenico S, Spada L The Mar Piccolo of Taranto: an interesting ecosystem for the study of the environmental problems This issue

Carlier A, Riera P, Amouroux J-M, Bodiou J-Y, Escoubeyrou K, Desmalades M, Caparros J, Grémare A (2007) A seasonal survey of the food web in the Lapalme Lagoon (northwestern Mediterranean) assessed by carbon and nitrogen stable isotope analysis. Estuar Coast Shelf Sci 73:299–315

Cibic T, Bongiorni L, Borfecchia F, Di Leo A, Franzo A, Giandomenico S, Karuza A, Micheli C, Rogelja M, Del Negro P. Ecosystem functioning nearby the largest steelworks in Europe and the main Italian naval base: the study case of the Mar Piccolo of Taranto This issue

Coplen TB (2011) Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun Mass Spectrom 25:2538–2560

Costanza R, d'Arge R, De Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'neill RV, Paruelo J (1997) The value of the world's ecosystem services and natural capital. Nature 387:253–260

Costanzo SD, O'Donohue MJ, Dennison WC, Loneragan NR, Thomas M (2001) A new approach for detecting and mapping sewage impacts. Mar Pollut Bull 42:149–156

Cresson P, Ruitton S, Fontaine MF, Harmelin-Vivien ML (2012) Spatio-temporal variation of suspended and sedimentary organic matter quality in the Bay of Marseilles (NW Mediterranean) assessed by biochemical and isotopic analyses. Mar Pollut Bull 64:1112–1121

D'ambra I, Graham WM, Carmichael RH, Malej A, Onofri A (2013) Predation patterns and prey quality of medusae in a semi-enclosed marine lake: implications for food web energy transfer in coastal marine ecosystems. J Plankton Res 35(6):1305–1312

De Niro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

Desrosiers G, Savenkoff C, Olivier M, Stora G, Juniper K, Caron A, Gagne J, Legendre L, Mulsow S, Grant J, Roy S, Grehan A, Scaps P, Silverberg N, Klein B, Tremblay J, Therriault J (2000) Trophic structure of macrobenthos in Gulf of St Lawrence and on the Scotian Shelf. Deep Sea Res II 47:663–697

Dolenec T, Lojen S, Lambasa Z, Dolenec M (2006) Effects of fish farm loading on seagrass *Posidonia oceanica* at Vrgada Island (Central Adriatic): a nitrogen stable isotope study. Isot Environ Health Stud 42:77–85

Franzo A, Auriemma R, Nasi F, Vojvoda J, Pallavicini A, Cibic T, Del Negro P Benthic ecosystem functioning in the severely contaminated Mar Piccolo of Taranto (Ionian Sea, Italy): focus on heterotrophic pathways This issue

Fry B (2006) Stable Isotope Ecology. Springer Ed, New York, **324 p**

Fry B, Wainright SC (1991) Diatom sources of ^{13}C-rich carbon in marine food webs. Mar Ecol Prog Ser 76:149–157

Gaston GR, Rakocinski CF, Brown SS, Cleveland CM (1998) Trophic functionin estuaries: response of macrobenthos to natural and contaminant gradients. Mar Freshw Res 49:833–846

Grall J, Le Loc'h F, Guyonnett B, Riera P (2006) Community structure and food web based on stable isotopes (δ^{15}N and δ^{13}C) analysis of a North Eastern Atlantic maerl bed. J Exp Mar Biol Ecol 338:1–15

Gray C, Baird DJ, Baumgartner S, Jacob U, Jenkins GB, O'Gorman EJ, Lu X, Ma A, Pocock MJO, Schuwirth N (2014) Ecological networks: the missing links in biomonitoring science. J Appl Ecol 51:1444–1449

Halpern BS, Waldbridge S, Selkoe KA, Kappel CV, Micheli F et al (2008) A global map of human impact on marine ecosystems. Science 319:948–952

Harmelin-Vivien M, Loizeau V, Mellon C, Beker B, Arlhac D, Bodiguel X, Ferraton F, Hermand R, Philippon X, Salen-Picard C (2008) Comparison of C and N stable isotope ratios between surface particulate organic matter and microphytoplankton in the Gulf of Lions (NW Mediterranean). Cont Shelf Res 28(15):1911–1919

Herry A, Diouris M, Le Pennec M (1989) Chemoautotrophic symbionts and translocation of fixed carbon from bacteria to host tissues in the littoral bivalve *Loripes lucinalis* (lucinidae). Mar Biol 101(3):305–312

Holtmann SE, Groenewold A, Schrader KHM, Asjes J, Craeymeersch JA, Duineveld GCA, van Bostelen AJ, van der Meer J (1996) Atlas of the zoobenthos of the Dutch continental shelf. Ministry of Transport, Public Works and Water Management: Rijswijk, The Netherlands, p 243

Iles AC, Rasmussen JB (2005) Indirect effects of metal contamination on energetics of yellow perch (*Perca flavescens*) resulting from food web simplification. Freshw Biol 50:976–992

Karuza A, Caroppo C, Cerino F, Monti M, Stabili L, Camatti E, Cibic T Planktonic trophic web in Mar Piccolo of Taranto (Ionian Sea, Italy) This issue

Katsanevakis S, Stelzenmüller V, South A, Sørensen TK, Jones PJS, Kerr S (2011) Ecosystem-based marine spatial management: review of concepts, policies, tools, and critical issues. Ocean Coast Manag 54:807–820

Krumins JA, van Oevelen D, Bezemer TM, De Deyn GB, Hol WHG, van Donk E, de Boer W, de Ruiter PC, Middelburg JJ, Monroy F et al (2013) Soil and freshwater and marine sediment food webs: their structure and function. Bioscience 63:35–42

Lassauque J, Lepoint G, Thibaud T, Francour P, Meinesz A (2010) Tracing sewage and natural freshwater input in a Northwest Mediterranean bay: evidence obtained from isotopic ratios in marine organisms. Mar Pollut Bull 60(6):843–851

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM, Bearhop S (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev 87:545–562

Logan JM, Jardine TD, Miller TJ, Bunn SE, Cunjak RA, Lutcavage ME (2008) Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. J Anim Ecol 77(4):838–846

Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Science 312:1806–1809

Mancinelli G, Vizzini S (2015) Assessing anthropogenic pressures on coastal marine ecosystems using stable CNS isotopes: state of the art, knowledge gaps, and community-scale perspectives. Est Coast Shelf Sci 156:195–204. doi:10.1016/j.ecss.2014.11.030

Mateo MA, Serrano O, Serrano L, Michener RH (2008) Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. Oecologia 157:105–115

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82:290–297

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102:378–390

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

Owens NJP (1985) Variations in the natural abundance of ^{15}N in estuarine suspended particulate matter: a specific indicator of biological processing. Estuar Coast Shelf Sci 20:505–510

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. Plos ONE 5: e9672

Parrott L (2010) Measuring ecological complexity. Ecol Indic 10:1069–1076

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies Annual Rev Ecol. System 18:293–320

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Pugnetti A, Acri F, Bernardi Aubry F, Camatti E, Cecere E, Facca C, Franzoi P, Keppel E, Lugliè A, Mistri M, Munari C, Padedda BM et al (2013) The Italian Long-Term Ecosystem Research (LTER-Italy) network: results, opportunities, and challenges for coastal transitional ecosystems. Transit Waters Bull 1:43–63

Rakocinski CF, Brown SS, Gaston GR, Heard RW, Walker WW, Summers JK (2000) Species-abundance-biomass responses by estuarine macrobenthos to sediment chemical contamination. J Aquat Ecosyst Stress Recover 7:201–214

Rau GH, Teyssie JL, Rassoulzadegan F, Fowler S (1990) ^{13}C/^{12}C and ^{15}N/^{14}N variations among size-fractionated marine particles: implications for their origin and trophic relationships. Mar Ecol Prog Ser 59:33–38

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Rubino F, Cibic T, Belmonte M, Rogelja M Microbenthic community structure and trophic status of sediments in Mar Piccolo of Taranto (Mediterranean, Ionian Sea) This issue

Savoye N, Aminot A, Tréguer P, Fontugne M, Naulet M, Kérouel R (2003) Dynamics of particulate organic matter δ^{15}N and δ^{13}C during spring phytoplankton blooms in a macrotidal ecosystem (Bay of Seine, France). Mar Ecol Prog Ser 255:27–41

Spiro B, Greenwood PB, Southward AJ, Dando PR (1986) ^{13}C/^{12}C ratios in marine invertebrates from reducing sediments: confirmation of nutritional importance of chemoautotrophic endosymbiotic bacteria. Mar Ecol Prog Ser 28:233–240

Strong DR, Frank KT (2010) Human involvement in food webs. Annu Rev Environ Resour 35:1–23

Umgiesser G, Scroccaro I, Alabiso G (2007) Mass exchange mechanisms in the Taranto Sea. Transit Waters Bull 2:259–71

Vafeiadou AM, Materatski P, Adão H, De Troch M, Moens T (2013) Food sources of macrobenthos in an estuarine seagrass habitat (*Zostera noltii*) as revealed by dual stable isotope signatures. Mar Biol 160(9):2517–2523. doi:10.1007/s00227-013-2238-0

Vander Zanden MJ, Rasmussen JB (2001) Variation in δ^{15}N and δ^{13}C trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066

Vermuelen S, Sturaro N, Gobert S, Bouquegneau JM, Lepoint G (2011) Potential early indicators of anthropogenically derived nutrients: a multiscale stable isotope analysis. Mar Ecol Prog Ser 422:9–22

Vizzini S, Mazzola A (2003) Seasonal variations in the stable carbon and nitrogen isotope ratios (^{13}C/^{12}C and ^{15}N/^{14}N) of primary producers and consumers in a western Mediterranean coastal lagoon. Mar Biol 142:1009–1018

Vizzini S, Mazzola A (2004) Stable isotope evidence for the environmental impact of a land-based fish farm in the western Mediterranean. Mar Pollut Bull 49:61–70

Vizzini S, Mazzola A (2006) The effects of anthropogenic organic matter inputs on stable carbon and nitrogen isotopes in organisms from different trophic levels in a southern Mediterranean coastal area. Sci Total Environ 368:723–731

Vizzini S, Savona B, Caruso M, Savona A, Mazzola A (2005) Analysis of stable carbon and nitrogen isotopes as a tool for assessing the environmental impact of aquaculture: a case study from the western Mediterranean. Aquac Int 13:157–165

Yokoyama H, Tamaki A, Koyama K, Ishihi Y, Shimoda K, Harada K (2005) Isotopic evidence for phytoplankton as a major food source for macrobenthos on an intertidal sandflat in Ariake Sound, Japan. Mar Ecol Prog Ser 296:115–128

Paper VI

FUNCTIONAL BIODIVERSITY OF MARINE SOFT-SEDIMENT POLYCHAETES FROM TWO MEDITERRANEAN COASTAL AREAS IN RELATION TO CONTAMINATION

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Abstract

In the present study Biological Traits Analysis (BTA) was used to identify shifts in functional features of infaunal polychaete assemblages associated with different levels of contamination in two impacted coastal areas: the harbor of Trieste (Adriatic Sea) and the Mar Piccolo of Taranto (Ionian Sea). The analysis was performed on 103 taxa, collected at four stations in each area. In spite of the decreasing taxa richness the trait categories and functional diversity is remained fairly stable. The anthropogenic stressors caused a decline in diversity but this was not clearly reflected in functional diversity, probably due to the reduction of species that contribute little to community functions or are functionally redundant in the analyzed polychaete assemblages. The most distinct trait patterns were shown for traits as mobility, environmental position, movement methods, and feeding habits in relation, principally, with levels of copper, mercury and total nitrogen in the sediments. The trait composition changed from a well-balanced functional structure toward highly motile species in the most severely contaminated site and sessile, tube-builders and suspension-feeders, from high to low contamination in relation also with organic enrichment. High motility of the species seems to be an essential trait for living in highly contaminated sediments. This could be linked to the ability to avoid hotspots of contaminants through active movements, impacting the sediment reworking and nutrient exchange. The results illustrate how BTA, offers an integrative approach to detect the functional adaptation related with contamination, in an area subjected to multiple anthropogenic stressors.

Keywords: Biological traits analysis; Polychaetes; Soft sediments; Contamination; Coastal areas.

Introduction

Coastal areas deserve proper attention regarding diffuse and synergistic forms of pollution. The latter is principally due to industrial activities that persist along coasts, usually near highly populated areas. Industrial areas are often characterized by the presence of large steelworks, oil refineries, shipbuilding and other industrial activities that are responsible for severe and persistent contamination due to heavy metals, asbestos, polycyclic aromatic hydrocarbons (PAHs), organic solvents, polychlorinated biphenyls (PCBs), and dioxins (Cardellicchio et al. 2016). Among coastal areas, the effects of diffuse and synergistic forms of pollution are even more emphasized in enclosed basins, where the water circulation is restricted and, therefore, the dilution effect of contaminants is limited (Trannum et al. 2004). Due to their capacity to retain pollutants from different sources, the sediments act as a sink in the aquatic environment reaching concentrations up to orders of magnitude higher than in the overlying water (Cardellicchio et al. 2007).

In soft sediments, benthic organisms, especially sessile or sedentary species due to their lack of or limited mobility, are exposed to high contamination levels and respond to these stress conditions both at the individual and community level through the variation in the total numbers, the selection of taxa and the elimination of the sensitive ones (Solis-Weiss et al. 2001). Thus, changes in environmental conditions, induced by anthropogenic drivers, strongly affect the benthic community structure and consequently also the overall ecosystem functioning (Harley et al. 2006; Doney et al 2012). Ecosystem functioning includes all the processes in the system as well as the chemical, physical and biological components involved (Naeem et al. 2002; Bremner 2008). Macrofauna is a key biological component that drives important processes such as nutrient cycling, sediment reworking, bio-irrigation and organic matter decomposition (Widdicombe et al. 2004; Bremner et al. 2006; Olsgard et al. 2008). Individual species can contribute to different ecosystem functions, and hence shifts in species composition due to either natural or human-mediated changes may have fundamental implications for ecosystem functioning (Gray et al. 2006; Tillin et al. 2006; Schratzberger et al. 2007). This depends on the biological features of the species that may decrease or increase in response to human influence (Oug et al. 2012; Krumhansl et al. 2016).

Studies on soft bottom species assemblages are fundamental in marine environmental monitoring and assessments of impacts from human activities. The majority of ecological studies focus on the structural aspect of species assemblages, such as abundance, biomass or diversity, but rarely assess the consequential functional progression of environmental variations. Species interact with and respond to their physical and chemical environment in various ways depending on their ability to do so. The maintenance and regulation of functions in an ecosystem is thus essentially dependent on the ecological roles and traits of the present taxa (Naeem 2002). The use of species composition might be inadequate for investigating processes that sustain an ecological system (Diaz and Cabido 2001), since the ecosystem processes are determined by the functional characteristics of the organisms involved, rather than by taxonomic identity (Grime 1997). Biological Trait Analysis (BTA) is a useful analytical approach to better understand the relationships between organisms and ecosystem functioning (Solan et al. 2004; Bremner et al. 2006; Gagic et al. 2015). BTA combines structural data of macrofaunal community (species abundance or biomass) with the information on functional features of species (Törnroos and Bonsdorff 2012). BTA relies on a comprehensive set of functional traits (e.g. feeding type, body size, and reproductive technique), which can serve as indicators for ecosystem functioning. Hence, the approach provides a link between species, environment and ecosystem processes.

The biological trait concept was originally developed for and used in freshwater systems (e.g. Olff et al. 1994; Charvet et al. 2000; Usseglio-Polatera et al. 2000), and only recently several studies have applied this approach also to marine benthic assemblages to describe their ecological functioning (e.g. Aarnio et al. 2011; Dimitiadis et al. 2012; Törnroos et al. 2014) as well as to investigate the effects of climate change (e.g. Neumann and Krönke 2011; Weigel et al. 2016). In contrast, the effects of chemical contamination on invertebrate biological features and consequently the changes in ecosystem functioning have been seldom considered (i.e. Oug et al. 2012; Krumhansl et. al. 2016). Other studies focused on the functional traits of only one of the macrofaunal phyla (e.g. bivalves and polychaetes) (Montalto et al 2015; Gambi et al. 2016; Otegui et al. 2016).

Polychaetes typically contribute to a high percentage of the total macrofaunal community diversity and abundance. Moreover, they occupy a large part of the available niches in the marine environment and dominate marine sediments in diverse forms and ways of life. In fact, the multi-faced response of polychaetes to environmental disturbance is probably a consequence of their high morpho-functional diversity (Giangrande 1997; Jumars et al 2015), which allows different species to occupy ecological niches along a gradient, from pristine to disturbed habitats (Giangrande 2005).

In this study, Polychaeta was chosen as a target group to illustrate the links between the environmental quality and their functional response to contamination. BTA was used to explore and characterize functional attributes of soft-sediment polychaete assemblages in two Mediterranean coastal basins subjected to different levels of contamination, to study potential alterations of ecosystem functioning and the functional adaptation of macrofaunal invertebrates to this environmental stress. In particular, we focused on two functional aspects (diversity and identity) of polychaete assemblages, potentially modified by contaminants and other environmental variables (grain-size and organic enrichment). In this study, we aimed to answer the following questions: 1) Does chemical contamination affect polychaete functional diversity? 2) Which traits among the functional features are more affected by differently contaminated sediments? 3) Which is the role of the considered environmental factors in structuring the assemblage and functional trait patterns?

Materials and methods

Study sites and sampling design

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

The Mar Piccolo of Taranto is an inner, semi-enclosed shallow sea located in the northern area of Taranto. It has a surface area of 20.72 km^2 and is divided by a promontory in two smaller basins named First (FI) and Second Inlet (SI). The area is characterized by shallow waters (maximum depth is about 13 m in FI). The FI is directly connected with the Mar Grande through two channels: the 'Navigabile' and the 'Porta Napoli'. The fluxes of water through the two inlets are generally weak and depend on the difference in density between the two basins. Most of the water inputs derive from numerous small surface watercourses and 34 submarine freshwater springs, called "Citri", which influence salinity and temperature of both inlets (Cardellicchio et al. 2016). As more than 80% of the province of Taranto is used for farming, in particular for the cultivation of wheat, cereal crops and fodder, freshwater inputs contain chemicals drained from the surrounding agricultural soils in the basin (Di Leo et al. 2010). The basin is generally characterized by low velocity currents (about 5–10 cm s^{-1}) driven by the sea tides, with the maximum reached in the two connecting channels with the Mar Grande (up to 30–40 cm s^{-1}) (Umgiesser et al. 2007). The two inlets are characterized by different levels of confinement, i.e. by a different degree of connection to the sea and of sea water renewal time (Canu et al. 2012). In particular, the SI, due to the low hydrodynamism and scarce water exchange with the nearby Mar Grande, represents the most confined part of the system (Cardellicchio et al. 2007).

Two sampling campaigns were performed in both areas, in February and April 2014 (in the Mar Piccolo); in May 2014 and March 2015 (in the Bay of Muggia). Sediment samples for grain-size, chemical analysis and macrofauna were taken at four stations in both areas. In the harbor of Trieste the stations were located near the major industrial activities of the area: St. TS1 between the port's cargo piers; St.

TS2 in an area dedicated to shipbuilding activities; St. TS3 close to the iron and steel plant and St.TS4 near the petroleum industry (Fig. 1a). St. TS2 and St. TS3, closest to the port and industrial activities, were characterized by high concentrations of contaminants (Table 1). In the Mar Piccolo of Taranto, sampling was carried out at four stations selected as representatives of different environmental and anthropogenic impacts. In the FI, St. TA1 represents the environmental features of the area in front of the "Navigabile" channel whereas St. TA2 of that nearby the military navy arsenal. In the SI, St. TA3 and St. TA4 were selected as representatives of the most enclosed part of the Mar Piccolo. The latter were chosen to follow an increasing distance from the main sources of industrial pollution (located in the FI) (Fig.1b). St. TA2 was the most contaminated station, due to high mercury and PCBs levels (Table 1).

Detailed data on contaminants are reported in Cibic et al. (under review) for Trieste sampling stations, and in Cibic et al. (2016) and Bellucci et al. (2016) for Taranto ones.

Polychaete sampling

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

Biological Trait Analysis

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

The polychaete species were coded based on their affinity for the chosen traits. For example, species were categorized by their feeding habits, as suspension feeder, surface-and subsurface deposit feeder, herbivore, predator and scavenger. For organisms that were strictly suspension feeders a value of 3 was assigned for suspension feeding and 0 was assigned for all other habits. For organisms that were suspension or deposit feeders, values of 1-2 were assigned for each of these habits (depending on the degree of affinity to these traits) and 0 for the rest of the habits. This is known as a "fuzzy coding" procedure (Chevenet et al. 1994) that allows for species adherence to multiple categories within each trait (0=no affinity, 1=low importance, 2=moderate high importance, 3=dominant). Traits for each taxon were derived from literature sources (i.e. Giangrande 1997; Rouse 2000 and Jumars et al. 2015) and databases (i.e. MarLIN 2006; Polytraits Team 2016). Taxonomical resolution was kept at the species level whenever possible but adjusted to genus or family when the information on traits was available only on a higher taxonomic level.

Sediment characteristics

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

Sediment subsamples of about 10-15 g were taken for grain-size analysis. Small pebbles and shell fragments (>2 mm) were separated from the sand and mud fractions by sieving at 2 mm. Each sample was disaggregated and the organic matter oxidized using 10% hydrogen peroxide (Carlo Erba, Rodano, Italy). The analyses were performed using a Malvern Mastersizer 2000 equipped with Hydro 2000s (Malvern Instruments Ltd., Malvern, UK). Data are expressed as percentages of sand, silt and clay. For Total Organic Carbon (TOC) and Total Nitrogen (TN) analyses, sediments were freeze-dried and grounded using a mortar, then a pestle and the fraction >250 µm was separated from the rest of the sample. Only the fraction <250 µm was used for the analysis. Triplicate of subsamples (~8–12 mg) were weighed directly in a silver capsule (5x9 mm^2) on a microultrabalance Mattel Toledo model XP6 (accuracy 1 µm). Before the TOC determination, subsamples were treated with increasing concentrations of HCl (0.1 N and 1 N) to remove carbonates (Nieuwenhuize et al. 1994). TOC and TN contents were measured using an elemental analyser CHNO-S Costech model ECS 4010 following the methods of Pella and Colombo (1973) and Sharp (1974). Capsule blanks were also analyzed. The precision of the analysis was about 3%. The values are expressed as mg C g dry mass^{-1}.

Data analysis

On trait profiles, univariate and multivariate analysis were performed to examine patterns and relationships with environmental factors. Trait category richness was calculated as the total number of categories expressed per sample. Functional diversity was measured as the diversity in distribution and range of expression of functional traits, with the latter reflecting characteristics of organisms that influence ecosystem processes (Petchey and Gaston 2002). To assess the functional diversity and functional identity of polychaete assemblages in each sample, the library FD in the R program ver. 3.1.3 was used (Laliberté et al. 2014; R Core Team 2016). The Functional Dispersion (FDis) metric, developed by Laliberté and Legendre (2010), was chosen as a diversity index, as it has analytical advantages in comparison to other indices. The metric for FDis is unaffected by species richness. This feature allows it to be used with more traits than species present, to be computed from any distance or similarity measure, and to be capable of taking species abundance into account (Laliberté and Legendre 2010), as it was required in this study. FDis describes the abundance-weighted mean distance of individual species to their group centroid (all species community) in the multivariate trait space. Larger FDis values imply a more functionally spread community in the multivariate trait space, and hence a higher functional diversity (Weigel et al. 2016). Difference in FDis among stations and sampling periods were tested by Kruskal-Wallis ANOVA test (STATISTICA 7 software).

To analyse changes in trait composition and expression, the functional identity as community level weighted means (CWM) of trait category expression was calculated. CWM was calculated in the FD library for each sampling station and period.

The Principal Coordinates Analysis (PCoA) was used to describe the main traits patterns and assess association among functional features. In the present case, distance-based redundancy analysis (dbRDA) was used as a direct gradient analysis related to PCoA, but the axes are constrained by the environmental information in order to extract inter-related variations. CWM matrix with a log (X+1) transformation was used and Euclidean distance was applied for both multivariate analyses (Kleyer et al. 2012).

In addition, in order to extend the functional information on the polychaete assemblages with species composition, the species abundance matrix was included in the statistical analysis of the study. The diversity was evaluated as the total number of taxa at a specific station/sampling. A vector fitting nMDS ordination was performed in order to illustrate the major structural faunal patterns and the significant ($p < 0.05$) relationships with environmental factors. Square root was used to transform the data matrix and Bray-Curtis similarity was applied. The matrix was created by selecting polychaete species that were significantly ($p < 0.05$) highlighted as indicator species of the whole assemblage in both areas (multi-level pattern analysis). This test was carried out using the library Indicspecies (De Cáceres 2013) in the R software ver. 3.1.3. The vector fitting nMDS plot was performed with the package Vegan (Oksanen 2007) in the R software ver. 3.1.3.

To assess differences in species composition and traits (CWM values) between areas and sampling periods, we used a PERMANOVA test, where 'area' and 'period' were fixed factors. Unrestricted permutations of row data and 9999 permutations were applied. A BIO-ENV analysis was performed to assess which environmental variables correlate best with the pattern of species composition and CWM values at both areas, using the average values of each abiotic variable. Data were normalized before entering the analysis and the BIO-ENV routine was calculated using the Spearman's coefficient. The two matrices were modified as follows: log (X+1) and square root transformations were used on the traits and species matrix, whereas Euclidean distance and Bray-Curtis dissimilarity were applied, respectively. PERMANOVA test, PCoA and dbRDA, and BIO-ENV analyses were performed using PRIMER 7 (PRIMER-E Ltd., Plymouth,UK) (Clarke et al. 2014).

Results

Environmental data

According to Shepard's (1954) classification, the sediment was clayey silt at all stations of the harbor of Trieste and Mar Piccolo of Taranto. In Trieste sites the percentage of sand (average values of the two sampling periods) ranged between 6.1±5.1% (St. TS1) and 18.0±0.4% (St.TS3) whereas at Taranto, the lower sand percentage (2.9%) and the highest one (14.4%) were recorded at St.TA4 and St.TA1, respectively.

In the harbor of Trieste, higher TOC values were observed in May 2014 than in March 2015. TOC contents ranged between 18.3±1.0 mg C g^{-1} at St. TS1 and 46.1±1.1 mg C g^{-1} at St.TS2 in May 2014, whereas the lower value at St. TS4 (22.3±0.3 mg C g^{-1}) and the maximum one at St.TS3 (47.8±2.5 mg C g^{-1}) were recorded in March 2015. Fairly constant TN contents were observed at all stations and periods (average value: 1.7±0.2 mg C g^{-1}). In Taranto sites, higher TOC contents characterized the FI (36.3±2.5 mg C g^{-1}) compared to the SI (27.3±7.0 mg C g^{-1}). On the contrary, the SI was distinguished by high values of TN, (3.4±0.8 mg C g^{-1}) compared to FI (2.6±0.8 mg C g^{-1}).

Biodiversity and functional diversity and identity

A total of 103 taxa of polychaetes were found at the Taranto and Trieste stations. The number of taxa varied from a minimum of 7 (at St. TA3) to a maximum of 45 (at St. TS4) in April and May 2014, respectively. No significant temporal variation was observed in either area, but a small difference in the number of taxa was detected between the two areas (higher in Trieste than Taranto). For the trait-category richness, no variations were observed between areas and sampling seasons (average: 26.3±1.2; range: 24-29) (Fig. 2a).

Further, FDis values did not vary among stations of either area or over time (average value: 4.6±0.4; range: 3.9-5.1). The uniformity of the functional traits between areas and sampling periods was confirmed by the PERMANOVA main test. In fact, the analysis carried on the CWM matrix showed there were no significant differences between them for either of the factors (area and season) (Fig. 2b).

The average values of CWM for all assemblages were plotted to visualize a functional meta-composition for the two study areas (Fig. 3). The polychaete assemblage for both areas showed higher proportions in five of the trait categories, in which the highly expressed CWM values were measured: 'iteroparous' (Iter), 'larval pelagic development' (Lpel), 'endofauna' (Endo), 'motile' (Mot) and 'burrower' (Burw). On the contrary, the less represented categories of traits (CWM values < 1) were: '6-10 years of adult longevity' (Al10), 'semelparous' (Sem), 'semi-continuous' (Scon), 'larval benthic development' (Lben), 'sessile' (Sess), 'swimmer' (Swim), 'tube-builder' (Tub), 'suspension feeder' (Susp), 'herbivore' (Herb) and 'scavenger' (Scav).

The PCoA performed on trait profiles indicated that the most distinctive patterns in contaminated sediments were obtained among 'life habit', 'reproductive frequencies', 'adult environmental position', 'Adult mobility', 'Adult movement method' and 'Adult feeding habit' (Fig. 4). The first axis highly distinguished (r=> ± 0.8) Burw and Endo organisms (right-hand side of the plot) from Sess, Epib and Susp ones (left-hand side of the plot). The second axis separated (r=> ± 0.8) Al6, Mot and Pred polychaetes (top of the plot) from Smot and Sdep ones (bottom of the plot). Both axes represented strong functional patterns by extracting 39.2 % and 33.2% of the variation in trait data, respectively.

Relationships between species or trait and environmental variables

The composition of polychaete assemblage differed between Taranto and Trieste (t= 2.1; p<0.01). Of the 20 species highlighted by the multi-level pattern analysis, only 7 were found in both areas (Fig. 5 and Table 3). Of the chemical vectors overlaid in the nMDS, only TN showed a significant (p<0.05) correlation to the species pattern distribution on the plot. This was also corroborated by the BIO-ENV analysis, in which the species patterns correlated with TN (r= 0.6) (Fig. 5).

Regarding the functional distribution related with the considered environmental variables, the BIO-ENV analysis illustrated that the functional expression was best correlated with sand and the levels of mercury (r = 0.5). In the dbRDA performed on the categories data, two principal components were identified (eigenvalue >1) which together explained 72.8% of the total variance, with the first and second factors explaining 39.2% and 33.2% of the total variance, respectively. The sand fraction, Cu and Hg were the predominant elements of the first factor, while the major contributor of the second one was TN (Fig. 6).

Discussion

The usefulness of the BTA depends on the selected traits and metrics (in our case abundance-based) to quantify the impact of contaminants on macrofaunal functional proprieties. The *a priori* selection of traits and categories is fundamental when performing biological traits analysis, because certain types of categories are more relevant in some circumstance than others (Bremner 2008). In this study, the included traits and categories were based on (i) the potential relation with contaminated sediments; (ii) expert judgement and personal knowledge on the investigated benthic community; and (iii) the availability of information on the traits for the collected benthic species.

Our results revealed that polychaete functional structure can be successfully used as a complement to traditional taxonomic information to describe the anthropogenic impacts through biological traits analysis. Differences in functional features were also depending on the index used: functional dispersions did not highlight any clear variation related to human pressures, on the contrary, a clear patter along a contamination level was obtained using functional identity. Overall, our results indicate that either the presence of contaminants or organic enrichment could contribute to the functional expression patterns in both areas.

Functional diversity and identity

The tested trait-richness and the FDis showed a clear pattern with species richness (see Fig. 2a, b) despite the variation of polychaete taxonomic diversity. The consequence of contamination to the reduction of species richness has been largely documented (Johnston et al. 2015 and reference therein). Also in our study, the most severely contaminated stations (St. TA2 and St. TS2) were characterized by low species richness compared to the less contaminated one (St. TS4). To some extent, the benthic ecosystem is able to adapt to anthropogenic pressure by trying to minimize its impact through the development of stress resistant communities that occupy new ecological niches (Cibic et al. 2012). In fact, in accordance with several studies on structural changes of macrofaunal communities in relation to human impact (e.g. Solis-Weiss et al. 2004; Lu and Wu 2007; Mutlu et al. 2010), at the stations closest to the main anthropogenic sources of contaminants, stress-tolerant species were observed in both areas (i.e. *Lumbrineris latreilli*, *Notomastus latericeus, Schistomeringos rudolphi,* and *Spirobranchus triqeuter*). However, this variation was not observed in functional diversity values. The perceived stability of these functional characteristics could be linked to functional redundancy in the polychaete assemblage, since higher species richness was not associated with higher FDis (Cadotte et al. 2011). Theory also suggests that, if there is evidence of functional redundancy in the macrofaunal community structure, the community may be less susceptible to changes in the ecosystem functioning caused by a species' loss (Gamfeldt et al. 2008). Indeed, the redundancy hypothesis states that if several species perform for the same function and thus express similar trait values, in case of one being eliminated, others remain to provide or potentially even increase functioning (Yachi and Loreau, 1999). Recent studies are in accordance with our results, which suggest that multiple anthropogenic stressors might cause declines in biodiversity but not affect the functional diversity, if they reduce species that contribute little to function or that are functionally redundant in these communities (McMahon et al. 2012).

Functional adaptation to anthropogenic stress

When BTA is used to assess human impact on polychaetes, it gives more insight into the contamination effects on the benthic invertebrates as confirmed by Oug et al. (2012); Piló et al. (2016); Krumhansl et al. (2016). The functional patterns were broadly concurrent with the distribution of species assemblages, indicating that the species changes along the contamination levels also resulted in changes in the ecological functioning of the assemblages (Oug et al. 2012). Overall, the two coastal areas were inhabited by different species of polychaetes (as shown by the PERMANOVA). In contrast, no statistical differences were found between the two study areas regarding the traits and their categories. The functional adaptations were more pronounced at the different level of contamination in the Mar Piccolo of Taranto than in the Gulf of Trieste. This may be due to diverse geomorphological characteristics of the areas: the Mar Piccolo is a semi-enclosed basin, with decreasing salinity, limited depth, and slow water renewal rates going from the first towards the second inlet and with a severe but localized contamination (Alabiso et al. 2006). Instead, the sediments in the harbour of Trieste are less contaminated likely due to the fact that the basin is a semi-enclosed system with high sea water renewal and contaminants' dilution and dispersion rates (in accordance with Cibic et al. under review).

The most distinctive patterns were obtained for 'reproductive frequencies', 'larval pelagic development', 'mobility', 'environmental positions', 'movement method' and 'feeding habits'. The main structuring environmental factors were the contaminants, in particular copper and mercury, and total nitrogen as an indication of level of eutrophication of the system. From these results, it could be inferred that ecological functioning changed from a well-balanced functional structure (no high CWM values for some categories were observed) in the less contaminated sitem, toward polychaetes being predominantly Endo, Mot, Burw, Sdep and Pred in the contaminated FI (St. TA1 and St.TA2) and Epib, Sess, Tub, Susp and Lben in the less contaminant sites but with the highest values of total nitrogen in the sediments (St.TA4) (see Fig. 4).

On a general basis, in accordance with Bonsdorff and Pearson (1999), Oug et al. (2012), Gusmao et al. (2016) the traits related to feeding and mobility were found to be the main responsible for functional differing between species assemblages. Our result are consistent with Selck et al. (1999) and Piló et al. (2016) where Sdf invertebrates were found predominantly in the contaminated sediments compared to Ssdf ones. Deposit-feeders normally prevail in low hydrodynamic conditions where smaller-sized particles at the sediment surface are found, representing a higher food source availability. In fact, these invertebrates showed an opportunistic behaviour. The increase of food supply could rapidly stimulate the growth, reproduction rate and survivorship of individuals. After the consumption of high amount of food and/or being consumed by predators, their numbers may decrease considerably, suggesting wide fluctuations (Rossi 2003). However, our values of Sdf decreased at the strongly contaminated site and Pred prevailed. This finding suggests that deposit feeders are likely killed by the ingestion of toxic sediments due to high contaminants concentrations as those observed at St. TA2 (Franzo et al. 2016). Previous studies have shown that the presence of contaminants creates harsh environment below the sediment surface affecting also Susp invertebrates (Gaston and Young 1992). In this study the ‘Susp’ modality was linked to lower contamination levels and to the high organic enrichment expressed as TN. The high TN content and the lower contamination level at St. TA4 revealed a situation of substantial organic enrichment likely ascribable to mussel farming that is widely spread and long-lasting over the SI of the Mar Piccolo of Taranto. The biodeposits (feces and pseudofeces) continuously produced by suspended bivalves settle to the bottom increasing the organic load (Karuza et al. 2016). This enrichment condition favored the Susp polychaetes such as *Pista lornensis* and *Lanice conchilega*. Zhang et al. (2015) reported that Susp taxa responded slightly more slowly to the increase of organic load compared to Sdep ones and thus this feeding typology has been observed in sediments with long-lasting and continuous bio-depositions. These results indicate that major organic loads from the water column could change ecosystem functioning and processes, principally the prokaryotic secondary production, in slightly contaminated sediments. In fact, Susp are fundamental players in the benthic-pelagic coupling, capturing large quantities of suspended organic matter and phytoplankton, and then incorporating them into the sediments through pseudo-faces, stimulating the microbial loop (Newell, 2004, Törnroos and Bonsdorff 2012).

Furthermore, in such an inhospitable environment as the most contaminated site of the Mar Piccolo (St. TA2), the trait 'Endo', 'Burw' and in particular 'Mot' were the most expressed functional adaptations, represented by the species *L. latreilli* and *Eunice vittata.* The distribution of contaminants in sediments is often patchy (Stockdale et al. 2009). The heterogeneity of contaminants concentration within the sediments varies the exposure of mobile benthic organisms to contaminants. Thus, the mobile organisms likely have a higher probability to survive because they are able to avoid hotspots of contaminants through their active movements (Ward et al. 2013). Their movement ability make them good bioturbators, able to increase nutrient exchange across the sediment surface and particularly the contamination dispersion from the first sediment centimeters to the deeper layers (De Vittor et al. 2016). On the contrary, 'Ses' and 'Tub' categories increased in the less contaminated site.

Larval dispersal plays an important role in the recovery processes in communities exposed to disturbance. Therefore, it has been identified as one of the most relevant traits to be included in the BTA (Pacheco et al. 2013). The dispersal capability of macrobenthic organisms during their benthic life stage contributes to the elasticity of these communities (Günther, 1992). Contamination can represent a significant threat for macrobenthic larvae when settling in the sediment, as higher concentrations of different contaminants are generally retained onto the sediment substrate than in the water mass above it (Tolun et al. 2001). However, in the pelagic environment, contaminants have only a slight effect on planktonic larvae due to their natural capability to live out of the sediment and disperse (Marchini et al. 2008). In fact, such a life history characteristic represents a typical survival strategy in the presence of a periodic disturbance, being therefore identified as typical of unstable environments (Paganelli et al. 2012). Contrary to what we expected, in the present study, organisms with planktonic larval type were not significantly associated with the contaminated sediments, in other words, the planktonic larval modality is widespread and dominant at all the investigated stations (more or less contaminated), and only a slight increase of 'Lben' was observed at the less contaminated ones. However, the fact that the majority of the species included in this study share the same planktonic larvae dispersal strategy, might have limited the sensitiveness of this trait to contamination. The predominance of this larval strategy may result from intrinsic adaptations of organisms to cope with the natural variability of these systems and, therefore, mask the potential indicator of this trait for anthropogenic factors (Piló et al. 2016).

The iteroparity as 'reproductive frequency' was the dominant reproductive modality coupled with 3-6 years of 'adult longevity' for the benthic invertebrates in impacted areas. This type of reproduction (i.e. invertebrates breeding several times per life time) and invertebrates with long life are considered to be the best life strategies to be selected in extreme environments with persistent and long-lasting contamination (Pianka 1970, Ranta et al. 2002).

Conclusion

In the present study for the first time, BTA was used to identify shifts in functional features of infaunal polychaete assemblages associated with different levels of contamination. The synergistic effect of chemical contaminants considered in this study seemed to not affect the polychaete functional diversity due to the functional redundancy. In spite of the decreasing taxa richness with increasing stress, the trait categories and FDis remained fairly stable. Mobility, environmental position, movement methods, and feeding habits were the traits more affected by contamination. Copper, mercury and total nitrogen resulted the most relevant environmental factors in structuring the polychaete assemblages and their functional trait patterns. By using solely the traditional taxonomic approaches these aspects would have likely not shown up. Integrative approaches that consider the sets of environmental features in relation to structural and functional macrofaunal patterns provide useful tools for understanding, monitoring and assessing ecosystem functioning in highly impacted areas.

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References

Aarnio, K., Mattila, J., Bonsdorff, E. (2011). Comparison of different sampling strategies in monitoring zoobenthos and classification of archipelago areas. *Boreal environment research*, 16(5).

Alabiso, G., Giacomini, M., Milillo, M., Ricci, P. (2006). Chemical-physical conditions in the Taranto sea system from 2002 to 2004. *Biologia Marina Mediterranea,* 13(1), 1055-1058.

Bellucci, L. G., Cassin, D., Giuliani, S., Botter, M., Zonta, R. (2016). Sediment pollution and dynamic in the Mar Piccolo of Taranto (southern Italy), insights from bottom sediment traps and surficial sediments. *Environmental Science and Pollution Research*, 23(13), 12554-12565.

Bremner, J. (2008). Species' traits and ecological functioning in marine conservation and management. *Journal of Experimental Marine Biology and Ecology*, 366(1), 37-47.

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.

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.

Cadotte, M. W., Carscadden, K., Mirotchnick, N. (2011). Beyond species, functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology*, 48(5), 1079-1087.

Canu, D. M., Solidoro, C., Umgiesser, G., Cucco, A., Ferrarin, C. (2012). Assessing confinement in coastal lagoons. *Marine pollutionbulletin*, 64(11), 2391-2398.

Cardellicchio, N., Buccolieri, A., Giandomenico, S., Lopez, L., Pizzulli, F., Spada, L. (2007). Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, Southern Italy). *Marine PollutionBulletin*, 55(10), 451-458.

Cardellicchio, N., Covelli, S., Cibic, T. (2016). Integrated environmental characterization of the contaminated marine coastal area of Taranto, Ionian Sea (southern Italy). *Environmental Science and Pollution Research*, 23(13), 12491-12494.

Charvet, S., Statzner, B., Usseglio-Polatera, P., Dumont, B. (2000).Traits of benthic macroinvertebrates in semi-natural French streams, an initial application to biomonitoring in Europe. *Freshwater Biology*, 43(2), 277-296.

Chevenet, F., Doléadec S., Chessel, D. (1994). A fuzzy coding approach for the analysis of long-term ecological data. *Freshwaterbiology*, 31(3), 295-309.

Cibic, T., Bongiorni, L., Borfecchia, F., Leo, A., Franzo, A., Giandomenico, S., Del Negro, P. (2016). Ecosystem functioning approach applied to a large contaminated coastal site, the study case of the Mar Piccolo of Taranto (Ionian Sea). *Environmental Science and Pollution Research*, 23(13), 12739-12754.

Cibic, T., Franzo, A., Celussi, M., Fabbro, C., Del Negro, P. (2012). Benthic ecosystem functioning in hydrocarbon and heavy-metal contaminated sediments of an Adriatic lagoon. *Marine Ecology Progress Series*, 458, 69-87.

Cibic, T., Franzo, A., Nasi, F., Auriemma, R., Del Negro, P. Benthic ecosystem functioning approach as a tool for sustainable management of a port area in the northern Adriatic Sea. *Frontiers in Marine Science* (under second revision).

Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M. (2014). *Change in marine communities, An Approach to Statistical Analysis and Interpretation.* PRIMER-E, Plymouth,

De Cáceres, M. (2013). How to use the indicspecies package (ver. 1.7. 1). *Centre TecnològicForestal de Catalunya, Catalonia.*

De Vittor, C., Relitti, F., Kralj, M., Covelli, S., Emili, A. (2016). Oxygen, carbon, and nutrient exchanges at the sediment–water interface in the Mar Piccolo of Taranto (Ionian Sea, southern Italy). *Environmental Science and Pollution Research*, 23(13), 12566-12581.

Di Leo, A., Cardellicchio, N., Giandomenico, S., Spada, L. (2010). Mercury and methylmercury contamination in Mytilusgalloprovincialis from Taranto Gulf (Ionian Sea, Southern Italy), risk evaluation for consumers. *Food and Chemical Toxicology*, 48(11), 3131-3136.

Dı́az, S., Cabido, M. (2001). Vive la difference, plant functional diversity matters to ecosystem processes. *Trends in ecology 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.

Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A., et al. (2012). Climate change impacts on marine ecosystems. *Annual review of marine science*, 4, 11-37.

Franzo, A., Auriemma, R., Nasi, F., Vojvoda, J., Pallavicini, A., Cibic, T., et al. (2016). Benthic ecosystem functioning in the severely contaminated Mar Piccolo of Taranto (Ionian Sea, Italy), focus on heterotrophic pathways. *Environmental Science and Pollution Research*, 23(13), 12645-12661.

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.

Gagic, V., Bartomeus, I., Jonsson, T., Taylor, A., Winqvist, C., Fischer, C., et al. (2015). Functional identity and diversity of animals predict ecosystem functioning better than species-based indices. *Proceedings of the Royal Society of London B, Biological Sciences*, 282, 20142620.

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.

Gamfeldt, L., Hillebrand, H., Jonsson, P. R. (2008). Multiple functions increase the importance of biodiversity for overall ecosystem functioning. *Ecology*, 89(5), 1223-1231.

Gaston, G. R., Young, J. C. (1992). Effects of contaminants of macrobenthic communities in the upper Calcasieu Estuary, Louisiana. *Bulletin of environmental contamination and toxicology*, 49(6), 922-928.

Ghirardelli, E., Pignatti, S. (1968). Conséquences de les pollutions sur les peuplements du "Vallone di Muggia" près de Trieste. *Rev. Int. Océanogr. Med*, 10, 111-1221.

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

Giangrande, A., Licciano, M., Musco, L. (2005).Polychaetes as environmental indicators revisited. *Marine Pollution Bulletin*, 50(11), 1153-1162.

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.

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

Günther, C. P. (1992). Dispersal of intertidal invertebrates, A strategy to react to disturbance of different scales? *Netherlands Journal of Sea Research*, 30, 45-56.

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.

Harley, C. D., Randall Hughes, A., Hultgren, K. M., Miner, B. G., Sorte, C. J., Thornber, C. S., et al. (2006). The impacts of climate change in coastal marine systems. *Ecology letters*, 9(2), 228-241.

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, 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.

Kleyer, M., Dray, S., Bello, F., Lepš, J., Pakeman, R. J., Strauss, B., Lavorel, S. (2012). Assessing species and community functional responses to environmental gradients, which multivariate methods? *Journal of Vegetation Science*, 23(5), 805-821.

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(1), 299-305.

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.

Lu, L., Wu, R. S. S. (2007). A field experimental study on recolonization and succession of subtidal macrobenthic community in sediment contaminated with industrial wastes. *Marine pollution bulletin*, 54(2), 195-205.

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.

MarLIN, (2006). BIOTIC - Biological Traits Information Catalogue. Avalable at: https//www.marlin.ac.uk/biotic. (Accessed at 25.06.2016).

McMahon, T. A., Halstead, N. T., Johnson, S., Raffel, T. R., Romansic, J. M., Crumrine, P. W. et al. (2012). Fungicide-induced declines of freshwater biodiversity modify ecosystem functions and services. *Ecology Letters*, 15(7), 714-722.

Montalto, V., Rinaldi, A., Sarà, G. (2015).Life history traits to predict biogeographic species distributions in bivalves.*The Science of Nature*, 102(9-10), 61.

Morri, C., Bellan-Santini, D., Giaccone, G., Bianchi, C. N. (2004). Principles of bionomy, definition of assemblages and use of taxonomic descriptors (macrobenthos). *Biologia Marina Mediterranea*, 11(Suppl 1), 573-600.

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.

Naeem, S., Loreau, M., Inchausti, P. (2002). Biodiversity and ecosystem functioning, the emergence of a synthetic ecological framework.In S. Naeem, M. Loreau, P. Inchausti (Eds.), *Biodiversity and ecosystem functioning* (pp 3-11).Oxford University Pres, Oxford, UK.

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.

Newell, R. I. (2004). Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs, a review. *Journal of Shellfish Research*, 23(1), 51-62.

Nieuwenhuize, J., Maas, Y. E., Middelburg, J. J. (1994).Rapid analysis of organic carbon and nitrogen in particulate materials. *Marine Chemistry*, 45(3), 217-224.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., et al. (2007). The vegan package. *Community ecology package*, 10, 631-637.

Olff, H., Pegtel, D. M., Van Groenendael, J. M., Bakker, J. P. (1994).Germination strategies during grassland succession. *Journal of Ecology*, 69-77.

Olsgard, F., Schaanning, M. T., Widdicombe, S., Kendall, M. A., Austen, M. C. (2008). Effects of bottom trawling on ecosystem functioning. *Journal of Experimental Marine Biology and Ecology*, 366(1), 123-133.

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, 94-105.

Otegui, M. B., Brauko, K. M., Pagliosa, P. R. (2016). Matching ecological functioning with polychaete morphology, Consistency patterns along sedimentary habitats. *Journal of Sea Research*, 114, 13-21.

Pacheco, A. S., Uribe, R. A., Thiel, M., Oliva, M. E., Riascos, J. M. (2013). Dispersal of post-larval macrobenthos in subtidal sedimentary habitats, Roles of vertical diel migration, water column, bedload transport and biological traits' expression. *Journal of sea research*, 77, 79-92.

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.

Pella, E., Colombo, B. (1973). Study of carbon, hydrogen and nitrogen determination by combustion-gas chromatography. *Microchimica Acta*, 61(5), 697-719.

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

Pianka, E. R. (1970). On r-and K-selection. *The American Naturalist*, 104(940), 592-597.

Piló, D., Ben-Hamadou, R., Pereira, F., Carriço, A., Pereira, P., Corzo, A., Carvalho, S. (2016). How functional traits of estuarine macrobenthic assemblages respond to metal contamination? *Ecological Indicators*, 71, 645-659.

Polytraits Team (2016). Polytraits, A database on biological traits of polychaetes. Available at http//polytraits.lifewatchgreece.eu (accessed 25.06.2016).

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Ranta, E., Tesar, D., Kaitala, V. (2002 Variability and Semelparity vs. Iteroparity as Life Histories. *Journal of Theoretical Biology*, 217, 391–396.

Rossi, F. (2003). Short-term response of deposit-feeders to an increase of the nutritive value of the sediment through seasons in an intertidal mudflat (Western Mediterranean, Italy). *Journal of experimental marine biology and ecology*, 290(1), 1-17.

Rouse, G. W. (2000). Polychaetes have evolved feeding larvae numerous times. *Bulletin of marine Science*, 67(1), 391-409.

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.

Selck, H., Decho, A. W., Forbes, V. E. (1999). Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete Capitella species I. *Environmental Toxicology and Chemistry*, 18(6), 1289-1297.

Sharp, J. H. (1974). Improved analysis for "particulate" organic carbon and nitrogen from seawater. *Limnology and Oceanography*, 19(6), 984-989.

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

Ozhan, E. (2001). Proceedings of the Fifth International Conference on the Mediterranean Coastal Environment, MEDCOAST 01, October 23-27, 2001, Hammamet, Tunisia. Volume 1.

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), 247-263.

Stockdale, A., Davison, W., Zhang, H. (2009). Micro-scale biogeochemical heterogeneity in sediments: A review of available technology and observed evidence. *Earth Science Reviews*, 92(1), 81-97.

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.

Tolun, L. G., Okay, O. S., Gaines, A. F., Tolay, M., Tüfekçi, H., Kıratlı, N. (2001). The pollution status and the toxicity of surface sediments in Izmit Bay (Marmara Sea), Turkey. *Environment International*, 26(3), 163-168.

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(8), 2221-2236.

Törnroos, A., Bonsdorff, E., Bremner, J., Blomqvist, M., Josefson, A. B., Garcia, C., et al. (2015). Marine benthic ecological functioning over decreasing taxonomic richness. *Journal of Sea Research*, 98, 49-56.

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.

Umgiesser, G., Scroccaro, I., Alabiso, G. (2007). Mass exchange mechanisms in the Taranto Sea. *Transitional Waters Bulletin*, 1(2), 59-71.

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.

Ward, D. J., Simpson, S. L., Jolley, D. F. (2013). Slow avoidance response to contaminated sediments elicits sublethal toxicity to benthic invertebrates. *Environmental science technology*, 47(11), 5947-5953.

Weigel, B., Blenckner, T., Bonsdorff, E. (2015).Maintained functional diversity in benthic communities in spite of diverging functional identities. *Oikos* 000, 1-13.

Widdicombe, S., Austen, M. C., Kendall, M. A., Olsgard, F., Schaanning, M. T., Dashfield, S. L., et al. (2004). Importance of bioturbators for biodiversity maintenance, indirect effects of fishing disturbance. *Marine Ecology Progress Series*, 275, 1-10.

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.

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.

http://.www.porto.trieste.it. Website of the Port Authority of Trieste. (accessed 20.09.2016).

Table 1: Coordinates, depths and contaminants values measured at the 4 stations located in the harbor of Trieste and in the Mar Piccolo of Taranto. PAHs = Polycyclic Aromatic Hydrocarbons; PCBs = Polychlorobiphenyls. (Cibic under review; Cibic et al. 2016 ; Bellucci et al. 2016)

Station	Latitude	Longitude	Depth	Cu	Hg	Pb	Zn	totalPCBs	Total PAHs
			m	mg kg-1				µg kg-1	
TS1	45° 38' 01	13° 45' 09	18.5	36.8	3.4	57.1	137.0	74.7	4870.0
TS2	45° 37' 47	13° 46' 11	15.0	112.0	4.4	388.0	770.0	907.0	19000.0
TS3	45° 37' 04	13° 46' 31	13.0	28.7	0.5	73.9	205.0	50.8	14950.0
TS4	45° 36' 41	13° 47' 04	10.5	16.6	0.1	4.0	57.0	1.0	46.0
TA1	40° 29′ 01	17° 14′ 46	11.2	180.3	0.9	80.0	231.0	164.9	1755.0
TA2	40° 28′ 46	17° 15′ 38	11.0	100.5	1.6	152.0	319.0	1067.6	1624.0
TA3	40° 28′ 57	17° 16′ 42	7.5	64.9	0.6	74.0	222.0	164.8	528.0
TA4	40° 28′ 57	17° 17′ 41	7.0	55.2	0.3	51.0	189.0	39.0	127.0

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

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

Table 3: Average and maximum abundance (ind. m^{-2}) of the species highlighted by multi-level pattern analysis ($p < 0.05$). In bold the species highly significant of the whole assemblage in both areas ($p < 0.01$). Station with the maximum observed abundance is indicated.

Species	**Trieste** Abundance ind. m^{-2}			**Taranto** Abundance ind. m^{-2}			**Station max abundance**
	Average	SD	Max	Average	SD	Max	
Ampharete acutifrons	3	7	20	0	0	0	TS4
Aonides oxycephala	1	2	7	6	10	23	TA1
Aricidea (Strelzovia) claudiae	7	8	20	0	0	0	TS4
Aricidea (Strelzovia) fragilis	10	13	40	0	0	0	TS4
Chaetozone zetlandica	1	4	10	1	2	3	TS4
Gallardoneris iberica	1	2	3	0	0	0	TS2, TS3, TS4
Glycera tesselata	3	6	17	0	0	0	TS3
Levinsenia gracilis	3	5	13	0	0	0	TS3
Lumbrineris latreilli	3	3	10	15	18	43	TA2
Marphysa bellii	3	3	7	7	14	40	TA1
Nephtys hystricis	3	5	10	0	0	0	TS2, TS4
Nereis rava	2	3	7	0	0	0	TS1, TS3
Notomastus latericeus	1	2	3	6	11	33	TA1
Paradoneis lyra	0	0	0	0	1	3	TA1
Poecilochaetus fauchaldi	0	1	3	10	18	47	TA1
Pseudoleiocapitella fauveli	20	20	57	62	118	310	TA1
Schistomeringos rudolphi	1	2	3	0	0	0	TS2
Pista lornensis	0	0	0	2	3	7	TA1,TA4
Serpula vermicularis	2	3	7	0	0	0	TS3, TS4
Spirobranchus triqueter	2	4	10	0	0	0	TS3

Figure captions

Figure 1. Location of the four sampling stations in the Mar Piccolo of Taranto and the harbor of Trieste. The main anthropogenic pressures that insist on their coasts are highlighted: the industrial area (*green- lined*), the military area (*red*), and the mussel farms (*blue*). The navigable channels are indicated by a dotted line.

Figure 2. Taxon and category richness. Total number of taxa (histogram) and trait categories (line) (a) and functional dispersion (FDisp) (b), for each sampling station and period.

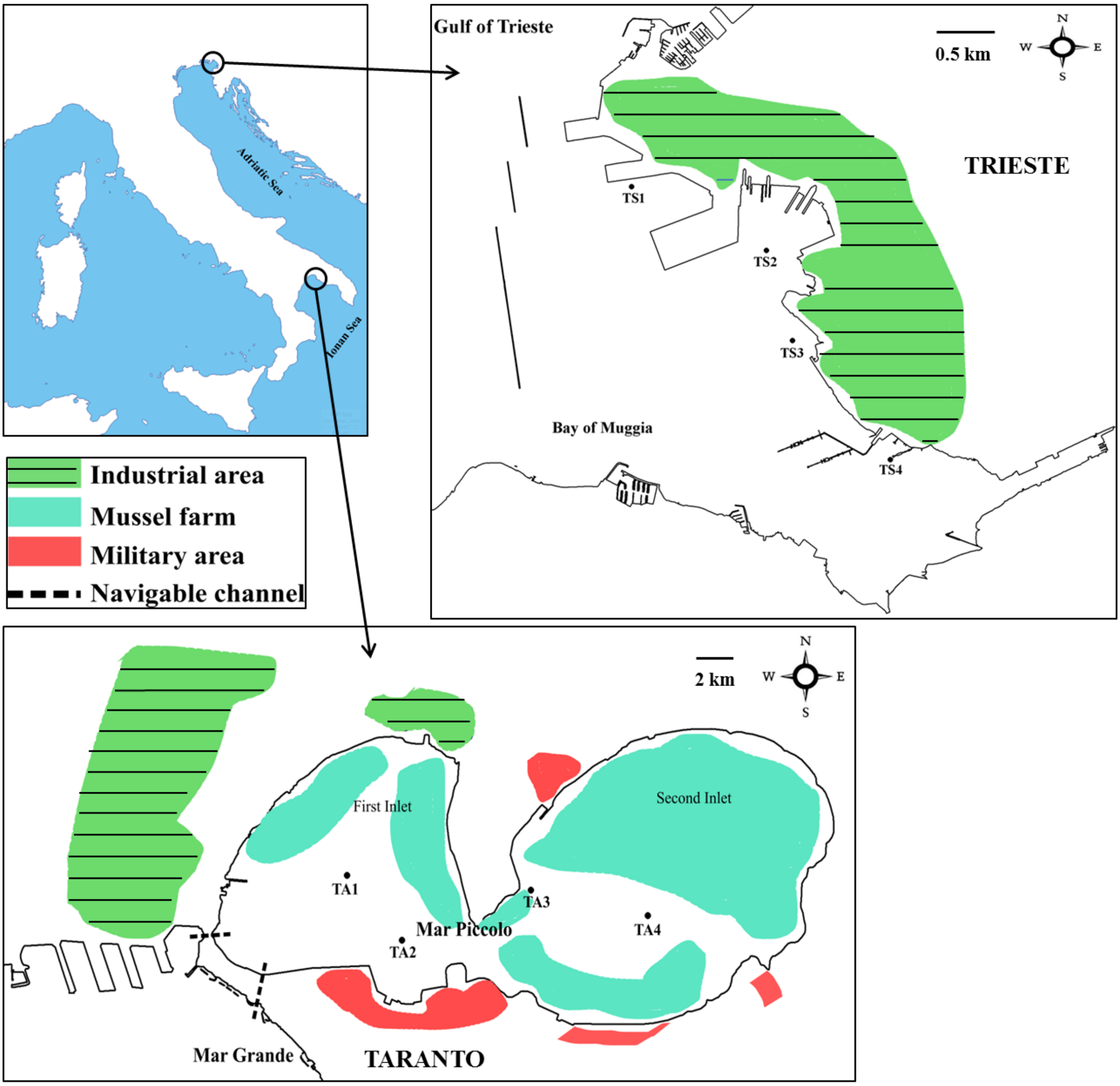
Figure 3. Community-weighted means of trait-category expression (CWM), average values of both areas. Color codes represent the trait affiliation; individual bars represent the trait category expression (for labels see Table 2).

Figure 4. Principal Coordinates Analysis on trait features of polychaete assemblages in Trieste and Taranto (for labels see Table 2).

Figure 5. nMDS ordination plot based on species highlighted by multi-level pattern analysis. Environment factors (black arrows) were plotted using vector fitting with ordination scores (stress value 0.50). In bold the vector which has significant ordination score. See Table 3 for complete species name. TOC=total organic carbon; TN=total nitrogen; PAHs = Polycyclic Aromatic Hydrocarbons; PCBs = Polychlorobiphenyls.

Figure 6. Distance based Redundancy Analysis (dbRDA) on polychaete assemblages in Trieste and Taranto: plot of stations and environmental factors. TOC =Total Organic Carbon; TN = Total Nitrogen; PAHs = Polycyclic Aromatic Hydrocarbons; PCBs = Polychlorobiphenyls. Zn and Pb vectors are overlaid.

Figure 1



Adriatic Sea
Ionan Sea
Gulf of Trieste
0.5 km
N
W
E
S
TRIESTE
TS1
TS2
TS3
TS4
Bay of Muggia
Industrial area
Mussel farm
Military area
Navigable channel
2 km
N
W
E
S
First Inlet
Second Inlet
TA1
TA2
TA3
TA4
Mar Piccolo
Mar Grande
TARANTO

Figure 2

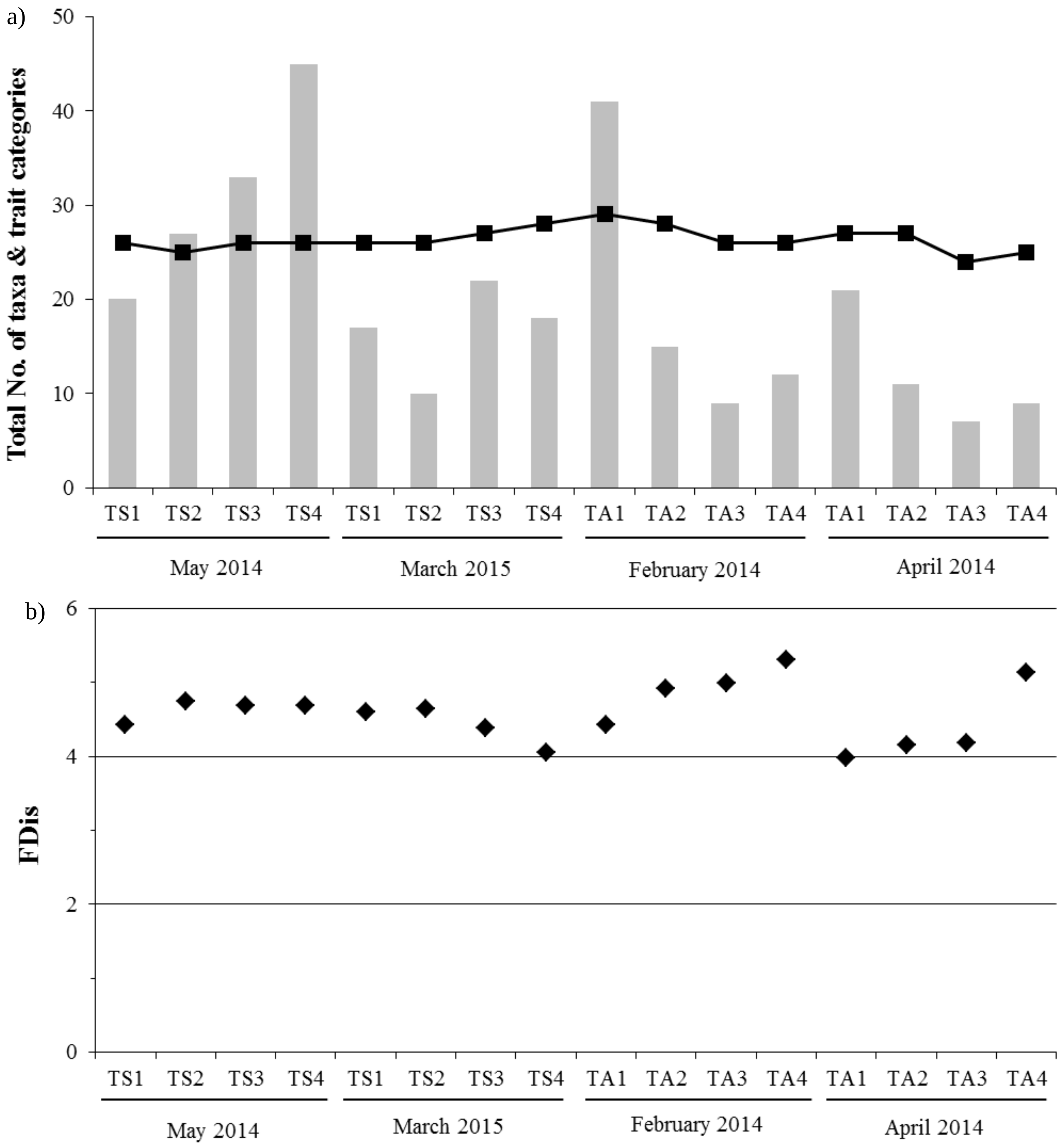

a)
Total No. of taxa & trait categories
0
10
20
30
40
50
TS1
TS2
TS3
TS4
TS1
TS2
TS3
TS4
TA1
TA2
TA3
TA4
TA1
TA2
TA3
TA4
May 2014
March 2015
February 2014
April 2014
b)
FDis
0
2
4
6
TS1
TS2
TS3
TS4
TS1
TS2
TS3
TS4
TA1
TA2
TA3
TA4
TA1
TA2
TA3
TA4
May 2014
March 2015
February 2014
April 2014

Figure 3

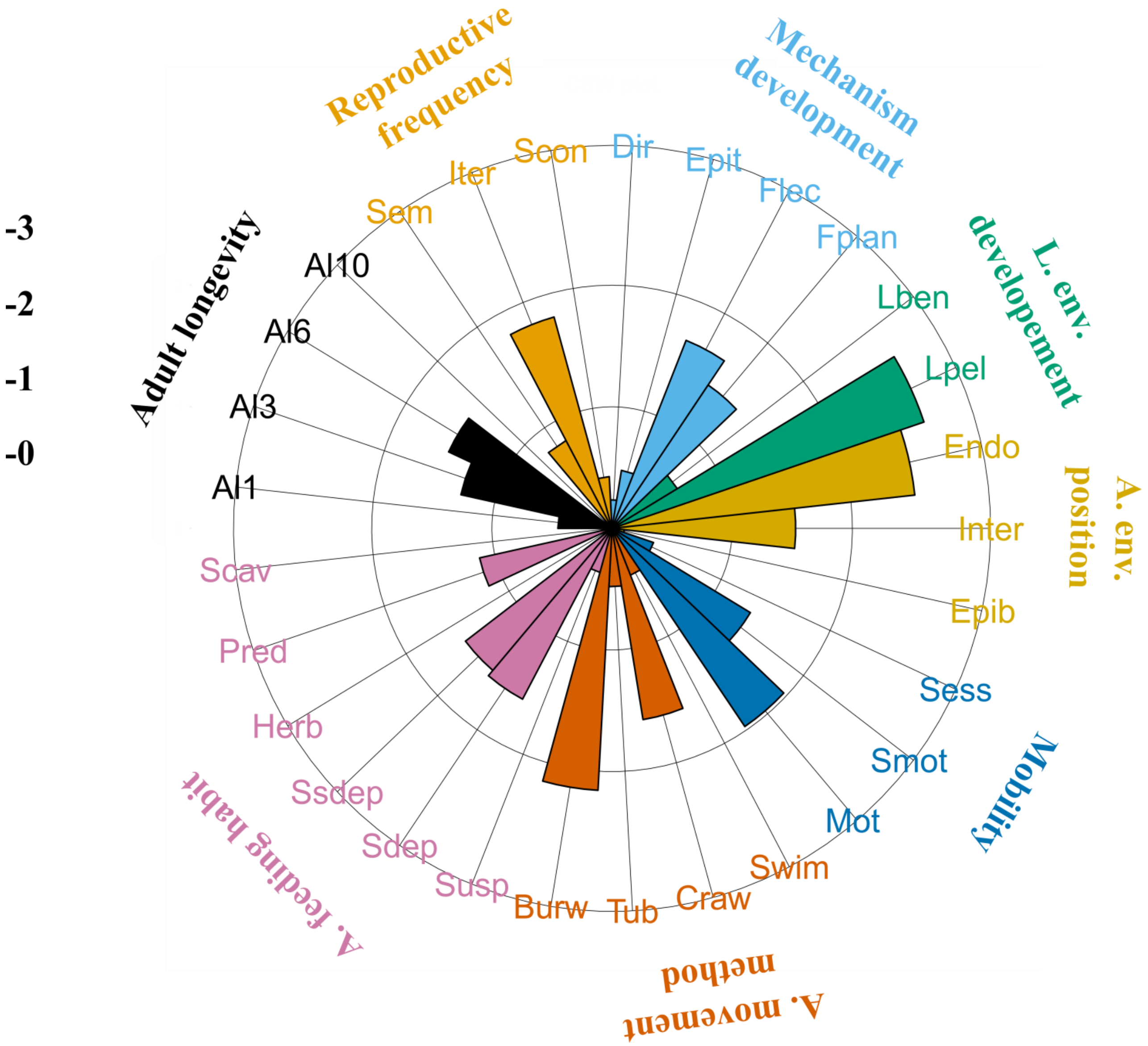

-3
-2
-1
-0
Reproductive frequency
Mechanism development
L. env. developement
A. env. position
Mobility
A. movement method
A. feeding habit
Adult longevity
Scon
Dir
Epit
Flec
Fplan
Lben
Lpel
Endo
Inter
Epib
Sess
Smot
Mot
Swim
Craw
Tub
Burw
Susp
Sdep
Ssdep
Herb
Pred
Scav
Al1
Al3
Al6
Al10
Sem
Iter

Figure 4

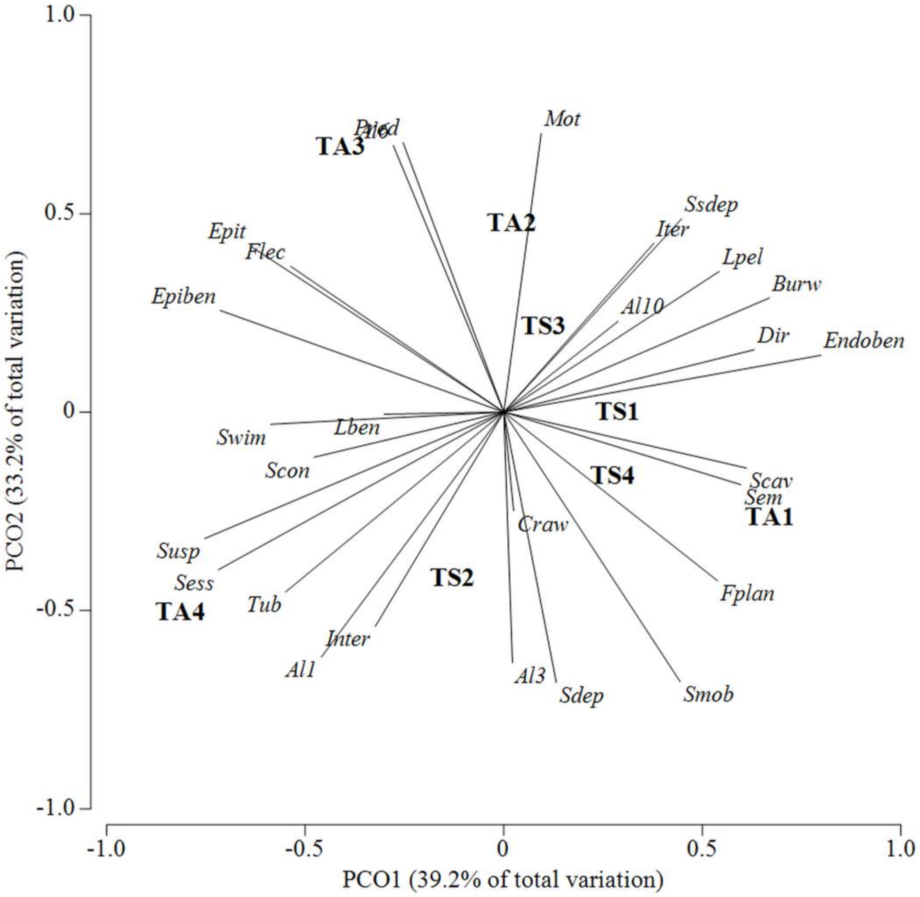

1.0
0.5
0
-0.5
-1.0
PCO2 (33.2% of total variation)
-1.0
-0.5
0
0.5
1.0
PCO1 (39.2% of total variation)
TA1
TA2
TA3
TA4
TS1
TS2
TS3
TS4
Pred
Al6
Mot
Ssdep
Iter
Lpel
Burw
Al10
Dir
Endoben
Epit
Flec
Epiben
Swim
Lben
Scon
Susp
Sess
Tub
Inter
Al1
Al3
Sdep
Smob
Craw
Fplan
Scav
Sem

Figure 5

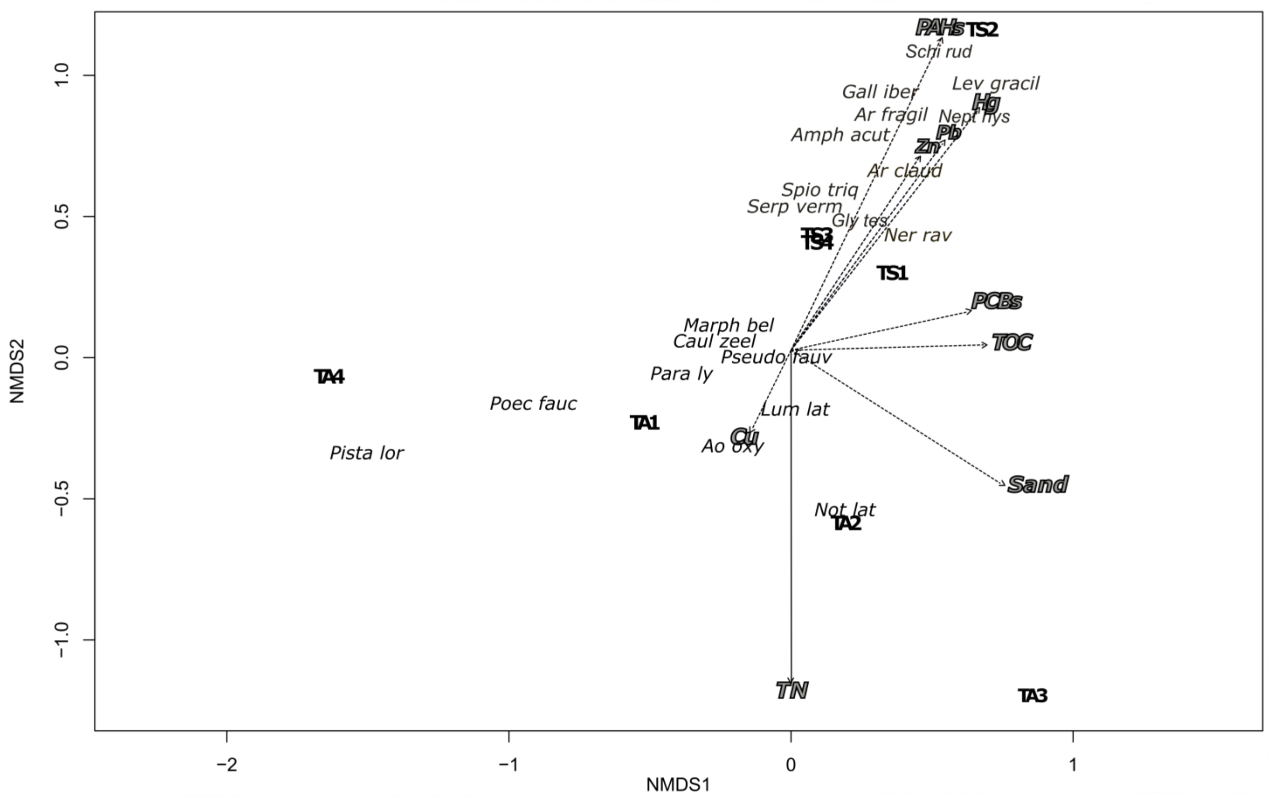
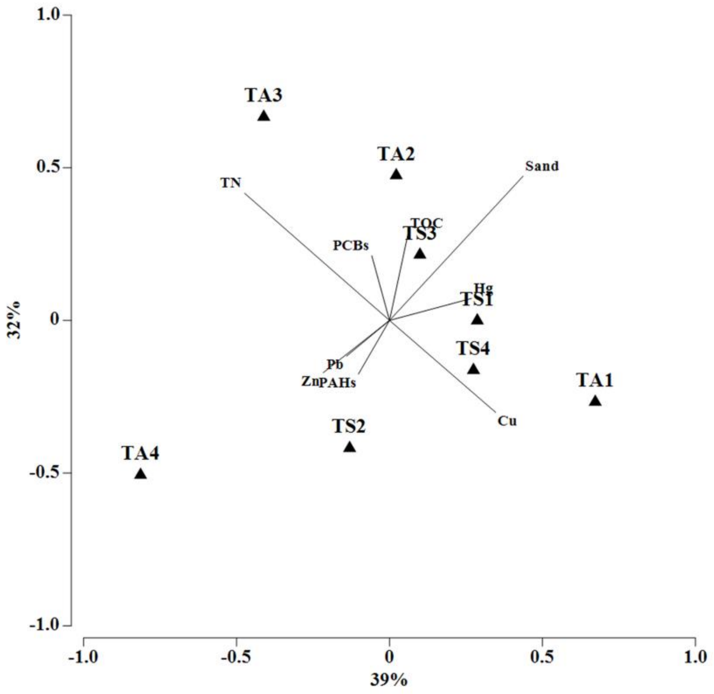

PAHs
TS2
Schi rud
Lev gracil
Gall iber
Hg
Ar fragil
Nept hys
Amph acut
Pb
Zn
Ar claud
Spio triq
Serp verm
Gly tes
TS3
TS4
Ner rav
TS1
PCBs
Marph bel
Caul zeel
TOC
Pseudo fauv
TA4
Para ly
Poec fauc
Lum lat
TA1
Cu
Pista lor
Ao oxy
Sand
Not lat
TA2
TN
TA3
1.0
0.5
0.0
−0.5
−1.0
NMDS2
−2
−1
0
1
NMDS1

Figure 6



TA3
TA2
TN
Sand
TOC
TS3
PCBs
Hg
TS1
TS4
TA1
Pb
Zn
PAHs
Cu
TS2
TA4
1.0
0.5
0
-0.5
-1.0
32%
-1.0
-0.5
0
0.5
1.0
39%

5. CONCLUSIONS

The overall aim of this thesis was to evaluate the effects of environmental disturbances on benthic soft-sediment macrofaunal communities. The findings indicate that macrofaunal communities respond to stress by decreasing in species diversity, but with only slight modification of the functional features and trophic structures of the assemblages. In fact, the macrofaunal communities adapt to environmental disturbances by minimizing the impacts through the success of stress-resistant species that occupy new ecological niches, and thus the entire communities may display a high resilience.

Overall, the results presented in this thesis highlight the importance of applying an integrated approach to study the impacts of different stressors on the benthic communities. Considering only structural variables (species, abundance and biomass) can lead to misleading conclusions. By combining the traditional measures with trophic pathways (stable isotope analysis) and functional trait information (biological trait analysis), it is possible to gain a more detailed and ecologically correct insight of the functioning of the benthic macrofaunal communities and their ecological functions. Functional and trophic attributes need to be evaluated in an integrated manner for a more accurate assessment of the consequently modifications of the community structure.

To conclude, the results of this thesis indicate that novel exploration of changes in benthic macrofaunal communities can provide important information on how an ecosystem can potentially withstand loss of species without losing in function and ecological services. In a world of increasing anthropogenic stress on the ecological systems, such information using the proper tools applied are essential for an adequate monitoring and assessments of ecosystem functioning in highly impacted areas.

6. FINANCIAL SUPPORT

My doctoral research was funded by four larger research projects. The temporal study conducted in the buffer zone of MPA-Miramare (**paper I**) was carried out as a part of the European Community INTERREG (Inter Regional) II-III Italy-Slovenia project. The study on Po River delta and on the Mar Piccolo of Taranto (**papers II, IV** and **VI**) were performed as part of the Project Bandiera RITMARE - La Ricerca Italiana per il Mare - coordinated by the National Research Council and funded by the Ministry for Education, University and Research within the National Research Programme 2011–2013. Two research projects financial support the study within the Port of Trieste: one was the Project Bandiera RITMARE, already described (**paper III**) and the other was the BALMAS project (Ballast Water Management System for Adriatic Sea Protection) (**paper VI**).

7. REFERENCES

Aleffi, F., Orel, G., Del Piero, D., Vio E., 1992. Oxygen condition in the Gulf of Trieste (High Adriatic). p. 431-440. In: *Marine Coastal Eutrophicatio.* Vollenweider, R.A., Marchetti, R., Viviani, R., (Eds). Elsevier.

Aleffi, I.F., Bettoso, N., Celio, M., Tamberlich, F., Predonzani, S., et al., 2006. Effects of suspended mussel culture on the macrozoobenthos in the Gulf of Trieste (northern Adriatic Sea). *Annales Series Historia Naturalis*, 16 (2), 135–146.

Ambrogi, R., Bedulli, D., & Zurijni, G. (1990). Spatial and temporal patterns in structure of macrobenthic assemblages. A three-year study in the northern Adriatic Sea in front of the Po River Delta. *Marine Ecology*, *11*(1), 25-41.

Assessment, M. E. (2005). Millennium ecosystem assessment. *Ecosystems and Human Well-Being: Biodiversity Synthesis, Published by World Resources Institute, Washington, DC*.

Balvanera, P., Pfisterer, A. B., Buchmann, N., He, J. S., Nakashizuka, T., Raffaelli, D., & Schmid, B. (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. *Ecology letters*, *9*(10), 1146-1156.

Barbier E.B., Hacker S.D., Kennedy C., Koch E., Stier A.C. et al. (2011) The value of estuarine and coastal ecosystem services. *Ecological Monographs* 8:169–193

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. *Marine Geology*, *222*, 135-158.

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. (2008) Species' traits and ecological functioning in marine conservation and management. *Journal of Experimental Marine Biology and Ecology* 366:37-47.

Bremner J., Rogers S.I. & Frid C.L.J. (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.

Cardellicchio, N., Annicchiarico, C., Leo, A., Giandomenico, S., & Spada, L. (2016). The Mar Piccolo of Taranto: an interesting marine ecosystem for the environmental problems studies. *Environmental Science and Pollution Research*, *23*(13), 12495-12501.

Cardellicchio, N., Buccolieri, A., Giandomenico, S., Lopez, L., Pizzulli, F., & Spada, L. (2007). Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, Southern Italy). *Marine Pollution Bulletin*, 55(10), 451-458.

Cardinale B.J., Palmer M.A. & Collins S.L. (2002) Species diversity enhances ecosystem functioning through interspecific facilitation. Nature 415:426-429.

Cardinale B.J., Duffy J.E., Gonzalez A., Hooper D.U., Perrings C. et al. (2012) Biodiversity loss and its impact on humanity. *Nature* 486:59-67.

Caroppo, C., Giordano, L., Palmieri, N., Bellio, G., Bisci, A., Portacci, G. & Hopkins, T. (2012). Progress toward sustainable mussel aquaculture in Mar Piccolo, Italy. *Ecology and Society*, 17(3).

Chapin Iii, F. S., Zavaleta, E. S., Eviner, V. T., Naylor, R. L., Vitousek, P. M., Reynolds, H. L., & Mack, M. C. (2000). Consequences of changing biodiversity. *Nature*, *405*(6783), 234-242.

Cohen, J. E. (1977). Food webs and the dimensionality of trophic niche space. Proceedings of the National Academy of Sciences, 74(10), 4533-4536.

Cibic, T., Acquavita, A., Aleffi, I.F., Bettoso, N., Blasutto., 2008. Integrated approach to sediment pollution: A case study in the Gulf of Trieste. *Marine Pollution Bulletin*, 56, 1650–1667.

Coma, R., Ribes, M., Gili, J. M., & Zabala, M. (2000). Seasonality in coastal benthic ecosystems. *Trends in Ecology & Evolution*, *15*(11), 448-453.

Creel, L. (2003). *Ripple effects: population and coastal regions* (pp. 1-7). Washington, DC: Population Reference Bureau.

Crema, R., Castelli, A., & Prevedelli, D. (1991). Long term eutrophication effects on macrofaunal communities in northern Adriatic Sea. *Marine Pollution Bulletin*, 22(10), 503-508.

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.

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

Di Leo, A., Cardellicchio, N., Giandomenico, S., Spada, L. (2010). Mercury and methylmercury contamination in Mytilus galloprovincialis from Taranto Gulf (Ionian Sea, Southern Italy), risk evaluation for consumers. *Food and Chemical Toxicology*, 48(11), 3131-3136.

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.

Drozina, R., 1999. Cenni su cinquanta e più anni della zona industriale di Trieste.

Elliott, M., & Quintino, V. (2007). The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin*, 54(6), 640-645.

Fain, A. M. V., Ogston, A. S., & Sternberg, R. W. (2007). Sediment transport event analysis on the western Adriatic continental shelf. *Continental Shelf Research*, *27*(3), 431-451.

Faresi, L., Bettoso, N., Aleffi, I.F., 2012. Vertical distribution of soft bottom macrozoobenthos in the Gulf of Trieste (northern Adriatic Sea). *Annales, Series Historia Naturalis*, 22, 123-132.

Fry, B. (2007). *Stable isotope ecology*. Springer Science & Business Media.

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. *Estuarine, Coastal and Shelf Science*, *60*(4), 673-694.

Ghirardelli, E., & Pignatti, S. (1968). Conséquences de les pollutions sur les peuplements du"Vallone di Muggia" près de Trieste. *Rev. Int. Océanogr. Med*, 10, 111-1221.

Griffiths, J. R., Kadin, M., Nascimento, F. J. A., Tamelander, T., Törnroos, A., Bonaglia, S., ... & Kotta, J. (2017). The importance of benthic-pelagic coupling for marine ecosystem functioning in a changing world. *Global Change Biology*. DOI: 10.1111/gcb.13642.

Grime J.P. (1974) Vegetation classification by reference to strategies. *Nature* 250:26-31.

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.

Heinänen, S., & Von Numers, M. (2009). Modelling species distribution in complex environments: an evaluation of predictive ability and reliability in five shorebird species. *Diversity and Distributions*, *15*(2), 266-279.

Holling, C. S. (1973). Resilience and stability of ecological systems. *Annual review of ecology and systematics*, *4*(1), 1-23.

Hooper, D. U., Chapin, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., ... & Schmid, B. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological monographs*, *75*(1), 3-35.

Hutchinson, G. E. (1957). The multivariate niche. In *Cold Spr. Harb. Symp. Quant. Biol* (Vol. 22, pp. 415-421).

Ieno, E. N., Solan, M., Batty, P., & Pierce, G. J. (2006). How biodiversity affects ecosystem functioning: roles of infaunal species richness, identity and density in the marine benthos. *Marine Ecology Progress Series*, *311*, 263-271.

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, 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.

Kennish, M. J., Brush, M. J., & Moore, K. A. (2014). Drivers of change in shallow coastal photic systems: an introduction to a special issue. *Estuaries and coasts*, *37*(1), 3-19.

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.

Krumins, J. A., van Oevelen, D., Bezemer, T. M., De Deyn, G. B., Hol, W. G., van Donk, E., ... & Soetaert, K. (2013). Soil and freshwater and marine sediment food webs: their structure and function. *Bioscience*, *63*(1), 35-42.

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.

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.

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.

Livingston, R. J. (2002). *Trophic organization in coastal systems*. CRC Press.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., ... & Jackson, J. B. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, *312*(5781), 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.

McCutchan, J. H., Lewis, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, *102*(2), 378-390.

McMahon, T. A., Halstead, N. T., Johnson, S., Raffel, T. R., Romansic, J. M., Crumrine, P. W., & Rohr, J. R. (2012). Fungicide-induced declines of freshwater biodiversity modify ecosystem functions and services. *Ecology Letters*, 15(7), 714-722.

Michener, R. H., & Schell, D. M. (1994). Stable isotope ratios as tracers in marine aquatic food web. Stable Isotopes in Ecology and Environmental Science 7 (Lajtha, K. and Michener, RH, eds.), 138-157.

Naeem, S. (2002). Ecosystem consequences of biodiversity loss: the evolution of a paradigm. *Ecology*, *83*(6), 1537-1552.

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.

Nordström, M. C. (2009). *Coastal food web structure and function: trophic interactions in shallow soft bottom communities*. Åbo Akademi University.

Occhipinti-Ambrogi, A., Savini, D., & 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*(1), 317-328.

Odum, E. P. (1969). The strategy of ecosystem development. *Sustainability: Sustainability, 164*, 58.

Olsgard, F., Schaanning, M. T., Widdicombe, S., Kendall, M. A., & Austen, M. C. (2008). Effects of bottom trawling on ecosystem functioning. *Journal of Experimental Marine Biology and Ecology*, *366*(1), 123-133.

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*, 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.

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

Paul, J. F., Scott, K. J., Campbell, D. E., Gentile, J. H., Strobel, C. S., Valente, R. M., ... & Ranasinghe, J. A. (2001). Developing and applying a benthic index of estuarine condition for the Virginian Biogeographic Province. *Ecological Indicators*, *1*(2), 83-99.

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

Pinka, E.R., 1970. On r- and K-Selection. *The American Naturalist*, 104, 592–597.

Prato, E., & Biandolino, F. (2005). Amphipod biodiversity of shallow water in the Taranto seas (north-western Ionian Sea). *Journal of the Marine Biological Association of the United Kingdom*, *85*(02), 333-338.

Quillien, N., Nordström, M. C., Schaal, G., Bonsdorff, E., & Grall, J. (2016). Opportunistic basal resource simplifies food web structure and functioning of a highly dynamic marine environment. *Journal of Experimental Marine Biology and Ecology*, *477*, 92-102.

Raffaelli, D. (2000). Trends in research on shallow water food webs. *Journal of Experimental Marine Biology and Ecology*, *250*(1), 223-232.

Reiss, H., & Kröncke, I. (2005). Seasonal variability of benthic indices: an approach to test the applicability of different indices for ecosystem quality assessment. *Marine Pollution Bulletin*, *50*(12), 1490-1499.

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

Rosenfeld, J. S. (2002). Functional redundancy in ecology and conservation. *Oikos*, 98(1), 156-162.

Russiani, S. (1992). Caratteristiche composizionali e tessiturali dei sedimenti dell'area portuale di Trieste. *Unpublished thesis dissertation. Istituto di Geologia e Paleontologia, Università degli Studi di Trieste.*

Sala, E., & Knowlton, N. (2006). Global marine biodiversity trends. *Annu. Rev. Environ. Resour.*, *31*, 93-122.

Schmid, B., Balvanera, P., Cardinale, B. J., Godbold, J., Pfisterer, A. B., Raffaelli, D., ... & Srivastava, D. S. (2009). Consequences of species loss for ecosystem functioning: meta-analyses of data from biodiversity experiments. *Biodiversity, Ecosystem Functioning, and Human Wellbeing: An Ecological and Economic Perspective*, 14-29.

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.

Simonini, R., Ansaloni, I., Pagliai, A. B., & 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: Journal du Conseil, 61*(6), 871-881.

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

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

Solis-Weis, V., Aleffi, I.F., Bettoso, N., Rossini, P., Orel, G., et al., 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, 247–263.

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.

Srivastava, D. S., & Vellend, M. (2005). Biodiversity-ecosystem function research: is it relevant to conservation? *Annu. Rev. Ecol. Evol. Syst.*, *36*, 267-294.

Tesi, T., Langone, L, Goni, MA, Miserocchi, S, & Bertasi, F (2008) Changes in the composition of organic matter from prodeltaic sediments after a large flood event (Po River, Italy). *Geochim Cosmochim Acta* 72:2100-2114.

Thrush, S. F., Hewitt, J. E., Dayton, P. K., Coco, G., Lohrer, A. M., Norkko, A., ... & Chiantore, M. (2009). Forecasting the limits of resilience: integrating empirical research with theory. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20090661.

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 recolonization of soft-bottom communities. *Journal of Experimental Marine Biology and Ecology*, *310*(1), 87-114.

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.

Vallarola, F. (2013). *Protected Adriatic.* E-book publication on Lulu platform (pp-121).

van der Linden P., Patrício J, Marchini A. Cid N., Neto J.M. et al. (2012) A biological trait composition to assess the functional composition of subtidal benthic communities in an estuarine ecosystem. *Ecological Indicators* 20:121-133.

Villnäs, A., J. Perus, and E. Bonsdorff. 2011. Structural and functional shifts in zoobenthos induced by organic enrichment - implications for community recovery potential. *Journal of Sea Research* 65: 8-18.

Violle C., Navas M-L., Vile D., Kazakou E., Fortunel C. et al. (2007) Let the concept of trait be functional. *Oikos* 116:882–892.

Weigel, B., Blenckner, T., & Bonsdorff, E. (2015). Maintained functional diversity in benthic communities in spite of diverging functional identities. *Oikos* 000, 1-13.

White, P. S., & Jentsch, A. (2001). The search for generality in studies of disturbance and ecosystem dynamics. In *Progress in botany* (pp. 399-450). Springer Berlin Heidelberg.

White, P. S., & Pickett, S. T. (1985). Natural disturbance and patch dynamics Pages 3-13 in S. T. A. Pickett and P.S. White. *The ecology of natural disturbance and patch dynamics*. Academic Press Inc.

Widdicombe, S., Austen, M. C., Kendall, M. A., Olsgard, F., Schaanning, M. T., Dashfield, S. L., & Needham, H. R. (2004). Importance of bioturbators for biodiversity maintenance: indirect effects of fishing disturbance. *Marine Ecology Progress Series*, *275*, 1-10.

Winemiller, K. O., & Polis, G. A. (1996). Food webs: what can they tell us about the world?. In *Food Webs* (pp. 1-22). Springer US.

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., ... & Sala, E. (2006). Impacts of biodiversity loss on ocean ecosystem services. *science*, *314*(5800), 787-790.

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.

Zanden, M., & Rasmussen, J. B. (2001). Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. *Limnology and oceanography*, *46*(8), 2061-2066.

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.

http://.www.porto.trieste.it. Website of the Port Authority of Trieste. (accessed 20.09.2016)

http://nettuno.ogs.trieste.it/ilter/. Website of the LTER C1 station in the Gulf of Trieste (accessed 20.10.2016)

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