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# BENTHIC ECOSYSTEM FUNCTIONING

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# ABSTRACT

Oceans and coastal areas in particular have been and continue to be affected by a large number of anthropogenic activities and pressures. Mankind depends on marine ecosystems for important and valuable goods and services, but human use has also altered the marine environment through direct and indirect means. There is a widespread degradation of marine habitats, depletion of resources and loss of biodiversity at the levels of genes, species and ecosystems. Because both natural systems and management approaches are evolving in time, continuous and integrative monitoring, evaluation, reporting and adaptive management are fundamental components for an effective marine management. The challenge is to determine achievable objectives and to develop realistic operational indicators against which effectiveness can be measured. The development of such operational objectives and the associated criteria and indicators is even more complicated when interactions among ecological, economical and societal factors are to be considered.

The coastal zone is a transition area between land and the open ocean and therefore it receives considerable amounts of freshwater, nutrients, dissolved and particulate organic matter, terrigenous inputs and pollutants. The sediments act as a sink for contaminants and frequently contain higher concentrations of pollutants compared to the water column. Since the benthic organisms are directly exposed not only to dissolved contaminants in the overlying water or in the pore water, but also to those adsorbed to sediment particles, they constitute one of the most effective tools for assessing the state of health of any given habitat. The identification of the stressors and the understanding of how their combined effect influences natural ecosystems may improve their management and protection. On a strictly ecological level, solid knowledge on the coastal ecosystem functioning is a fundamental prerequisite for understanding how humans interact with the environment. This complex subject derives from the integration of structural variables (physical-chemical characterization of the marine environment and its biological communities) and functional ones (i.e. the major metabolic processes such as primary production, respiration as well as degradation of the organic matter), which together describe how carbon and energy flow through the system.

The main aim of this doctoral research project was to assess the benthic ecosystem functioning in areas subjected to multiple and diffuse impacts. For that reason I have selected three study areas influenced by natural stress sources or impacted to a different extent by contaminants: the Aeolian Islands, the port of Trieste and the Mar Piccolo of Taranto.

The Aeolian Islands, originated by the volcanic activity and characterised by continuous gas emissions and presence of thermal vents on the surrounding seabed, represent a good natural laboratory for global warming and carbon capture and storage (CCS) leakage

studies. The aim of this work was to investigate to what extend the high $CO_2$ alone and in combination with the high temperature affects the microphytobenthic abundance and its community composition (Chapter 1). A further objective was to detect a possible change in the primary production rate in relation with these two variables. Higher microphytobenthic densities were recorded at the vent stations and values were even more pronounced in relation with high temperature. On the other hand, high microphytobenthic abundances were coupled with low community richness and diversity. The gross primary production estimates were strongly coupled with the microphytobentic abundance values. We observed several diatoms with morphological deformities and others with occluded microstructures due to the heavy silicification of diatom frustules that did not allow their taxonomic identification.

The Port of Trieste represents an area with a diffuse sediment contamination as it is subjected to diverse impacts: the shipbuilding activities, the iron and steel plant, the petroleum industry and the port activities. The aim of this study (Chapter 2) was to detect any variation in the structure of the microbenthic community, both as active phototrophic and resting microbenthos, and the possible differences in the primary production and oxygen consumption at stations subjected to different anthropogenic impacts. The total microbenthic abundance did not vary among stations. On the other hand, the structure of the community gave more information. In particular, the analysis based on the active and resting microbenthic community structure highlighted a clear separation among stations close to the productive activities from the more distant ones and the reference station, indicating a contamination effect on those communities. Species that are more tolerant to pollution were also observed in high numbers (*Nitzschia* cf. *commutata*).

As an example of an area severely contaminated by heavy metals and synthetic organic compounds, the Mar Piccolo of Taranto was chosen. Firstly, the microphyto- and macrozoobenthic communities were investigated and a marked influence of contamination on the abundance and biodiversity of those two communities was detected (Chapter 3). The presence of stress-resistant species and highly reduced abundance of some sensitive ones was also noted, indicating poor conditions of the benthic ecological status in the Mar Piccolo. To further asses the overall trophic status of the benthic ecosystem, an integrated study of the active phototrophic and resting microbenthic stages, combined with the main photoautotrophic pathways, was conducted (Chapter 4). The results showed that chemical contamination affected to a higher extent the active microphytobenthos than the resting one. Furthermore, although the Mar Piccolo is very shallow, the benthic system was scarcely productive, likely as a consequence of the accumulated contaminants in the surface sediments that probably interfere with the proper functioning of the benthic ecosystem. The study was further extended to the biota and its functioning in the water column, and its interaction with the benthic ecosystem. The abundance of planktonic phototrophs was investigated and associated with the physical-chemical features of the water column and primary production (Chapter 5). Contrary to the benthic ecosystem, the pelagic one was very productive and a clear seasonal succession of different sized plankton phototrophs was observed. To have a better overall view and provide some insights into the benthic-pelagic coupling, the primary production and heterotrophic prokaryotic production in the water column were integrated with those in the sediments and related to the origin of the organic matter pools based on the analysis of stable isotopes (Chapter 6). Heavy metals and polychlorobiphenyls (PCBs) were also analysed in the surface sediment layer and their effect on the overall ecosystem functioning due to the frequent sediment resuspension was considered.

# RIASSUNTO

Gli oceani ed in particolare le zone costiere sono soggetti a molteplici pressioni antropiche. Il genere umano dipende dagli ecosistemi marini per i beni ed i servizi che questi offrono, ma lo sfruttamento antropico ne ha alterato la struttura e funzionalità, portando ad uno stato di diffuso degrado, all'esaurimento delle risorse naturali e dalla perdita di biodiversità a livello di geni, specie ed ecosistemi. Poiché i sistemi naturali si evolvono nel tempo, per una gestione efficace delle aree marine è necessario un monitoraggio continuo ed integrativo, oltre a strumenti di valutazione e gestione adattativa. La sfida consiste, quindi, nell’individuare obiettivi raggiungibili e nello sviluppare realistici indicatori operativi mediante i quali misurare l’efficacia delle azioni. Lo sviluppo di questi strumenti è notevolmente complicato quando vengono considerate anche le interazioni tra i fattori ecologici, economici e sociali.

L’area costiera è una zona di transizione tra terra e mare che riceve una considerevole quantità di acqua dolce, nutrienti, sostanze organiche disciolte e particolate, input terrigeni ed inquinanti. I sedimenti agiscono come sito di accumulo dei contaminanti, pertanto di norma esibiscono concentrazioni più elevate rispetto alla colonna d'acqua. Poiché direttamente esposti sia ai contaminanti disciolti nelle acque di velo ed interstiziale, sia a quelli adsorbiti dalle particelle dei sedimenti, gli organismi bentonici costituiscono uno degli indicatori più efficaci per valutare lo “stato di salute” di un determinato habitat. L'identificazione dei fattori di stress e la comprensione di come la loro azione sinergica influenzi gli ecosistemi naturali sono due aspetti che potrebbero migliorare la gestione e la protezione dell’ambiente marino. Da un punto di vista strettamente ecologico, una solida conoscenza del funzionamento dell’ecosistema costiero costituisce un prerequisito fondamentale per la comprensione di come gli esseri umani interagiscono con l'ambiente. Questo tema complesso necessita dell'integrazione delle variabili strutturali (caratterizzazione chimico-fisica dell'ambiente marino e delle sue comunità biologiche) con quelle funzionali (i principali processi metabolici quali la produzione primaria, la respirazione e la degradazione della materia organica), che insieme descrivono il flusso di carbonio e di energia attraverso il sistema.

L’obiettivo principale del presente progetto di dottorato è stato quello di valutare il funzionamento dell'ecosistema bentonico in aree soggette a impatti antropici multipli e diffusi. Per questo motivo sono state scelte tre aree di studio soggette a stress naturale o ad un diverso grado di contaminazione: le Isole Eolie, il porto di Trieste e il Mar Piccolo di Taranto.

Le Isole Eolie sono caratterizzate da continue emissioni di gas e di acque termali dal fondale marino a causa dell’attività vulcanica che interessa l’arcipelago. Per queste loro peculiarità sono considerate un laboratorio naturale ideale per gli studi sul riscaldamento

globale e sull’impatto potenziale sull’ecosistema che potrebbero avere accidentali fuoriuscite di $CO_2$ dai siti di stoccaggio CCS (Carbon Capture and Storage). Lo scopo di questo lavoro è stato quello di indagare in che misura l'elevata concentrazione di $CO_2$, da sola e in sinergia con l'alta temperatura, influenza l'abbondanza e la composizione delle comunità microfitobentoniche (Capitolo 1). Un ulteriore obiettivo è stato quello di rilevare un’eventuale variazione del tasso di produzione primaria in relazione a queste due variabili. Sono state osservate densità maggiori di microfitobenthos nelle stazioni con emissioni di gas e abbondanze ancora più elevate quando la disponibilità di $CO_2$ era associata ad alte temperature. Seppur numerosa, la comunità in esame è risultata tuttavia scarsamente strutturata, come indicato dai minimi di ricchezza e diversità. Le stime di produzione primaria hanno seguito lo stesso andamento dell’abbondanza. Sono state inoltre osservate diverse specie di diatomee con deformità morfologiche e altre con microstrutture occluse dovute alla notevole silicizzazione dei frustuli, un fenomeno che non ha permesso la loro identificazione tassonomica.

Il porto di Trieste rappresenta un'area soggetta ad una contaminazione diffusa e multipla che deriva da diverse attività produttive: i cantieri navali, l'impianto siderurgico, l'industria petrolifera e varie attività portuali. Lo scopo di questo studio (Capitolo 2) è stato quello di determinare le variazioni nella struttura delle comunità microbentoniche, sia del microbenthos fototrofo attivo che di quello costituito dagli stadi di resistenza, assieme alle possibili differenze nei processi di produzione primaria e respirazione in stazioni sottoposte a differenti impatti antropici. Non è stata notata una differenza significativa nell’abbondanza totale microbentonica tra le stazioni indagate, mentre la struttura delle comunità è risultata più informativa. In particolare, l'analisi basata sulla struttura delle comunità microfitobentoniche attive e di resistenza, ha evidenziato una netta separazione tra le stazioni posizionate vicino alle attività produttive da quelle più lontane, compresa la stazione di riferimento, indicando una risposta della comunità ascrivibile alla contaminazione. Nei pressi delle attività produttive sono stati inoltre osservati in numero elevato organismi appartenenti a specie più tolleranti all'inquinamento (*Nitzschia* cfr. *commutata*).

Come esempio di un’area fortemente contaminata da metalli pesanti e composti organici di sintesi (policlorobifenili-PCB), è stato scelto il Mar Piccolo di Taranto. Le comunità microfitobentonica e macrozoobentonica sono state indagate ed una marcata risposta alla contaminazione è stata osservata in termini di variazioni nell’abbondanza e nella biodiversità dei popolamenti (Capitolo 3). La presenza di specie resistenti allo stress concomitante con ridotte abbondanze di taxa sensibili, hanno indicato un pessimo stato ecologico del sistema bentonico in alcune aree del Mar Piccolo. Per valutare ulteriormente lo stato trofico dell'ecosistema bentonico in questo sito, è stato inoltre condotto uno studio volto ad integrare le forme microbentoniche attive fototrofe e quelle di resistenza con i principali processi metabolici ad esse associati (Capitolo 4). I risultati hanno evidenziato che la contaminazione chimica influenza in misura maggiore il microfitobenthos attivo rispetto agli stadi di resistenza. Inoltre, anche se il Mar Piccolo è un ambiente poco profondo, il sistema bentonico è risultato scarsamente produttivo, a causa dell’elevata concentrazione di contaminanti accumulati nei sedimenti superficiali che probabilmente interferiscono con il suo corretto funzionamento. Lo studio è stato quindi esteso al funzionamento dell’ecosistema pelagico e alla sua interazione con quello bentonico. L'abbondanza del plancton autotrofo è stata messa in relazione con le caratteristiche chimico-fisiche della colonna d'acqua e la produzione primaria (Capitolo 5). Contrariamente all’ecosistema bentonico, quello pelagico è risultato molto più produttivo ed è stata osservata una chiara successione stagionale di plancton fototrofo appartenente a diverse classi di taglia. Nell’accoppiamento tra il comparto bentonico e quello pelagico la produzione primaria e la produzione eterotrofa procariotica in colonna d'acqua sono state integrate con quelle stimate nei sedimenti e messe in relazione con l'origine della sostanza organica basata sull'analisi degli isotopi stabili (Capitolo 6). Analizzando, inoltre, i metalli pesanti e PCB nello strato superficiale dei sedimenti è stato valutato il loro effetto sul funzionamento dell’ecosistema legato alla frequente risospensione dei sedimenti.

# INTRODUCTION

Humanity has benefited enormously from the rapid economic and technological developments of the last century but it is now paying this progress by the detriment of natural systems and the sustainability of resources and ecosystem functions. Our oceans and coastal areas in particular have been and continue to be affected by a heavy burden of anthropogenic pressures (Katsanevakis et al. 2011). Humans impact natural systems in a multitude of ways, yet the cumulative effect of multiple stressors on ecological communities remains largely unknown (Crain et al. 2008). Historically, habitat loss and over-exploitation of key species were the main human impacts on landscapes, but in the last century, the list has grown to include pollution, invasive species, and climate change among many other stressors (Crain et al. 2008 and the references therein). Humans depend on marine ecosystems for important and valuable goods and services, but human use has also altered the marine environment through direct and indirect means. There is a widespread degradation of marine habitats, depletion of resources and loss of biodiversity at the levels of genes, species and ecosystems. No areas of the ocean, even the most remote ones, are completely untouched by human impacts.

While ecological research has begun to document the individual effects of various stressors on species and ecosystems, research into the cumulative and interactive impacts of multiple stressors is less frequent. Urgently needed is a strategic approach to predict the development of community structures and the functional responses of ecosystems to multiple human-derived stressors (Breitburg et al. 1998; Halpern et al. 2007; Halpern et al. 2008). The dynamics and regulation of food webs cannot be understood, however, unless one simultaneously considers processes at the level of individual populations in concert with those acting over the domain of the entire community. All populations are embedded in an ecological context, i.e. processes that accrue at the population level permanently change properties of the whole system (Gaedke 1995).

Because both natural systems and management approaches are never static, continuous and interactive monitoring, evaluation, reporting and adaptive management are fundamental components for effective marine management. The challenge is how to develop realistic operational objectives and indicators against which effectiveness can be measured. This can be quite complicated as the focus of marine planning and management strategies are not only single species but also habitats and whole ecosystems. In addition to this, it may also be relevant to set multiple objectives taking into account interactions among ecological, economical and societal factors. This makes the development of operational objectives and the associated criteria and indicators even more complicated (Katsanevakis et al. 2011).

Understanding how multiple stressors affect natural systems will improve our ability to manage and protect these systems, as well as contribute to the understanding of fundamental ecological principles. An over-reliance on ecosystem structural features, such as diversity, in quality indicators therefore makes the detection of the anthropogenic stress more challenging (Breitburg et al. 1998) and a more integrated approach, including also the assessment of the ecosystem functionality, is therefore needed.

In my doctoral study I tried to implement an integrated approach for the evaluation of the benthic ecosystem functioning with emphasis on the microphytobenthic communities and their main processes in differently stressed environments and when possible, I further considered their interaction with other components of the benthos and also the plankton.

## THE BENTHIC ECOSYSTEM FUNCTIONING

Biogeochemical cycles that occur in the coastal areas as a result of the interplay between physical, geological, chemical and biological processes, affect the distribution of elements between living and non-living reservoirs on Earth. Living organisms both react to changes in the environment and affect environmental change (Thornton 2012). Indeed, an ecosystem may be defined as an open dynamic system, composed of “subunits” that are interlinked by a complex web of “connections”. The former can be intended as forms of C storage, represented by both the inorganic ($CO_2$ and its ionic forms) and the organic pool of C (non living organic matter and the biomass of biological communities). These components of the system are interlinked by flows of C and energy which are represented by processes of organic matter production (as primary production) and transformation (as mineralisation) and energy dissipation (as respiration). In the middle, there is the myriad of trophic relationships among organisms (e.g. predator-prey interactions) along the food web and which are influenced by the reciprocal balance between bottom up and top down control mechanisms. 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 (Franzo 2013).

The coastal zone is a transition area between land and the open ocean and therefore it receives considerable amounts of freshwater, nutrients, dissolved and particulate organic matter, terrigenous inputs and pollutants. As it is 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 (Gazeau et al. 2004). Shallow coastal photic systems are among the most productive on the planet (Odum 1983) on the account of a diverse array of benthic primary producers including microalgae (diatoms and cyanobacteria), macroalgae, and seagrasses (Kennish et al. 2014).

Aside from light, temperature and nutrients, that are key parameters regulating system production, coastal processes are largely influenced by physical factors such as horizontal transport, sediment composition and resuspension. Rapid sinking of phytoplankton blooms, efficient filtration of the water column by benthic fauna and a tidal energy subsidy can determine a tight benthic-pelagic coupling that leads to a high local benthic production (Kennish et al. 2014). Moreover, microbial mediated processes in sediments can enhance nutrient availability for primary production in benthic and pelagic habitats and become important in regulating the relative magnitude of benthic versus pelagic primary production (Kennish et al. 2014). Apart from phytoplankton and local benthic production, terrestrial matter carried by coastal rivers represents a non negligible contributor to coastal organic matter pools.

The benthic organisms inhabiting the sediments can be divided regarding their size into macrobenthos (> 1000 µm), meiobenthos (38 – 1000 µm), microbenthos (20 – 200 µm), nanobenthos (2 -20 µm) and picobenthos (0.2 – 2 µm). Otherwise, according to their trophic category they can be divided into animals and phototrophic organisms and are all interlinked with each other by a dense web of connections represented by the processes responsible for the flows of C and energy through the system. The microbial loop is a major sub-system within the marine food chain and its role in affecting ecosystem functioning has raised an increasing interest since its first description by Azam et al. (1983). The microbenthic loop encompasses organic matter, bacteria, microphytobenthos, protozoa and meiofauna and the state of its structure and dynamics has been recently proposed as a sensitive indicator of change occurring in the marine ecosystem (Vassallo et al. 2006).

In unvegetated sediment systems microphytobenthos (MPB) are at the base of the food web (Admiraal 1984; Middelburg et al. 2000) and when suspended by water movements, MPB contributes also to the pelagic primary production (de Jonge et al. 2012). As an important oxygen producer via photosynthesis, MPB are ‘indirectly’ controlling important biogeochemical processes in the sediment (Sundbäck 2000) like nitrification, denitrification, mineralization, sulfate reduction and others (Froelich et al. 1985). Usually the scale between oxic and anoxic layers in shallow sediments is within a few millimeters. Thus, any change in the trophic state (autotrophy *vs* heterotrophy) can have large effects on nutrient cycling and mineralization processes (Engelsen et al. 2008). MPB therefore have the capacity to buffer against nutrient discharges and hence counteract coastal eutrophication (McGlathery et al. 2007).

# BENTHIC ECOSYSTEMS SUBJECTED TO ENVIRONMENTAL ALTERATIONS

Shallow coastal photic systems rank among the most heavily impacted aquatic ecosystems, being affected by a wide range of anthropogenic activities. Multiple anthropogenic disturbances create both acute and insidious problems for many biotic communities and habitats that can compromise the stability and resiliency of these systems and their long-term integrity. Among other stressors, chemical contaminants can cause severe changes in 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 (Tolun et al. 2001). 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). For that reason the benthic organisms constitute the most effective group for assessing the state of health of any given habitat. Due to their scarce ability to avoid different stressors, they are forced to react synergetically without any escape possibility (Solis-Weiss 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 (Cibic et al. 2012b). Stress-resistant species can therefore replace the more sensitive ones within a community that consequently maintains its original functioning. For example, a well-mixed, high-energy area may have a high carrying capacity to absorb organic wastes without adverse effects being detected (McLusky and Elliott 2007). A highly specialised 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. 2012b).

Laboratory studies are seldom able to predict long-term ecological effects of severe contamination by different chemical compounds, especially with regard to functional interactions among organisms and between trophic levels (Carman et al. 1995). Several meio- and macrofaunal species are known to exert a strong top-down control on MPB biomass (Coull 1999; Weerman et al. 2011) and as the predators and prey are exposed simultaneously to toxicants in the field, the direct influence of contaminants on predators can lead to cascading indirect effects on resistant species in other trophic levels. Resource availability may be likewise influenced by contaminants, which may in turn modify important ecosystem functions (e.g. decomposition rates, oxygen dynamics and nutrient recycling) (Fleeger et al. 2003).

Many studies report changes in abundance or diversity of microbial communities (in the broad sense) in chronically polluted marine environments. Other fundamental aspects, such as how pollution affects primary production, respiration rates or other microbial processes have been seldom analysed in chemically polluted environments (Cibic et al. 2012b).

## MICROPHYTOBENTHOS

Microphytobenthos (MPB) constitute the only autochthonous source of primary production on sediment in the absence of macroscopic vegetation (Larson and Sundback 2008 and the references therein). As the principal oxygen producers in coastal environments, MPB allow the aerobic degradation of both autochthonous and settled organic matter in sediments. Benthic microalgae regulate sediment-water nutrients fluxes and might reduce the population capable of having an active metabolism (Risgaard-Petersen 2003). MPB are the key to the carbon cycle in littoral environments as producers of new organic matter that can enter the benthic and pelagic trophic web (Barranguet 1997). MPB includes the microscopic aquatic algae living on or close to the bottom. Most are eukaryotic (diatoms, dinoflagellates, phytoflagellates, etc.), but some prokaryotic photosynthetic organisms, such as cyanobacteria, contribute to the benthic community as well (MacIntyre et al. 1996). In temperate areas, the microphytobenthic communities are mainly composed of benthic diatoms that dominate at least on muddy and to some extent, sandy sediments (Admiraal 1984; Cibic and Blasutto 2011; Cibic et al. 2007; Round 1971; Underwood et al. 2005). Diatoms are the most species-rich group of algae with the number of extant species estimated to be at least 30,000 and probably ca. 100,000 (Mann and Vanormelingen 2013), ranging in size from a few micrometres to a few millimetres and existing either as single cells or as chains of connected cells. Today they generate most of the organic matter that serves as food for life in the sea (Armbrust 2009). It is estimated that diatoms account for 40 to 45% of net oceanic productivity (approximately 20 Pg C $yr^{-1}$; 1 Pg = 1015 g) or almost a quarter of the carbon fixed annually on Earth by photosynthesis (Mann 1999; Sarthou et al. 2005). They are ecologically widespread and have global significance in the carbon and silicon cycles, and are used increasingly in ecological monitoring, paleoecological reconstruction, and stratigraphic correlation (Mann 1999). They are also particularly promising indicators of organic and inorganic pollutants since they are typically one of the most abundant life forms in aquatic environments and occupy the base of the food chain (Torres et al. 2008). They occur in all types of aquatic ecosystems; their assemblages are diverse according to the environmental status and therefore contain considerable ecological information. They are characterised by one of the shortest generation times of all biological indicators that allows them to rapidly respond to environmental changes (De la Rey et al. 2004). Diatoms reproduce primarily by mitotic divisions interrupted infrequently by sexual events. They bloom quickly, increasing in cell number by many orders of magnitude in just a few days.

Microscopic counting of a microalgal community ensures detailed insight into the species composition, which enables a more profound functional analysis and provides the basis for the subsequent approaches (Gaedke 1995). It is of great importance that we separate the counting of viable cells (whole frustules with chloroplasts) from the total cell counts (cleaned samples where all frustules are counted). The total cells count provides taxonomic confidence while the viable cells count offers ecological reliability. Both can be used in bioassessment based on specific assessment objectives. It would be especially necessary for regions with large amount of depositional areas where the accumulation of dead diatoms might potentially be high (Gillett et al. 2009). Dividing the viable diatoms according to their living forms can give us additional information about the environment they live in, like changes in sediment grain size, the presence of higher hydrodynamism in the area, contaminants within the surface sediments, high organic load and others. The following division by Round (1981) is commonly used: erect (forms attached to the substrate by mucilage stalks, pads, or peduncles), adnate (forms growing with the valve face adherent to the substrate and having a limited motility), motile (biraphid species that can move freely on the substrate), tube-dwelling (naviculoid and nitzschioid diatoms living in mucilage tubes of their own making), tychopelagic (diatoms that are loosely associated with the substrate), and planktonic (truly planktonic species which settled on the substratum from the water column).

Also the planktonic resting stages contribute to the overall MPB as they accumulate on the seafloor of coastal areas where, like seeds of terrestrial plants, can reach densities of millions of cysts per square metre of bottom (Rubino et al. 2000). Furthermore, the microalgal resting stages can give us additional information on the past and present environmental status of an area and its degree of pollution, eutrophication and other influences (Liu et al. 2012).

Microphytobenthos have been studied in several multiple-stressor experiments and despite the observed changes in diversity and primary production, MPB seem to be less sensitive to stressors compared to sediment bacteria and meiofauna (Larson et al. 2007; Sundbäck et al. 2007) Variations in abundance and community structure are not the only detected changes in stressful environments as diatoms can respond also on the individual level. For instance, diatoms, if exposed to different kinds of stress during reproductive processes, can change in different ways their cell outline and striation pattern, producing teratological forms. These modifications can be slight, leading to difficulties in establishing a threshold between normal and teratological cells, or so marked that it is very difficult to recognize whether an unknown form is teratological or whether it belongs to a new species or variety. Teratological forms appear as an accidental effect of environmental stress, which can be both physical and chemical. Environmental factors like UV, pH, heavy metal contamination (e.g. Hg, Cd and Pb), toxic compounds (PAH and pesticides) and others can be involved. Presence of teratological forms may therefore be a valid indicator of ecosystem health as it was also shown that their frequency and severity are related to the magnitude of the stress (Falasco et al. 2009).

At the individual level, a diatom to withstand high contamination levels has to resort to different defence mechanisms. For instance, a (Morin et al. 2012) may be driven by limitation of the influx, storage of the contaminant in the cytosol in insoluble form, neutralization of oxidative stress and active expulsion out of the cell. Exposure enhances the production of polysaccharidic exudates (e.g. extracellular polymeric substances, EPS) able to bind contaminants outside of the cell, in general proportionally to the concentration of toxicant exposed, thus leading to immobilization of the complexes outside the cell in a less bioavailable form (Morin et al. 2012). MPB can also survive in both dark and anoxic conditions by respiring nitrate and quickly starting to produce oxygen again when environmental conditions improve (Kamp et al. 2011). Furthermore, microalgae react to toxicant stress with a change in cellular macromolecule content, generally increasing their lipid content (Podemski and Culp 2001).

## PRIMARY PRODUCTION AND RESPIRATION

The role of sediments in nutrient cycling has primarily been studied from the standpoint of heterotrophic processes, such as bacterial and faunal activities. This may seem natural as the majority of marine sediments exist in constant darkness or dim light, but there are, however, large coastal areas shallow or clear enough to enable photosynthesis to occur at the sediment surface. Thus, for ecologically relevant results, nutrient and oxygen exchange between sediment and water in shallow waters must be measured in both light and darkness to account for the influence of photosynthetic organisms (Sundbäck 2000). Awareness of the importance of the microphytobenthic activity has been considerably heightened in previous decades as in some systems their production can equal or even exceed that of the pelagic phytoplankton (Underwood and Kromkamp 1999). Shallow-water coastal sediments are recognised as highly productive areas which function as active buffer zones in the transport of nutrients, terrigenous inputs and contaminants between land and sea (Gazeau et al. 2004; Sundbäck 2000). The factors that affect primary production are well understood in broad terms to be light availability, microalgal biomass, and environmental variables such as temperature and nutrients that affect the efficiency of conversion of absorbed light into fixed carbon (Platt and Sathyendranath 1988). Global estimates of phytoplankton productivity are numerous but on the other hand, estimates of global microphytobenthic productivity are rare. The Mediterranean region in particular is poorly documented (Gazeau et al. 2004). Benthic microphytes have by far the widest spatial and temporal distributions, even though sea grasses and floating macroalgae can locally be important autochthonous primary producers for these sediments (Sundbäck

2000). In addition, the importance of microbenthic primary producers is not restricted to the warm season (Sundbäck et al. 1996), as is the case for rooted macrophytes and ephemeral floating macroalgae.

Both photosynthesis and chemosynthesis contribute to the primary production of the oceans, however oxygenic photosynthesis is by far the dominant process in terms of the amount of carbon fixed and energy stored in organic compounds. Almost all ecosystems in the ocean are fuelled by organic carbon and energy which was initially fixed by oxygenic photosynthesis (Thornton 2012). Gross primary production (GPP) refers to the total rate of organic carbon production by autotrophs, while respiration refers to the energy-yielding oxidation of organic carbon back to carbon dioxide. 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). The organic matter produced by the MPB is transferred very rapidly and efficiently to the bacteria and into the benthic food chains (Cook et al. 2004; Miller et al. 1996) which makes MPB a very important resource for a wide array of benthic grazers and deposit feeders (McLusky and Elliott 2007). Secondary production (SP) typically refers to the growth rate of heterotrophic biomass. Only a small fraction of the organic matter ingested by heterotrophic organisms is used to grow, the majority being respired back to dissolved inorganic carbon and nutrients that can be reused by autotrophs. Therefore, SP in the ocean is small in comparison to NPP (Bender et al. 1987).

Different methods are regularly used to measure rates of primary productivity, but the $^{14}C$ method is the most sensitive technique especially when the production is low.Other techniques are based on gas exchange, the use of isotope tracers, or chlorophyll fluorescence. The microphytobenthos is usually limited to the surface 2 or 3 mm of sediment, therefore high resolution measurements are required (Revsbech 1989). Primary productivity is usually expressed as the production of oxygen or the assimilation of inorganic carbon into organic carbon over time. In sediments, profiles and changes in oxygen concentration over time may be performed using oxygen microelectrodes (Revsbech et al. 1983).

For example, combining appropriately the incubations of sediment cores in dark and light conditions, the use of microelectrodes may give an indication of the oxygen availability within the sediments and the rate of its consumption/production. However, in specific environmental conditions such as a high amount of sedimentary organic matter, the oxygen availability depends not only upon the biological activity (i.e. respiration) but also upon abiotic factors as the reduction of chemical compounds (through redox reactions), introducing a potential error. Considering these methodological issues, only a wise combined use of the $^{14}C$ incorporation technique and $O_2$ microprofiling allows to estimate the trophic status of the ecosystem, i.e. if net autotrophy or heterotrophy prevails.

# STUDY SITES AND THEIR MAIN ENVIRONMENTAL FEATURES AND ISSUES

## Aeolian Islands - Elevated $CO_2$ concentrations and temperature

The Aeolian archipelago (Tyrrhenian Sea, Italy) is a ring-shaped volcanic arc, composed of seven islands and 10 seamounts, where the subduction-related volcanic activity is still present (Tassi et al. 2009). The majority of emissions are of gas only, although at some points a mixture of geothermal water and seawater in different proportions is released (Tassi et al. 2009).

This type of study areas are gaining importance as the $CO_2$ concentrations are unsustainably rising in the atmosphere and since 1970 the cumulative emissions of $CO_2$ increased by 40%. About 60% of these anthropogenic $CO_2$ emissions have been removed by sinks (ocean and vegetation uptake) and stored in natural carbon cycle reservoirs. The ocean alone has absorbed about 30% of the emitted anthropogenic $CO_2$ causing ocean acidification that, based on models, will range from 0.06 to 0.32 in decrease of pH at the end of the 21th century (IPCC 2014). The interest towards $CO_2$ vent areas is even higher, because they can be used as natural laboratories to study the impact of $CO_2$ leakage from Carbon Capture and Storage (CCS) systems, a key technology for the disposal of $CO_2$ derived from power plants and other industrial sources (Lewicki et al. 2007). A sudden gas release from those reservoirs would inevitably influence the marine organisms at least on the local level as they would be exposed to very high $p\mathrm{CO}_2$ (IPCC 2005). Regarding this particular aspect, only a few studies on the MPB communities have been published (Dias et al. 2010; Johnson et al. 2015; Johnson et al. 2013; Raghukumar et al. 2008). Studies considering also functional aspects are even more limited (Fu et al. 2007; Wenzhöfer et al. 2000) at the $CO_2$ vent sites.

## Port of Trieste - Multiple and diffuse contamination

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 $36 \cdot 10^6$ tons of crude oil discharged in 2001) and finally the expansion of the commercial docks. Because of this variety of anthropogenic impacts, the Port of Trieste has been under intensive investigation to assess the chemical quality status of the sediments and the water column and was declared a Site of National Interest (SIN) by Decree of the Italian Ministry of the Environment in 2003.

The sensitivity and quick response of benthic diatom communities to metal and petroleum hydrocarbon contamination was previously observed by Cunningham et al. (2003) and recently by Potapova et al. (2016). Although several studies on the microphytobenthic communities have been carried out in the Gulf of Trieste (Cibic et al. 2007; Cibic et al. 2012a; Franzo et al. 2015; Franzo et al. 2014), no previous research has focused on its harbour area and in particular on the MPB response to high contamination levels associated with the main productive activities carried out within the Port of Trieste.

## Mar Piccolo of Taranto - Severe contamination

Since 1960s the city of Taranto and its coastline have been subjected to the industrialization process that has caused profound environmental changes. The Mar Piccolo is an enclosed ecosystem strongly exploited for intensive mussel commercial fishery and affected by industrial, agricultural, and sewage inputs (Caroppo et al. 2012). The industrial zone is characterized mainly by the presence of the largest steelworks in Europe (ILVA) 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 dioxin. These activities have heavily affected the coastal marine environment, especially the sediment quality: the benthic sediments contain pollutants at concentrations that often exceed those of the overlying water column by several orders of magnitude (Cardellicchio et al. 2015). For these reasons, the Taranto area is classified as Site of National Interest”, established by National Law 426 (1998), and included in the “National Environmental Remediation and Restoration Projects.” Previous studies on the benthic trophic web in the Mar Piccolo have been mainly focused on the macroscopic components. To the best of our knowledge, this study has produced the first data on microphytobenthos, primary production and oxygen consumption rates in sediments of the Mar Piccolo of Taranto.

# GOALS OF THE THESIS

The purpose of this study was to contribute to promote ecosystem-based management and its application in coastal areas, grounded on a better understanding of the ecosystem functioning in the presence of specific types of stress. In particular, it aimed to assess the benthic ecosystem functioning in areas subjected to different impacts. Ecological parameters of the sediment were defined through: a) definition of structural characteristics of sediments in the investigated areas, b) evaluation of the benthic community structure in the investigated areas, c) evaluation of energy storage processes (primary production) and dissipation (respiration), d) identification of taxa resistant to pollutants and their trophic role in the investigated areas, e) assessment of ecosystem functioning in the investigated areas.

Therefore, the main objectives of my doctoral research project were to implement an integrated approach to the evaluation of the benthic ecosystem functioning with emphasis on the microphytobenthic communities and their functioning in differently stressed environments and when possible, to further consider their interaction with other benthic and also planktonic communities. To achieve these goals I have studied the microphytobenthic communities, their abundance and community composition and identified stress-resistant species as well as diatoms with possible morphological deformities. I have implemented these data with primary production and respiration rates, estimating the trophic status of the benthic ecosystem.

To achieve that, three study areas subjected to different degrees of natural or human pressures were chosen:

- An area characterised by high $CO_2$ and temperature that can serve as natural laboratory for global warming and CCS leakage studies (Aeolian Islands).
- A port area where the contamination of the sediments is very widespread (Port of Trieste).
- An area in the vicinity of industrial settlements subjected to localized but severe contamination by heavy metals and polychlorobiphenyls (Mar Piccolo of Taranto).

My doctoral thesis is a collection of scientific papers; papers from Chapters 3, 4, 5 and 6 have been already published, while papers from Chapters 1 and 2 are submitted for publication.

**Chapter 1** - The aim of this work was to investigate to what extent the high $CO_2$ alone and in combination with the high temperature affects the microphytobenthic abundance and its community composition in a site naturally affected by these stressors. A further objective was to detect a possible change in the primary production rate in relation with these two variables.

**Chapter 2** - A study focused on detecting any variation in the structure of the microbenthic community, both active phototrophic and resting microbenthos, and the possible differences in the primary production and respiration at stations subjected to different anthropogenic impacts.

**Chapter 3** - This study investigated the microphyto- and macrozoobenthic communities with the aim to detect the influence of contamination on the abundance and biodiversity of those two communities.

**Chapter 4** - An integrated study of the active phototrophic and resting microbenthic stages, combined with the main photoautotrophic pathways.

**Chapter 5** - This study was further extended to the biota and its functioning in the water column, and its interaction with the benthic ecosystem.

**Chapter 6** - This study provided a better overall view and some insights into the benthic-pelagic coupling as the primary production and heterotrophic prokaryotic production in the water column were integrated with those in the sediments and related to the origin of the organic matter pools based on the analysis of stable isotopes.

My doctoral research was funded by three larger research projects. The study conducted in the Mar Piccolo of Taranto was 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. The study within the Port of Trieste was performed as part of the characterisation plan of this SIN area: “Piano di Caratterizzazione Ambientale dell’Area Marino-Costiera del Sito di Interesse Nazionale di Trieste” whereas the study conducted at the Aeolian Islands was carried out within the WP4 (assessment of the impact of leakage on benthic organisms and marine ecosystems) of the Project $ECO_2$ - Sub-seabed $CO_2$ Storage: Impact on Marine Ecosystems.

# CHAPTER 1

## Rogelja M., Cibic T., Pennesi C., De Vittor C.: Microphytobenthic community composition and primary production in relation to high $CO_2$ concentration and temperature in the Aeolian Islands (Tyrrhenian Sea, Italy)

*Submitted to Marine Environmental Research*

# Microphytobenthic community composition and primary production in relation to high $CO_2$ concentration and temperature in the Aeolian Islands (Tyrrhenian Sea, Italy)

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## Abstract

Sediment samplings were performed to investigate the microphytobenthic community and photosynthetic activity adaptations to higher $CO_2$ concentrations and temperature in the Aeolian Islands during a three-year period (2012–2014). Higher microphytobenthic densities were recorded at the vent stations and values were even more pronounced in relation with high temperature. The gross primary production estimates strongly depended on microphytobentic abundance values reaching up to $45.79 \pm 6.14$ mgC $m^{-2}$ $h^{-1}$. High abundances were coupled with low community richness and diversity. Motile diatom living forms were predominant at all stations and the greatest differences among vent and reference stations were detected on the account of the tychopelagic forms. Morphological deformities of diatom silica walls in relation to higher $CO_2$ were also observed. A significant influence of the $CO_2$ and temperature on the phototrophic community was highlighted suggesting the Aeolian Islands as a good natural laboratory for global warming and CCS leakage studies.

## Highlights

- Microphytobenthic abundance was positively influenced by high $CO_2$ and temperature.
- Photosynthetic activity was stimulated by $CO_2$ and temperature.
- Vents were characterized by low community richness and diversity.
- Tychopelagic diatoms species were absent at the vents due to high hydrodynamics.
- Morphological deformities and more silicified diatom frustules were detected.

**Keywords:** Benthic diatoms, Primary production, Biodiversity, Temperature, Microphytobenthos, $CO_2$, Hydrothermal vent, Aeolian Islands

## 1 Introduction

The Aeolian archipelago (Tyrrhenian Sea) is characterised by $CO_2$ and temperature diverse conditions and offers a good natural study area for research on shallow benthic (Maugeri et al. 2009) and pelagic systems (Karuza et al. 2012). This type of study areas are gaining importance as the $CO_2$ concentrations are unsustainably rising in the atmosphere and since 1970 the cumulative emissions of $CO_2$ increased by 40%.  About 60% of these anthropogenic $CO_2$ emissions have been removed by sinks (ocean and vegetation uptake) and stored in natural carbon cycle reservoirs. The ocean alone has absorbed about 30% of the emitted anthropogenic $CO_2$ causing ocean acidification that, based on models, will range from 0.06 to 0.32 in decrease of pH at the end of the 21th century (IPCC 2014). Firstly, the acidification studies were focused mainly at the calcifying organisms like corals (Cohen and Holcomb 2009; Pandolfi et al. 2011), foraminifera (Dias et al. 2010) and coccolitophores (Beaufort et al. 2011; Doney et al. 2009), because of the direct effect of lower pH on their ability to maintain the external calcium carbonate skeletons (Orr et al. 2005). More attention to the effects of acidification on non-calcinated organisms followed as more complex shifts in marine ecosystem composition and function were highlighted (Fabry et al. 2008; Hall-Spencer et al. 2008; Pörtner 2008; Riebesell 2004). The interest towards $CO_2$ vent areas is even higher, because they can be used as natural laboratories to study the impact of $CO_2$ leakage from Carbon Capture and Storage (CCS) systems, a key technology for the disposal of $CO_2$ derived from power plants and other industrial sources (Lewicki et al. 2007). The CCS process allows us to separate the $CO_2$ from the industrial and energy-related sources and to isolate it long-term from the atmosphere, transporting it to a storage location i.e., geological formations on land or under the seabed. One of the possibilities is also the direct injection of the $CO_2$ into the water column by a fixed pipeline or moving ship. The latter technology or a sudden gas release from geological reservoirs triggered by failure of the storage seal or the injection well, earthquakes or volcanic eruptions, or accidentally punctured reservoirs by subsequent drilling activity, would inevitably influence the marine organisms at least on the local level as they would be exposed to very high $pCO_2$ (IPCC 2005). The possible effects of such $CO_2$ releases  were already studied on viruses and prokaryotes (Karuza et al. 2012; Rastelli et al. 2015; Tait et al. 2015), zooplankton (Halsband and Kurihara 2013) and also higher taxonomical levels (Basallote et al. 2012; Kita et al. 2013; McConville et al. 2013; Murray et al. 2013). However, even though the microphytobenthos (MPB) are useful bio-indicators, being representative of the conditions at sampling site, ubiquitous and easy to sample (Desrosiers et al. 2013) the research conducted on the microalgae in relation with different $pCO_2$ is very limited. Regarding this particular aspect, only a few studies on the MPB communities have been published (Dias et al. 2010; Johnson et al. 2015; Johnson et al. 2013; Raghukumar et al. 2008). Benthos, in contrast to nekton and plankton, remains in place and thus, subjected to different environmental stresses, reacts to those synergetically without any escape possibility. The resulting composition of species, replacements, elimination, diversity or abundance changes, can give a proper idea of environmental events affecting the area (Solis-Weiss et al. 2001). Studies considering some functional aspects are even more limited (Fu et al. 2007; Wenzhöfer et al. 2000) at the $CO_2$ vent sites. Even though primary production estimates measured in situ with the $^{14}C$ technique are commonly used to have an overview of the benthic trophic state (Cibic et al. 2012; Krause-Jensen et al. 2012; Rubino et al. 2015), we found no such studies on vents to this date. Therefore, in this study we upgrade the information gained from data on MPB with the main photoautotrophic pathway, the primary production. The variation in pH can affect algal growth in different ways: it can change the distribution of carbon dioxide species and carbon availability, alter the availability of trace metals and essential nutrients, and at extreme pH levels potentially cause direct physiological effects (Chen and Durbin 1994). Laboratory and mesocosm studies of diatom growth and photosynthesis under elevated $pCO_2$ showed very diverse responses (Gao et al. 2012). Predominately the effect on growth was stimulative (Kim et al. 2006; King et al. 2011; Low-Decarie et al. 2011; Yang and Gao 2012) but in some cases a negative effect on growth was shown (Ihnken et al. 2011; Low-Decarie et al. 2011; Torstensson et al. 2012). Other effects were reported, like decreased silicification of diatom frustules (Mejía et al. 2013) and selection of larger diatom genera (Johnson et al. 2013) under higher $pCO_2$, both very important responses, as the diatoms represent the world's largest contributors to biosilicification. In the oceans, silicon is a major limiting nutrient for diatom growth and hence is a controlling factor in primary productivity (Martin-Jézéquel et al. 2000). The differences in frustules' thickness reflect characteristics of the resident diatom communities as they have been shaped over time in response to the prevailing ecological, chemical and physical settings (Baines et al. 2010). High temperatures have been proven to induce significant effects on the composition of the MPB community, causing changes in relative abundances of the major groups and/or at the single species level and can provoke heterotrophy (Cartaxana et al. 2015). The dominance of smaller sized diatoms has been recorded under higher environmental temperature (Falkowski and Oliver 2007; Winder et al. 2009). The combined effect of different variables found at the Aeolian Islands, like high $pCO_2$, temperature, concentration of metals and biogenic elements (especially Si) could shift the frustules silicification process and selection to more or less silicified diatom species and therefore their cumulative effect should be investigated.

The majority of the previous studies on the effects of $pCO_2$ and temperature on any benthic or pelagic community were conducted in vitro or in mesocosms. In this field study we assessed the effects of high $CO_2$ and temperature on the microphytobenthic community in the sediments of the Aeolian Islands. In particular the aims of this work were: i) to investigate to what extend the high $CO_2$ alone and in combination with the high temperature affects the microphytobenthic abundance and its community composition; ii) to detect a possible change in the primary production rate in relation with high $CO_2$ and temperature; iii) to identify other differences among microphytobenthic assemblages such as different cell size, morphological deformities or diverse degrees of silicification of the diatom frustule.

Therefore, our study gives new insights to the overall pattern of microphytobenthic community response to the extreme environment of shallow hydrothermal vents.

## 2 Material and methods

### *2.1 Study area*

The Aeolian archipelago (Tyrrhenian Sea, Italy) is a ring-shaped volcanic arc, composed of seven islands and 10 seamounts, associated with the Peloritanian–Calabrian orogenic belt. Panarea is the smallest (3.3 $km^2$) of the islands and it represents the emergent part of a wide stratovolcano that is more than 2000 m high and 20 km long where the subduction-related volcanic activity is still present (Tassi et al. 2009). In the early 1980's researchers began to conduct gas geochemistry surveys of the area (Caliro et al. 2004), showing that the system was relatively stable in both gas chemistry and flux rates ($7\text{-}9 \times 10^6$ l/d). The majority of emissions are of gas only, although at some points a mixture of geothermal water and seawater in different proportions is released. In November 2002 this area entered in an explosive phase and several new vents appeared but after three months the flux rates began to decrease towards the pre-outburst conditions (Tassi et al. 2009).

### *2.2 Sampling*

Samplings were carried out during four sampling campaigns in the following periods: June and October 2012, May 2013 and May 2014. A total of five stations were sampled: three near Basiluzzo Island, characterised by different sediment colour and gas emission, and two NE of Panarea Island on a sampling site referred as "Hot-Cold" (Table 1) where the sediments are subjected to very different temperatures at a distance of approximately 1 m one from another (Figure 1).

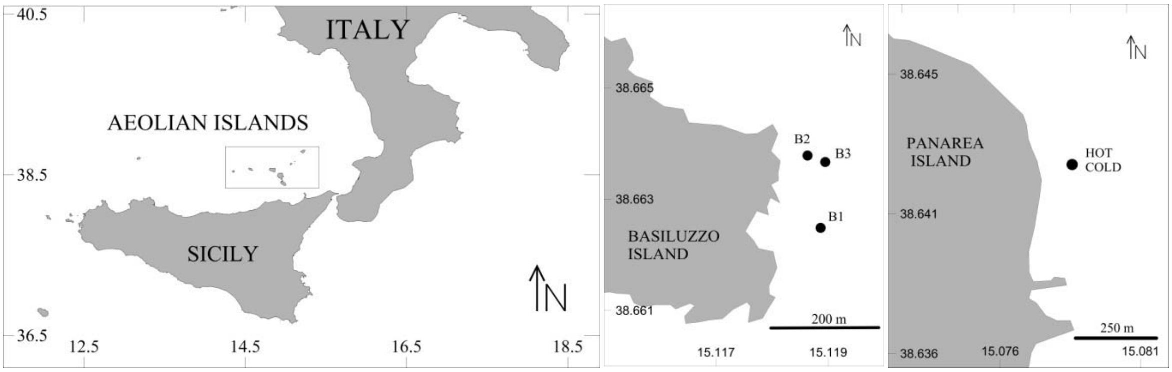

Fig. 1 Map of the study areas showing the sampling stations B1, B2 and B3 at Basiluzzo Island and stations HOT and COLD at the Hot-Cold sampling site near Panarea Island.

Gas emissions were mainly composed of $CO_2$ with a mean value of 98 vol. % and $H_2S$ that varied from 0.4 to 4.0 vol. %. Other detected gases like $N_2$, $H_2$, He and $CH_4$ were represented in very low volumes (0.4 vol. %, 1100 vol. ppm, 11 vol. ppm and 10 vol. ppm, respectively) (Voltattorni et al. 2009). At the vents St. B1 and B3 pH values measured at the sediment surface were below 6.0 whereas at the reference St. B2 they were around 7.5 (Queirós et al. 2014). At the sampling site Hot-Cold pH values were around 7.1 in the overlaying water (unpublished data).

Table 1 Location, depth and main hydrothermal characteristics of the sampling stations at the Basiluzzo and Hot-Cold sampling sites. (*) Yes, enriched in $CO_2$ (5-11 mg $m^{-3}$ $s^{-1}$ flux).

| Station | Latitude | Longitude | Depth (m) | Gas emission | Thermal vent |
|---|---|---|---|---|---|
| B2 (reference) | 38° 39.827' | 15° 07.118' | 14.0-16.0 | no | no |
| B3 (gray vent) | 38° 39.82' | 15° 07.137' | 21.0 | yes ($CO_2$ 97%) | no |
| B1 (red vent) | 38° 39.749' | 15° 07.132' | 14.0-15.0 | yes ($CO_2$ 98%) | no |
| HOT | 38° 38.536' | 15° 04.714' | 11.9 | yes | yes*(40 ± 5°C) |
| COLD | 38° 38.536' | 15° 04.714' | 11.9 | yes | no |

At each sampling, temperature and salinity profiles were registered using a CTD multiparametric probe model Sea-Bird Electronics 19plus SEACAT (Sea-Bird Electronics, Inc., Bellevue, Washington, USA). Photosynthetically available radiation (PAR) was recorded *in situ*, using a Profiling Natural Fluorometer PNF-300A (Biospherical Instruments Inc., San Diego, CA, USA). PAR at the bottom was expressed as the percentage of the measured irradiance with respect to the surface irradiance (%PAR).

At all sampling stations and during all campaigns, sediment samples were collected by scuba divers with cut-off plastic syringes (2.6 cm i.d.). In total 7 sediment cores were sampled: one was dedicated to the grain-size analysis, three to the microphytobenthic abundance (ABU) and community composition, and three to the microphytobenthic primary production (PP). Once on board, the sediment cores were immediately extruded and only the uppermost sediment layer (~0.5 cm) was subsampled for the above mentioned analyses. PP was estimated only in October 2012 at the Hot-Cold sampling site and in May 2013 at the Basiluzzo sampling site.

### *2.3 Sample analyses*

#### *2.3.1 Sediment grain size*

Sediment samples (about 100 g each) were oven dried at 105 °C for 24 h, kept in a drier and then dry-sieved through a battery of sieves spaced at 1 phi (Φ) unit interval (where $\Phi = -\log_2$ of the particle diameter expressed in mm) following the Wentworth (1922) scale. A battery of sieves with the opening range from 2 mm (-1) to 63 µm (4) was used and therefore shaken for 30 minutes with an Endecotts Octagon 200 test sieve shaker. Grain size classes are expressed in dry weight percentage (%).

#### *2.3.2 Microphytobenthic abundance (ABU) and community composition*

For the quantitative determination of microphytobenthos, the uppermost sediment layer obtained from three distinct sediment cores was fixed with 10 mL of 4% formaldehyde buffered solution ($CaMg(CO_3)_2$) in pre-filtered seawater (0.2 µm filter) and stored at 4°C until processing. For each sampling three replicates were counted for each station. After manual stirring, 125 µL aliquots of the sediment suspension were drawn off and placed in a counting chamber. To obtain an estimate of the total viable cells, only cells containing pigments and not empty frustules were counted under a Leitz inverted light microscope (Leica Microsystems AG) using a 32X objective (320X final magnification) (Utermöhl 1958). MPB community was divided into major taxonomic groups and within the group of diatoms more precise species identification was attempted. Diatoms frustule cleaning was performed by oxidizing subsamples with two time volume of hydrogen peroxide (35%) and ≈1 mL of sulphuric acid (95-97%) (CEN 2003). Between single treatments, the subsamples were heated over a Bunsen burner until boiling and left under the hood for 24 hours. After that, they were washed with Milli-Q water and left to settle for at least 24 hours between single washings, until reaching a pH close to 7. All samples were then mounted on permanent slides in Naphrax$^{TM}$ (Northern Biological Supplies Ltd. Ipswich, UK) and observed under 1000x final magnification using a light microscope (LM) (Leica DM2500, Germany) equipped with a digital camera (Leica DFC490, Germany). Aliquots of the same subsamples were also mounted on filters, placed on aluminium stubs, gold coated using a S150A sputter coater (Edwards High Vacuum, Crawley, West Sussex, UK) and observed using the scanning electron microscope (SEM) Leica Stereoscan 430i (Leica Cambridge Ltd, Cambridge, UK). The microalgal taxonomy was based on Van Heurck (1899), Hustedt (1933), Hendey (1976), Germain (1981), Dexing et al. (1985), Richard (1987), Simonsen (1987), Round et al. (1992), Tomas (1997), Witkowski et al. (2000), Hein et al. (2008), Lobban et al. (2012), ProtistCentral (Jordan et al. 2009-2015) and the AlgaeBase website (Guiry and Guiry 2015). The qualitative identification of diatoms was carried out to the genus and, when possible, to the species level. The viable diatoms were further divided into the following living forms (Round 1981): erect (forms attached to the substrate by mucilage stalks, pads, or peduncles), adnate (forms growing with the valve face adherent to the substrate and having a limited motility), motile (biraphid species that can move freely on the substrate), tube-dwelling (naviculoid and nitzschioid diatoms living in mucilage tubes of their own making), tychopelagic (diatoms that are loosely associated with the substrate), and planktonic (truly planktonic species which settled on the substratum from the water column).

### *2.3.3 Primary production (PP)*

The uppermost layer of the three sediment cores was homogenised and re-suspended in 100 mL of overlying filtered seawater (0.22 µm filter) and 500 µL of $^{14}C$ ($NaH^{14}CO_3$, Amersham Pharmacia Biotech, UK) were added to reach a final activity of 1 µCi $mL^{-1}$ (37 kBq $mL^{-1}$) (Steemann Nielsen 1952). After stirring, only seawater with resuspended diatoms and not the coarse sediment was transferred into 9 glass vials containing 9 mL. Three light and three dark samples were fixed on a rosette, lowered at the bottom and incubated for 1 h. To the three remaining dark samples, 100 µL of HCl 5N were added immediately to stop the PP activity by lowering the pH to less than 2 (Cibic and Virgilio 2010). The other replicates were stopped after incubation by adding the same volume of the acid solution. Samples were brought to the laboratory, transferred to glass scintillation vials and left open under hood overnight. Subsequently, 10 ml of the Scintillation cocktail Ultima GoldXR (Packard BioScience, USA) was added and the samples were analysed on a QuantaSmart TRI-CARB 2900 TR Liquid Scintillation Analyzer (Packard BioScience, USA) including quenching correction, obtained using internal standards. Disintegrations per minute (DPM) were calculated. DPM obtained from dark samples were subtracted from DPM derived from light samples (Jönsson 1991; Lignell 1992; Steemann Nielsen 1952). To convert DPM into gross primary production (PP), expressed as mg C $m^{-2}$ $h^{-1}$, the protocol by Cibic et al. (2008b) was followed.

## *2.4 Data analyses*

Descriptive statistics was used to process data on ABU and community composition.

The Kruskal-Wallis test using Statistica 10 (Statsoft Inc. 2011) and the pairwise comparisons using Tukey and Kramer (Nemenyi) test in R (Development Core Team 2013) were applied to show differences in ABU among stations.

The species abundance matrix was previously standardised and root-transformed and then the diatom community structure was analysed with non-parametric multivariate tools. For the similarity or dissimilarity distances among samples the Bray-Curtis coefficient was used. A two-way crossed ANOSIM permutation test was performed to determine the statistical differences between groups of samples among stations and sampling periods. To define a possible correlation with the abiotic variables (bottom temperature, depth or sediment grain size) the RELATE routine was used.

The SIMPER routine was performed to calculate the average similarity (S*)* of samples at each site, but also the average dissimilarity (δ) of all pairs of inter-site samples. Univariate diversity analysis was applied to benthic diatom abundances considering richness *d*; (Margalef 1986), equitability *J'*, (Pielou 1966), diversity *H'*; (Shannon and Weaver 1949) and dominance λ; (Simpson 1949) and graphical/distributional plots, i.e. the *k* dominance curves were also built using the software package PRIMER© version 6.1.9. (Plymouth Marine Laboratory, U.K.).

## 3 Results

### *3.1 Abiotic parameters*

At the bottom, water temperature did not vary remarkably among stations or sampling periods, showing similar values during samplings in June 2012, May 2013 and 2014 (19.0 ± 0.1, 18.0 ± 0.4 and 17.5 ± 0.5°C, respectively) with only October 2012 reaching 23.4 ± 0.0°C. Salinity was even less variable with an average (over stations and sampling periods) value of 37.9 ± 0.3. The %PAR was quite high, always above 22% with the average irradiance at all stations equal to 619 µE $m^{-2}$ $s^{-1}$.

### *3.2 Sediment grain-size*

Grain-size was quite variable due to the strong hydrodynamism of the area (Figure 2). Sediments were mainly composed of coarse and medium-coarse sand. The gravel fraction varied among stations and also among samplings ranging from 0.4 to 21.1%. The contribution of the mud fraction (silt + clay) was negligible at all stations (0.15 ± 0.1%).

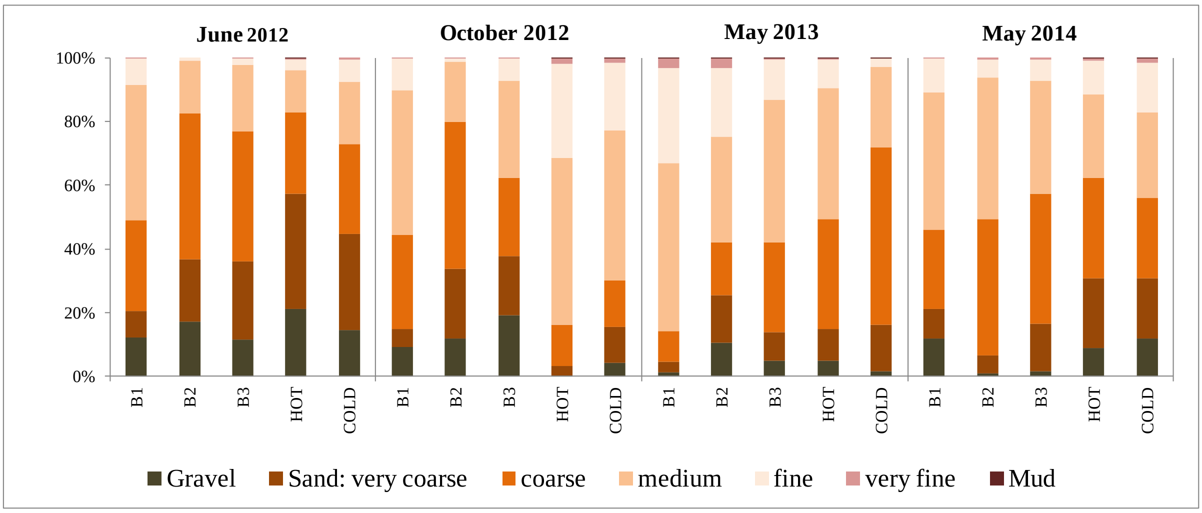

Fig. 2 Grain-size at the sampling stations during the four sampling periods, expressed in percentage: Gravel (>2 mm); Sand: very coarse (> 1 mm), coarse (> 500 µm), medium (>250 µm), fine (>125 µm), very fine (>63 µm); Silt and clay (mud) (≤63 µm).

### *3.3 Microphytobenthic abundance (ABU)*

At the Basiluzzo site the highest ABU values were reached in October 2012, intermediate values were observed in May 2013 and 2014, while the lowest ones were obtained in June 2012 (Figure 3a). A significant difference in ABU was detected among these stations (Kruskal-Wallis test, H (2, N=36) = 9.98, p = 0.0068). The highest ABU were consistently recorded at St. B1 reaching the absolute maximum in October 2012 (20045 ± 2344 cells cm$^{-2}$) when values about four times higher compared to St. B2 (4904 ± 1232 cells cm$^{-2}$) were measured. The pairwise comparisons using Tukey and Kramer (Nemenyi) test showed a significant difference between St. B1 and St. B2 (p = 0.0055). On the other hand, the difference between St. B2 and St. B3 was not marked (p = 0.5599) as the ABU at St. B3 differed only in October 2012 reaching twice higher values (9668 ± 1491 cells cm$^{-2}$) compared to St. B2. The absolute minimum in ABU was detected at St. B3 in June 2012 (1617 ± 177 cells cm$^{-2}$).

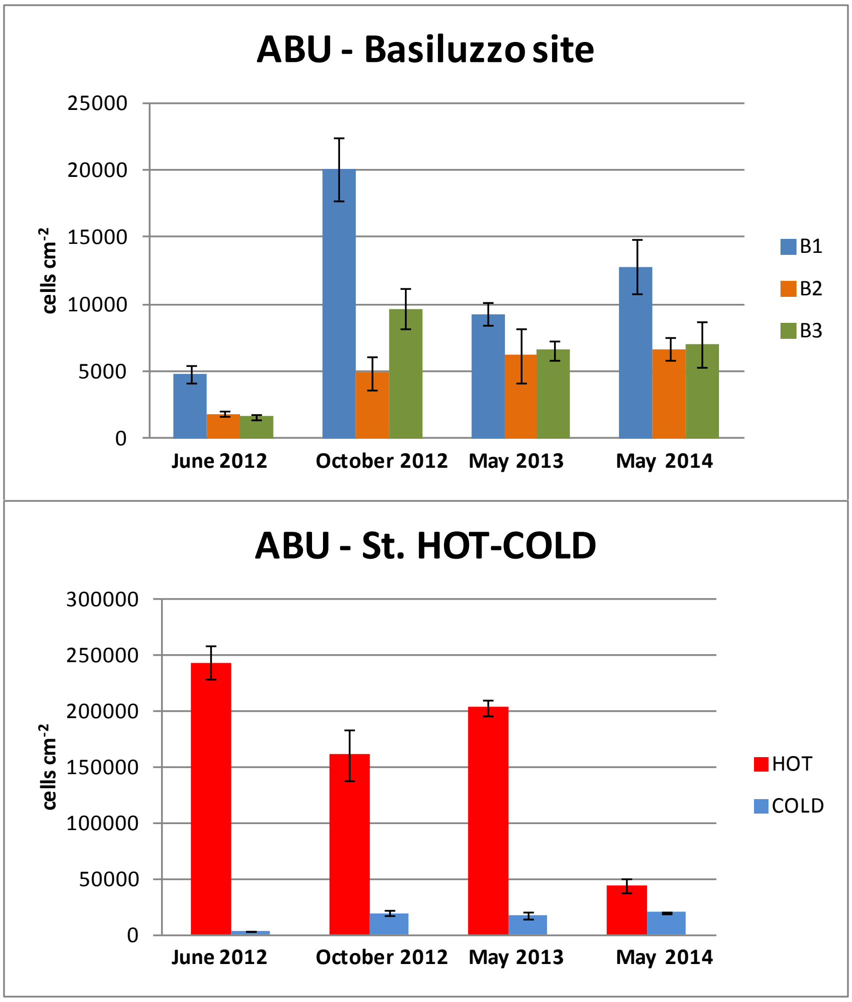

Fig. 3 Microphytobenthic total abundance (ABU) at a) the Basiluzzo sampling site at b) St. HOT and St. COLD during the four sampling periods.

NE of Panarea Island, very remarkable differences in the ABU were recorded between St. HOT and COLD throughout the study (Kruskal-Wallis test: H (1, N= 16) =11.29412, p =0.0008). In June 2012 values at St. HOT reached 243361 ± 14384 cells cm$^{-2}$ and were almost 100 times higher compared to those observed at St. COLD (2510 ± 29 cells cm$^{-2}$) (Figure 3b). Even though the difference in ABU was much lower in October 2012 and May 2013, in both periods the ABU at St. HOT was still about 10 times higher compared to that at St. COLD. In May 2014 the ABU at St. HOT was only twice higher compared to St. COLD, which maintained about the same cell density as in previous periods. Taking into account only the total values of ABU, St. COLD can be considered reasonably comparable to the vent St. B1 at the Basiluzzo site (Kruskal-Wallis test: H (1, N= 20) =1.005952 p = 0.3159).

### *3.4 Microphytobenthic community composition*

Overall, Bacillariophyta dominated the MPB community with a relative abundance (RA) of 97.4% (Figure 4). The lowest percentages were reached in May 2013 at St. B2 (92.0%) and St. B3 (92.5%) mainly on the account of undetermined Phytoflagellates. At St. HOT Bacillariophyta represented more than 99% of the total RA during all samplings (Figure 4b).

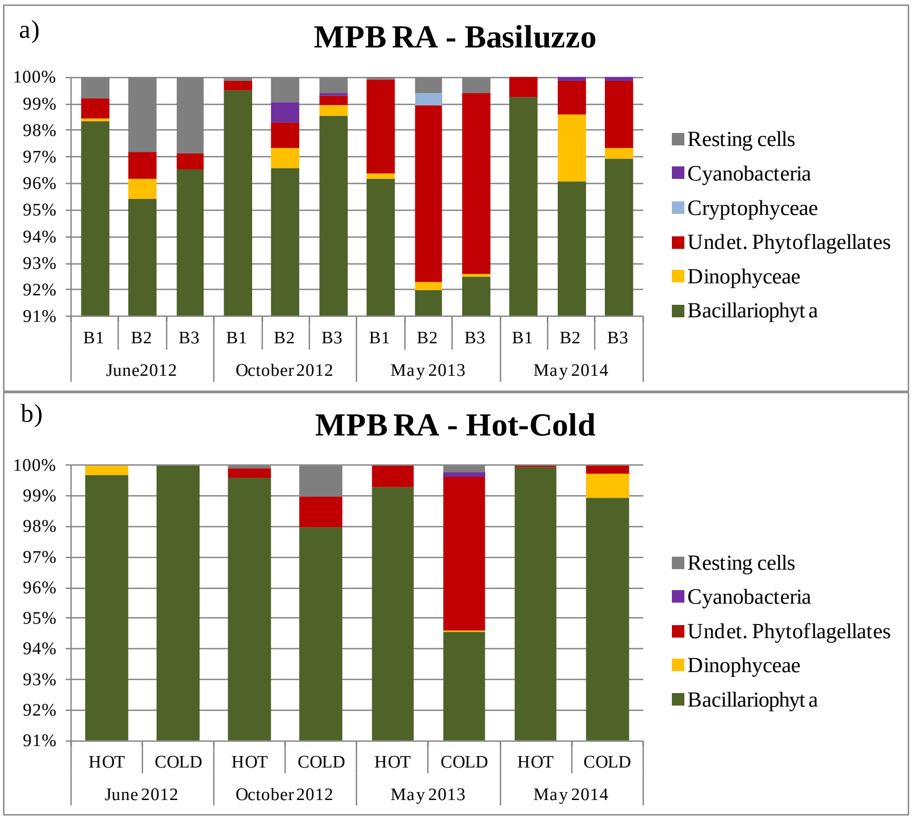

Fig. 4 Microphytobenthic relative abundance (MPB RA) at a) the Basiluzzo and b) the Hot-Cold sampling site: the y-axis starts from 91% to better evidence the contribution of smaller groups.

Since at both sampling sites the MPB community was mainly composed of diatoms and that the other groups were identified only at a very low taxonomic level, the further analyses were focused on the diatom community only. Considering the Basiluzzo and Hot-Cold sites together, a total of 116 taxa belonging to 55 diatom genera were identified (Table 4).

At St. B1 the most abundant genus was *Fragilaria* with a mean RA (of four samplings) of 36.82% that reached its maximum in October 2012 with an RA of 70.7%. The genus *Amphora* was the second most abundant (14.5%), followed by *Diploneis* (9.7%) and *Mastogloia* (8.0%). At the other vent St. B3, the genus *Amphora* was the most abundant (20.6%), followed by *Fragilaria* (15.7%) and genera like *Navicula* (7%), *Nitzschia* (4.5%) and *Ceratoneis* (4.2%) in lower values (Figure 5).

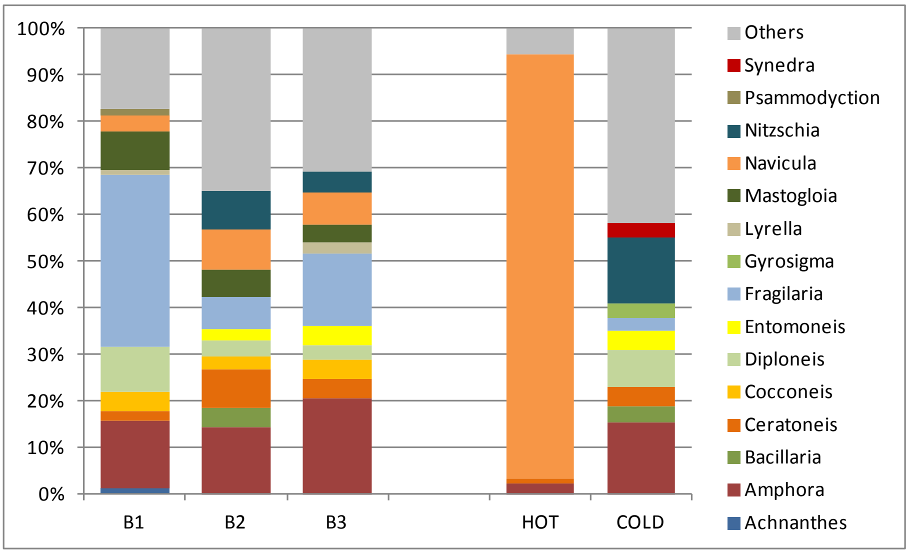

Fig. 5 Diatom genera relative composition (mean at four samplings) at the five sampled stations. Only the ten most abundant genera present at each station (>1% of total abundance) are shown while the less abundant ones are grouped in Others.

At the reference St. B2 genera were more evenly distributed (*Amphora* 14.5%, *Navicula* 8.9%, *Nitzschia* 8.2%, *Ceratoneis* 8.5%) with *Fragilaria* reaching only 6.8% of ABU.

At the vent stations larger diatoms species were present (Plate 1 and 4) compared to the St. B2 (Plate 2). At St. HOT only a small number of genera was found (Plate 3). The predominant genus was *Navicula* and the species *Navicula* cf *cancellata* alone exceeded 23.62% of the total MPB abundance. Two still undetermined species were also present in high abundances (*Navicula* sp.1 with 9.1% and *Navicula* sp.2 with 39.5%) together with a few species belonging to the genera *Amphora* (2.4%), *Ceratoneis* (1.1%) and *Proschkinia* (1%). At St. COLD *Amphora* (15.4%), *Nitzschia* (14.1%) and *Diploneis* (8.1%) were the most abundant genera (Plate 4). Compared to the nearby St. HOT, the abundance of *Navicula* cf *cancellata*, *Navicula* sp. 1 and *Navicula* sp. 2 was lower or negligible (2.2%, 4.4% and 1.4% respectively). To our knowledge, *Navicula* sp. 1 was not described to this date and further taxonomy work is needed for this species determination. On the other hand, *Navicula* sp. 2 showed heavily silicified frustules that rendered difficult its identification. Whenever present, the diatom species with elongated frustules (*Nitzschia longissima*, *Ceratoneis closterium*) also showed teratological changes expressed as curved apices of the valves (Plate 2 i,k,l).

At the Basiluzzo site the diversity indices (Table 2) revealed the lowest diversity at St. B1, the highest at St. B2, and an intermediate value at St. B3. The vent stations were not only characterized by a lower diversity but also by an uneven distribution of species with a small number of predominant ones. At St. HOT and COLD diversity indices showed an opposite pattern. At St. HOT richness and diversity were very low along with high values of dominance, due to the extremely reduced number of species observed at this site. Conversely, at St. COLD high diatom richness and diversity were calculated with an even species distribution. There was no evident temporal pattern in the variation of Shannon (*H'*) diversity index at the sampling stations either at Basiluzzo or at Hot-Cold sampling site.

Table 2 Mean values (of the four sampling periods) and standard deviation of diversity indices at the Basiluzzo and Hot-Cold sampling sites: S-number of species; N-number of diatoms; d-Margalef's index; J'Pielou's index; H'Shannon-Wiener diversity index (Log2) and Simpson's dominance index ($\lambda$).

| **Station** | **S** | **N** | **Richness** | **Equitability** | **Diversity** | **Dominance** |
|---|---|---|---|---|---|---|
| | | | D | J' | H' | Λ |
| **B1** | 23 ± 2 | 10293 ± 6277 | 2.51 ± 0.28 | 0.57 ± 0.16 | 1.80 ± 0.53 | 0.29 ± 0.21 |
| **B2** | 32 ± 6 | 3476 ± 1620 | 3.86 ± 0.44 | 0.81 ± 0.03 | 2.79 ± 0.13 | 0.09 ± 0.01 |
| **B3** | 25 ± 5 | 4630 ± 2727 | 2.91 ± 0.42 | 0.73 ± 0.15 | 2.34 ± 0.49 | 0.17 ± 0.11 |
| **HOT** | 11 ± 5 | 131354 ± 70634 | 0.9 ± 0.51 | 0.44 ± 0.07 | 1.02 ± 0.21 | 0.44 ± 0.09 |
| **COLD** | 34 ± 8 | 11452 ± 6356 | 3.68 ± 0.88 | 0.82 ± 0.08 | 2.88 ± 0.25 | 0.09 ± 0.03 |

At the Basiluzzo sampling site, the *k*-dominance curves were more even, but with slightly steeper curves at St. B1 and more sinusoidal profiles at the reference St. B2 (Figure 6).

The curves at St. HOT revealed a typical shape of a low-diversity community, in contrast to the semi-sinusoidal profile of the curves at St. COLD, typical of a high-diversity community. At St. HOT a cumulative dominance of 80% was attained with only 4 taxa and in October 2012 with just 2 different taxa (Figure 7).

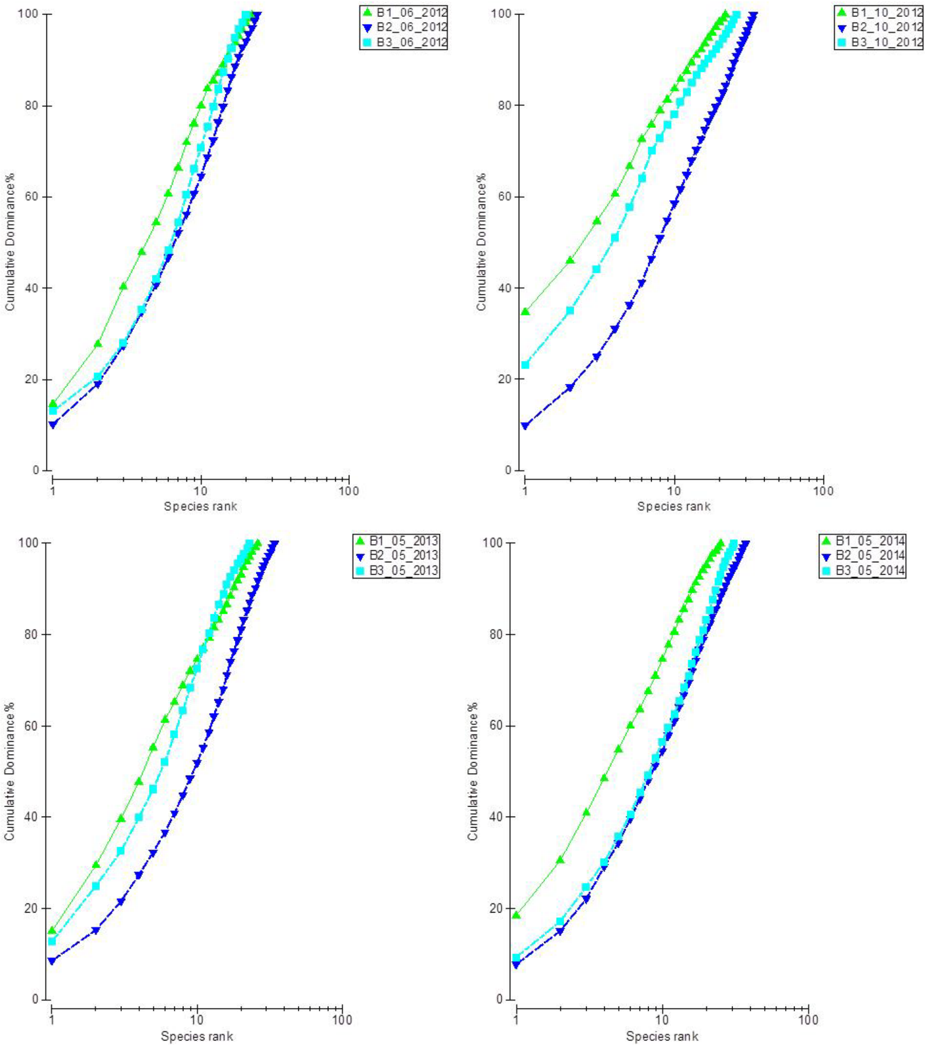

Fig. 6 *k*-Dominance curves calculated on the species abundance matrix at the Basiluzzo site.

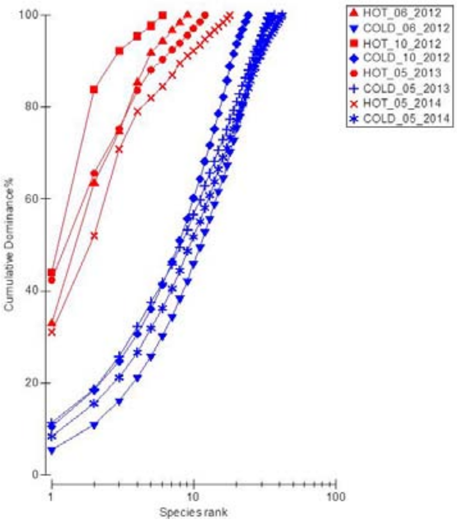

Fig. 7 *k*-Dominance curves calculated on species abundance matrix at St. HOT and COLD.

The two-way crossed ANOSIM revealed that differences in the structure of the diatom assemblages among sites were significant ($R$ = 0.921, p = 0.1 %); whereas the difference among sampling periods was not ($R$ = 0.297, p = 6.5 %).

The SIMPER routine was performed to calculate the average similarity (S) among the diatom communities at each site influenced by different sampling periods, and also the average dissimilarity (δ) between pairs of stations (Table 3). The average similarity percentages ranged from 46.6 to 62.7% with the genus *Amphora* contributing the most at St B2, B3 and COLD while at St. B1 and St. HOT *Fragilaria* predominated at the first station and *Navicula* sp. 2 at the latter one. The lowest intra-site dissimilarities occurred at the Basiluzzo sampling site (45.1 – 50.3 %) where the genus *Fragilaria* contributed the most at St. B1 and *Amphora* at St. B2 and St. B3. The highest inter-site dissimilarities were obtained between St. HOT and all the others stations (77.5 – 84.2 %) due to *Navicula* sp. 2.

Table 3 SIMPER analysis; In gray are shown the similarity values of diatom communities within each site depending on the sampling period (%) and below the diagonal are reported the dissimilarity values between pair of sites (%).

| | **B1** | **B2** | **B3** | **H** | **C** |
|---|---|---|---|---|---|
| **B1** | 62.74 | | | | |
| **B2** | 50.31 | 54.30 | | | |
| **B3** | 45.06 | 46.74 | 49.59 | | |
| **HOT** | 83.02 | 84.15 | 84.00 | 55.22 | |
| **COLD** | 63.83 | 58.97 | 63.94 | 77.56 | 46.63 |

The RELATE routine showed no correlation of the diatom community structure to water temperature at the bottom, depth or sediment grain size (ρ = 0.14, p =22.9%).

At the Basiluzzo site, the motile forms were the most represented during all study periods with the highest percentage at St. B2, followed by vents at St. B3 and B1 with RA of 34.65%, 30.33% and 26.98%, respectively (Figure 8a). The erect, adnate and motile forms showed little or no differences among the three stations. The tychopelagic form was more abundant at St. B2 with 13.92% compared to St. B1 and B3 with 2.01% and 5.27% respectively. *Bacillaria paxillifera, Ceratoneis closterium* and *Paralia sulcata* were the main representatives of this form. On the other hand, the planktonic form, mainly on the account of the genus *Fragilaria*, was more abundant at St. B1 (37.76%) and less abundant at St. B2 (7.41%). The values at St. B3 were again in-between the latter two (16.28%).

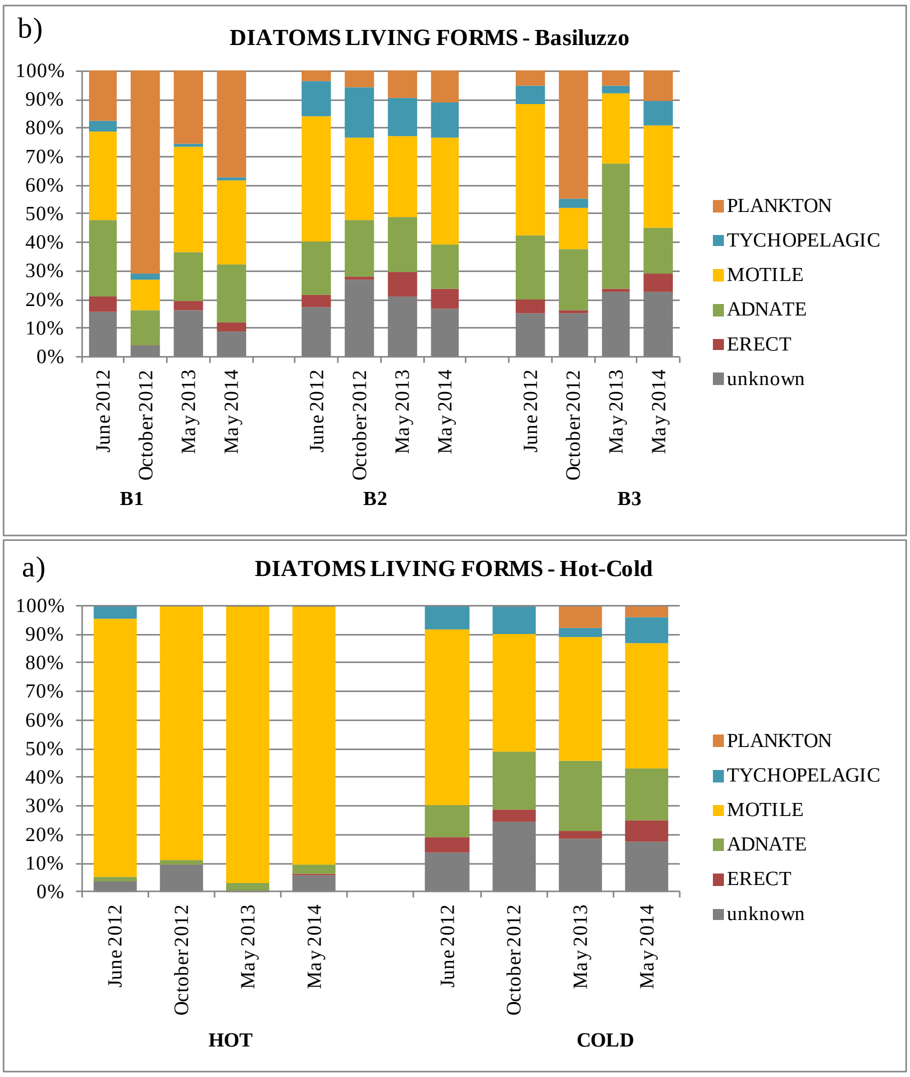

Fig. 8 Relative abundance (in percentage) of the diatom living forms at a) the Basiluzzo and b) the Hot-Cold sampling site during the four sampling periods. The unknown group gathers undetermined Pennate and Centric diatoms.

Even more pronounced were the differences between St. HOT and COLD where the motile form clearly predominated with an RA of 91.47% and 46.36%, respectively (Figure 8b). All the other living forms were very under represented at St. HOT. The RA of diatom living forms at the St. COLD were comparable to those at the reference St. B2 at the Basiluzzo sampling site.

### *3.5 Primary production (PP)*

This MPB metabolic process was estimated in October 2012 at Hot-Cold and in May 2013 at the Basiluzzo sampling site. The observed PP rates strongly depended upon the MPB abundance (Figure 9). At St. HOT primary production rates were six times higher (45.79 ± 6.14 mgC $m^{-2}$ $h^{-1}$) than at St. COLD (7.41 ± 0.13 mgC $m^{-2}$ $h^{-1}$). The PP rates at the

Basiluzzo stations also followed the abundance pattern and were comparable with the value estimated at St. COLD.

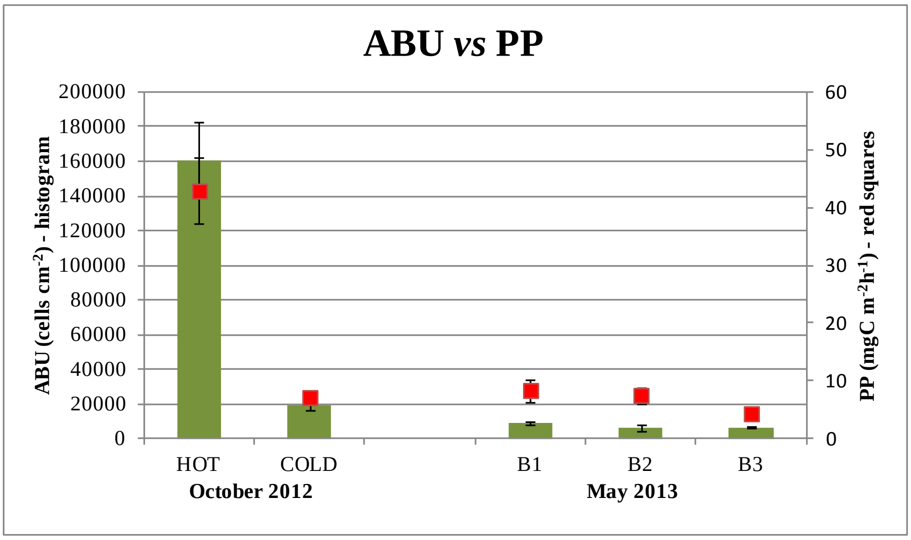

Fig. 9 Microphytobenthic total abundance (ABU) coupled with microphytobenthic primary production (PP) rates at Hot-Cold in October 2012 and at the Basiluzzo sampling site in May 2013.

## 4 Discussion

In this study area the grain size, particularly the sand fraction, was quite variable among stations and sampling periods. This is mainly due to the strong hydrodynamics of this area, especially at the smaller scale, responsible for the continuous changing of the sediment bottom morphology. Even though depth, grain size and light availability have been previously demonstrated as important selection factors (Cibic et al. 2007; Miles and Sundbäck 2000), they did not show a significant role (RELATE, $\rho = 0.14$, $p = 22.9\%$) on the community composition at this study site. The light availability was not a limiting factor at the sampling stations since the measured irradiance at the bottom, with respect to the surface irradiance, was always above 22%, meaning that both depth and water transparency ensured enough light penetration to the sea bottom throughout the study period and hence the development of an active microalgal community.

Our study on the effects of $CO_2$ and temperature on MPB communities revealed very distinct differences in their structure and functioning among the sampled stations situated close to Panarea and Basiluzzo Islands. While according to our results the effect of the temporal variation on MPB proliferation was weak (two-way crossed ANOSIM test: $R$ = 0.297, p = 6.5 %), the $CO_2$ concentration and temperature were the only significant variables (two-way crossed ANOSIM test: $R$ = 0.921, p = 0.1 %) for the MPB development. At the Basiluzzo sampling site, the influence of $CO_2$ emissions on the ABU was particularly evident at St. B1 (p = 0.0055). Here, the cell densities were from two to even four times higher compared to the reference St. B2. Similarly to our findings, Johnson et al. (2013) reported higher MPB abundances with increasing $CO_2$ levels on biofilms that colonised slides anchored nearby the Vulcano Island coast. On the other hand, we have not observed such difference in ABU between St. B2 and B3. Even though St. B3 is a few meters deeper than the other two sites, the irradiance and temperature were similar to those measured at St. B2 and B1. Furthermore, previous research carried out at the same sampling stations revealed just a slight difference in the composition of the released gas (94% $CO_2$ at St. B1 and 91% $CO_2$ at St. B3) (Weber et al. 2011) but a difference in the $CO_2$ out-flow that was weaker or less constant at St. B3 (Queirós et al. 2014). The gas variability in concentration and its composition may therefore influence the communities' structure, as already hypothesised by other authors (Johnson et al. 2013; Kerrison et al. 2011). Besides, other substances like reduced compounds ($H_2S$, $CH_4$, $H_2$), nutrients ($SiO_3^{2-}$, $NH_4^+$, $NO_3^-$, $PO_4^{3-}$) or metals ($Mn^{2+}$, $Fe^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $Pb^{2+}$, etc.) could have influenced the ABU (Tarasov et al. 2005). For instance Admiraal et al. (1979) reported tolerance to a different degree in individual benthic diatom species to the toxic effects of $H_2S$.

The grazing effect of meiofauna is also a very important factor for the overall microphytobenthic abundance (Montagna et al. 1995). It is very likely that the high MPB abundance at St. HOT was a further result of a very reduced meiofaunal pressure at this station (unpublished data) as meiofauna probably cannot cope with the extreme conditions of the thermal vents. Meiofaunal abundance and diversity were reported to be negatively influenced by vents also in the study by Zeppilli and Danovaro (2009) in the Pacific Ocean and even more by Colangelo et al. (2001) whose research was carried out nearby the Panarea Island.

In this study, the MPB was mainly composed of diatoms, with some representatives of undet. Phytoflageallates, Dinophyceae, Cyanobacteria and resting cells that all together never exceeded 8% of ABU. At this taxonomic level, Cyanobacteria showed no evident response to $CO_2$ concentration changes and since their abundance was negligible at St. HOT we assume that they could not cope with the extremely high temperature. In some studies an increase of Cyanobacteria was observed in presence of higher $CO_2$ (Cartaxana et al. 2015; Hutchins et al. 2007; Kranz et al. 2010). However, we could not detect a positive response of Cyanobacteria to $CO_2$, in accordance with the results of Johnson et al. (2013), who also found that this microalgal group remained scarcely represented (<2%) at all stations. This may be due to the fact that besides the high $CO_2$ concentration, Cyanobacteria need also higher water temperatures to proliferate (Watermann et al. 1999). Indeed, in the experiments performed by Cartaxana et al. (2015); Hutchins et al. (2007) and Kranz et al. (2010) microalgae were incubated at 24-25°C, whereas at the Eolian Islands the temperature at the bottom was around 18°C, both in our study and in that of Johnson et al. (2013). Also Tortell et al. (2002) reported a community dominated by diatoms under high $CO_2$ conditions (pH 7.9) before they shifted to the non-siliceus phytoplankton taxa, particularly *Phaeocystis*, when lowering the $CO_2$ concentration (pH 8.5). Other studies (Gao et al. 2012; Kim et al. 2006; King et al. 2011; Riebesell et al.

1993; Schippers et al. 2004; Wu et al. 2010) also confirmed the diatom affinity for higher $CO_2$ levels.

Focussing on diatoms, at St. B1 and to some degree at St. B3, small-sized *Fragilaria* predominated over the otherwise most represented genera (*Amphora*, *Navicula* and *Nitzschia*) at the reference station and contributed the most to the average dissimilarity among the Basiluzzo stations. Other taxa that contributed to the average dissimilarity between St. B1 and B2 were *Diploneis*, *Amphora*, *Cocconeis* and *Mastogloia* for St. B1 and *Bacillaria paxillifera* and *Nitzschia longissima* for the reference St. B2. The dissimilarity between St. HOT and St. COLD was mostly due to the account of only three species belonging to the genus *Navicula* (*Navicula* cf *cancellata*, *Navicula* sp 1, *Navicula* sp 2). Interestingly, high densities of *Navicula* species were observed in areas with remarkable concentrations of $H_2S$ (up to 4% in volume) such as those encountered at St. HOT. This gas is highly toxic to all living organisms (Bagarinao 1992), and the dominance of this genus only at this particular site indicate its high tolerance to $H_2S$. Previous research from Cibic et al. (2008a) on MPB response to sediment pollution in the Gulf of Trieste reported the genus *Navicula* to be more resistant to stress as it dominated in the diatom community with more than 69% of abundance at the polluted sites. Previously, *Navicula* showed to be less sensitive to pollution compared to two other species during an experiment on sediment toxicity (Moreno-Garrido et al. 2007). Also other studies obtained similar conclusions (Agatz et al. 1999; Cunningham et al. 2005; Palmer 1969), so further efforts should be directed to the understanding of the ecophysiological adaptations of this particular genus that has a wide ecological valence.

Johnson et al. (2013) reported the presence of large and chain-forming pennates (*Toxarium* and *Grammatophora*) at stations with higher $CO_2$ concentration. In our study, the genus *Toxarium* was not encountered and *Grammatophora* was only present at the reference station but never exceeded 1.8% of ABU. Notwithstanding, we observed many other larger genera like *Surirella* sp., *Campylodiscus* sp., *Biddulphia* sp. and *Diploneis* sp. (plate 1) at the Basiluzzo vent stations. The presence of larger representatives of some species was also noticed by Raghukumar et al. (2008) at the white-coloured sediment stations of the hydrothermal vent in the Azores.

To the best of our knowledge, there are no reports in the literature on the effects of naturally occurring high temperature on benthic diatom assemblages. However, studies on microphytoplankton historical data (Winder et al. 2009), fossil records (Falkowski and Oliver 2007) and dinoflagellate cyst fossils (Finkel et al. 2007) indicate that high temperature promotes smaller sized cells. According to the hypothesis by Winder et al.(2009) the abiotic drivers affect the size structure of planktonic communities and warmer climate favours small-sized diatom cells with a high surface area to volume ratio. The diatom cells observed at St. HOT were mainly small, rarely exceeding 30 µm, and we can infer that the cell size reduction is an adaptive strategy linked to the high temperature.

Despite higher abundances at the Basiluzzo vent stations, very low species diversity and richness were obtained, in agreement with Johnson et al. (2013). Just a few dominant species represented the main share of the abundance value. This was even more pronounced at St. HOT where a very low mean number of taxa over the study period (11 ± 5) was calculated, indicating that only a few taxa were able to adapt to these extreme environmental conditions. Raghukumar et al. (2008) observed that the hydrothermal vent with lower temperature (~37°C) in the Azores was characterised by higher MPB diversity compared to the one with higher temperature (~60°C). Also Tarasov et al. (2005) summed up that diversity (bacterio-, phyto, holo- and zooplankton) in shallow thermal vents is low compared to background conditions even though the abundance and biomass are higher. On the other hand, Estradas-Romero and Prol-Ledesma (2014) showed higher species richness of benthic diatoms at the hydrothermal vent sites compared to control locations off the Mexican coast.

The motile diatoms were the most represented living forms at all stations not only considering their mean share in ABU but also the number of taxa. Motile forms were well represented at St. HOT with 91.5 ± 3.3% of RA but in correspondence with the lowest number of observed taxa (S = 8.8 ± 3.8). Here, the genus *Navicula* alone constituted 89.5 ± 3.7% of ABU with only three different species (*Navicula* cf c*ancellata*, *Navicula* sp.1 and *Navicula* sp. 2). Interestingly, St. COLD, situated just one meter from St. HOT, displayed a completely different picture. Here the motile group was represented by a mean number of 26.3 ± 7.1 taxa reaching the absolute maximum of all the investigated stations. Erect and adnate diatoms were quite unvarying among stations and sampling periods, but the presence of tychopelagic (living loosely associated with the substrate) and planktonic forms indicated different assemblages at the station level. The highest abundance of tychopelagic forms, namely *Bacillaria paxillifera*, *Ceratoneis closterium*, and *Paralia sulcata* at the reference St. B2 and their almost complete absence at the vent stations was probably due to their inability to remain attached and therefore they were likely swept away by the bubbles arising from the vents. On the other hand, the small planktonic *Fragilaria*, with its chain-forming strategy that prevented them from being swept away, were able to proliferate in this high $CO_2$ conditions at St. B1.

In our study we observed another peculiarity linked to this particular environment i.e. a difference in the degree to which diatom frustules were silicified. The amount of silica in a diatom species can vary substantially, by up to 4-fold (Martin-Jézéquel et al. 2000). Durbin (1977) showed that clones of diatoms of a given size grown at a lower temperature had increased silicification relative to those grown at a higher temperature, implying that frustules were thicker. The frustules we observed under the light microscope at the vents stations, and especially at St. HOT, appeared more silicified than those from other marine environments (Cibic, personal comment). Especially the frustules of the species *Navicula* sp. 2 were so silicified that the microstructures looked occluded with unrecognizable specifics needed for the taxonomic identification. We applied different diatom frustule cleaning methods with subsequent SEM observations to exclude any possible methodological error, but the result was unchanged. This made the determination of some species very difficult or even impossible. The higher silicification of the frustules at the vents is in accordance with the results of Cibic and Blasutto (2011) who reported that among all the tested diatoms some species of the genus *Navicula* displayed a high degree of membership with $SiO_2$ (92.5) compared to other inorganic nutrients.

Another feature, observed only during this study, was the frustule deformity in some species that are characterised by elongated apices (*Nitzschia longissima*, *Ceratoneis closterium* and *Gyrosigma fasciola*). Several of these cells presented curved frustule apices that looked like broken (Plate 2 i,k,l). It is well known that some substances present in solution in the marine waters, act as toxicant for some organisms such as diatoms causing morphological deformities of the frustule (Dickman 1998; Laws 2003). Unfortunately, we do not have data on the gas geochemical composition from these vents to link them to the observed deformities. Only by performing specific experiments it would be possible to identify what particular compound mainly affects the induced changes. However, we hypothesize that the main driver could be the low pH levels. In fact, similar morphological deformities of the frustule were already observed by Cibic and Virgilio (2011) during an experiment that aimed to standardise sample acidification with HCl in primary production estimates. In their samples *Cylindrotheca fusiformis* presented deformed apices following a mild HCl treatment (0.1N, pH ~ 4) while none were observed in the reference treatment.

Primary production (PP) rates followed the same dynamics as ABU and showed slightly higher values at the Basiluzzo vent stations. The higher PP rates at both vents St. B1 and B3, compared to the reference station suggest a positive influence of the $CO_2$ on this process. Even though the PP estimates were carried out only once at each station, they still give us a good indication of this photoautotrophic pathway. Our data are in accordance with those reported by Chen and Durbin (1994) who, through an experiment on two species of *Thalassiosira*, showed that the carbon uptake rates of the natural assemblages were enhanced lowering the pH from ambient levels (pH 8.8 to 8.9) to lower levels (pH 8.0 to 8.3). Furthermore, elevated $CO_2$ concentrations under low or moderate levels of PAR have been shown to enhance photosynthesis or growth of both phytoplankton and macroalgae as summed up by Gao et al. (2012).

There is very little literature on the combined effects of $CO_2$ and high temperature on the MPB communities from in situ studies. Previous research on MPB biomass and photosynthetic performance under increased $CO_2$ and temperature carried out experimentally or *in vivo*, has led to various conclusions. Cartaxana et al. (2015) showed a detrimental effect of temperature on MPB biomass and PP under control (pH 8.0) and elevated $CO_2$ (pH 7.4). Chl *a* concentrations and PP values were higher at lower temperatures (18°C) and pH (pH 7.4) and lower at higher temperatures (24°C) regardless of the pH, indicating temperature as the most influencing factor. In addition, the laboratory experiments on *Navicula directa* carried out by Torstensson et al. (2012) showed that elevated temperature alone promoted effective quantum yield of photosynthesis and increased this species growth rates. The authors found no synergistic effects between temperature and $pCO_{2.}$ However, in their study the used temperature range was very low (0.5 – 4.5°C) compared to the temperatures at the Aeolian Islands. Our results were more straightforward as ABU and PP were both significantly promoted by higher temperatures at St. HOT.

## 5 Conclusions

Our study showed a significant influence of the gas vents with high $CO_2$ concentration, and even more a selective response to combined effect of high $CO_2$ and temperature at the hydrothermal vents, on the MPB community composition and total abundance. This influence was also mirrored in increased PP rates, especially at the hydrothermal vent. Even though the MPB diversity was lowered, the adapted species, especially those belonging to the genus *Navicula*, were capable to survive in very high cell densities and take advantage of the continuous nutrients outflow from the vents showing a high photosynthetic activity. Further studies should be carried out to determine the influence of other specific variables present in this extreme environment, like trace gases, nutrients and metals on the MPB community. In this study several diatoms with morphological deformities were also found that are an indicator of generic stress. Moreover, new diatoms species were observed in this study and their identification is ongoing.

We have to point out that the MPB communities at the natural laboratory of Basiluzzo had time to adapt to high $CO_2$ conditions and a highly specific community has developed. In contrast, the MPB communities present in an area where a leakage from a shallow water CCS site could occur would not have the possibility to rapidly adapt to a $CO_2$ outburst. Furthermore, sooner or later the carbonates present in the sediments of a CCS site would likely dissolve and their buffer effect (Cibic and Virgilio 2010) would cease, causing a direct impact on diatom cells, both in terms of their morphology and metabolic functioning. For this reason, in case of a leakage the initial MPB community would most probably change over time and shift to a high-$CO_2$ adapted photosynthetic community that would thereafter provide the basis of an alternative trophic web.

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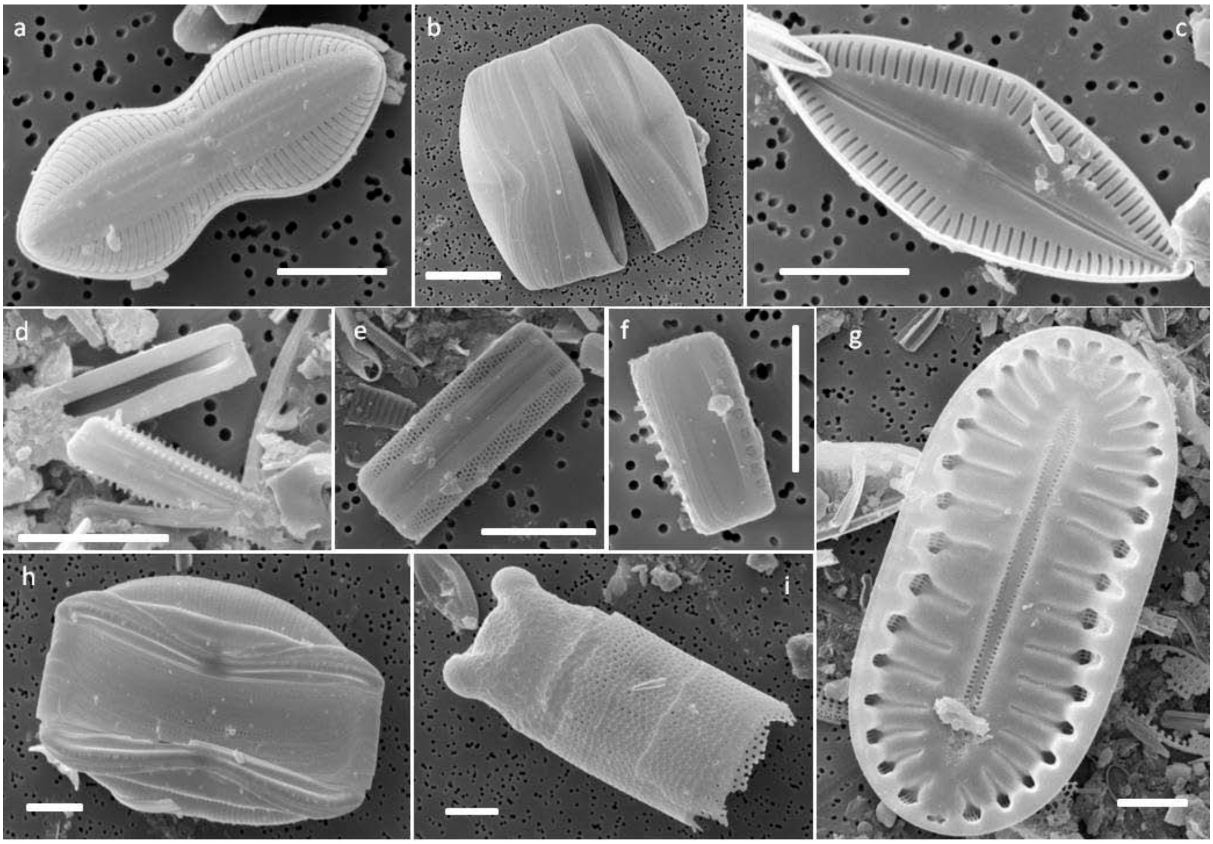

Plate 1 Benthic diatoms at St. B1 and B3 close to Basiluzzo Island.
Large species found at the vent stations B1 and B3: (a) *Diploneis* cf *stroemi*, external valve view, SEM; (b) *Amphora* sp., external view, SEM; (c) *Navicula palpebralis*, internal valve view, SEM; (d) *Fragilaria* sp., girdle view, SEM; (e) *Grammatophora* sp., girdle view, SEM; (f) *Fragilaria* sp., girdle view, SEM; (g) *Surirella* sp. internal valve view, SEM; (h) *Amphora* sp., external girdle view, SEM; (i) *Biddulphia* sp., external girdle view, SEM. (Scale bars = 10 µm (a,c,d,e,f,g,h,i); 20 µm (b).

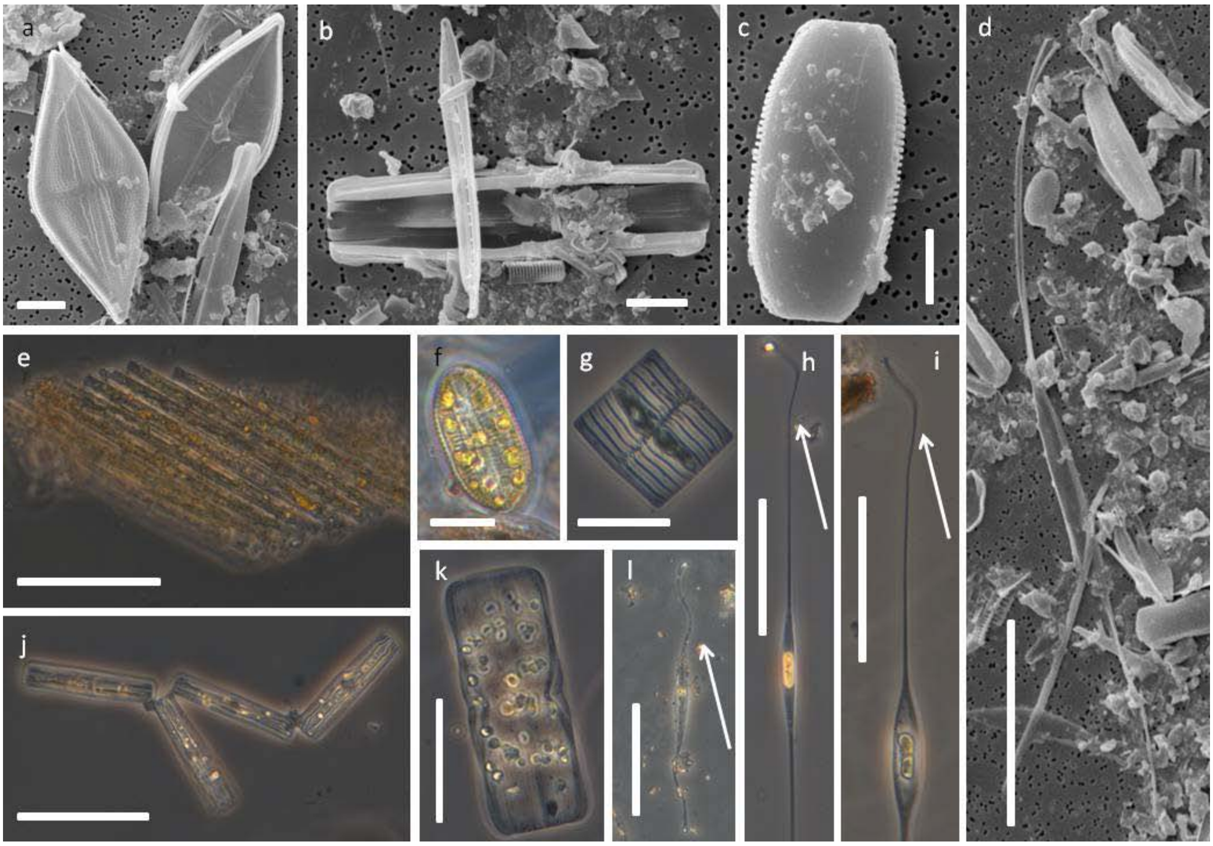

Plate 2 Benthic diatoms at the reference St. B2 close to Basiluzzo Island.
(a) *Mastogloia jelinecki* internal and external valve view, SEM; (b) *Nitzschia spathulata* in valve and gridle view, SEM; (c) *Amphora* sp. external girdle view, SEM; (d) *Nitzschia longissima*, valve view, SEM; (e) A colony of *Bacillaria paxilifera*, LM; (f) *Diploneis* sp., LM; (g) dividing *Hyalosira interrupta*, LM. (h,i) *N. longissima* with deformed frustule apices (arrow), LM. (j) A colony of *Grammatophora* sp., LM. (k) *Undatella* cf *quadrata*, LM. (l) *Gyrosigma* sp. with curved frustule apices (arrow), LM. Scale bars = 10 µm (a,b,c); 20 µm (f,g); 50 µm (d,e,h,i,j,k,l).

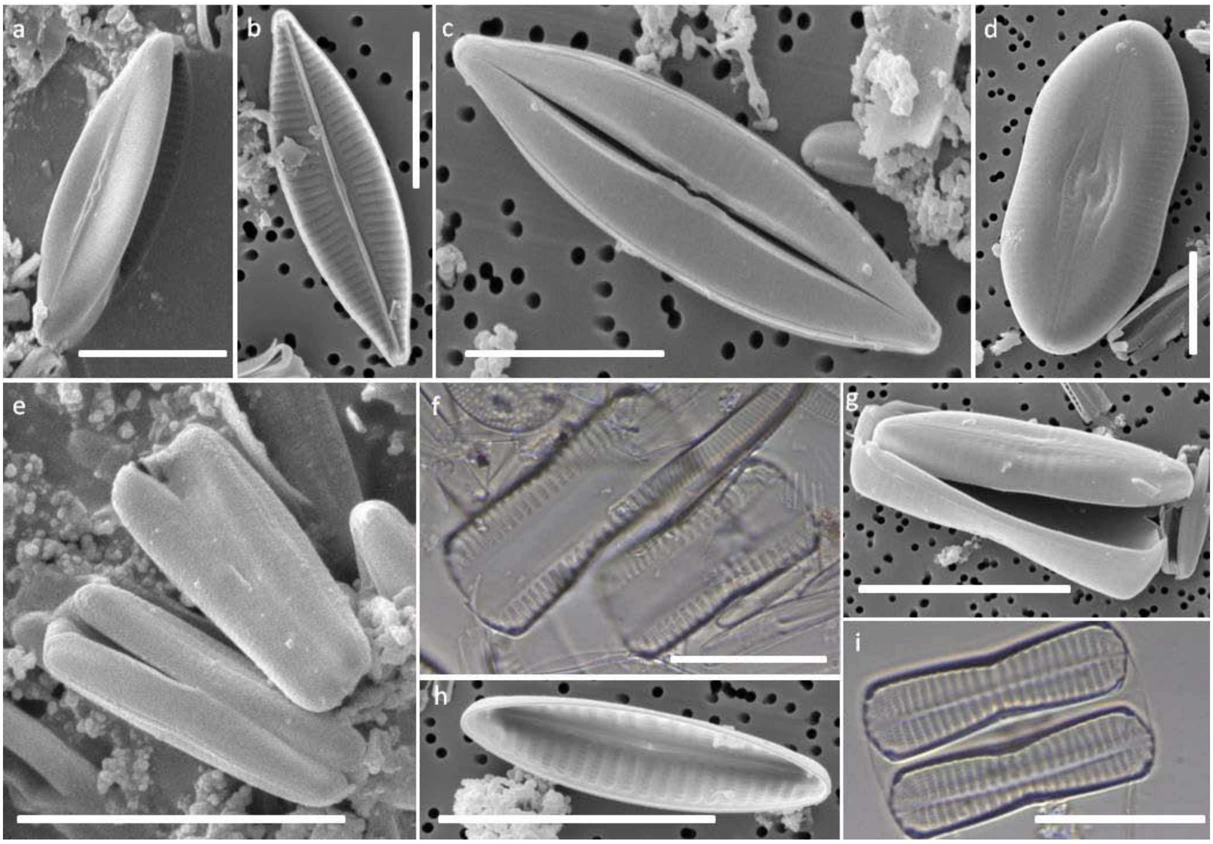

Plate 3 Benthic diatoms at St. HOT northeast from the Panarea Island.
(a-c) *Navicula* sp. 1 in external (a,c) and internal (b) valve view, SEM. (d) Heavily silicified *Diploneis* sp. with effaced frustule micro-structures, external view SEM. (e) *Navicula* sp. 2 in girdle view showing teratologic characteristics of the frustules, SEM. (f-i) *Navicula* cf *cancellata* in girdle view, LM (f), valve view of the opened frustule, SEM (g), internal part of the valve, SEM (h) and cell division, LM (i). Scale bars = 10 µm (a,b,c,d,e); 20 µm (f,g,h,i).

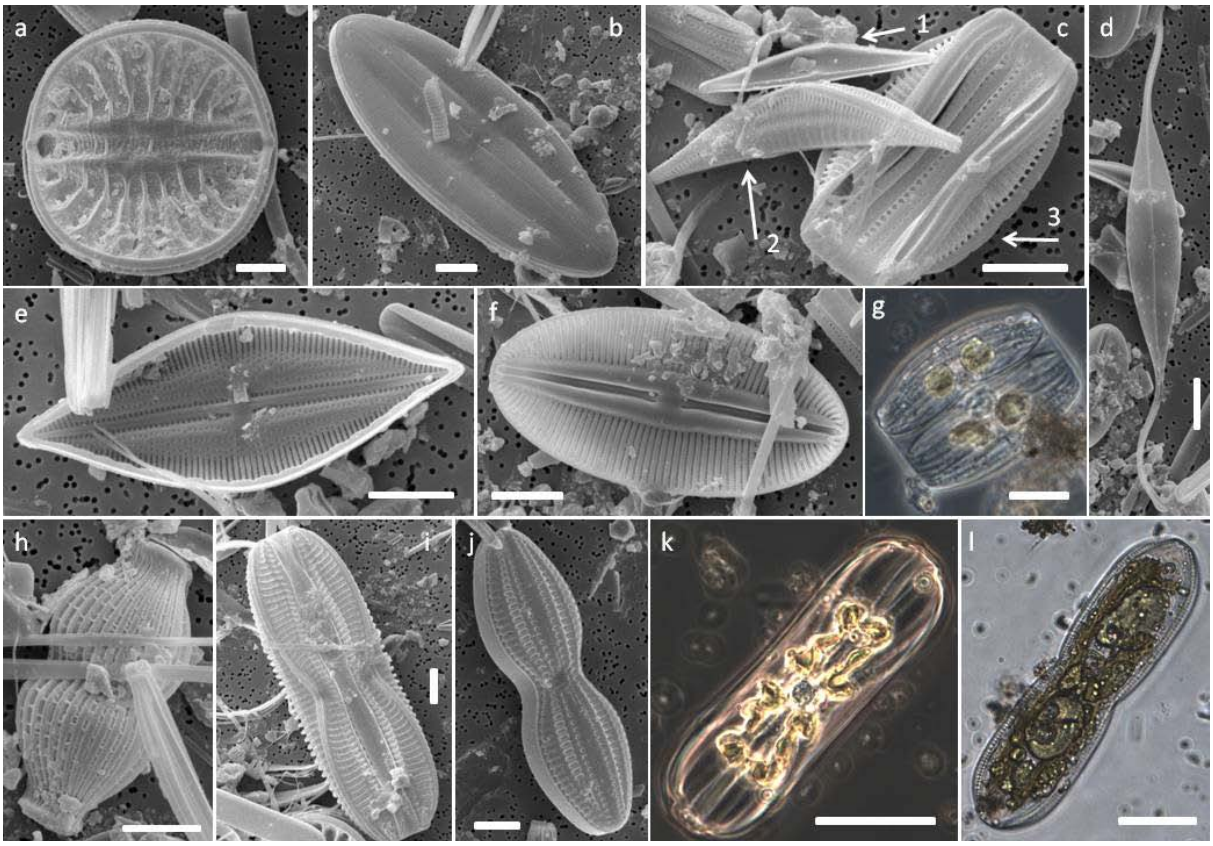

Plate 4 Benthic diatoms at St. COLD northeast from the Panarea Island.
Large and heavy genera of benthic diatoms like (a) *Campylodiscus* sp., internal valve view, SEM; (b) *Lyrella* sp., external valve view, SEM; (c) *Navicula* sp. (arrow 1), *Rhopalodia* sp. in valve view (arrow 2) and *Amphora* sp. (arrow 3), SEM; (d) *Gyrosigma* sp., girdle view, SEM; (e) *Mastogloia jelinecki* internal valve view, SEM; (f) *Diploneis* cf *smithii* internal valve view, SEM; (g) *Amphora* sp. during cell division, LM (h) *Amphora bigibba*, external view SEM; (i) *Amphora* sp., external view, SEM; (j) *Diploneis crabro* internal valve view, SEM; (k) *Amphora* sp., LM; (l) *Nitzschia* sp. LM. Scale bars = 10 µm (a,b,c,d,e,f,g,h,i,j); 20 µm (g); 50 µm (k,l).

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# CHAPTER 2

## Rogelja M., Cibic T., Rubino F., Belmonte M., Del Negro P.: Active and resting microbenthos and main functional processes within a harbour area in the northern Adriatic Sea, Italy

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# Active and resting microbenthos and main functional processes within a harbour area in the northern Adriatic Sea, Italy

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## Abstract

To gain new insight into the benthic ecosystem functioning of an area subjected to multiple and diffuse contamination, in June 2013 seven stations were sampled within the Port of Trieste, and a reference station within a marine natural reserve. Even though no major differences in total abundances of active microbenthos were observed among stations, a selection of stress-resistant species nearby the main productive activities was highlighted, with *Nitzschia* cf. *commutata* being the most represented. The diatom genus *Gyrosigma*, that becomes opportunistic in organically enriched sediments, was likely too sensitive for the high contamination levels and its specimens were reduced from the assemblage. The highest densities of the resting microbenthos were registered near the industrial area, with key species like some potentially toxic *Alexandrium*, the heterotrophs and some calcareous-walled species, *i.e. Calciodinellum albatrosianum*, that were clearly linked to contamination. The photosynthetic capability of the microphytobenthic community did not seem to be affected by contamination at stations close to the port and industry infrastructure whereas light availability at the seafloor confirmed to be the main driver of this photoautotrophic process. In contrast, the reduced thickness of the oxic layer and the maximum oxygen consumption rates observed at the same stations were likely ascribable to high TOC contents and the presence of metals in reduced chemical form. Although our results are necessarily case-specific, depending upon the particular features of the Port of Trieste, we believe they may have broader implications and be extended to other contaminated areas in which similar findings are expected.

**Keywords:** Microphytobenthos, Plankton's Resting stages, Benthic primary production, Benthic respiration, Anthropogenic pollution, Port of Trieste

## 1 Introduction

The concern regarding the impacts of wastewater and effluents of urban, industrial and agricultural activities is constantly growing. This problem is even more pressing when the environment is characterised by low hydrodynamism typical of a shallow and enclosed bay subjected to constant sediment resuspension caused by port activities. The sediments act as a sink for contaminants and frequently contain higher concentrations of pollutants compared to the water column (Tolun et al. 2001) causing a negative effect on the biological diversity and the functioning of coastal ecosystems (Araújo et al. 2010). Benthic organisms living in the sediment or on its surface may be exposed not only to chemical compounds dissolved in the overlying water or in the pore water, but also to contamination by direct contact with toxic substances adsorbed to sediment particles or by ingestion of those particles (Macken et al. 2008). 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. A highly specialised 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. 2012b). Monitoring the impact of contaminants on aquatic life forms is challenging due to the different degree of sensitivity of organisms to a given contaminant, and so is the assessment of the long-term effects of persistent contaminants on the ecosystem as they are bioaccumulated along the trophic chain. Marine microalgae are a particularly promising indicator of organic and inorganic contamination since they are one of the most abundant life forms in aquatic environments and represent the base of the food chain (Torres et al. 2008). Diatoms in particular occur in all types of aquatic ecosystems, their assemblages are diverse according to the environmental status and therefore contain considerable ecological information. They are characterised by one of the shortest generation times of all biological indicators that allows them to rapidly respond to environmental changes (De la Rey et al. 2004). Consequently, they have been increasingly used as indicators of different kinds of stress. For instance, microphytobenthic response to different nutrient concentrations was studied for the assessment of the impact of river inflows (Cibic et al. 2012b) and sewage discharge(Cibic et al. 2008a). Furthermore, changes in the community structure have been observed in areas devoted to mussel farming (Franzo et al. 2014). The sensitivity and quick response of benthic diatom communities to metal and petroleum hydrocarbon contamination was also observed by Cunningham et al. (2003) and recently by Potapova et al. (2016). However, most of the ecotoxicological studies on diatoms are experimental and restricted to small study areas and more in-situ tests on larger spatial scales are needed (Rimet 2012).

Plankton’s resting stages (commonly named cysts) offer a different point of view of the ecosystem status. They are produced by planktonic organisms in the water column but sink to the bottom where they accumulate like seeds of terrestrial plants, reaching densities of millions of cysts per square metre of bottom (Rubino et al. 2000). For this reason, cysts analysis allows us to gain information complementary to that obtained by holobenthos (organisms spending the whole life cycle at the sea bottom). The microalgal resting stages can give us additional information on the past environmental status of an area due to their capacity to remain viable for even centuries (Ribeiro et al. 2011). In port areas there is also a high potential for the introduction of non-indigenous marine microalgae via ship's ballast water, as nearly all known harmful algal bloom species have been documented in viable form in ship's ballast water (Hallegraeff 2015). Beside living cells, cysts can be introduced in ship tanks during ballast intake, or they may be produced inside these tanks during voyages because of the unfavourable conditions (Casas-Monroy et al. 2013; Steichen et al. 2014). Until now, only a few studies are available concerning the resting stages assemblages in the bottom sediments of the northern Adriatic (Nichetto et al. 1995a; Nichetto et al. 1995b; Rubino et al. 2000) and, at a broader geographical level, even less numerous are studies where they are combined with active stages of microbenthos (Rubino et al. 2015).

Investigations on the microbenthic communities subjected to multiple stressors in the natural environments are gaining importance but are still very limited compared to the increasing number of laboratory studies. Moreover, by integrating the microbenthic structural data with benthic processes like primary production and oxygen consumption, a more detailed view of the functioning of a given benthic ecosystem can be obtained. While in some studies a marked influence of contamination on these functional processes have been reported (Cibic et al. 2015; Rubino et al. 2015) in others the microbenthic community did not seem to be affected by the high organic load or contamination, reaching high densities and photosynthetic rates (Cibic et al. 2008a; Cibic et al. 2012b). Since conflicting results arise from the literature, new studies are needed to shed light on these aspects.

Several studies on the microbenthic communities and their functional processes have been conducted in the Gulf of Trieste and related to different anthropogenic pressures, but very little information is available within its port area subjected to multiple and diffuse anthropogenic pressures.

Therefore, to gain new insight into the benthic autotrophic pathways, the aims of this study were: a) to investigate the active and resting microbenthic community structure; b) to estimate benthic processes like primary production and oxygen consumption c) to relate them to the main anthropogenic pressures in the area and, finally, d) to update the knowledge on the plankton's resting stage assemblages in the northern Adriatic Sea.

## 2 Materials and methods

### *2.1 Study site*

The Gulf of Trieste, located at the north-western end of the Adriatic Sea, is a shallow embayment of about 500 $km^2$ and a coastline of about 100 km. It is almost completely surrounded by land except to the southwest, where it is limited by an imaginary line connecting Punta Tagliamento in Italy with Punta Salvore in Croatia and it is isolated from the rest of the Adriatic Sea by a sill from Grado to the Salvore peninsula (Ogorelec et al. 1991); 10% of its area is < 10 m deep and the maximum depth is about 25 m. Average salinity range is from 33 to 38 PSU at the surface and from 36 to 38.5 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). The main physical factors influencing the characteristics of the composition, evolution and persistence of marine life in the Gulf of Trieste, are winds and the stratification of the water column.

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 (F.V.G. 1985) 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 hydrodynamism.

The Port of Trieste is subjected to different anthropogenic pressures and therefore it represents a complex study site. For this study, the sampling stations were situated nearby the major productive activities of the port area, all positioned at the north-eastern part of the bay of Muggia (Fig. 1); St. 1 between the port's cargo piers, St. 2 in an area dedicated to the shipbuilding activities, St. 3 close to the iron and steel plant and St.4 near the petroleum industry. St. 5 and 6 were positioned on the other side of the bay where there is a residential area, and finally St. 7 was located in the centre of the bay, right behind a breakwater. The three stations closest to the port and industrial activities (St. 1, 2, 3) were characterised by high concentrations of contaminants that exceeded the legal limits. At St. 2, in particular, total heavy hydrocarbons reached 1050 mg $kg^{-1}$, total Polychlorobiphenyls (PCBs) 907μg $kg^{-1}$ and total Polycyclic Aromatic Hydrocarbons (PAHs) 1900 μg $kg^{-1}$ of sediment dry mass. Also several heavy metals, namely As, Hg, Pb, Cu and Zn reached high values at this station: 23.8 mg $kg^{-1}$, 4.4 mg $kg^{-1}$, 388.0 mg $kg^{-1}$, 112.0 mg $kg^{-1}$ and 770.0 mg $kg^{-1}$, respectively. At St. 1 and 3, total heavy hydrocarbons and PAHs in particular, exceeded the legal limits. On the other hand, at all the other stations, especially those close to the residential area contaminant concentrations were below the legal limit (unpublished data).

### *2.2 Sampling*

The above mentioned seven stations, located within the Port of Trieste were sampled in June 2013. In the same period the reference St. C1 (45° 42.050’ N, 13° 42.600’ E) (Fig. 1), situated within the Marine Reserve of Miramare, was also sampled. This area has been under protection since 1986 and is therefore sheltered from all direct human activities and boat traffic. At each station, 5 virtually undisturbed sediment cores were collected by an automatic KC Haps bottom corer (KC-Denmark) using polycarbonate sample tubes (13.3 cm i.d. with a sample area of 127 $cm^2$); 1 sediment core was used for oxygen microprofiling, while from the 4 remaining cores the uppermost oxic layer was sampled, homogenised and subsampled for active and resting microbenthos, primary production, grain-size and chemical variables. Because of an inconvenience it was not possible to obtain sediments for resting stage analysis from samples collected at St. 5, and a core devoted to the $O_2$ microprofiling from St. 4. Macroalgae were totally absent at all sampling stations. Bottom water samples were collected by means of a 2-L horizontal Niskin bottle. At the moment of sampling Photosynthetic 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 water 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).

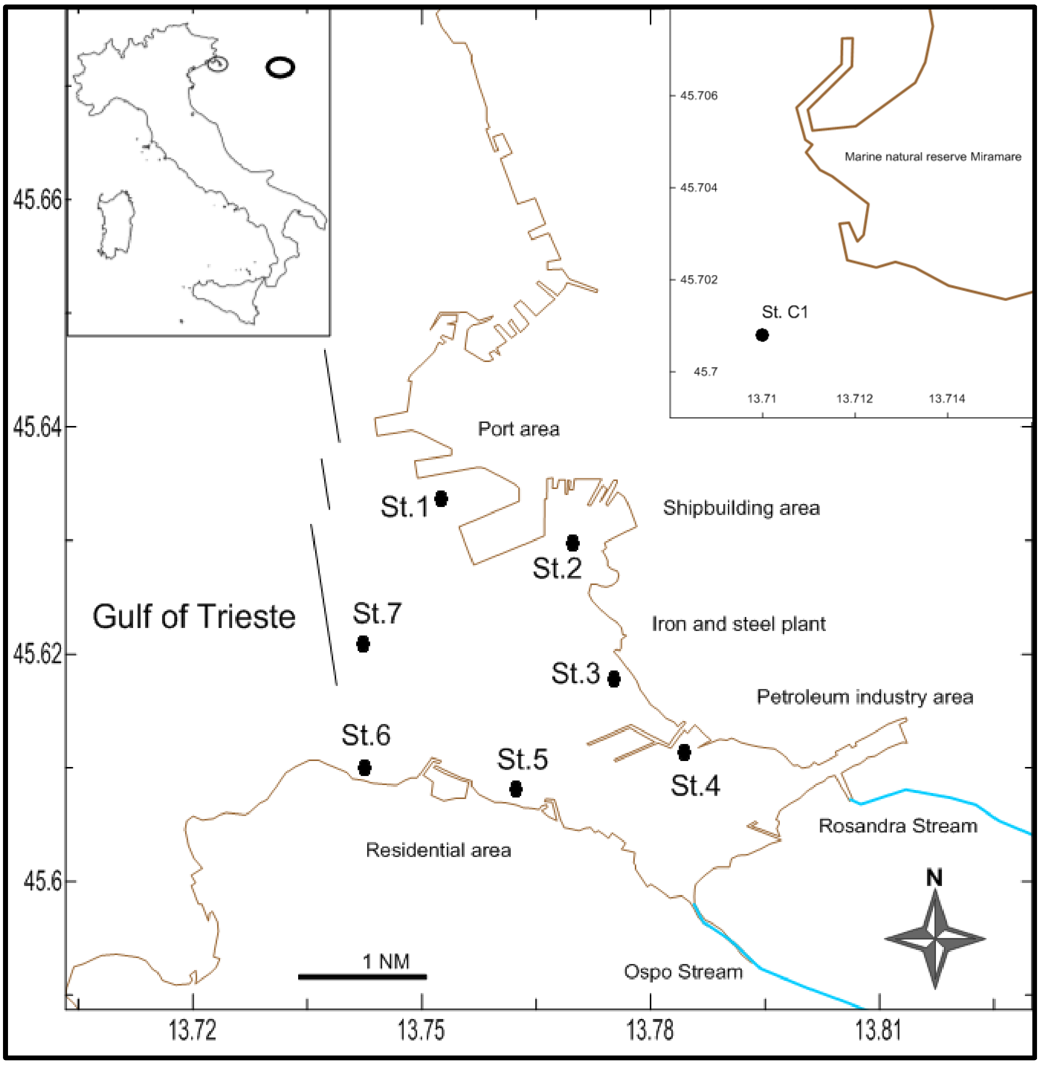

Fig. 1 Location of the sampling stations within the Port of Trieste and the reference St. C1.

## 2.3 Sample analyses

### 2.3.1 Sediment grain-size and Total Organic Carbon (TOC)

For each station, aliquots (10-15 g) of homogenised sediment were collected for grain-size analyses and processed as described by Cibic et al. (2007b). 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) and treated with increasing concentrations of HCl (0.1N and 1N) to remove carbonates (Nieuwenhuize et al. 1994). TOC was determined according to the methods of Pella and Colombo (1973) and Sharp (1974).

### *2.3.2 Active microbenthic abundance and community structure*

For the analyses of the active microbenthos (or microphytobenthos, MPB), 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 $CaMg(CO_3)_2$ (Carlo Erba, Rodano, Italy), in pre-filtered bottom seawater (0.2 µm filters) (Schleicherb& Schuell, Dassel, Germany). After manual stirring, 20-µL aliquots of the sediment suspension were drawn off from the slurries and placed into a counting chamber (Thalassia, Trieste, Italy). 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) (Utermöhl 1958). The microalgal taxonomy was based on Round et al. (1992) and the AlgaeBase website (Guiry and Guiry 2015). The qualitative identification of microphytobenthic assemblages was carried out to the genus and, when possible, to the species level using floras of Van Heurck (1899), Hendey (1976), Germain (1981), Dexing et al. (1985), Ricard (1987), Round et al. (1992), Tomas (1997) and Witkowski et al. (2000). Planktonic, tychopelagic (loosely associated with the sediment, commonly found in the water column) and benthic species were distinguished (Cibic et al. 2007d). Quantitative data are reported as cells $cm^{-3}$ of wet sediment (cells $cm^{-3}$) and as Relative Abundance (RA) for the main *taxa*.

### *2.3.3 Resting microbenthic abundance and community structure*

From each sampling site, three aliquots of wet sediments (from 1.9 to 2.2 $cm^3$, depending on the quantity of available sediment) were treated in the laboratory, according to the sieving technique proposed by Montresor et al. (2010). The obtained samples were ultrasonicated for 1 minute and then screened through sieves with different mesh sizes (200, 75, 20, 10 µm) (Endecott's LTD steel sieves, ISO3310-1, London, England) using natural filtered (0.45 µm) sea water. The different fractions were screened again through the sieve battery, obtaining two fine-grained fractions (10-20 µm and 20-75 µm) containing protistan cysts and a >75 µm fraction with larger dinoflagellate resting stages. The material retained onto the 200 µm mesh was discarded. No chemicals were used to dissolve the sediment particles to preserve calcareous and siliceous cyst walls.

Qualitative and quantitative analyses were carried out under an inverted microscope (Zeiss Axiovert 200M) equipped with a Nikon Coolpix 990 digital camera, using 32x and 40x objectives (320x and 400x final magnifications). Both full (*i.e.* with cytoplasmic content) and empty (*i.e.* already germinated) cysts were enumerated. As a general rule, at least 200 full cysts were counted per sample to obtain density values as homogeneous as possible and to evaluate rare species too. This was not possible for the samples from St. 6 due to the coarser sediments and in that case only 100 full cysts were counted. The >75 µm fractions were examined in their entirety. Data from St. 5 are not available.

All resting stage morphotypes were identified on the basis of published descriptions, the Modern Dinocyst Key website (https://www.marum.de/en/Modern_Dinocyst_Key.html) and germination experiments. Identification was performed to the species level when possible. As a rule, the modern biological names were used. For dinoflagellate cyst morphotypes whose active stage is unknown, the paleontological (fossil) names were used.
Quantitative data are reported as cysts cm$^{-3}$ of wet sediment (cysts cm$^{-3}$) and as Relative Abundance (RA) for the main *taxa*.
For germination experiments single viable cysts were isolated using a micropipette and placed into Nuclon microwells (NalgeNunc International, Roskilde, Denmark), containing ≈1 mL of natural sterilised sea water. Cysts were incubated at 21°C, 12-12 h light-dark cycle and an irradiance of 80 μmol photons m$^{-2}$ sec$^{-1}$ and examined daily until germination, up to a maximum of 30 days. The incubation conditions were chosen on the basis of previous studies proven to be effective for a large number of species (Moscatello et al. 2004; Rubino et al. 2010; 2009).
Hereafter we use the terms active and resting microbenthos to differentiate the active cells of photoautotrophic microbenthos (microphytobenthos, MPB) from resting stages (cysts) of autotrophic and heterotrophic microplankton in the sediments.

### *2.3.4 Microphytobenthic primary production (PP)*

PP was estimated in the laboratory from $^{14}$C-incubation of slurries. For each sampling station, 10 cm$^{3}$ of homogenised surface sediment was withdrawn 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 21 glass vials containing 9 mL which were divided as follows: 3 replicates to assess the sediment matrix effect, 3 dark replicates and 3 replicates for each of the 5 light intensities used. In a thermostatic chamber the samples were incubated at the *in situ* temperature measured at each station (Table 1), under a gradient of light intensities (20-50-100-200-300 μE m$^{-2}$ s$^{-1}$) and 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. (2008b). PPs is referred to data obtained at *in situ* light conditions whereas PPp is referred to the potential primary production, *i.e.* the highest value obtained under a gradient of light intensities.

### *2.3.4 $O_2$ consumption rates*

For each station, an intact sediment core for $O_2$ consumption estimates in darkness was collected and transferred to a cryostatic system to maintain the *in situ* temperature. A stable water flow above the sediment surface was established by a pressure-regulating air pump which maintained a diffusive boundary layer of 300-500 µm and kept the overlying water at atmospheric oxygen saturation at all times. 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 consumption were calculated as described by Cibic et al. (2007c).

### *2.4 Statistical analyses*

All univariate and multivariate statistical analyses were performed using PRIMER software v.6 (PRIMER-E Ldt, Plymouth, UK) and STATISTICA v.7.

The PRIMER "DIVERSE" routine was used on untransformed data to calculate taxonomic richness (S), Margalef richness *d* (Margalef 1986), Shannon diversity *H'* (Shannon and Weaver 1949), equitability *J'* (Pielou 1966), and dominance *λ* (Simpson 1949). For active microbenthos the analysis was carried out only for diatoms and for resting microbenthos only for full cysts.

Bi-dimensional representations of the statistical comparisons among samples were obtained by non parametric multidimensional scaling (nMDS) separately for the diatom and the cysts *taxa*. The data were previously standardised and fourth-root transformed to down-weight the effect of common species on the analysis.

A one-way analysis of similarity permutation test (PRIMER ANOSIM) was performed to determine the statistical difference, for the active microbenthos, among three groups of stations, identified on the basis of their location: St. 1, 2, 3, 4: close to the port; St. 5, 6: close to the residential area; St. 7: in the centre of the bay and the reference St. C1. Since for the resting microbenthos, data from St. 5 are not available, the groups of stations were identified as follows: St. 1, 2, 3, 4: close to the port; St. 6, 7, C1: others, in order to analyse anyway the influence of the port activities on the resting stage assemblages.

To highlight interactions between abiotic and biotic variables a Spearmann rank correlation analysis (R) was performed using STATISTICA v.7

A Principal Component Analysis (PCA) was carried out on environmental data in order to visualise the trends of main abiotic variables (depth, %PAR, sand fraction, TOC and major contaminants: Hg, Pb, PAH and PCBs). The biotic components 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 and present contaminants upon each considered benthic taxon. Two separate analyses were carried out, one for some selected species (RA>1%) of the active microbenthos and PP estimates and one for some selected species (RA>1%) of the resting microbenthos. St. C1 was not considered in this analysis because contaminant concentration data were not available.

## 3 Results

### 3.1 Physical-chemical data

Bottom sea 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, going from 37.07 to 37.23. The lowest PAR at the bottom was measured at St. 2 due to the 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 1). %PAR (benthic PAR as a percentage of the surface irradiance) was negatively correlated to the stations' depth ($R = -0.762$, $p < 0.05$) and reached lower percentages at the deepest St. 1 and 7).

**Table 1** Physical data measured at the bottom layer of the water column (above the double line) and Sand, expressed in %, and TOC (Total Organic Carbon) measured in the sediments (under the double line) at the seven sampling sites within the port area and at the reference St. C1 in June 2013. %PAR = benthic PAR as a percentage of the surface irradiance, TOC = Total Organic Carbon. For the latter, values are averages of three replicates ± SD.

| | Stations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | St. 1 | St. 2 | St. 3 | St. 4 | St. 5 | St. 6 | St. 7 | St. C1 |
| Depth(m) | 18.5 | 15.0 | 13.0 | 10.5 | 11.5 | 8.5 | 19.5 | 17.0 |
| Temperature (°C) | 17.30 | 17.47 | 17.84 | 18.07 | 17.86 | 23.01 | 17.29 | 17.33 |
| $O_2$% | 80.7 | 83.7 | 88.5 | 89 | 90.5 | 107 | 80.6 | 89 |
| Salinity (psu) | 37.23 | 37.20 | 37.14 | 37.07 | 37.14 | 35.03 | 37.22 | 37.21 |
| surface PAR (µE $m^{-2}$ $s^{-1}$) | 1539.4 | 589.4 | 1678.5 | 2358.8 | 1840.9 | 467.4 | 1087.1 | 1253.3 |
| bottom PAR (µE $m^{-2}$ $s^{-1}$) | 23.9 | 14.2 | 36.7 | 61.6 | 116 | 57.3 | 20.9 | 37.5 |
| %PAR | 1.5 | 2.4 | 2.2 | 2.6 | 6.3 | 12.3 | 1.9 | 3 |
| Sand % | 7.0 | 14.2 | 17.1 | 11.3 | 10.6 | 40.2 | 8.8 | 9.0 |
| TOC (mg C g-1) | 28.6 ± 1.2 | 40.8 ± 1.9 | 51.1 ± 2.2 | 22.3 ± 1.8 | 18.9 ± 0.2 | 8.6 ± 0.5 | 19.9 ± 0.7 | 14.4 ± 0.3 |

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 1). Mean

TOC content reached 51.1 ± 2.2 mg $g^{-1}$ at St. 3, thrice the value recorded at the reference station (C1).

### *3.2 Active microbenthic community structure*

Total MPB abundances were high, exceeding 200,000 cells $cm^{-3}$, and comparable at St. 1, 2, 3, 4 and C1 (Fig. 2a), whereas the highest density was observed at St. 5 with 263,800 ± 16,215 cells $cm^{-3}$. At St. 6 and 7 the microalgal densities were approximately half of that obtained at the first four stations.

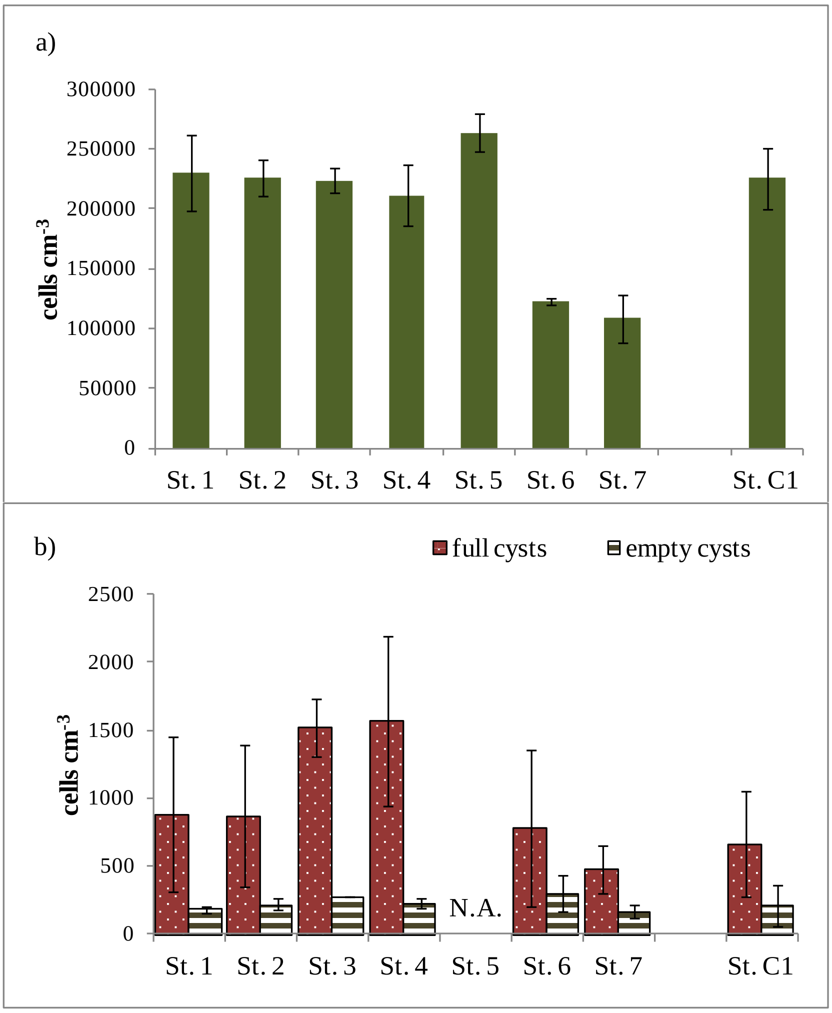

**Fig. 2** a) Active and b) resting microbenthic abundances at the seven sampled stations within the port area and at the reference site C1. To better highlight the different dynamics for active and resting microbenthos, the Y-scale for the two communities are diverse. N.A. = not available.

The MPB community was dominated by diatoms (> 99.7 % at all the investigated stations) at the expense of other groups such as dinoflagellates or phytoflagellates. Focusing on diatoms only and considering all the sampled stations together, a total of 50 taxa belonging to 26 genera were identified (Appendix 1). *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 (Fig. 3). At this station the genus *Gyrosigma* was also represented with the highest number of species (S=7), namely *G. acuminatum*, *G. attenuatum*, *G. balticum*, *G. fasciola*, *G. macrum* and others. The tychopelagic species *Ceratoneis closterium* was also observed in high densities, especially at the deeper St. C1, 7 and 1 with RAs reaching 15.3, 10.2 and 8.3%, respectively. A similar pattern was observed for another tychopelagic species, *i.e. Paralia sulcata*.

*Nitzschia* cf. *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, at the reference site and in the centre of the bay of Muggia its percentage did not exceed 1.5%. Overall, the genus *Nitzschia* was well-represented by different species, namely *N. fasciculata*, *N. lorenziana*, *N. sigma*, all quite abundant at the more contaminated stations close to the port and industrial areas.

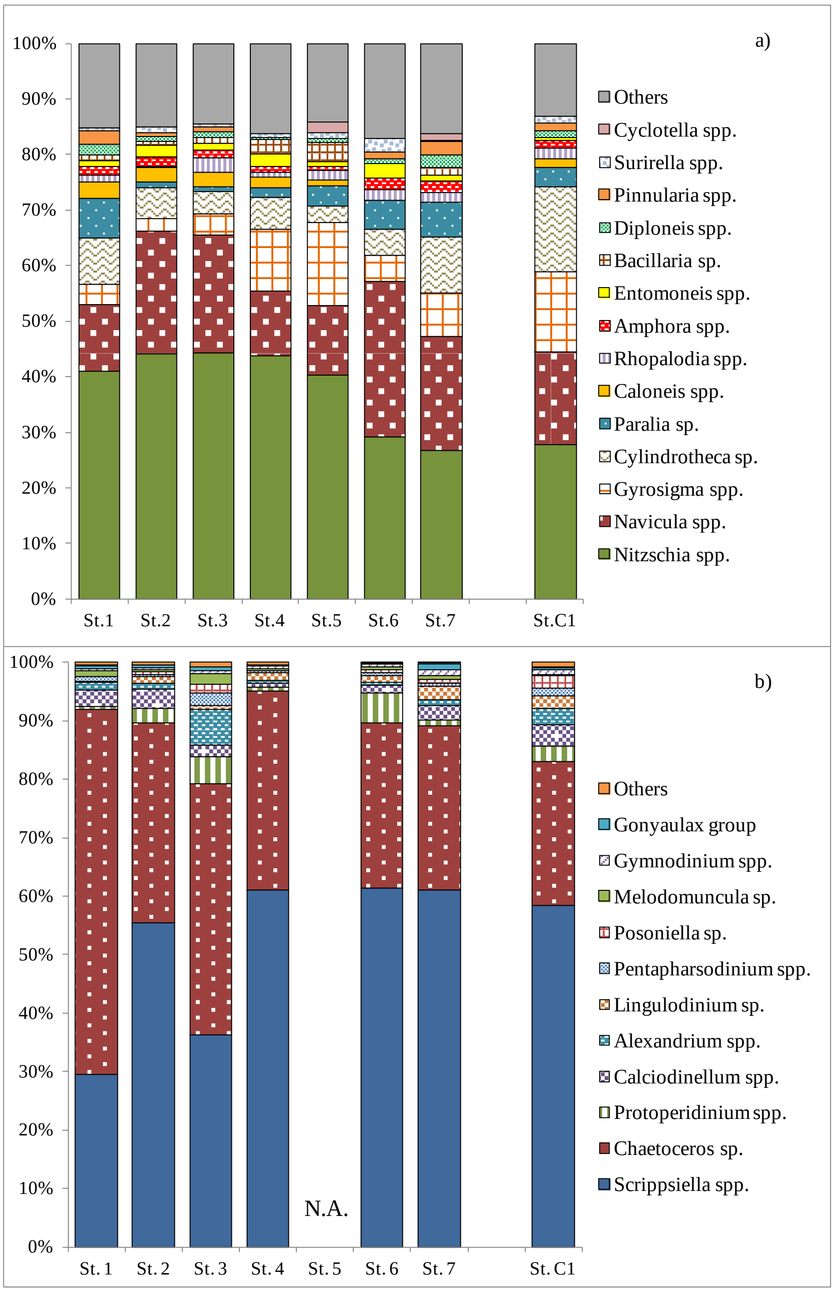

**Fig. 3** Relative abundance (RA) of a) diatom genera of active microbenthos and b) main taxa of resting microbenthos at all the stations investigated.within the port area and at the reference St. C1. Only the taxa with an RA >1%, recorded at least at one station, are shown while the less abundant ones are grouped in 'Others'. N.A. = not available.

Resting microbenthic densities ranged for full forms from 478 ± 119 (St. 7) to 1569 ± 621 (St. 4) cysts cm$^{-3}$ (Fig. 2b). Empty cysts resulted by far less abundant, reaching the highest density of 298 ± 131 cysts cm$^{-3}$ at St. 6.

Considering both full and empty forms, a total of 51 different cyst morphotypes were identified (Appendix 2). Except for one, all were produced by dinoflagellates. The only diatom taxon was identified as *Chaetoceros* sp.2. For some species of dinoflagellates more than one cyst morphotype was identified, so the total number of taxa was 46. This is the case of *Lingulodinium polyedrum* (2 morphotypes) and *Scrippsiella acuminata* complex (5 morphotypes).

*Chaetoceros* sp.2 and *S. acuminata* resulted by far the most abundant taxa, reaching together 68% of the total abundance considering all the investigated stations. Analysing the single sampling stations, *Chaetoceros* sp.2 reached an RA of 63% at St. 1 (Fig. 3b) while *S. acuminata* represented 51% of the total abundance at St. 7.

Another important species was *Scrippsiella* sp.1 with an RA of 19% calculated over the investigated stations, while all the other taxa never exceeded an RA of 2%. The cysts of *Dissodinium pseudocalani* were collected only at St. C1 (full cysts), and those of *Gymnodinium uncatenatum* were collected at St. 4, 7 and C1 (full cysts). At St. 2 we found empty cysts of *L. polyedrum* with very short spines. Finally *Alexandrium minutum*, a known PSP toxic species, resulted widespread in the study area, including the reference site (St. C1) but its full cysts reached an RA of 4% at St. 3. Its empty cysts were present at St. C1 and very abundant at St. 6 (RA= 11%).

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 (ʎ = 2.63) likely due to the prevalence of *Nitzschia* cf. *commutata* at this site (Table 2). At St. 7 the diatom community resulted more evenly distributed (J’ = 0.84) compared to the other stations.

Regarding the community level, the species richness of the resting microbenthos resulted quite high in the study area with an average value of 25.4 ± 1.5. The reference site C1 hosted the highest number of taxa (S=28). In contrast, low values of ecological diversity (H') and evenness (J') in the entire study area (Table 2).

**Table 2** Univariate diversity indices calculated on the active and resting microbenthic assemblages at the sampled stations within the port area and the reference St. C1. For active microbenthos, only diatom taxa were considered. N=number of organisms (average values); S=species richness; d=Margalef's index; J'=Pielou's index and H'=Shannon-Wiener's diversity index (Log2); λ=Simpson's index

| station | N | S | d | J' | H' | λ |
|---|---|---|---|---|---|---|
| active microbenthos (diatoms) | | | | | | |
| St. 1 | 195600 | 35 | 2.791 | 0.785 | 2.792 | 0.085 |
| St. 2 | 195600 | 34 | 2.709 | 0.713 | 2.514 | 0.132 |
| St. 3 | 195000 | 39 | 3.120 | 0.742 | 2.717 | 0.097 |
| St. 4 | 179400 | 32 | 2.563 | 0.774 | 2.681 | 0.111 |
| St. 5 | 229600 | 41 | 3.240 | 0.816 | 3.030 | 0.072 |
| St. 6 | 103600 | 35 | 2.944 | 0.756 | 2.687 | 0.107 |
| St. 7 | 90800 | 27 | 2.277 | 0.843 | 2.779 | 0.082 |
| St. C1 | 195000 | 31 | 2.463 | 0.806 | 2.769 | 0.090 |
| resting microbenthos (full cysts) | | | | | | |
| St. 1 | 879 | 25 | 3.541 | 0.3971 | 1.278 | 0.4409 |
| St. 2 | 874 | 24 | 3.396 | 0.5102 | 1.622 | 0.2756 |
| St. 3 | 1523 | 26 | 3.412 | 0.5958 | 1.941 | 0.248 |
| St. 4 | 1569 | 27 | 3.533 | 0.4275 | 1.409 | 0.3009 |
| St. 6 | 780 | 24 | 3.454 | 0.4987 | 1.585 | 0.2668 |
| St. 7 | 478 | 24 | 3.728 | 0.4764 | 1.514 | 0.3439 |
| St. C1 | 667 | 28 | 4.152 | 0.5283 | 1.76 | 0.2941 |

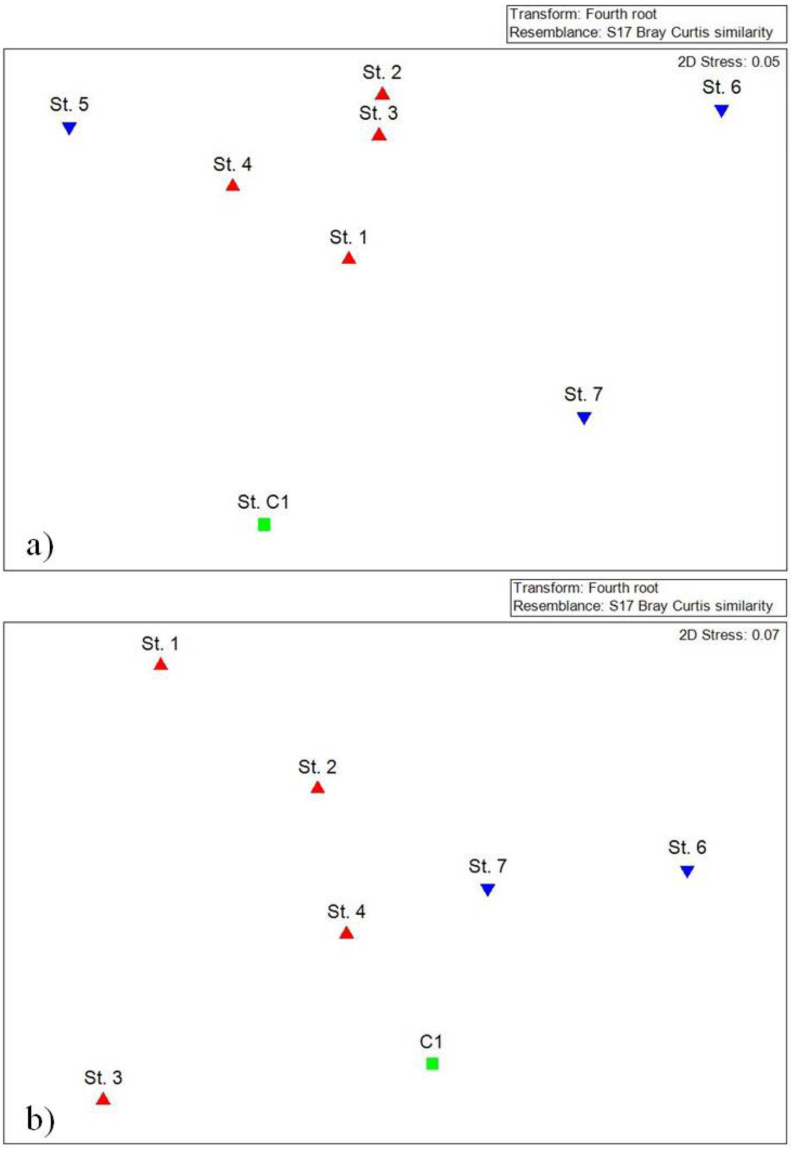

**Fig. 4** Non parametric Multidimensional scaling (nMDS) of a) active and b) resting microbenthos (full cysts) at the 7 sampled stations within the port area and the reference St. C1. Data for resting stages at St. 5 are not available. Red triangle = stations close to the port, Inverted blue triangle = stations close to the residential area and centre of the bay, Green square = reference St. C1

According to the multivariate analysis (nMDS) based on MPB, the stations close to the NE coast (St. 1 to 4) that are more influenced by the port activities, resulted clearly differentiated from St. 6, 7 and from the reference site (C1), whereas the position of St. 5 was far from the others (Fig. 4a). The MDS based on full cysts also highlighted a division between station close to the port (on the left) and all the others (on the right) (Fig. 4b).

The analysis of similarity showed a significant difference in the structure of the diatom assemblages among three groups of stations: those close to the port (St. 1, 2, 3 and 4), those close to the residential area (St. 5 and 6) and the station in the centre of the bay with the reference station ($R_{ANOSIM}$ = 0.600, p = 0.01). Since for the resting microbenthos data from St. 5 are not available, when cysts assemblages were tested for similarity, they were labelled as follows: those close to the port (St. 1, 2, 3 and 4) and all the other stations in another group (St. 6, 7 and C1). The differences between these two groups were less pronounced ($R_{ANOSIM} = 0.481$, p = 0.057).

### *3.3 Benthic processes*

The PPs rates, that are the rates obtained in laboratory exposing the microalgae to *in situ* light and temperature conditions, were uncoupled with MPB abundance values, particularly at St. 1 and 2 (Fig. 5), while at the other stations the PPs followed to a higher degree the total densities. At all stations the PPs values were considerably lower compared to the PPp (Fig. 6): the microalgal community could be much more photosynthetically active with a higher light availability at the bottom, as indicated by the potential primary production, i.e. the highest value obtained under a gradient of light intensities ranging from 20 to 300 μE $m^{-2}$ $s^{-1}$.

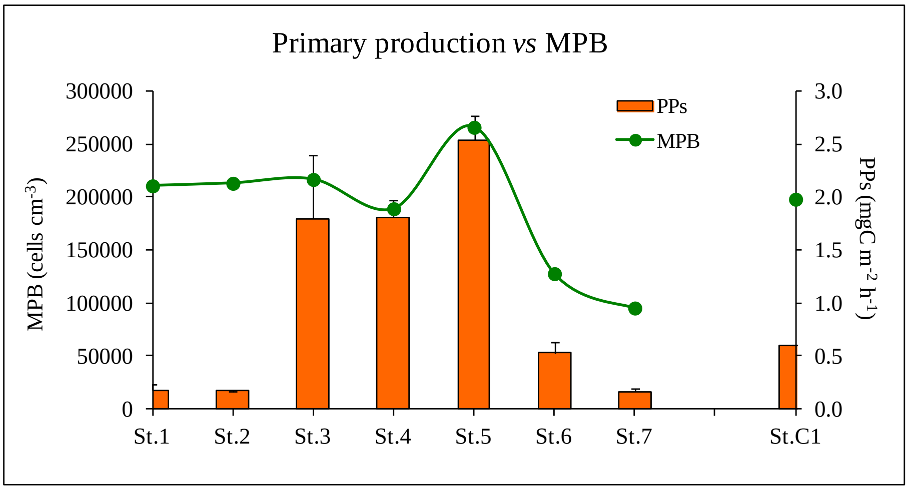

**Fig. 5** Primary production rates obtained *in situ* and active microbenthic abundances at the seven sampled stations within the port area and at the reference St. C1.

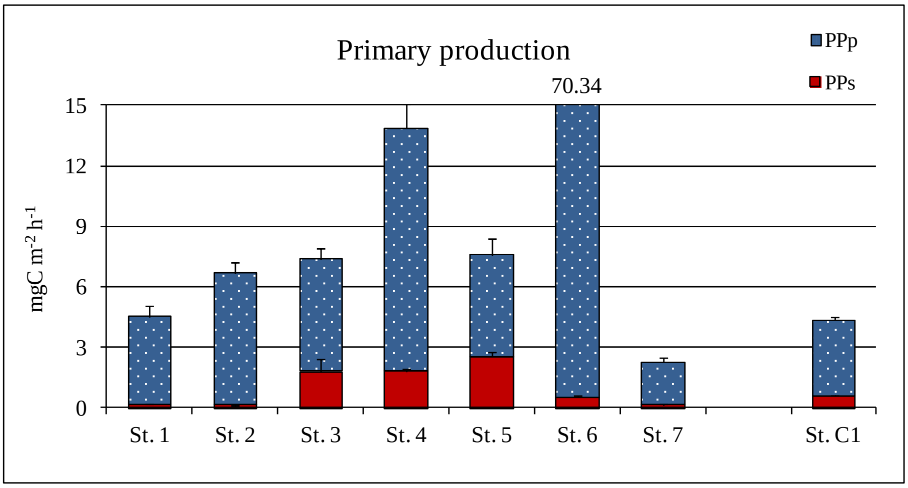

**Fig. 6** Primary production rates obtained at *in situ* light and temperature conditions (PPs) in comparison to the highest potential rates obtained under a gradient of light intensities (PPp) at the eight investigated stations.

Indeed, at all stations by exposing the microalgae to increasing light intensities, gradually higher rates were obtained following a curve which inclination was clearly depending upon the depth of the site: the deeper the station, the lower the rate (Fig. 7). In fact at the deepest St. 7 (19.5 m) the maximum rate did not exceed 3.23 ± 0.57 mg C $m^{-2}$ $h^{-1}$ whereas at St. 4 (10.5 m) a PP rate of 17.46 ± 1.42 mg C $m^{-2}$ $h^{-1}$ was estimated at 300 μE $m^{-2}$ $s^{-1}$. At the maximum irradiance the MPB community did not seem to be light saturated at any station except for the shallowest one. In fact, at St.6 the absolute maximum was obtained at 200 μE $m^{-2}$ $s^{-1}$ (58.60 ± 8.41 mg C $m^{-2}$ $h^{-1}$) while the rate dropped by increasing the irradiance up to 300 μE $m^{-2}$ $s^{-1}$.

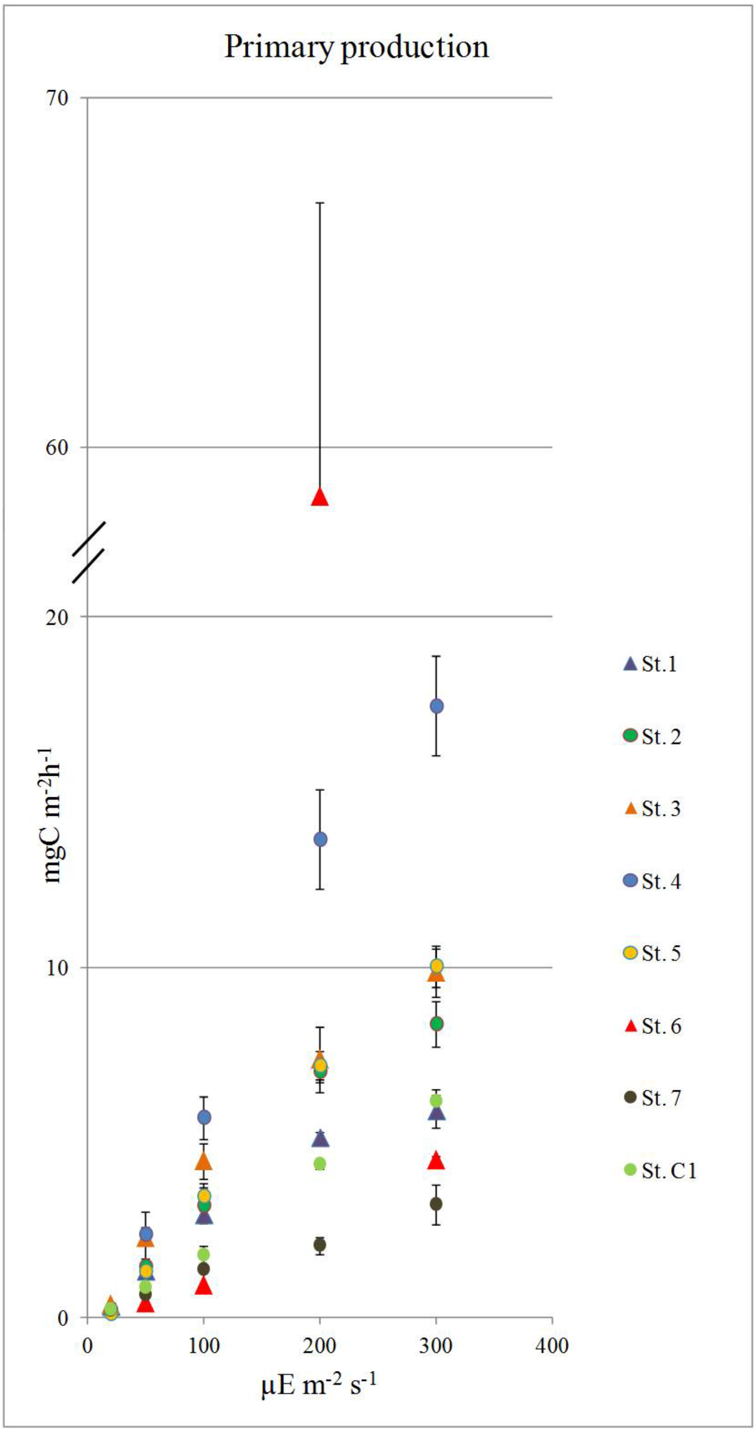

**Fig. 7** Primary production rates (average of three replicates with SD) obtained exposing the MPB community to *in situ* temperature and increasing light intensities (from 20 to 300 µE $m^{-2}$ $s^{-1}$) at the seven stations within the port area and at the reference site.

Oxygen microprofiles revealed that the oxygen penetration depth was quite diverse among stations (Fig. 8). Particularly at St. 3 the thickness of the oxic layer was highly reduced and the oxygen was completely depleted within the top 0.8 mm. Similarly, only the top millimeter of the sediment was oxic at St. 2. In contrast, oxygen penetration at the centre of the bay and at the reference St. C1 reached 2.5 mm, while an intermediate situation was obtained at St. 5 and 6. A higher variability among replicates was observed for microprofiles measured at St. 6 and St. C1; in the first case it probably depended upon the higher percentage of sand which facilitates horizontal gas diffusion whereas in the second case it could be due to bioturbation. Oxygen consumption rates were calculated from the microprofiles performed in the darkness. The maximum oxygen consumption was observed at St. 3 (-1.81 ± 0.19 µmol $O_2$ $cm^{-3}$ $h^{-1}$), followed by that obtained at St. 2 (-1.07 ± 0.23 µmol $O_2$ $cm^{-3}$ $h^{-1}$) whereas at the other stations more modest rates were obtained that were comparable to the consumption calculated at the reference site (-0.21 ± 0.02 µmol $O_2$ $cm^{-3}$ $h^{-1}$).

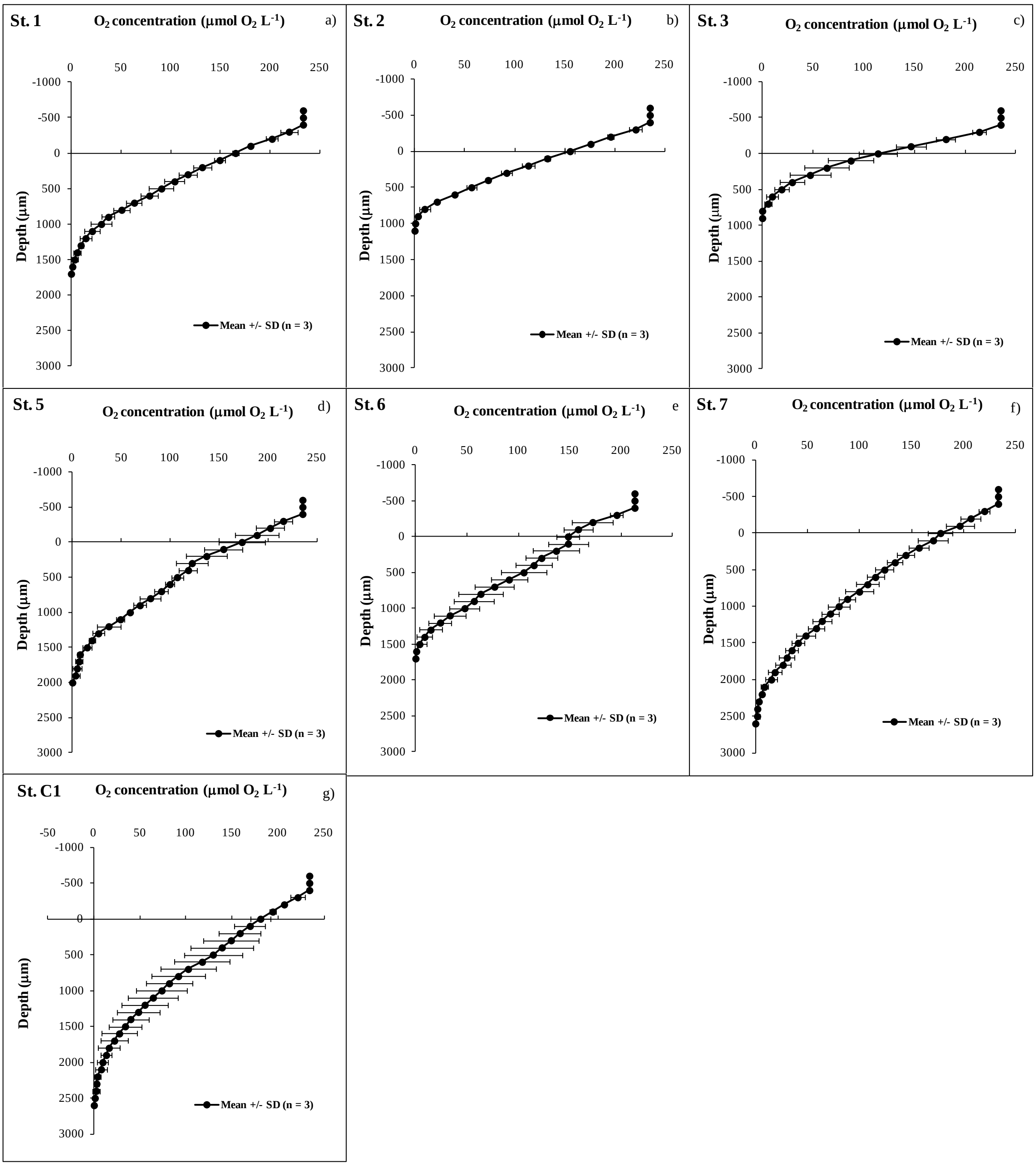

**Fig. 8** $O_2$-microprofiles estimated at the stations within the Port of Trieste (a-f) and at the reference site (g). Data from St. 4 are not available.

The ordination plot of the considered parameters on which the diatom species were plotted accounted for 81.8% of total variance (Fig. 9a). The principal component axis 1 (PC1) explained 55.7% of total variance whereas the component axis 2 (PC2) explained 26.2% of the remaining variance.

Hg, Pb, PAHs and PCB were predominant elements of the first factor, while the major contributor for the second was the sediment grain size. *Nitzschia* cf. *commutata* was plotted in correspondence with the contaminants and *Nitzschia lorenziana* was also positioned close to TOC and depth. Plotting the samplings on the PCA factor-plane 1 x 2 (Fig. 9b), separated St. 2 and 3 from the others in quadrant II, St. 1 was positioned with the other deep station, St.7 in quadrant I. In quadrant III, only St. 6 was positioned whereas St. 4 and 5 were located in quadrant IV.

The ordination plot of the considered parameters on which the cysts taxa were plotted, accounted for 79.25% of total variance (Fig. 9c). The principal component axis 1 (PC1) explained 53.40% of total variance whereas the component axis 2 (PC2) explained 25.85% of the remaining variance. Hg, Pb, PAHs and PCB were again predominant elements of the first factor, while the major contributors for the second one were the depth and grain size. The species *Calciodinellum albatrosianum* was plotted in correspondence with the contaminants whereas the majority of the considered species (*Scrippsiella acuminatum*, *Alexandrium minutum* and *Scrippsiella* sp. 1) were plotted nearby the grain size. The PCA factor-plane 1 x 2 (Fig. 9d) separated the most contaminated stations on the left and the others on the right of the axis, with St. 2 being more distant.

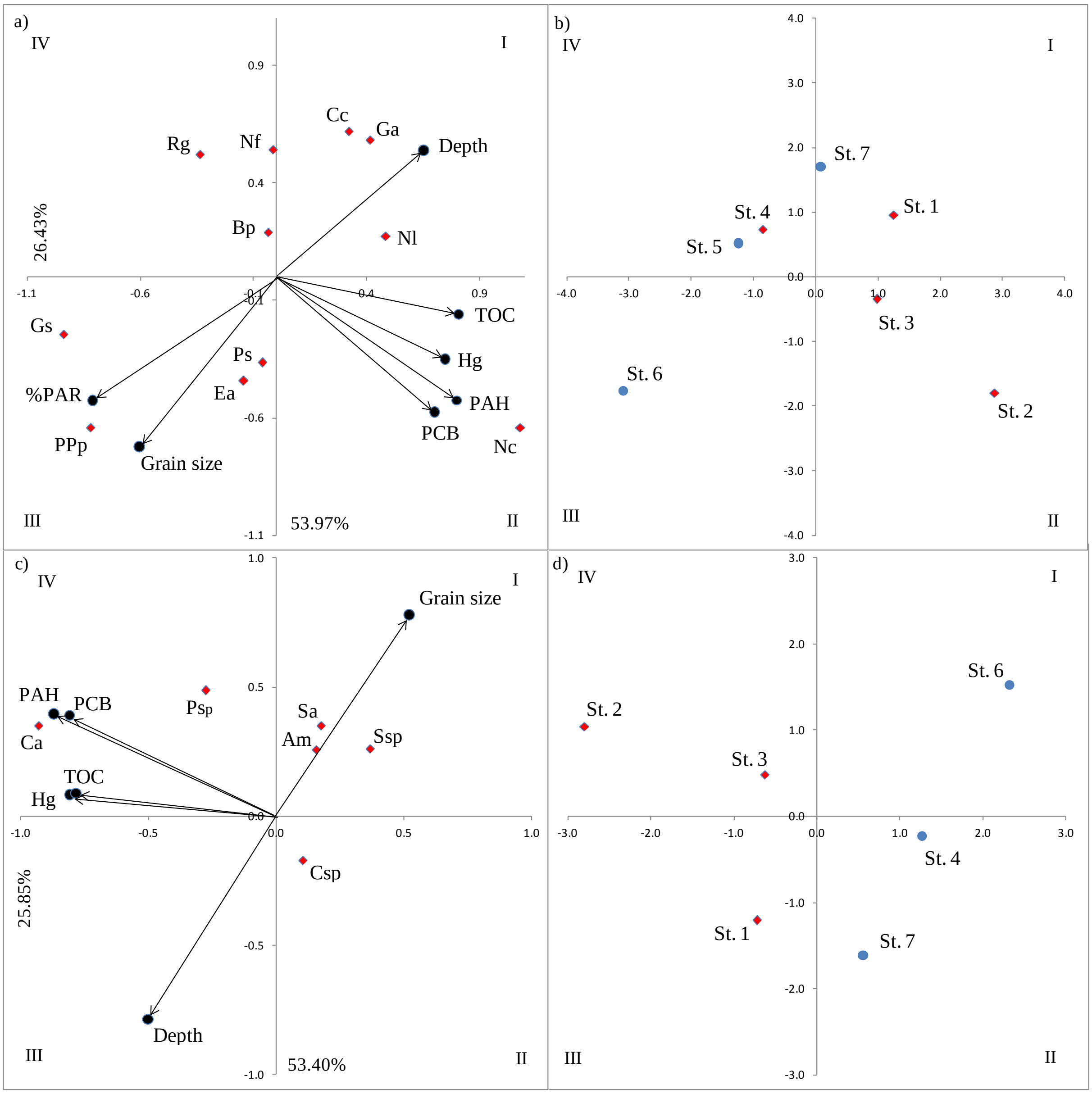

**Fig. 9** Principal component analysis (PCA) ordination diagram of the sampling stations within the port area based on the selected variables. b) Scatter diagram plotting factors 1 and 2 of samplings a) on which the main diatom species (RA>1%) are projected as additional variables; d) Scatter diagram plotting factors 1 and 2 of samplings c) on which the main taxa of full cysts (RA>1%) are projected as additional variables. For the latter data from St. 5 are not available. PPp = potential rates of primary production, Bp = *Bacillaria paxillifera*, Cc = *Ceratoneis closterium*, Ea = *Entomoneis alata*, Ga = *Gyrosigma acuminatum*, Nc = *Nitzschia* cf. *commutata*, Gs = *Gyrosigma spenceri*, Nf = *Nitzschia fasciculata*, Nl = *Nitzschia lorenziana*, Ps = *Paralia sulcata*, Rg = *Rhopalodia gibba*, Csp = *Chaetoceros* sp.2, Sa = *Scrippsiella acuminata*, Ss = *Scrippsiella* sp.1, Ca = *Calciodinellum albatrosianum*, Psp = *Protoperidinium* sp.1, Am = *Alexandrium minutum*

## 4 Discussion

In this study, the analytical effort was focused mainly on the micro-sized phototrophic components of the benthic compartment. The sediment, in fact, acting as a repository of both natural and anthropogenic compounds from the water column, can be used as a record of past activities that have taken place in the area. In addition, benthic organisms, due to their limited mobility, are exposed to environmental changes and respond to 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).

The abiotic variables were quite alike among the sampled stations and only St. 6 differed primarily for its reduced depth (8.5 m) and the sediment grain-size. In fact, due to a higher sand fraction (about 40%) the sediment was classified as sandy silt whereas at all the other stations it was clayey silt. The grain-size is considered one of the most important factors influencing the colonisation of sediments by MPB (Round et al. 1992). Besides the higher temperature (23.0%) and %PAR (12.3) due to the lower depth, St. 6 was also characterised by lower salinity (35.0 PSU) indicating a fresh water outflow in the vicinity. In contrast, St. 2 and 3 differed from the other stations for a higher TOC that likely derived from the nearby productive activities (shipbuilding and steel plant).

### *4.1 Active microbenthic abundance and community composition*

Overall, the total abundance of MPB in the port area was of the same order of magnitude as that previously estimated in another sublittoral site of the Gulf of Trieste (Cibic et al. 2012a). The highest value was observed at St. 5 while at St. 6 and 7 the microalgal densities were approximately half of that obtained at the first four stations. At St. 6 this was likely due to the different grain-size composition, i.e. the higher percentage of sand that in turn derives from a higher hydrodynamism that thwarts the attachment of diatom cells to the sea bottom. On the other hand, at St. 7 a greater depth (19.5 m) and lower irradiance at the bottom did not support the development of the microbial phototrophic community. The overall high abundance of MPB at the stations close to the port and industry infrastructure suggests that at this level, the community was not sensitive to the contaminants accumulated in the sediments and resorts to defense mechanisms against these harmful compounds. For instance, in presence of toxic substances such as heavy metals diatoms produce more extracellular polymeric substances (EPS) to protect themselves from the harsh environment (Sheng et al. 2010 and references therein). EPS, which may function as a protective barrier against toxic compounds as well as enhance the uptake of favorable ones, can therefore decrease the vulnerability of microbial mats to toxic compounds (Sundbäck et al. 2007). Cibic et al. (2012b) infer that the extremely abundant MPB community in a contaminated channel of the Grado-Marano lagoonal system was due to the extrusion of high amounts of EPS that allowed the diatoms to cope with the contaminants. However, as the concentration of toxic substances exceeds a threshold, its effect on the stimulation of EPS production become less significant (Sheng et al. 2005). Indeed, in severely contaminated environments the diatom community cannot survive and extremely low abundances are encountered (Rubino et al. 2015).

Although there were no remarkable differences in the total abundance of MPB at St. 1, 2, 3 and 4 compared to the reference one, the community structure at the investigated stations was quite diverse. Overall, the MPB community was mainly represented by Bacillariophyta in accordance with other studies in this area (Cibic et al. 2007a; 2012a; Franzo et al. 2015). At the stations within the port (St. 1 - 4) the RA of the genus *Nitzschia* was always above 40.1% in contrast to the more distant St. 6, 7 and the reference St. C1 where it never exceeded 29.0%. Especially at St. 2 and 3, we observed some diatom species belonging to this genus that are tolerant to pollution. For instance, *Nitzschia* cf. *commutata* was the most abundant species at those two stations with 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 where a higher TOC content was measured. The affinity of the genus *Nitzschia* towards sediments enriched with organic matter was also suggested by its high correlation with TOC ($R = 0.86$, $p < 0.05$). Moreover, in the PCA, the position of another species belonging to this genus, i.e. *N. lorenziana*, projected nearby TOC, corroborates the same assumption. However, if on one hand we observed a certain degree of affinity of *N. commutata* for the organic enrichment, on the other hand at the same stations, the most severe contamination was observed. Indeed, in the PCA this species was plotted in correspondence with the highest concentrations of the considered contaminants. There is an objective problem in the difficulty of separating the effect of contaminants from other factors, especially eutrophication (Potapova et al. 2016) or in our case the organic enrichment. However, the high RA of this species at St. 2 and 3 reasonably indicates that *N. commutata* can be considered a tolerant species.

In the investigated area, the genus *Gyrosigma* was well represented by several species. Since *Gyrosigma* has been defined as a nutrient loving genus (Hunter 2007), we expected to encounter numerous specimens belonging to this genus at the organic-enriched port stations, as already reported in a previous study (Franzo et al. 2014). However, at St. 1, 2 and 3 *Gyrosigma* was present in low abundances (RA = 3.2 ± 1%) compared to the stations where low contaminant levels were measured (St. 4 and 5) (RA = 13.1 ± 2.6%) but with a still quite high TOC content. This finding suggests that *Gyrosigma* cannot cope with the contamination levels at the port stations, regardless of the higher organic load. This is further indicated by the significant negative correlation between this genus and the considered contaminants (Hg: $R = -0.96$, Pb: $R = -0.89$, PAH: $R = -0.89$, PCB: $R = -0.96$, for all $p < 0.05$).

A higher RA of *Paralia sulcata*, reaching 7.2% at St. 1 and 6.3% at St. 7, was observed at the deeper stations that have a lower light availability at the bottom, in accordance with Cibic et al. (2012a). The tychopelagic diatom *Paralia sulcata* appears both in the plankton and the benthos, and has a competitive advantage under low light conditions (McQuoid and Nordberg 2003). The higher RA of *Ceratoneis closterium* at the deeper stations St. 7 and C1 was probably a consequence of a lower hydrodynamism at these stations, allowing a better development of this tychopelagic taxon. Even though St. 7 is positioned in the centre of the bay, it is sheltered by a breakwater that prevents the direct sediment resuspension caused by the port traffic. Interestingly, the tychopelagic species *P. sulcata*, *C. closterium* and *B. paxillifera* did not seem to be affected by contamination at the port stations. In a severely contaminated site these species, that thrive just above the sediments, were the only ones able to survive the extremely high concentrations of PCBs and heavy metals (Rubino et al. 2015).

### *4.2 Resting microbenthic abundance and community structure*

The resting microbenthos showed density and species richness values comparable to those registered in other sites inside and outside the Mediterranean. See Aydin and Uzar (2014), Aydin et al. (2015), Fertouna-Bellakhal et al. (2014) Satta et al. (2014) for recent Mediterranean studies and Baula et al. (2011), Liu et al. (2012) and Orlova and Morozova (2013) for recent extra-Mediterranean studies.

In this study we identified 50 different cyst morphotypes produced by dinoflagellates, corresponding to 46 taxa, due to the production of more than one cyst type for some species, and one resting spore produced by the diatom *Chaetoceros* sp.2. Only two remained determine as Dinophyta spp., while all the others were identified to the genus level at least. A very interesting comparison can be made with previous studies carried out in the Gulf of Trieste and also in the port area. This comparison allows us to update the knowledge on the resting stage assemblages in the area and infer also some changes that could have occurred during the recent years. The earliest study conducted by Nichetto et al. (1995b) reported 27 dinocyst types besides 6 unidentified "Cyst types". In the latter study some sampling stations roughly correspond to St. 1, 4 and 7 from the present study, plus the reference St. C1. Even though quantitative data are not reported, the authors indicated that the most abundant species were *Lingulodinium polyedrum* (as *Gonyaulax polyedra*) and *Scrippsiella acuminata* (as *S. trochoidea*), *i.e.* two of the most represented also in this study. Interestingly, the authors reported some species recorded in the water column as active stages but not in their resting phase in the sediments. Among them the following cyst producers were present: *Alexandrium tamarense*, *A. minutum*, *Protoperidinium conicum and P. pentagonum* (= *P. shanghaiense*) that were also identified in this study. During a previous study in the Gulf of Trieste conducted by Rubino et al. (2000) 19 dinocyst morphotypes, with total densities of 382 full cysts and 830 empty cysts cm$^{-3}$ of wet sediment were observed. Conversely, in both studies some *Protoperidinium* cyst types were reported that were not found in our samples, *i.e. P. claudicans, P. compressum, P. divaricatum*, and *P. leonis*.

In the present study, a high variability was registered among the three replicates of the samples at each station, both for full and empty cysts, although the latter showed much less variability. This could be simply due to the low amount of the oxic layer of the sediment available for the analysis. In fact, the total densities in the anoxic layer were much more constant (data not shown). The other possible explanation could be the high "natural" variability of the system, as highlighted by some following considerations. Nevertheless, the replication of the counts, together with the limit of 200 full cysts counted per sample, ensured an accurate analysis of the resting stage assemblages.

The lowest densities and species richness values were registered at St. 7, exactly like for the active microbenthos. But for the resting component of the microbenthos, the greater depth and especially the lower light intensity at the sea bottom, cannot explain these findings. The key factor must be searched among the water column conditions. At this station the lowest % of sand was found, and this indicates a reduced hydrodynamism that can influence the planktonic community (e.g. for the supply of nutrients) and consequently the resting stage production. The highest densities were registered at the highly contaminated stations close to the port and the industrial area, in particular at St. 3 and 4, where high species richness was also observed.

Besides *Chateoceros* sp.2, *Scrippsiella acuminata* and *Scripppsiella* sp.1 that were widespread and dominant across the entire study area, some species have reached high densities only at stations close to the port. Just like for the active microbenthos in these polluted areas, the structure of the community resulted altered. According to Liu et al. (2012), the increase of heterotrophic species in the sediments could be linked to the chemical pollution. Indeed at St. 3 and 4 some heterotrophs like *Oblea rotunda*, and many *Protoperidinum* species were recorded with the highest densities in this study. Besides them, also *Alexandrium minutum*, *A. tamarense*, *Alexandrium* spp., many calcareous species of the family Thoracosphaeraceae and *Pentapharsodinium dalei*, showed high densities at these stations, while *Lingulodinium polyedrum* was abundant at all stations. *Alexandrium* is a dinoflagellate genus that includes many potentially toxic species like *A. minutum* and *A. tamarense*. It is well known that the production of toxic compounds is an allelopathic character linked to competition and predation (Hakanen et al. 2014 and the references therein). Allelopathy is considered a major factor influencing the plankton's structure, so that the presence of numerous cysts of toxic species in polluted sediments can be a signal of the alteration of the phytoplanktonic community, in synergy with the effects of chemical pollutants. Other important species recorded at these sites are good indicators of eutrophic conditions.In fact *L. polyedrum* is a HAB species (Faust and Gulledge 2002) whose encystment is also induced by the presence of heavy metals (Okamoto et al. 1999), while *P. dalei* is linked to the increase of phosphate in upper waters (Zonneveld et al. 2012). It seems clear that a signal of chemical pollution or change in the trophic status of the system can be observed in the resting stage assemblages in the port area, even though in some other studies this was not apparent (Rubino et al. 2015). Another indicator of stress for the ecosystem was highlighted by the low values of the indices of ecological diversity registered throughout the entire study area. In particular the values of the evenness (J') and Shannon diversity (H') were very low compared to other polluted and confined areas, like the Mar Piccolo of Taranto (Rubino et al. 2015), the Bizerte Lagoon (Fertouna-Bellakhal et al. 2014) or the Homa Lagoon (Aydin and Uzar 2014).

### *4.3 Benthic ecosystem functioning*

The productivity of the microalgal community inhabiting surface sediments is known to be strongly depended upon the light availability at the bottom (Sundbäck et al. 2000). In our study area, primary production rates estimated at *in situ* light and temperature conditions (PPs) were close to zero at the deeper stations (St. 7, 1, C1). Overall, PPs and bottom PAR were significantly correlated ($R = 0.88$, $p < 0.05$). In contrast, the microalgal community at the shallower St. 6 was extremely active, as indicated also by the oxygen oversaturation at the water column bottom layer (107.1%). The highest PP rate estimated in this site was about twice higher than the maximum one previously measured in the Gulf of Trieste (Cibic et al. 2008b; Franzo et al. 2015). However, this major photosynthetic rate was likely ascribable to the lower depth (8.5 m *vs* 17 m) and therefore a higher light availability at the seafloor.

We observed an uncoupling between the PPs rate and total abundance that was more evident at St. 1 and 2, where the microalgal densities of about $2.1 \cdot 10^{5}$ cell cm$^{-3}$ did not resulted in equally high PPs rates. The PP rates mainly depend upon the photosynthetic efficiency of the microalgal cells, i.e. on their capability to perform photosynthesis in optimal conditions of light, temperature and nutrients. According to Franzo et al. (2015) this uncoupling could be induced by a low light availability at the sea floor during samplings, such as that which occurred at St. 2 due to cloudy weather conditions. This uncoupling further indicates that the MPB biomass and PP are not interchangeable in surface sediments (Forster et al. 2006). In fact, not all the chlorophyll *a* present in the sediments is likely to be active at once (Cahoon and Cooke 1992) and as cells move in and out of the photic sediment layer, remarkable and rapid changes in primary production rate can occur (Pinckney and Zingmark 1991).

Except for St.6, at the other stations light inhibition conditions were not achieved exposing the microalgae to a light gradient up to 300 µE m$^{-2}$ s$^{-1}$, a light intensity that is hardly reached at the seafloor at these depths. At St. 6 the maximum PPp was reached at 200 µE m$^{-2}$ s$^{-1}$ and photoinhibition was observed at 300 µE m$^{-2}$ s$^{-1}$. As benthic diatoms are not accustomed to high amounts of light as it is the phytoplankton, they tend to avoid overexposure to potentially damaging light levels by migrating deeper within the sediments (Consalvey et al. 2004). They are therefore able to track optimum light levels within the sediments and maximise absorption whilst minimising photoinhibition (Admiraal 1984). This leads to an energetically cheap, rapid and plastic response to optimise productivity over photophysiological adaptations (Serôdio et al. 2001). Van Leeuwe (2008) assumed that diatoms inhabiting sandy sediments depend more heavily on xanthophyll cycling than the ones on muddy sediments. In sandy sediments light penetrates deeper and also the diatoms vertical migration is more restricted. We can therefore infer that the diatom community at the sandier St. 6 did not have the possibility to optimise the exposure to the highest light intensity during incubation and so photoinhibition occurred.

Overall, the photosynthetic capability of the active microalgal community did not seem to be affected by contamination at St. 1, 2 and 3. In fact, PPp rates estimated at these stations were comparable to those obtained at the other stations and light availability at the seafloor confirmed to be the main driver of this photoautotrophic process.

The oxygen penetration at the most contaminated stations close to the port and industry infrastructure was very limited (0.8 – 1.5 mm) but the reduced thickness of the oxic layer was comparable to other similarly impacted sites (Cibic et al. 2012b; Rubino et al. 2015). Consequently, also the maximum oxygen consumption was observed at St. 2 and especially at St. 3. These high rates were likely ascribable to a lesser extent to the respiration of the benthic communities inhabiting the surface sediments, and to a greater extent to high TOC contents and the presence of metals in reduced chemical form. The thickness of the oxic layer at the other stations was comparable to that obtained in summer during a previous study at the reference St. C1 (Franzo et al. 2015).

## 5 Conclusions

The clear separation based on active and resting microbenthic community structure between stations close to the port and industry infrastructure (St. 1 - 4) from those nearby the residential area and in the centre of the bay clearly indicates that these benthic communities are affected by contamination. Even though no major differences in total abundances of the active microbenthos were observed among stations, a selection of stress-resistant species in the contaminated sites was highlighted, with *Nitzschia* cf. *commutata* being the most represented. The diatom genus *Gyrosigma*, that becomes opportunistic in organically enriched sediments, was likely too sensitive for these contamination levels and its specimens were reduced from the assemblage. The resting component of the microbenthos showed a clear response to the environmental conditions as well. The highest densities were registered near the industrial area, with key species like some potentially toxic *Alexandrium*, the heterotrophs and some calcareous-walled species, *i.e. Calciodinellum albatrosianum*, that were clearly linked to high contamination levels. Moreover, some changes in the community structure were detected, in comparison with previous studies, as well as the constant presence and dominance of taxa like *Lingulodinium polyedrum*, *Scrippsiella* spp. and *Protoperidinium* spp.

The photosynthetic capability of the active microalgal community did not seem to be affected by contamination at stations close to the port and industry infrastructure whereas light availability at the seafloor confirmed to be the main driver of this photoautotrophic process. In contrast, the reduced thickness of the oxic layer and the maximum oxygen consumption rates observed at the same stations were likely ascribable to high TOC contents and the presence of metals in reduced chemical form.

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.), we believe they may have broader implications and be extended to other contaminated areas in which similar findings are expected.

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# CHAPTER 3

Rogelja M., Nasi F., Cibic T., Auriemma R., Prato E., Del Negro P.: Response of two benthic communities (microphyto and macrozoobenthos) to synergistic effect of contaminants in the Mar Piccolo of Taranto

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# RESPONSE OF TWO BENTHIC COMMUNITIES (MICROPHYTO-, AND MACROZOOBENTHOS) TO SYNERGISTIC EFFECT OF CONTAMINANTS IN THE MAR PICCOLO OF TARANTO

## *RISPOSTA DI DUE COMUNITÀ BENTONICHE (MICROFITO-E MACROZOOBENTHOS) AGLI EFFETTI SINERGICI DI CONTAMINANTI NEL MAR PICCOLO DI TARANTO*

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### Abstract

In order to study the benthic ecological status of the Mar Piccolo of Taranto sediment samples were collected in June 2013 from four stations inside the two Inlets. For microphyto- and macrozoo- benthic communities total abundances were obtained and qualitative analyses were carried out to the lowest possible taxonomic level. Diversity indices were applied to benthic diatom taxa and macrofaunal species. This preliminary study showed that both the abundance and biodiversity of the investigated benthic communities were affected by sediment contamination. Moreover, the presence of stress-resistant species and very reduced abundance of some sensitive ones most likely indicates poor conditions of the benthic ecological status in the Mar Piccolo.

**Key-words:** microphytobenthos, macrozoobenthos, Mar Piccolo.

## 1 Introduction

Since 1960s the city of Taranto and its coastline have been subjected 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 dioxin. In fact, previously collected data on organic and inorganic pollutants in the Mar Piccolo have shown high levels of contamination and stress conditions on different communities (Cardellicchio *et al*., 2007; Spada *et al*., 2012). 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. 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). Previous studies on the benthic trophic web in the Mar Piccolo have been mainly focused on the macroscopic components, both the macrophyto- and the macrozoobenthic communities. As far as the heterotrophic benthic fraction is concerned, several studies have been centred on Amphipods (Prato and Biandolino, 2005) and only a few on Polychaetes (Prato *et al.*, 2000). In contrast, to the best of our knowledge, there are no data available on microphytobenthos. In this preliminary study we synoptically investigated the abundance and biodiversity of two benthic communities (microphytobenthos and macrozoobenthos) to try to assess how these autotrophic and heterotrophic communities respond to a synergistic effect of contaminants.

## 2 Materials and methods

The Mar Piccolo is a shallow, nearly enclosed basin connected through two narrow canals with the Mar Grande and widely with the Gulf of Taranto. It consists of two naturally divided basins, the First Inlet and the Second Inlet, with maximum depths of 13 and 10 m and a surface area of 8.28 km$^2$ and 12.43 km$^2$, respectively. The features of the Mar Piccolo in the Gulf of Taranto have been exhaustively described by Prato and Biandolino (2005). The sedimentation in this area is mainly influenced by land runoff, numerous submarine springs, small streams, sewage outfalls and industrial discharges rather than by marine currents. The sediment composition in both Inlets is clayey silt (SPICAMAR, 2003). The study was carried out in June 2013 when samples were collected in triplicate by a van Veen grab for macrozoobenthos and scuba divers using polycarbonate liners for microphytobenthos, at 4 stations from the Mar Piccolo (St. 2: 40.48361 N, 17.24611 E; St. 4: 40.47639 N, 17.26028 E; St. 5: 40.4825 N, 17.27833 E; St. 6: 40.4825 N, 17.295 E). For the sample processing, analysis and determination of the microphytobenthos and macrozoobenthos the methods described by Cibic *et al*., (2012) and Gambi *et al*., (2003) were followed. Univariate diversity analyses (PRIMER software v. 5) were applied to benthic diatom density and macrobenthic abundance.

## 3 Results

Microphytobenthic abundances in the uppermost layer of the sediments were 9200±1732 cells $cm^{-3}$ at St. 4, 22700±2722 cells $cm^{-3}$ at St. 5, 42000±3132 cells $cm^{-3}$ at St. 6 and 42700±8861 cells $cm^{-3}$ at St. 2 (Tab. 1). At all four stations diatoms were the most abundant taxonomic group even though their percentage decreased at St. 4 and St. 5 on the account of a higher incidence of filamentous Cyanobacteria, undetermined Phytoflagellates and resting spores. At stations situated in the Second Inlet (St. 5 and St. 6), the abundance of Cyanobacteria was higher, reaching about 10%. The two stations situated in the center of the basins (St. 2 and St. 6) were characterized by the highest microphytobenthic abundances, but differed markedly in community composition. Focusing solely on diatoms, the univariate diversity analysis confirmed the highest values of richness and diversity at St. 2 (d=3.55, H'=3.06), that is the site closest to the canal connected with the Mar Grande. The other three stations followed with remarkably lower values.

**Table 1** Microphytobenthic abundance and community composition at the four sites sampled in the Mar Piccolo.

*Abbondanza e composizione della comunità microfitobentonica nei quattro siti campionati nel Mar Piccolo.*

| Taxonomic group (cells $cm^{-3}$) | St.2 | St.4 | St.5 | St.6 |
|---|---|---|---|---|
| Bacillariophyta | 38700 | 6200 | 17100 | 36000 |
| Cyanobacteria | 400 | 700 | 2400 | 4700 |
| resting spores | 2100 | 1000 | 1600 | 400 |
| undet. Phytoflagellates | 1200 | 1000 | 1100 | 800 |
| Dinoflagellates | 300 | 300 | 500 | 100 |
| TOTAL ABUNDANCE | 42700 | 9200 | 22700 | 42000 |

The most abundant genus was *Nitzschia* spp. reaching the highest percentage at St. 6 (more than 58% of the total microphytobenthic abundance) and was therefore responsible for the lowest value of evenness (J' =0.59) and the highest dominance (λ' = 0.28) compared to the other stations. The total abundance of macrozoobenthos ranged from 589 ind. $m^{-2}$ at St. 4. to 1911 ind. $m^{-2}$ at St. 2. Overall, Polychaetes were the most abundant taxonomic group (42%), followed by Molluscs with 39% and Crustaceans with 13% of total abundance. The high number of Polycheates derived from their frequent occurrence at St. 2 (56% of total abundance), while at the others stations Molluscs represented the most abundant group. Only a few Crustaceans were observed at St. 2 (4.4%) but at the other stations their abundances increased considerably. Echinoderms were encountered only at St. 2 and St. 4 (4% and 1% of total abundance, respectively). At St. 2 more representatives belonging to the groups of Anthozoans, Sipunculids and Nemerteans were determined, leading to a higher richness at this station. In terms of community composition, the taxonomic group of Polychaetes was represented by 59 identified species, followed by Molluscs with 26 and Crustaceans with 21 species. “Others” and Echinoderms with 6 and 3 species respectively, followed far behind. Many species collected at the St. 2 were typically marine (i.e. the Bivalves *Plagiocardium papillosum* and *Pitar rudis* or the Echinoderms *Amphiura chiajei* and *Ophiotrix quinquemaculata*) in contrast to St. 4, 5 and 6 where different species typical of paralic environments occurred, namely *Abra segmentum*, *Cerastoderma glaucum*, *Gammarus aequicauda* and *G. insensibilis*. Especially at St. 4 but also at St. 6 we observed different epibenthic species or species related to the presence of algae (invasive Bivalve *Arcuatula senhousia* or other Bivalves as *Modiolarca subpicta* and *Modiolula phaseolina*, the Amphipods *Gammarus* sp. and *Leuchotoe* sp. or the alien Isopod *Paracereis sculpta*). At least at the St. 4 this could be potentially related to the low oxygen concentration and occurrence of contaminants in the sediments at these stations. Indeed, almost all endofaunal species observed at these sites are reported as dominant or prominent in areas that are polluted or enriched by organic material. These species are Bivalves *Corbula gibba* and *Parvicardium exiguum*, Polychaetes *Lumbrineris latreillei*, *Notomastus latericeus* and *Capitellidae* undet (Gray and Elliot, 2009).

**Table 2** Ecological parameters measured in the four sampling sites: S-number of species; N-number of organisms; d-Margalef’s index; J’Pielou’s index and H’Shannon-Wiener diversity index (Log2).
*Parametri ecologici misurati nelle quarto stazioni di campionamento: S = numero di taxa e N = numero di individui, d = Margalef’s index; J’Pielou’s index and H’Shannon-Wiener diversity index (Log2).*

| | S | N | d | J' | H' |
|---|---|---|---|---|---|
| **St.2** | 56 | 321 | 9,530 | 0,683 | 3,964 |
| **St.4** | 27 | 105 | 5,587 | 0,879 | 4,179 |
| **St.5** | 31 | 111 | 6,370 | 0,837 | 4,146 |
| **St.6** | 25 | 176 | 4,642 | 0,815 | 3,787 |

Due to the presence of such taxa these communities are likely considered stressed by anthropogenic impact. Diversity indices at all four stations were quite comparable (Tab. 2). Numbers of species (S) and individuals (N) at St. 4, 5 and 6 were half the number compared to the St. 2. In fact the lowest value of evenness (J’) was calculated at the St. 2 due to the dominance of individual species (the Polychaete *Notomastus formianus* and the Mollusc *Abra alba*).

## 4 Conclusions

This preliminary study showed that the abundance and biodiversity on of microphytobenthos and macrozoobenthos was highly affected by the poor environmental status of this marine ecosystem. For both communities the total abundances were quite low and characterized by a reduced biodiversity, especially at the heavily contaminated site nearby the military navy arsenal. However, the benthic communities at the St. 2, where the exchange of marine waters from the Mar Grande is facilitated through the canals, differed from those at the other investigated stations for the higher number of taxa and total abundance. Overall, abundances were still low compared to other similar environments. For instance, the microphytobenthic abundance did not reach half of the value of the much deeper stations close to the steelworks in the Port of Trieste (Cibic, personal comment).

Moreover, the presence of stress-resistant species and very reduced abundance of some sensitive ones most likely indicates poor conditions of the benthic ecological status in the Mar Piccolo.

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# CHAPTER 4

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

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# Microbenthic community structure and trophic status of sediments in the Mar Piccolo of Taranto (Mediterranean, Ionian Sea)

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## Abstract

This study aimed to assess the benthic ecosystem trophic status in a heavily polluted marine area and the response of the microbenthic community to multiple and diffuse anthropogenic impacts, integrating information coming from the active and resting (plankton's cysts) components of microbenthos. Two sampling campaigns were carried out in the period 2013-2014 and four sampling sites at different levels of industrial contamination were chosen within the 1st and 2nd Inlet of the Mar Piccolo of Taranto. The chemical contamination affected to a higher extent the active microbenthos than the resting one. In the central part of the 1st Inlet, characterized by more marine features, thrive a very rich and biodiverse microbenthic community. In contrast, at the polluted site near the military navy arsenal extremely low densities (9576 ± 1732 cells $cm^{-3}$) were observed for active microbenthos, but not for the resting community. Here, the high level of contamination selected for tychopelagic diatom species, *i.e.* thriving just above the surface sediments, while the other life forms died or moved away. Following the adoption of a 10 µm mesh, for the first time resting spores produced by small diatoms of the genus *Chaetoceros* were found. Our results further indicate that although the Mar Piccolo is very shallow, the benthic system is scarcely productive, likely as a consequence of the accumulated contaminants in the surface sediments that probably interfer with the proper functioning of the benthic ecosystem.

**key-words**: microphytobenthos; plankton; resting stages; benthic primary production; benthic respiration; confined marine basin; Ionian Sea

## 1 Introduction

In shallow water areas (particularly in estuaries and lagoons) where the bottom substratum is soft, most important biochemical processes take place in the sediment, where dense microbenthic communities drive central ecosystem functions, such as primary production, decomposition and nutrient cycling (Larson and Sundbäck 2008). Most of these microbial organisms are eukaryotic (diatoms, dinoflagellates, phytoflagellates, etc.), but also some prokaryotic photosynthetic organisms, such as cyanobacteria, contribute to the benthic community (MacIntyre et al. 1996). Microbenthic populations, especially in shallow waters, are often enriched by planktonic species. Active stages settle to the bottom also in the presence of turbulence, tidal currents and water column stratification, although with lower speed. Plankton supply microbenthos with resting stages (commonly named cysts) that, produced in the water column, sink to the bottom. In the sediments the cysts can remain viable for decades and more (Ribeiro et al. 2011), overcoming the unfavourable conditions in the water column and resting quite inert, with only a moderate metabolic activity (Binder and Anderson 1990). Once the conditions return optimal, they can germinate and, together with the settled active stages, they can be resuspended in the water column, entering the planktonic community again (Delgado 1989; Boero et al. 1996; McIntyre et al. 1996; Bravo and Figueroa, 2014).

Microphytobenthos (MPB) constitute the only autochthonous source of primary production on sediment in the absence of macroscopic vegetation (Larson and Sundbäck, 2008 and references therein). As the principal oxygen producer in coastal environments, MPB allow the aerobic degradation of both autochthonous and settled organic matter in sediments. Benthic microalgae regulate sediment-water nutrients fluxes and might reduce the population of nitrifying bacteria capable of having an active metabolism (Risgaard-Petersen 2003). MPB are key to the carbon cycle in littoral environments as producers of new organic matter that can enter the benthic and pelagic trophic web (Barranguet 1997). In sublittoral sediments, pennate diatoms usually account for the major part of the total microphytobenthic abundance (up to 97%) (Cibic et al. 2007a).

On the other hand, plankton's resting stages play a major role in the plankton dynamics, due to multiple reasons. They accumulate on the seafloor of coastal areas where, like seeds of terrestrial plants, can reach densities of millions of cysts $m^{-2}$ of bottom (Rubino et al. 2000). These huge amounts act as a reservoir of biodiversity for the plankton, conferring a high resilience to the species of an area. The encystment/excystment dynamics is a further example of benthic/pelagic coupling in coastal areas that severely influences the productivity of the ecosystem. The excystment, in particular, refuels the "active" community in the water column and, if conditions are particularly favourable, this can lead to planktonic blooms or harmful effects such as anoxia, fish kills and toxic episodes (Boero 1994).

Benthic diatoms and plankton's resting stages have been proven to be excellent bioindicators and are increasingly being used in marine biological monitoring (Cibic et al.

2008a; Kim et al. 2009; Wang et al. 2011). Diatoms have short generation times and they rapidly respond to environmental changes, while the structure of the encysted planktonic community in the sediments mirrors that of the active populations in the water column, so the integration of the information coming from benthic diatoms and cysts can provide early warnings of both increased pollution and successful habitat restoration in the pelagic and benthic compartments.

These organisms are sensitive to contamination and each *taxon* has a specific tolerance for heavy metals and organic compounds (Pospelova et al. 2002; Cunningham et al. 2003). The resulting composition of species, replacements, eliminations, diversity or abundance changes can give a proper idea of the recent history of environmental events affecting an area (Cibic and Blasutto 2011 and references therein; Liu et al. 2012).

Previously collected data on organic and inorganic pollutants in the Mar Piccolo have shown high levels of contamination, mainly heavy metals and polychlorinated biphenyls (PCBs), and stress conditions on different communities (Cardellicchio et al. 2007; Spada et al. 2012). 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. 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. 2012b). Previous studies on the benthic trophic web in the Mar Piccolo have been mainly focused on the macroscopic components. To the best of our knowledge there are no data available on microphytobenthos, while the plankton's cyst bank is well documented (Belmonte et al. 1995; Rubino et al. 1996; 1998; 2009). Therefore, the aim of this study was to investigate the response of the community to multiple and diffuse anthropogenic impacts and assess the benthic ecosystem trophic status in a heavily polluted area. Another focus of this study was to verify the hypothesis that some types of cysts suffer sediment oxic conditions.

## 2 Material and methods

### *2.1 Study site and sampling*

The study area has been exhaustively described by Cardellicchio et al. (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 1st Inlet, St. IE (depth: 11.2 m; 40° 29' 01'' N, 17° 14' 46'' E) and St. 1I (depth: 11.0 m; 40° 28' 46'' N, 17° 15' 38'' E) should summarize the environmental features of the area in front of the Navigable Channel and that one nearby the military navy arsenal, respectively. In the 2nd Inlet, St. 2B (depth: 7.0 m; 40° 28' 57'' N, 17° 16' 42'' E) and St. 2C (depth: 8.0 m; 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 1st Inlet) and, at the same time, a more pronounced influence of mussel farms.

**Fig. 1** Map of the Taranto marine area. ■ the four sites sampled in the Mar Piccolo in June 2013 and April 2014. The mussel plants in the 1st Inlet are intended only to the collection of the larvae and juveniles' growth.

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$^2$). At St. 1E and 1I (1st Inlet) macroalgae were absent, while at St. 2B and 2C (2nd Inlet), when present, scuba divers avoided them to maintain the same sampling conditions at all sites. One sediment core was immediately processed for primary production estimates, one was dedicated to oxygen microprofiling. The three remaining cores were brought to the laboratory, partially extruded and the oxic sediment layer (0-0.5 cm ca) of each core was collected, homogenised and used for the grain size analysis as well as the quantitative and qualitative determination of microbenthos. The same homogenised oxic sediment was also subsampled for the determination of heavy metals ans PCBs as detailed in Cibic et al. (this issue).

During each sampling, seawater temperature, dissolved oxygen and salinity along the water column were measured using a Seabird 19 Plus Seacat probe whereas the Photosynthetic Available Radiation (PAR) was registered by a PNF-300 Profiling Natural Fluorometer (Biospherical Instruments Inc.). PAR was converted to %PAR, that is the benthic PAR expressed as the percentage of surface irradiance.

### *2.1.1 Sediment grain size*

Aliquots (10-15 g) of homogenised oxic sediment were collected from each station and subjected to grain-size analysis. Each sample was disaggregated and the organic matter oxidized using 10% hydrogen peroxide (Carlo Erba, Rodano, Italy). Small pebbles and shell fragments were subsequently separated from sand and mud fractions by sieving at 2 mm. The analyses were performed using a Malvern Multisizer 2000S. Data are expressed as percentages of sand, silt and clay.
To estimate the water content of oxic and anoxic sediments, both in June and April an aliquot from each homogenised sample (~2 cm$^3$ of wet sediment) was weighed and dried overnight at 105°C.

### *2.1.2 Abundance and species composition of microbenthic active stages*

For the analyses of microphytobenthos, 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 $CaMg(CO_3)_2$ (Carlo Erba, Rodano, Italy), in pre-filtered bottom seawater (0.2 µm filters) (Schleicher&Schuell, Dassel, Germany). After manual stirring, 20 µL aliquots of the sediment suspension were drawn off from the slurries and placed into a counting chamber (Thalassia, Trieste, Italy). Only cells containing pigments and not empty frustules were counted under a Leitz inverted light microscope (Leica Microsystems AG, Wetzlar, Germany) using a 32x objective (320x final magnification) (Utermöhl 1958). The microalgal taxonomy was based on Round et al. (1992) and the AlgaeBase website (http://www.algaebase.org/). The qualitative identification of microphytobenthic assemblages was carried out to the genus and, when possible, to the species level using floras of Van Heurck (1899), Hendey (1976), Germain (1981), Dexing et al. (1985), Ricard (1987), Round et al. (1992), Tomas (1997) and Witkowski et al. (2000). Planktonic, tychopelagic (loosely associated with the sediment, commonly found in the water column) and benthic species were distinguished (Cibic et al. 2007b). Microphytobenthos data are expressed as cells cm$^{-3}$.

### *2.1.3 Abundance and species composition of microbenthic resting stages*

The study of the resting stage assemblages was differentiated in the two campaigns. During the first one (June 2013), oxic and anoxic sediments were obtained for each sampling site from the same cores used for the microphytobenthos. Due to the extreme thinness of the oxic layer, the amount of sediments was not enough to obtain replicates as for the microphytobenthos. This preliminar analysis of resting stage assemblages in oxic and anoxic sediments was performed to ascertain any possible differences in their structure caused by oxygen concentration. During the second campaign (April 2014), an *ad-hoc* sampling was carried out by scuba diving to estimate the structure of resting stage assemblages. In this case, since no noticeable differences were observed between the oxic and anoxic layers in the samples collected in June, no differentiation was made during the sampling and surface sediments (top 2 cm) were collected in triplicate at each station. To allow a comparison between the resting stage assemblages in the two campaigns, the data from oxic and anoxic sediments of June 2013 were merged, obtaining cyst densities homogeneous to those calculated for the April 2014 campaign (*i.e.* considering the top of the sediment without differentiation between oxic and anoxic layers).

From each sample, an aliquot (≈3 cm$^{3}$ of wet sediment) was treated in the laboratory, according to the sieving technique proposed by Montresor et al. (2010). The samples from the June 2013 campaign were screened through a 200, 75, 20 μm mesh battery (Endecott's LTD steel sieves, ISO3310-1, London, England) using natural filtered (0.45 μm) sea water. The retained fraction was ultrasonicated for 1 minute and screened again through the sieve battery, obtaining a fine-grained fraction (20-75 μm) containing protistan cysts and a >75 μm fraction with larger dinoflagellate resting stages. The material retained onto the 200 μm mesh was discarded. For the samples obtained during the second campaign, a 10 μm mesh was added to retain also the small diatom resting spores. No chemicals were used to dissolve the sediment particles to preserve calcareous and siliceous cyst walls.

Qualitative and quantitative analyses were carried out under an inverted microscope (Zeiss Axiovert 200M) equipped with a Nikon Coolpix 990 digital camera, using 32x and 40x objectives (320x and 400x final magnifications). Both full (*i.e.* with cytoplasmic content) and empty (*i.e.* already germinated) cysts were enumerated. The quantitative data on the community structure refer to full cysts only. In contrast, for the analysis of oxic and anoxic sediment layers, also empty forms were considered. When possible, at least 200 full cysts were counted per sample to obtain density values as homogeneous as possible and to evaluate rare species too. The >75 μm fractions were examined in their entirety.

All resting stage morphotypes were identified on the basis of published descriptions and germination experiments. Identification was performed to the species level when possible. As a rule, the modern biological names were used. For dinoflagellate cyst morphotypes whose active stage is unknown, the paleontological (fossil) names were used.

Quantitative data are reported as cysts cm$^{-3}$ of wet sediment (cysts cm$^{-3}$).

For germination experiments single viable cysts were isolated using a micropipette and placed into Nuclon microwells (NalgeNunc International, Roskilde, Denmark), containing ≈1 mL of natural sterilised sea-water. Cysts were incubated at 22°C, 10-14 h light-dark cycle and an irradiance of 80 μmol photons m$^{-2}$ sec$^{-1}$ and examined daily until germination, up to a maximum of 30 days. The incubation conditions were chosen on the basis of previous studies proven to be effective for a large number of species (Moscatello et al. 2004; Rubino et al. 2009; 2010).

Hereafter we use the terms active and resting microbenthos, to differentiate the active cells of both autotrophic and heterotrophic microbenthos from resting stages (cysts) of autotrophic and heterotrophic microplankton in the sediments.

### *2.1.4 Microphytobenthic primary production (PP)*

PP was estimated *in situ* from $^{14}$C-incubation of slurries. Five cm$^3$ of homogenised surface sediment were drawn off from the slurries, resuspended in 95 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 10 glass vials containing 9 mL which were divided as follows: 3 replicates to assess the sediment matrix effect, 3 replicates in dark and 4 replicates in light conditions. The first three samples were stopped by adding 200 µL of 5N HCl (final HCl concentration 0.11N) and the pH was estimated using a litmus test. The remaining 4 light and 3 dark samples were fixed on a rosette, lowered at the sea-bottom and incubated for 1 hour. After 1 hour, carbon incorporation was stopped by adding 200 µL of 5N HCl, too (Cibic and Virgilio 2010). Subsequently, samples were treated as described in detail by Cibic et al. (2008b).

### *2.1.5 $O_2$ consumption rates*

At each station one intact sediment core for $O_2$ consumption estimates in darkness was sampled and transferred to a cryostatic system to maintain the *in situ* temperature. A stable water flow above the sediment surface was established by a pressure-regulating air pump which maintained a diffusive boundary layer of 300-500 µm and kept the overlying water at atmospheric oxygen saturation at all times. Steady-state $O_2$ microprofiles were obtained 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 were recorded with the UnisenseProfix 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. (2007c).

## *2.2 Statistical analysis*

All univariate and multivariate statistical analyses, were performed using PRIMER software v.6 (PRIMER-E Ldt, Plymouth, UK) and STATISTICA v.7. The "DIVERSE" routine was used to calculate taxonomic richness (S), diversity (H’, Shannon and Weaver 1949), equitability (J’, Pielou 1966), and dominance (λ, Simpson 1949). For active microbenthos the analysis was carried out only for diatoms.

Cluster analysis (complete linkage) (Clarke and Warwick 2001) was performed on the Bray-Curtis similarity matrices of the means (*taxa vs* stations and seasons) based on total abundances both of active and resting microbenthos.

To investigate the influence of pollutants (PCBs, Hg and Pb) on the structure of the microbenthic community and the considered benthic processes, a data matrix was constructed with active and resting microbenthos mean abundance, primary production, $O_2$ consumption, sum of 28 PCBs, Hg and Pb values in surface sediments as variables and all samplings of June and April as samples.

Bi-dimensional representations of the statistical comparisons among the samples were obtained by non-parametric multidimensional scaling (nMDS) superimposing the hierarchical clustering with a cut at 50% and 65% of similarity for benthic diatoms and plankton's resting stages, respectively.

The data were previously fourth root transformed to down-weight the effect of common species on the analysis.

To detect which *taxa* were mainly responsible for the discrimination of sub-clusters, a 1-way similarity percentage procedure (the SIMPER routine) was used with a cut-off at 90% dissimilarity.

The variation in trends between sampling periods, inlets and position (central *vs* outer stations) was subsequently tested for significance with an analysis of similarity (ANOSIM), to test *a priori*-defined groups (subgroupings based on the above mentioned factors) against random groups in ordinate space. The $R_{ANOSIM}$ statistic values, generated by ANOSIM in PRIMER, are a relative measure of separation of the *a priori*-defined groups. A value of zero (0) indicates that no difference exists among groups, while the value 1 indicates that all samples within groups are more similar to each other than any samples from different groups. Only statistically significant data are presented.

In order to visualise how contamination could affect the considered biotic variables, a Principal Component Analysis (PCA) was carried out using STATISTICA v.7. The structure of microbenthic community (microphytobenthos and resting stages) and functional parameters (primary production, $O_2$ consumption) together with some major contaminants (sum of 28 PCBs, Hg and Pb) were simultaneously ordinated using first and second autovectors and first and second principal components.

## 3 Results

### *3.1 Physical data*

The grain-size analysis revealed that in June the percentage of sand was higher in the 1st Inlet (St. 1E = 18.5% and St. 1I = 16.2%) than in the second one (St. 2B = 7.5% and St. 2C = 2.2%). In contrast, in April comparable percentages were obtained among the first three stations (St. 1E = 14.4%, St. 1I = 13.2% and St. 2B = 14.3%) whereas in the centre of the 2nd Inlet the sandy fraction barely reached 3%. However, according to the Shepard's (1954) classification, at all stations the sediment was clayey silt.

At the bottom layer of the water column, temperature varied between 20.61 and 22.24°C in June and between 15.44 and 16.72°C in April, the higher values being measured at the shallower stations (Table 1). Lower salinity was registered in April compared to June due to a major freshwater input in this month; lower salinity was consistently measured in the shallower 2$^{nd}$ Inlet. In June the bottom layer of the 2$^{nd}$ Inlet was oversaturated in oxygen, while in April oversaturated conditions were observed in the whole Mar Piccolo.

**Table 1** Physical data measured at the bottom layer in the Mar Piccolo during the two sampling campaigns. %PAR= percentage of irradiance measured at the bottom vs. surface irradiance

| **site** | **Depth** m | | **temperature** °C | | **salinity** psu | | **O$_2$%** | | **bottom PAR** µmolphotons | | **surface PAR** µmolphotons m$^{-}$ | | **%PAR** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jun 13 | Apr 14 | Jun 13 | Apr 14 | Jun 13 | Apr 14 | Jun 13 | Apr 14 | Jun 13 | Apr 14 | Jun 13 | Apr 14 | Jun 13 | Apr 14 |
| 1E | 11.2 | 11.2 | 20.61 | 15.44 | 38.37 | 37.45 | 100.8 | 105.9 | 188.2 | 163.7 | 2790.3 | 2120.5 | 6.74 | 7.72 |
| 1I | 11.0 | 11.5 | 20.64 | 15.74 | 38.33 | 37.47 | 98.5 | 110.4 | 183.7 | 146.3 | 2466.4 | 2005.5 | 7.45 | 7.29 |
| 2B | 7.0 | 7.5 | 22.24 | 16.72 | 37.88 | 36.86 | 159.2 | 129.2 | 356.5 | 261.0 | 2302.8 | 2321.3 | 15.48 | 11.24 |
| 2C | 8.0 | 8.1 | 21.00 | 16.59 | 38.00 | 36.81 | 112.6 | 124.6 | 238.9 | 174.7 | 2334.9 | 2224.0 | 10.23 | 7.86 |

### *3.2 Microbenthos abundance and community structure*

Overall, for the active microphytobenthos 35 genera and 49 different species were determined in the two study periods and four sampling stations (Appendix 1). The biodiversity was higher in April 2014, when 38 species were identified with representatives in all the observed genera. In June 2013, 33 species were determined that were included in only 26 genera. Some diatom genera, *e.g. Auricula*, *Fragilaria*, *Frustulia*, *Grammatophora*, *Mastogloia*, *Melosira* and others, were present exclusively in April 2014. Some of them are predominantly freshwater (*Fragilaria*, *Frustulia*) (Round et al. 1992) and their occurrence could be due to the higher input of freshwater inflow in that period. Most of the identified diatom species were epipelic with some tychopelagic specimens (*Paralia sulcata*, *Bacillaria paxillifera* and *Ceratoneis closterium*) and epiphytic or planktonic free-living *taxa* (*Synedra* sp., *Fragilaria* sp.). A few filamentous Cyanobacteria from the genera *Oscillatoria* sp., *Anabaena* sp., *Spirulina* sp. were also identified. Finally, undetermined Phytoflagellates, naked dinoflagellates and *Prorocentrum* sp. were rarely encountered during both sampling campaigns.

Resting stages produced by plankton represented a small percentage of the microbenthic assemblages (Fig. 2b). Considering both viable and germinated forms, a total of 62 *taxa* were identified at least to the genus level (Appendix 2). All the 50 *taxa* identified from the first campaign were produced by dinoflagellates, while in the second campaign, among the 51 *taxa*, also small resting spores of diatoms were found, representing 21% of the resting microbenthic community. Two different types were observed, here reported as *Chaetoceros* sp.1 and *Chaetoceros* sp.2.

In June, the active microbenthos abundance in the uppermost sediment layer was extremely low at St. 2B (23103±2722 cells $cm^{-3}$), and particularly at St. 1I (9576±1732 cells $cm^{-3}$), whereas higher and comparable densities were found in the center of the 1st and 2nd Inlets (St. 1E = 43186±8861 cells $cm^{-3}$; St. 2C = 42245±3132 cells $cm^{-3}$) (Fig. 2a). A quite different pattern was observed in April, particularly due to a major total abundance detected at St. 1E (286559±47964 cells $cm^{-3}$). At St. 2C a comparable value to that of June was obtained, while still low densities were found at St. 1I and 2B (18990±3309 and 17570±3309 cells $cm^{-3}$, respectively). The resting microbenthos showed sharper differences between the two campaigns. In June the densities were very low, ranging from 241 cysts $cm^{-3}$ at St. 2C to 395 cysts $cm^{-3}$ at St. 2B (Fig. 2b). In April the lowest and highest densities were registered again at St. 2C (697±68 cysts $cm^{-3}$) and St. 2B (491±132 cysts $cm^{-3}$), but in general, the abundance at all the stations was almost twice compared to June. Overall, benthic diatoms were the most abundant group at all four stations, although their percentage decreased at St. 1I and St. 2B on the account of a higher incidence of undetermined Phytoflagellates and filamentous Cyanobacteria, especially in June (Fig. 2a). In the first sampling campaign, the abundance of Cyanobacteria was higher in the 2nd Inlet (St. 2B and St. 2C), reaching a relative abundance (RA) of over 10%.

Focusing on active diatoms, in June *Nitzschia* was the most abundant genus, followed by *Navicula*, *Amphora* and *Cocconeis*. The two stations situated in the center of the basins (St. 1E and St. 2C) were characterized by comparable microbenthos abundances, but they differed markedly in the community structure (Table 2). At St. 2C *Nitzschia* alone accounted for 68.6% of the whole diatom assemblage whereas at St. 1E it reached an RA of 26.1%. Although this genus was very abundant at St. 2C, it was represented by a few species, mostly the dominant *Nitzschia* cf. *angularis*, with an RA of 42.8%.

**Table 2** Univariate diversity indices calculated on the active and resting microbenthic assemblages at the four stations in the Mar Piccolo, in the two sampling campaigns. For active microbenthos only diatom *taxa* were considered. N= number of individuals; S= species richness; H'= Shannon-Wiener's index; J'= Pielou's index; ʎ= Simpson's index

**active microbenthos (Diatoms)**

| | **June 2013** | | | | | **April 2014** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **S** | **H'** | **J'** | **ʎ** | **N** | **S** | **H'** | **J'** | **ʎ** |
| **1E** | 31200 | 31 | 3.051 | 0.888 | 0.062 | 252800 | 53 | 2.994 | 0.754 | 0.092 |
| **1I** | 6000 | 10 | 2.125 | 0.923 | 0.140 | 13875 | 24 | 2.345 | 0.738 | 0.164 |
| **2B** | 15300 | 11 | 2.006 | 0.836 | 0.179 | 11300 | 17 | 2.275 | 0.803 | 0.158 |
| **2C** | 33000 | 12 | 1.864 | 0.750 | 0.221 | 30400 | 22 | 2.543 | 0.823 | 0.110 |

**resting microbenthos**

| | **June 2013** | | | | | **April 2014** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **S** | **H'** | **J'** | **ʎ** | **N** | **S** | **H'** | **J'** | **ʎ** |
| **1E** | 619 | 26 | 1.680 | 0.516 | 0.403 | 1385 | 36 | 1.799 | 0.502 | 0.274 |
| **1I** | 644 | 26 | 1.617 | 0.496 | 0.402 | 1360 | 37 | 1.946 | 0.539 | 0.288 |
| **2B** | 951 | 22 | 1.214 | 0.393 | 0.518 | 1586 | 35 | 1.763 | 0.496 | 0.317 |
| **2C** | 498 | 28 | 1.363 | 0.409 | 0.505 | 1337 | 33 | 1.665 | 0.476 | 0.327 |

In contrast, at St. 1E more than ten species belonging to the same genus were identified, namely *Nitzschia* cf. *angularis*, *N. commutata*, *N. tryblionella*, *N. lorenziana*, *N. punctata*, *N. longissima*, *N. vermicularis* and others. At the latter station an overall higher biodiversity was observed. In June, the highest values of richness and diversity were in fact obtained at St. 1E where the most abundant species, *Paralia sulcata*, reached a RA of only 8.5%. In contrast, the highest dominance was calculated at St. 2C due to a major abundance of *Nitzschia* cf. *angularis* at this site.

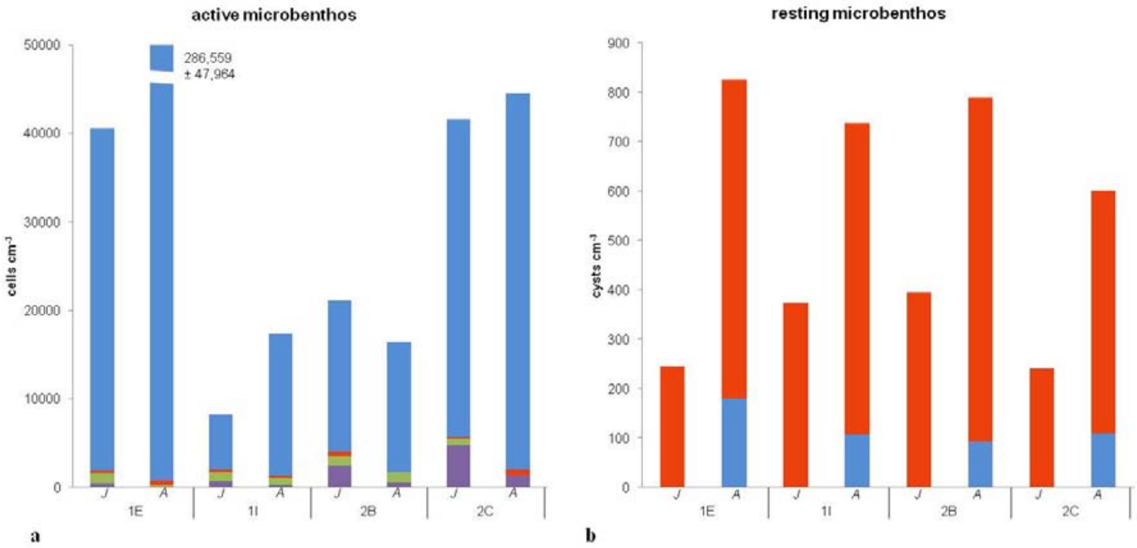

**Fig. 2** Abundance recorded for the active (a) and resting microbenthos (b) during the two sampling campaigns in the Mar Piccolo of Taranto. 🟦 diatoms; 🟥 dinoflagellates; 🟩 undet. phytoflagellates; 🟪 cyanobacteria;

In April, *Nitzschia* was again the dominant genus, followed by *Ceratoneis*, *Navicula* and *Bacillaria*. Compared to the other three stations, St. 1E was not only characterized by a major total microbenthos density, but also the highest richness in diatom *taxa* (Table 2). At this site, *Nitzschia* reached an RA of 37.5%, mainly due to the presence of *Nitzschia* cf. *angularis*, *N. sigma*, *N. lorenziana*, *N. fasciculata*, *N. longissima*, *N. sigmoidea* and others. However, the most abundant species encountered at this station was *Ceratoneis closterium*, with an RA of 18%. At St. 2C, *Nitzschia* accounted for 38.8% and was represented by *Nitzschia dubia*, *N. fasciculata* and *N.* cf. *angularis*.

The differences among stations and between the sub-basins too, were more relevant than those induced by the sampling season. According to the multivariate analysis, the stations 1E and 1I were clearly distinct, regardless the season (Fig. 3a). Also the sites 2B and 2C resulted well separated, even though they grouped together in the hierarchical cluster (cut-off at 50% of similarity), confirming an even more homogeneous community in the 2$^{nd}$ Inlet.

The main differences between the groups 1E and 1I were due to the higher density of *Ceratoneis closterium*, *Nitzschia* cf. *angularis* and *Bacillaria paxillifera* at St. 1E (SIMPER analysis; average dissimilarity 76.4%).

The groups 1E and 2$^{nd}$ Inlet (average dissimilarity 71.2%) differed again because of *C. closterium*, *Nitzschia* cf. *angularis*, *B. paxillifera* and *Paralia sulcata* much more abundant at St. 1E. Finally, differences between 1I and 2$^{nd}$ Inlet (average dissimilarity 66.9%) were due to several species belonging to the genus *Nitzschia*, namely *Nitzschia* cf. *angularis*, *Nitzschia dubia*, *N. fasciculata* that were more abundant at St. 2B and 2C compared to 1I.

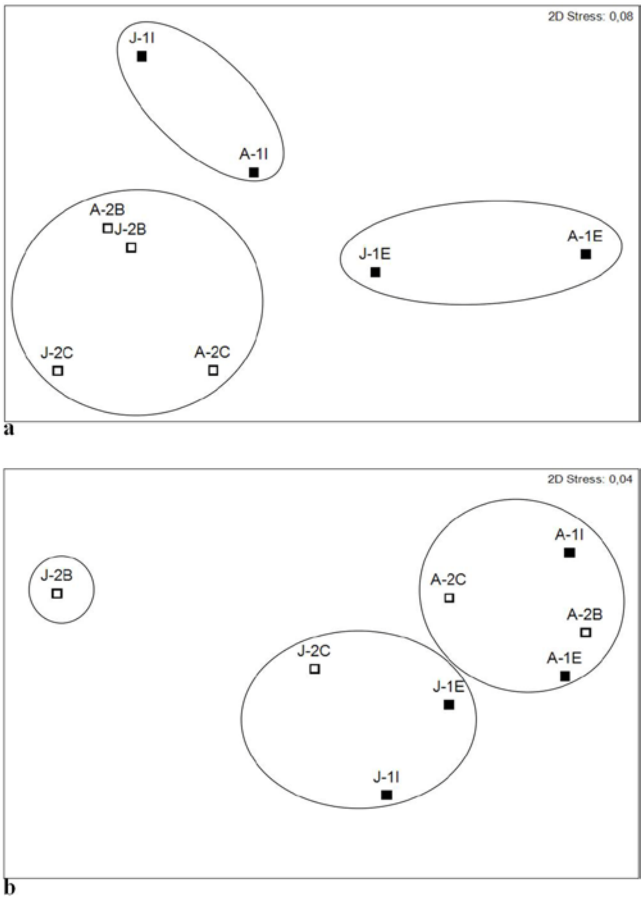

**Fig. 3** nMDS (non-metric Multi Dimensional Scaling) representation of the samples of active (a) and resting (b) microbenthos collected during the two sampling campaigns of June 2013 (J) and April 2014 (A) in the Mar Piccolo of Taranto. The cluster analysis at 50% and 65% of similarity is superimposed for active and resting microbenthos respectively. ■ 1st Inlet; □2nd Inlet

Regarding the resting microbenthos, in both campaigns, the dinoflagellate *Scrippsiella trochoidea* resulted by far the most abundant species, reaching 67% of the total abundance in June and 49% in April, when the detection of *Chaetoceros* spores decreased their RA. Other few *taxa* reached a high RA in both campaigns; they were *Calciodinellum operosum*, *Pentapharsodinium tyrrhenicum*, *Posoniella tricarinelloides* and *Scrippsiella* sp.1. Together they accounted for an RA of 23% in June and 17% in April when also other dinoflagellate *taxa* resulted important in the structure of the community, namely *Gonyaulax* group, *Gymnodinium impudicum* and *Lingulodinium polyedrum* that represented 5% of the total abundance of resting stages.

Both the species richness and the ecological diversity were markedly lower in the resting community, compared to the active one, with few dominant species characterizing the resting stage assemblages, as also indicated by the high values of the Simpson's index (Table 2). Unlike the active diatoms, little differences in the resting stage assemblages were registered between the two sub-basins of the Mar Piccolo, with higher species richness and ecological diversity in the 1st Inlet.

As a general pattern, in each sampling season, resting stages were slightly more abundant in the 2nd Inlet, and, as already observed for the active community, the sharpest differences resulted between the stations located at the center of the two sub-basins. In fact, in June *S. trochoidea* accounted for 62% of total abundance at St. 1E and 70% at St. 2C. In April, this species contributed for 43% to the total abundance at St. 1E and for 52% at St. 2C. An opposite trend was observed for resting spores of *Chaetoceros* spp., that were generally more abundant in the 1st Inlet (23% *vs* 19%).

At the community level, there were apparent differences linked to the sampling season. The samples from the two campaigns were clearly separated from each other (Fig. 3b). Only the sample collected at St. 2B in June remained isolated, because of its high abundance and low diversity, compared to the others from the same campaign.

The SIMPER calculated a higher similarity (81%) inside the April group of samples compared to June (63%), while only 38% of dissimilarity resulted between the two seasons. This value, lower than those registered for the active microbenthos, again, was probably due to the presence of the same few *taxa* that were very abundant in the two sampling periods (*i.e. Scrippsiella trochoidea*, *Scrippsiella* sp.1, *Gymnodinium impudicum* and *Gonyaulax* group). Instead, the species mostly responsible for this dissimilarity were *Chaetoceros* sp.2, *Alexandrium* sp.9, *Oblea rotunda*, *Pentapharsodinium tyrrhenicum*, *Posoniella tricarinelloides* and *Melodomuncula berlinensis*. All of these *taxa* were more abundant in April and together accounted for 30% of the recorded dissimilarity.

### *3.3 Resting microbenthos in oxic* vs *anoxic sediments*

Oxic layers of sediments collected during the campaign of June 2013 contained always more cysts than anoxic ones (Fig. 4). This was observed both for viable and germinated forms. The only exception was found at St. 1E, where total viable forms were slightly more abundant in the anoxic layer. The main responsible were the calcareous walled cysts of the dinoflagellate family of Thoracosphaeraceae.

Considering the total densities of viable forms, among the different dinoflagellate genera or group of species considered in this analysis, *i.e. Gonyaulax*, *Gymnodinium*, *Pentapharsodinium*, *Polykrikos*, *Protoperidinium*, *Scrippsiella* and "calcareous cysts", this latter not including *Scrippsiella* and *Pentapharsodinium*, the values were always higher in oxic sediments, with an RA variation ranging from 24% (calcareous cysts) to 57% (*Gymnodinium*). For germinated forms, *Pentapharsodinium* cysts showed an inverse pattern with 77% of variation, while all the other groups, again resulted more abundant in the oxic sediments, with variations going from 32% (calcareous cysts) to 67% (*Polykrikos*).

### *3.4 Functional parameters*

#### *3.4.1 Primary production*

In June primary production rates (PP) estimated *in situ* varied between 0.06 ± 0.01 mg C $m^{-2}$ $h^{-1}$ at St. 1I and 0.69 ± 0.08 mg C $m^{-2}$ $h^{-1}$ at St. 1E. Similarly, also in April very low rates (< 0.5 mg C $m^{-2}h^{-1}$) were estimated at all four stations, particularly at St. 1I, where values were close to zero (Fig. 5a).

#### *3.4.2 Oxygen consumption*

Oxygen microprofiles showed very different patterns according to the sampling period (Fig. 6). In June oxygen penetration was maximum at St. 1E, reaching about 3 mm. In the centre of the 2nd Inlet, the oxic layer was about 2.4 mm thick, whereas at St. 2B, and particularly at St. 1I, oxygen was completely depleted within the top 1.5 mm.

In contrast, in April, surface sediments of the Mar Piccolo were more oxygenated. At St. 2C oxygen was present up to 3.2 mm deep, at St. 1I and St. 2B the microprofiles showed a similar pattern although at the latter station a higher variability among replicates was observed. Only in the centre of the 1st Inlet oxygen was depleted within the top 2 mm, showing an opposite pattern between June and April compared to the other stations.

Oxygen consumption rates were calculated from the microprofiles performed in darkness. With the exception of St. 1E, at the other stations higher oxygen consumption was observed in June than in April (Fig. 5b). In June the oxygen minimum (as absolute value) was measured at St. 1E (-0.13 ± 0.03 µmol $O_2$ $cm^{-3}$ $h^{-1}$) while the highest rate was calculated at St. 2B (-0.48 ± 0.09 µmol $O_2$ $cm^{-3}$ $h^{-1}$). In contrast, in April, the rates ranged between -0.14 ± 0.03 µmol $O_2$ $cm^{-3}$ $h^{-1}$ at St. 2C and -0.21 ± 0.02 µmol $O_2$ $cm^{-3}$ $h^{-1}$ at St. 1I and were much more comparable among sites.

Oxygen data were converted to mg C $m^{-2}$ $h^{-1}$ to allow an estimation of the trophic status (autotrophy or heterotrophy) of the system (Fig. 5c, d). 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 (Cibic et al. 2012b and references therein). In our case, the incubation time was one hour, and therefore a GPP rate was measured. We attempted to estimate NPP by subtracting CR, assessed as oxygen consumption in the dark.

Overall, the benthic system of the Mar Piccolo resulted net heterotrophic in both sampling periods and at all sites. In June, rates varied between -3.70 mg C $m^{-2}h^{-1}$ at St. 1E and -8.17 mg C $m^{-2}h^{-1}$ at St. 2B, and in April between -3.62 mg C $m^{-2}h^{-1}$ at St. 1E and -6.56 mg C $m^{-2}h^{-1}$ at St. 1I.

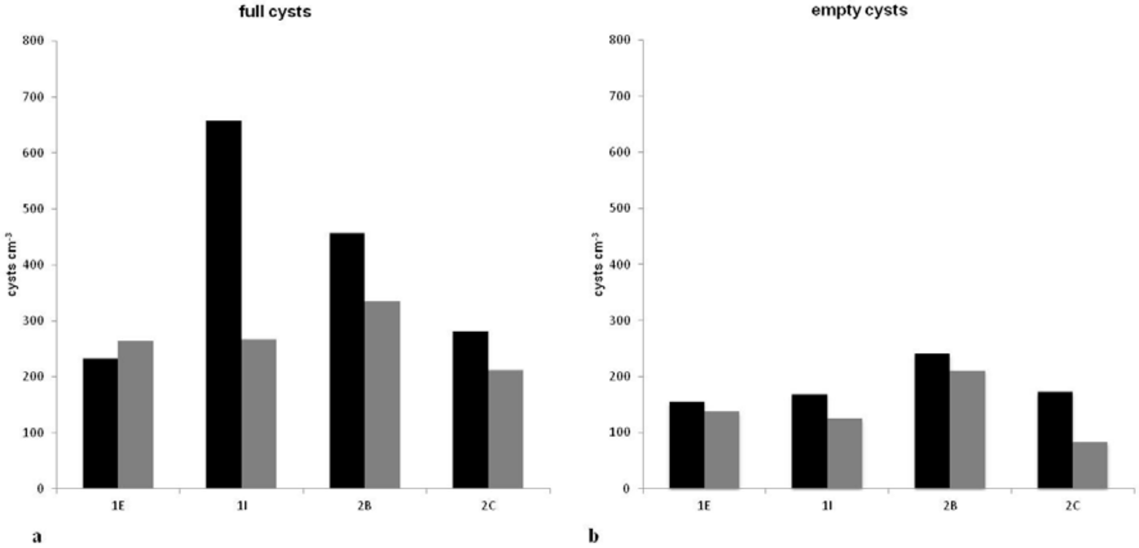

**Fig. 4** Abundance of full (a) and empty (b) cysts of plankton during the June 2013 campaign in the Mar Piccolo of Taranto. ■ oxic sediment layer; ■ anoxic sediment layer

### *3.5 Influence of pollutants on the microbenthos and benthic processes*

According to ANOSIM, the structure of the total microbenthic community (active + resting) did not significantly change between the two sampling periods under the influence of the environmental parameters and pollutants. Therefore, the June and April data were pooled for further analysis. Thus, it was possible to observe a significantly different response of the communities at the central sites (St. 1E and 2C) *vs* the outer ones (St. 1I and 2B) ($R_{ANOSIM}$ = 0.635, $p < 0.05$) that grouped separately in the nMDS plot (data not graphically shown).

The ordination plot of the considered parameters accounted for 63.4% of the total variance (Fig. 7). The principal component axis 1 (PC1) explained 42.8% of the total variance, whereas the PC2 axis explained 20.6% of the remaining variance. Pb, Hg and the sum of PCBs were the predominant elements of the first factor, while the major contributors of the second one were $O_2$ consumption and active microbenthos (AMB). Plotting all the samplings on the PCA factor-plane 1x2, they could be separated into three distinct groups, positioned along an increasing gradient of contamination (from quadrant III to quadrant II). Both June and April samplings, carried out at the central stations (1E and 2C), were gathered in the same group, confirming the output of the ANOSIM analysis.

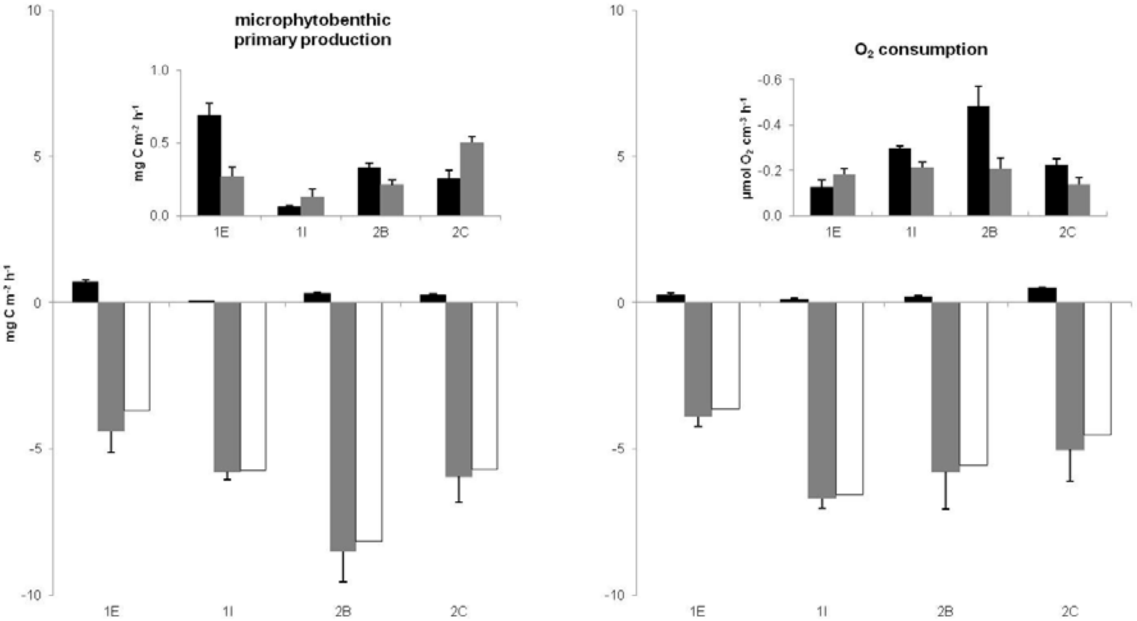

**Fig. 5** (a) Primary production rates estimated *in situ* at the four sites in the two sampling campaigns in the Mar Piccolo of Taranto. ■ June 2013; ▨ April 2014 (b) Oxygen consumption estimated in darkness and at *in situ* temperature at the four sites in the two sampling campaigns in the Mar Piccolo of Taranto. ■ June 2013; ▨ April 2014 (c,d) Rates of Gross Production (from $^{14}$C data), Consumption (converted from $O_2$ consumption data) and Net Production (estimated) in c) June 2013 and d) April 2014 ■ gross production; ▨ $O_2$ consumption in darkness; ☐net production (estimated)

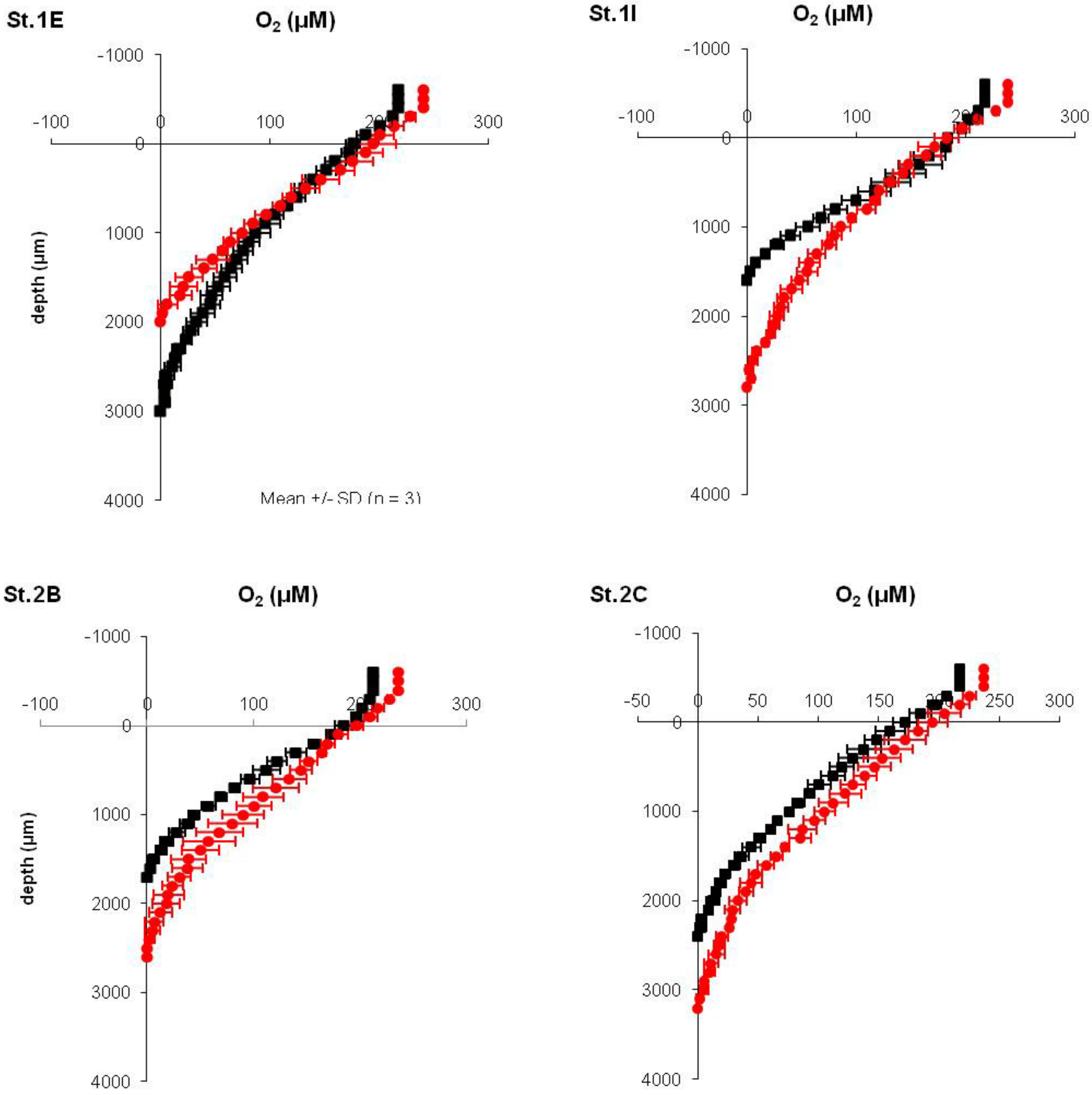

**Fig. 6** $O_2$-microprofiles estimated at the four stations in the two sampling campaigns in the Mar Piccolo of Taranto ■ June 2013; 🟥 April 2014

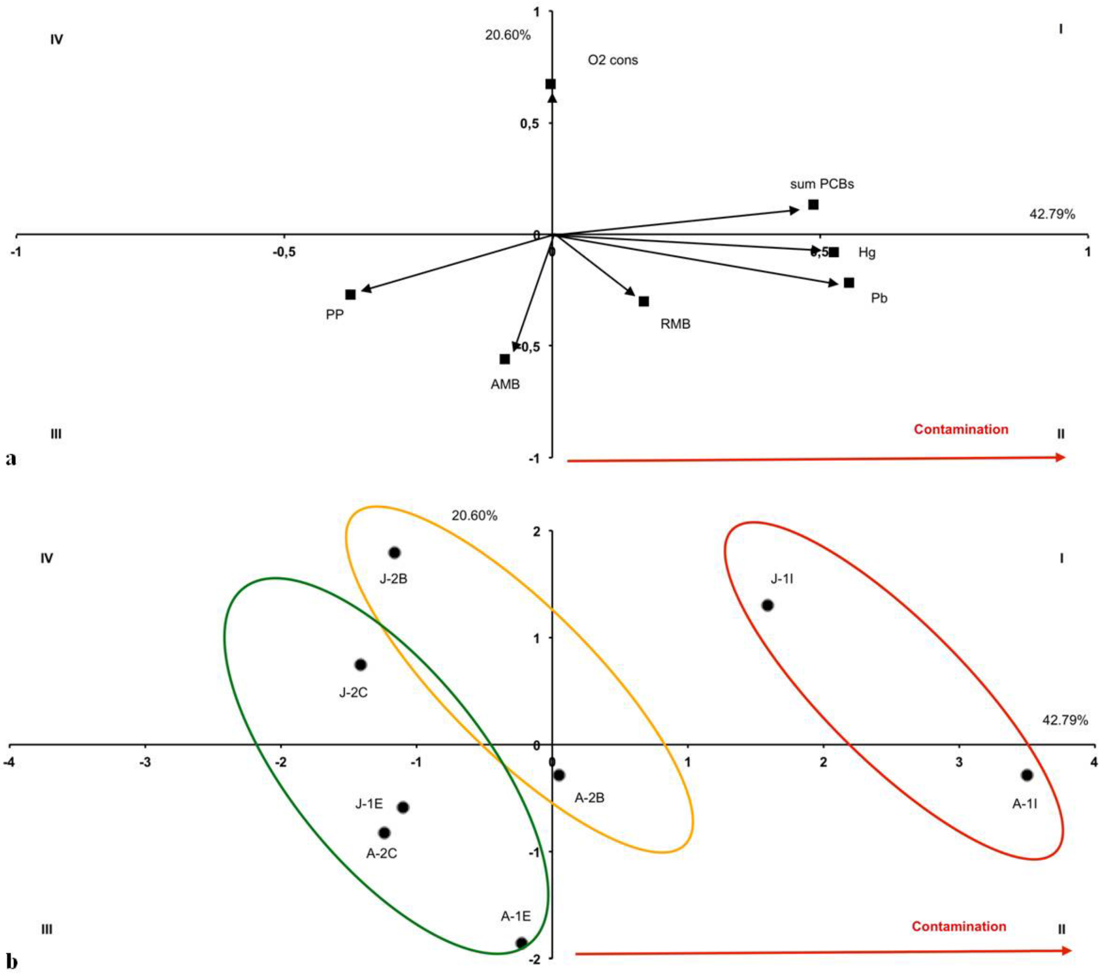

**Fig. 7** PCA ordination diagram based on the selected variables (a). Scatter diagram plotting factors 1 and 2 of samplings carried out in June 2013 and April 2014 (b) in the Mar Piccolo of Taranto. $O_2$ cons: oxygen consumption; sum PCBs: sum of the 28 considered polychlorobyphenyls; AMB: active microbenthos; RMB: resting microbenthos; PP: primary production

## 4 Discussion

### *4.1 Microbenthic community composition*

Compared to phytoplankton, the literature on microphytobenthos (MPB - here reported also as active microbenthos) is rather poor due to the major complexity of sampling and difficulties in cell identification in muddy or sandy matrices. Although the ecological role of the active microbenthos is widely recognized, until now only few coastal areas and lagoons have been investigated in Europe. In the last decades, the literature on microphytobenthic distribution in the Mediterranean basin was limited to Spanish and French coasts (Delgado, 1989; Barranguet et al., 1996; Barranguet, 1997; Riaux-Gobin et al., 1998). Only recently, other European sites have been studied, such as the Tagus estuary in Portugal (Jesus et al., 2009 and references therein) and the French Atlantic coast

(Guarini et al., 2004 and references therein) where the importance of active microbenthos as primary producers in coastal and lagoonal ecosystems has been highlighted. Along the Italian coasts, MPB have been studied in soft sediment samples of the Gulf of Trieste (Cibic et al. 2012a and references therein), the Venice lagoon (Facca e Sfriso 2007 and references therein), the Adriatic coast from Ancona to the Po delta (Totti 2003) and the Lesina lagoon (Gambi et al. 2003). To the best of our knowledge this is the first study reporting data on MPB of the Mar Piccolo of Taranto.

Except for St. 1E in April, in the rest of the basin total microbenthos abundances did not exceed 5· 10$^{4}$ cells cm$^{-3}$ during the study period. These densities are quite low for a shallow environment, such as the Mar Piccolo, where the maximum depth is about 12 m. Both in June and April, the seafloor was well illuminated as confirmed by PAR profiles registered at each station (Table 1). In fact, the percentage of irradiance measured at the bottom did not drop below 6.7%, indicating that light availability was not a limiting factor for the development of the microphytobenthic community. Comparing our abundances to those reported from other sublittoral areas and in the same months, MPB reached much higher values in other regions (Cibic et al. 2007a and references therein). For instance, in an 8-m-deep site located nearby Tautra Island (Norway) MPB varied between 13:610$^{4}$ cells cm$^{-3}$ in April and 14.8 · 10$^{4}$ cells cm$^{-3}$ in June 2005 (Cibic et al. 2007c), while at a 21-m-deep station located in the centre of the Gulf of Trieste up to 15· 10$^{4}$ microphytobenthic cells cm$^{-3}$ were reported in June 2001 (Cibic et al. 2009).

Despite the fact that the Mar Piccolo is a small basin (surface area of 20.72 km$^{2}$), different situations were highlighted at the four chosen sampling sites. Within the surface sediments of the central part of the 1st Inlet thrive a very rich and biodiverse active microbenthic community that may become quite abundant in spring, when the environmental conditions are optimal for the development of the benthic diatom assemblage (low temperature and increasing irradiance). The higher number of *taxa* and total abundance observed at this site are likely ascribable to the input of marine waters from the Mar Grande through the canals that are closest to St. 1E. Therefore, among the four sites, St. 1E was characterised by more marine features. This was confirmed by the presence of typically marine diatom *taxa* such as *Ceratoneis closterium*, *Psammodictyon panduriforme*, *Rhopalodia gibba*, *Nitzschia longissima*, *Gyrosigma fasciola* and others. At the other stations their density was very reduced or they completely disappeared from the assemblage. Moreover, especially in April some diatom species appeared very viable and full of pigments (*Bacillaria paxillifera*, *Caloneis liber*, *Nitzschia* cf. *levidensis*, *Toxarium hennedyana*, *Pinnularia* spp.) and several organisms belonging to the genera *Nitzschia* and *Caloneis* were in vegetative division, suggesting that the community in this site was in the logarithmic phase of growth.

At the station close to the military navy arsenal (St.1I) extremely low densities of the active microbenthos were observed both in June and April. Comparing this station to other sublittoral sites, such low abundances were seldom observed before, not even in low light and/or stress conditions (Cibic, personal comment). Cibic et al. (this issue) reported the concentrations of 13 heavy metals and 28 congeners of polychlorobiphenyls from the surface sediments of the same four stations investigated in this study. We infer that at St. 1I the contamination level is so high, or rather, the synergistic effect among various contaminants is such as to interfere with the proper development of the microphytobenthic community. In the lagoonal system of Grado-Marano (northern Adriatic Sea), despite a severe contamination by hydrocarbons and heavy metals, exceptionally high microphytobenthic densities, over half a million cells cm$^{-3}$ (up to 50 times higher compared to that at the polluted St. 1I), were found (Cibic et al. 2012b). In that lagoon MPB was not only abundant but also extremely biodiverse and active, suggesting that this community was not in the least influenced by the presence of contaminants. In contrast, at St. 1I, apart the very low abundance, the few encountered diatoms contained scarce pigments in damaged frustules. Interestingly, the few observed viable diatoms were tychopelagic, namely *Paralia sulcata* and *Bacillaria paxillifera*. *P. sulcata* is a non-motile centric diatom, living loosely linked with the sediments (Round 1985), while *B. paxillifera* forms colonies whose characteristic movement makes the cells slide forward while adhering to one another for the full length of the raphe (Hendey 1976). At St. 1I both species were moderately abundant in April, indicating that at this site diatoms with a specific life strategy were preferentially selected, *i.e.* those that thrive just above the surface sediments rather than within them. In fact, subsurface sediments are likely too contaminated to become a favourable environment to be colonised by benthic diatoms. This could represent an adaptation strategy of the benthic diatom assemblage to severe contamination nearby the navy arsenal. At this site, besides the above mentioned species, few other diatoms were observed, one of them was *Nitzschia* cf. *commutata*. This species was present in high relative abundance in sediments from the port area of Trieste, up to 22.5% in the most impacted area (data not published) indicating that this species is tolerant to pollution.

The two sites investigated in the 1st Inlet clearly differed, as shown also by the multivariate analysis, and *B. paxillifera* was one of the few *taxa* indicated by the SIMPER as responsible for the dissimilarity among the sites.

On the basis of all this, the great dissimilarity observed between St. 1E and 1I could be ascribed to a cumulative effect of two factors: the marine influence at St. 1E and the high level of contamination at St. 1I. This could explain also the more homogeneous patterns observed in the 2nd Inlet in both the sampling seasons. Indeed, a certain degree of influence of the contamination from the St. 1I to the closest site in the 2nd Inlet, *i.e.* St. 2B, could be inferred by the quite comparable structure of the communities. In fact, although St. 2B is located in the 2nd Inlet and is shallower than St. 1I, the active microbenthic communities at the two stations were characterised by very low abundance and ecological diversity and a lower dissimilarity, compared to that between the two sites in the 1st Inlet. Moreover they were closer in the nMDS, even though in different clusters. Due to the hydrological features of the Mar Piccolo, it is likely that the uppermost sediment layer in the navy arsenal, enriched in contaminants, has been resuspended by tidal currents, transported into the 2nd Inlet and settled in its north-western part, where St. 2B is located (De Pascalis et al. this issue). However, compared to the 1st Inlet in June, a higher RA of Cyanobacteria was observed at this site. Most of the them were filamentous Cyanobacteria, but some cells of *Oscillatoria* spp. and *Spirulina* spp. were also encountered.

In June, filamentous Cyanobacteria were even more abundant (3300 ± 1873 cells cm$^{-3}$) in the centre of the 2nd Inlet whereas the genus *Anabaena* reached up to 1500 cells cm$^{-3}$. The major density of Cyanobacteria was likely due to the more lagoonal features of the 2nd Inlet (higher freshwater input and lower salinity); higher temperatures, as that registered in June, also favoured the development of these microalgae in the surface sediments.

As widely expected, a very different picture came out from the analysis of the resting microbenthos. Since the first studies conducted on the benthic resting stage assemblages produced by planktonic species in the Mar Piccolo (Rubino et al. 1996; 1998), it has been evident the role of this confined basin as reservoir of biodiversity for the plankton. During this study this has been confirmed, even though lower densities and diversity were registered, at least in the June 2013 campaign. This could be explained according to the seasonal fluctuations that characterize the plankton dynamics in coastal waters. In fact, even if the sediments act as a tank where resting stages accumulate over the time, nonetheless, the very surface layer is continuously modified by the arrival and leaving of the cysts according to the encystment/excystment dynamics (Rubino et al. 2009; Anglés et al. 2012). We hypothesise that before June 2013 an important recruitment occurred from the sediments into the water column for the phytoplanktonic community, with the consequent decrease of the cysts' densities. In April 2014 this recruitment had probably not yet occurred and the densities resulted much higher. This explanation is consistent with the little increment of the ecological diversity from June 2013 to April 2014, because the entire biodiversity remains stored in the sediments, even if the densities fluctuate. Anyway, the global diversity resulted lower than that registered in previous studies in the Mar Piccolo (Rubino et al. 2009 reported 76 dinocyst morphotypes only at St. 1E) and comparable to other similar sites in the Mediteranean (Rubino et al. 2010; Satta et al. 2010; 2014). A few dinoflagellate species resulted dominant in both the sampling periods and they were all produced by the family Thoracosphaeraceae; in April 2014 also an important contribution was given by little spores of two diatom species of the genus *Chaetoceros*. This finding is of paramount importance for the benthic-pelagic coupling because concomitantly, in the water column high densities (over $3\cdot 10^{6}$ cells L$^{-1}$) of small *Chaetoceros* cells were found (Karuza et al. this issue). This is the first observation of these diatom spores in the Mar Piccolo and it was consequent to the adoption of a 10 μm mesh during the treatment. Now further studies are needed to investigate the assemblage of these resting stages and its role in the plankton dynamics in this basin, also with the adoption of more suitable methods to detect the presence of small diatom spores (*e.g.* McQuoid et al. 2002).

Active and resting microbenthic communities showed rather different responses to the environmental conditions, that the multivariate analysis revealed as differences among the sites and between the sampling seasons. Active microbenthos spend all its life in the sediments, while resting stages come from the pelagos. This allowed us to integrate the information coming from these two compartments of the ecosystem and acquire a more complete knowledge about the feedback of the organisms to the environmental conditions, both natural and linked to human activities.

For instance, the sediment contamination at St. 1I, did not seem to influence the resting assemblage as it did for the active microbenthos. In fact, the densities and species richness were comparable to those at the other sites. Indeed, the ecological diversity resulted the highest in April 2014. Recently, many studies attempted to discover the environmental signals encoded in the benthic resting stage assemblages (Shin et al. 2010 and references therein). Chemical pollution has been correlated to a decrease of cyst abundance and the increase of the importance of cysts produced by heterotrophic species (Liu et al. 2012). Our results, clearly did not support this hypothesis. Only in some heterotrophic species the signal of pollution was evident. In fact, among the *Protoperidinium* cysts, many were present exclusively or with at least higher densities at St. 1I but, in our opinion this is not enough to support the pollution signal hypothesis. As already stressed for the active microbenthos, it is clear that in a complex environment like the Mar Piccolo, there is a network of causal and correlation factors acting to promote the response of the single species and the entire communities. For resting stage assemblages produced by plankton, the matter is even more complex because these factors must be related to both the water column and the sediment conditions (*e.g.* nutrient enrichment, temperature, salinity, sedimentation rate, sediment grain-size, bottom oxygen concentration, pollution). It is evident that in many cases this response should be site-specific and only merging much knowledge as possible from many different scenarios it would be possible to achieve general patterns. As already hypothesized by Shin et al. (2010) for some embayments in Korea and Japan, freshwater inputs can influence the structure and distribution of dinocyst assemblages in the sediments. The Mar Piccolo receives freshwater by small rivers and, most of all, by submarine springs that in some areas of the basin can lower the salinity below 36 psu (Rubino personal comment). Also bottom water oxygen concentration could be a major factor in the Mar Piccolo. At St. 1I, in average, the lowest values were registered. Moreover, particularly in June 2013, the oxic layer of the sediments was very thin and this could explain the good conservation of dinocysts, *i.e.* the high abundance and the higher density registered in the oxic layer *vs* the anoxic one. According to Zonneveld (1997) organic-walled cysts are sensitive to the exposure to oxic conditions that can hardly alter the structure of cyst assemblages. In the Mar Piccolo this factor did not seem to be important in surface sediments. Organic-walled cysts were always more abundant in oxic sediments, both full/viable cysts and empty/germinated ones. Even in the very thin oxic layer at St. 1I, the density was by far higher than in the anoxic layer. This result, again, leads to consider the specificity of each study site as the main element to be regarded trying to correlate environmental variables to the structure and distribution of resting stage assemblages.

### *4.2 Functional parameters*

The active microbenthic community has a key ecological role in the aquatic ecosystem, being responsible for the nutrient and oxygen flux regulation at the water-sediment interface and contributing significantly to the primary production (MacIntyre et al. 1996), even though, generally, planktonic productivity is much considered and studied. Martin et al. (1987) and Longhurst et al. (1995) estimated that 0.7% of the total oceanic production and 2.4-3.7% of the continental shelf production is due to benthic microalgae. There is the need to evaluate the magnitude and distribution of benthic microalgal production and biomass in continental shelf and other subtidal, neritic ecosystems and to consider the broader implications of this evaluation (Cahoon, 1999 and references therein). Along the Italian coasts, microphytobenthic primary production (PP) has been previously estimated in soft sediments of the lagoonal system of Grado-Marano (Blasutto et al. 2005, Cibic et al. 2012b) and in the Gulf of Trieste (Cibic et al. 2008a, b). In the first study carried out in May 2002, the authors have reported mean PP values of 5.1 ± 3.3 mg C $m^{-2}$ $h^{-1}$ in the Grado lagoon (1.4 m < depth < 2 m) and 2.5 ± 1.3 mg C $m^{-2}$ $h^{-1}$ in the Marano lagoon (0.7 m < depth < 1.7 m). In the second study, a PP value of 1.7 ± 0.32 mg C $m^{-2}$ $h^{-1}$ has been estimated in January 2011 in a lagoonal site at a depth of 3.6 m. Over a two-year study carried out in a 17-m-deep site in the Gulf of Trieste, PP rates up to 34.59 ± 7.66 mg C $m^{-2}$ $h^{-1}$ have been estimated (Cibic et al. 2008b). In surface sediments with a high organic load due to sewage discharge, the PP rate estimated at a depth of ca. 22 m equalled 4.9 ± 0.3 mg C $m^{-2}$ $h^{-1}$ in June 2004 (Cibic et al. 2008a). All these rates are remarkably higher compared to those estimated in the Mar Piccolo of Taranto, where values were < 0.7 mg C $m^{-2}$ $h^{-1}$ at all stations and both sampling campaigns. These rates are particularly low for a shallow basin with a maximum depth inferior to 12 m. Especially in April at St. 1E, where the surface sediments were colonised by an abundant microphytobenthic community, a higher PP value was expected. We infer that the microphytobenthic community conveyed the C flux primarily to the production of new biomass at the expense of other primary production pathways (*i.e.* extrusion of extracellular polymeric substances). Moreover, among diatoms several epiphytic *taxa* were present with a relatively high RA (species belonging to the genera *Synedra*, *Achnanthes*, *Licmophora*) that are capable of heterotrophic metabolism (Tuchman et al. 2006) and, therefore, could have not been photosynthetically active.

At St. 1I and 2B, minima PP rates were a result of low microphytobenthic densities. Furthermore, at St. 1I rates close to zero were likely ascribable to the presence of one or more synthetic organic substances that might have interfered with the liquid scintillation analysis applied in this study. For instance, glutaraldehyde is known to induce autofluorescence in cells fixed for florescence microscopy (Collins and Goldsmith 1981) and has also been found to interfere with primary production estimates (Cibic and Virgilio 2010).

In April surface sediments of the Mar Piccolo were more oxygenated than in June, as generally occurs after the winter mixing. The only exception was St. 1E, where an opposite pattern was observed. We assume that in the centre of the 1st Inlet the oxygen in the surface sediments was partly consumed by the abundant microbenthic community that was not particularly photosynthetically active and likely respired some of the produced oxygen. At St. 1E also the macrobenthic community reached a relative maximum of abundance (Franzo et al. this issue), contributing to a major oxygen consumption in this site.

Due to low PP rates, the benthic system of the Mar Piccolo resulted net heterotrophic. However, in the estimate of the trophic state we did not consider the contribution of macroalgae, potentially a major primary producer but with a patchy distribution and strong biomass fluctuations according to the seasonal cycle. They were absent in the 1st Inlet, while at St. 2B and 2C a large coverage was detected (Cibic et al. this issue). Therefore our estimate of the primary production (and consequently of the trophic state) in sediments of the 1st Inlet could represents a quite good approximation of the actual rate, whereas it was likely underestimated in the 2nd Inlet.

## 5 Conclusion

This is the first attempt to integrate information coming from active and resting microbenthos in a marine coastal area, aiming to assess the benthic ecosystem trophic status and the response to multiple and diffuse environmental and anthropogenic impacts.

At the four sampling sites different situations were highlighted.

In particular, the chemical contamination in the Mar Piccolo seems to affect to a higher extent the active microbenthos than the resting one, likely due to the coming of this latter from a different domain, *i.e.* the pelagos, and its higher resistance to the conditions in the sediments. For the first time many resting spores produced by small diatoms of the genus *Chaetoceros* were found. This result was concomitant to the detection of high densities of small cells in the water column. Further studies are needed to identify the species and quantify their role in the plankton dynamics in the Mar Piccolo.

Within the surface sediments of the central part of the 1st Inlet thrive a very rich and biodiverse microbenthic community that may become quite abundant in spring, when the environmental conditions are optimal for its development. In contrast, at the military navy arsenal extremely low densities were observed for active microbenthos, but not for the resting community. The high level of contamination in the most impacted area of the Mar Piccolo selected for tychopelagic diatom species, *i.e.* loosely associated with the sediments and thriving just above the surface sediments, while the other life forms died or moved away. Finally, in the 2nd Inlet, characterised by more lagoonal features, filamentous Cyanobacteria were quite abundant whereas the benthic diatom community was dominated by *Nitzschia* that alone accounted for almost 70% of the whole assemblage but was represented by a few species.

Our results further indicate that although the Mar Piccolo is very shallow, the benthic system is scarcely productive, likely as a consequence of the accumulated contaminants in the surface sediments that probably interfere with the proper functioning of the benthic ecosystem.

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## Appendix 1

List of the active microbenthos *taxa* and counting categoriesrecovered from surface sediments in the Mar Piccolo of Taranto.

A distinction was made considering the dimensions of different species belonging to the genus *Navicula*: spp. 1 < 20 µm, 20 µm < spp. 2 < 40 µm, spp.3 > 40 µm

J: June 2013 campaign; A: April 2014 campaign

| **taxon** | **Life mode** | **J** | **A** |
|---|---|---|---|
| **Diatoms** | | | |
| *Achnanthes* sp. | Epiphytic | | ● |
| *Amphora* cf. *bigibba* Grunow ex A.Schmidt | Epiphytic, epilithic, epipelic | ● | |
| *Amphora ostrearia* Brébisson | Epiphytic, epilithic, epipelic | | ● |
| *Amphora* spp. | Epiphytic, epilithic, epipelic | ● | ● |
| *Auricula* cf. *insecta* (Grunow) A.Schmidt | Epipsammic | | ● |
| *Auricula* sp. | Epipsammic | | ● |
| *Bacillaria paxillifera* (O.F.Müller) T.Marsson | Tychopelagic | ● | ● |
| *Biddulphia biddulphiana* (J.E.Smith) Boyer | Epiphytic | | ● |
| *Caloneis liber* (W.Smith) Cleve | Epipelic | ● | ● |
| *Caloneis* cf. *ventricosa* (Ehrenberg) F.Meister | Epipelic | | ● |
| *Caloneis* sp. | Epipelic | ● | ● |
| *Campylodiscus* sp. | Epipelic | ● | ● |
| *Ceratoneis closterium* Ehrenberg | Tychopelagic | ● | ● |
| *Cocconeis* spp. | Epipsammic, epiphytic | ● | ● |
| *Diploneis bombus* (Ehrenberg) Ehrenberg | Epipelic | ● | ● |
| *Diploneis* spp. | Epipelic | ● | ● |
| *Entomoneis alata* (Ehrenberg) Ehrenberg | Epipelic | ● | ● |
| *Entomoneis* sp. | Epipelic | ● | ● |
| *Fragilaria* cf. *acus* (Kützing) Lange-Bertalot | Freshwater, planktonic | | ● |
| Fragilariaceae | Mostly freshwater, planktonic | | ● |
| *Frustulia* sp. | Freshwater, brackish, epipelic | | ● |
| *Grammatophora* cf. *serpentina* Ehrenberg | Epiphytic | | ● |
| *Gyrosigma* cf. *acuminatum* (Kützing) Rabenhorst | Epipelic | ● | ● |
| *Gyrosigma attenuatum* (Kützing) Rabenhorst | Epipelic | ● | |
| *Gyrosigma* cf. *balticum* (Ehrenberg) Rabenhorst | Epipelic | ● | |
| *Gyrosigma fasciola* (Ehrenberg) J.W.Griffith & Henfrey | Epipelic | ● | ● |
| *Gyrosigma macrum* (W.Smith) J.W.Griffith & Henfrey | Epipelic | ● | ● |
| *Gyrosigma* spp. | Epipelic | ● | ● |
| *Licmophora* sp. | Epipelic, epiphytic | ● | ● |
| *Mastogloia* sp. | Epipelic | | ● |
| *Melosira jurgensi* C.Agardh | Epipelic | | ● |
| *Navicula cancellata* Donkin | Epipelic | | ● |
| *Navicula* cf. *complanata* (Grunow) Grunow | Epipelic | ● | |
| *Navicula* spp. 1 | Epipelic | ● | ● |
| *Navicula* spp. 2 | Epipelic | ● | ● |
| *Navicula* spp. 3 | Epipelic | ● | ● |
| *Nitzschia* cf. *angularis* W. Smith | Epipelic | ● | ● |
| *Nitzschia* cf. *clausii* Hantzsch | Epipelic | ● | |
| *Nitzschia* cf. *commutata* Grunow | Epipelic | ● | ● |
| *Nitzschia* cf. *dubia* W. Smith | Epipelic | ● | ● |
| *Nitzschia fasciculata* (Grunow) Grunow | Epipelic | ● | ● |
| *Nitzschia insignis* Gregory | Epipelic | | ● |
| *Nitzschia longissima* (Brébisson) Ralfs | Epipelic | ● | ● |

| | | | |
|---|---|---|---|
| *Nitzschia lorenziana* Grunow | Epipelic | ● | ● |
| *Nitzschia sigma* (Kützing) W.Smith | Epipelic | | ● |
| *Nitzschia sigma* var. *rigida* Grunow ex Van Heurck | Epipelic | | ● |
| *Nitzschia sigmoidea* (Nitzsch) W.Smith | Epipelic | | ● |
| *Nitzschia* cf. *tryblionella* Hantzsch | Epipelic | ● | ● |
| *Nitzschia vermicularis* (Kützing) Hantzsch | Epipelic | ● | ● |
| *Nitzschia* spp. | Epipelic | ● | ● |
| *Paralia sulcata* (Ehrenberg) Cleve | Epipelic, tychoplagic | ● | ● |
| *Pinnularia* cf. *claviculus* (Gregory) Rabenhorst | Epipelic | ● | |
| *Pinnularia* sp. | Epipelic | ● | ● |
| *Pleurosigma* cf. *angulatum* (Queckett) W. Smith | Epipelic | | ● |
| *Pleurosigma* sp. | Epipelic | | ● |
| *Psammodictyon panduriforme* (W.Gregory) D.G.Mann | Epipelic | ● | ● |
| *Rhopalodia gibba* (Ehrenberg) Otto Müller | Epipelic, epiphytic | ● | ● |
| *Surirella* cf. *ovata* Kützing | Epipelic | ● | |
| *Surirella* sp. | Epipelic | ● | ● |
| *Pseudostaurosira* cf. *parasitica* (W.Smith) Morales | Mostly freshwater | ● | ● |
| *Synedra* cf. *pelagica* Hendey | Epiphytic, free-living | ● | |
| *Synedra* cf. *pulchella* Kützing | Brackish, epiphytic | ● | |
| *Synedra* cf. *ulna* (Nitzsch) Ehrenberg | Epiphytic, free-living | ● | ● |
| *Synedra* sp. | Epipelic, epiphytic | ● | ● |
| cf. *Synedra* | Epipelic, epiphytic | ● | ● |
| *Toxarium hennedyanum* (Gregory) Pelletan | Epipelic | | ● |
| *Toxarium undulatum* Bailey | Epipelic | ● | |
| *Tropidoneis longa* (Cleve) Cleve | Epipelic | ● | ● |
| *Tryblionella* cf. *levidensis* W.Smith | Epipelic | | ● |
| *Tryblionella littoralis* (Grunow) D.G.Mann | Epipelic | | ● |
| *Tryblionella punctata* W. Smith | Epipelic | ● | |
| **Dinoflagellates** | | | |
| Gymnodiniales sp. | | ● | ● |
| *Prorocentrum* sp. | | ● | ● |
| undet. Dinophyta | | ● | ● |
| **Cyanobacteria** | | | |
| cf. *Anabaena* sp. | | ● | |
| cf. *Oscillatoria* sp. | | ● | ● |
| cf. *Spirulina* sp. | | ● | ● |
| | | | |
| undet. Cyanophyta | | ● | ● |
| undet. Phytoflagellates | | ● | ● |

Appendix 2
List of the resting microbenthos *taxa* recovered from surface sediments in the Mar Piccolo of Taranto
J: June 2013 campaign; A: April 2014 campaign
● indicates the finding as full cyst
○ indicates the finding as empty cyst
# successful germination during present or previous studies

| ***taxon*** | **J** | | **A** | | |
|---|---|---|---|---|---|
| **Diatoms** | | | | | |
| *Chaetoceros* sp.1 | | | ● | | |
| *Chaetoceros* sp.2 | | | ● | ○ | |
| **Dinoflagellates** | | | | | |
| *Alexandrium minutum* Halim | ● | ○ | ● | ○ | # |
| *Alexandrium tamarense* (Lebour) Balech | | ○ | ● | | # |
| *Alexandrium* sp.7 | ● | | | | |
| *Alexandrium* sp.9 | | | ● | ○ | |
| *Calciodinellum* cf. *albatrosianum* (Kamptner) Janofske & Karwath | ● | ○ | ● | ○ | |
| *Calciodinellum operosum* (Deflandre) Montresor | | | ● | | # |
| *Calciperidinium asymmetricum* Versteegh | | ○ | ● | | # |
| *Cochlodinium polykrikoides* Margalef | | | ● | ○ | |
| *Diplopelta parva* (Abé) Matsuoka | | | | ○ | |
| *Diplopsalis lenticula* Bergh | ● | ○ | ● | ○ | # |
| *Dissodinium* sp. | ● | | | | # |
| *Follisdinellum splendidum* Versteegh | ● | ○ | ● | | |
| *Gonyaulax* group | ● | ○ | ● | ○ | |
| *Gymnodinium impudicum* (Fraga & Bravo) G. Hansen & Moestrup | ● | ○ | ● | ○ | # |
| *Gymnodinium nolleri* Ellegaard & Moestrup | ● | | | | # |
| *Gymnodinium uncatenatum* (Hulburt) Hallegraeff | ● | | ● | | # |
| *Gymnodinium* sp.6 | ● | ○ | ● | ○ | # |
| *Kryptoperidinium foliaceum* (F. Stein) Lindemann | | | ● | | |
| *Lingulodinium polyedrum* (Stein) Dodge | ● | ○ | ● | ○ | # |
| *Melodomuncula berlinensis* Versteegh | ● | ○ | ● | ○ | |
| *Nematodinium armatum* (Dogiel) Kofoid & Swezy | | | | ○ | # |
| *Oblea rotunda* (Lebour) Balech ex Sournia | ● | | ● | ○ | # |
| *Pentapharsodinium dalei* Indelicato & Loeblich | ● | | ● | ○ | # |
| *Pentapharsodinium tyrrhenicum* Montresor, Zingone & Marino | ● | ○ | ● | ○ | # |
| *Polykrikos hartmannii* Zimmermann | ● | | ● | | # |
| *Polykrikos schwartzii* Bütschli | ● | ○ | ● | ○ | |
| *Posoniella tricarinelloides* (Versteegh) Streng et al. | ● | ○ | ● | ○ | # |
| *Protoperidinium claudicans* (Paulsen) Balech | | | ● | | # |
| *Protoperidinium compressum* (Abé) Balech | ● | ○ | ● | ○ | # |
| *Protoperidinium conicum* (Gran) Balech | ● | | ● | ○ | # |
| *Protoperidinium leonis* (Pavillard) Balech | | ○ | | | |
| *Protoperidinium oblongum* (Aurivillius) Parke & Dodge | ● | ○ | ● | | # |
| *Protoperidinium parthenopes* Zingone & Montresor | ● | | | ○ | # |
| *Protoperidinium pentagonum* (Gran) Balech | | ○ | ● | | # |

| | | | | | |
|---|---|---|---|---|---|
| *Protoperidinium quinquecorne* (Abé) Balech | ● | ○ | | | # |
| *Protoperidinium subinerme* (Paulsen) Loeblich III | | ○ | | ○ | |
| *Protoperidinium thorianum* (Paulsen) Balech | ● | ○ | ● | ○ | # |
| *Protoperidinium* sp.1 | ● | ○ | ● | ○ | |
| *Protoperidinium* sp.4 | ● | ○ | ● | ○ | |
| *Protoperidinium* sp.5 | ● | ○ | ● | ○ | |
| *Protoperidinium* sp.6 | ● | ○ | | | |
| *Protoperidinium* sp.7 | ● | | | | |
| *Pyrophacus horologium* Stein | ● | ○ | ● | ○ | # |
| *Scrippsiella bicarinata* Zinssmeister et al. | ● | | | | # |
| *Scrippsiella lachrymosa* Lewis | ● | ○ | ● | ○ | # |
| *Scrippsiella precaria* Montresor & Zingone | ● | | | | |
| *Scrippsiella ramonii* Montresor | ● | ○ | ● | ○ | # |
| *Scrippsiella spinifera* Honsell & Cabrini | ● | ○ | ● | | # |
| *Scrippsiella trifida* Lewis | | | ● | | |
| *Scrippsiella trochoidea* (Stein) Loeblich | ● | ○ | ● | ○ | # |
| *Scrippsiella* sp.1 | ● | ○ | ● | ○ | # |
| *Scrippsiella* sp.4 | ● | | ● | | # |
| *Scrippsiella* sp.5 | ● | | ● | | # |
| *Scrippsiella* sp.6 | ● | ○ | ● | ○ | # |
| *Scrippsiella* sp.8 | ● | ○ | ● | ○ | # |
| *Scrippsiella* sp.9 | | ○ | | | |
| *Warnovia* cf. *rosea* (Pouchet) Kofoid & Swezy | ● | ○ | ● | | |
| Dinophyta sp.2 | | | ● | | |
| Dinophyta sp.28 | | | | ○ | |
| Dinophyta sp.33 | | ○ | | | |

# CHAPTER 5

## Cibic T., Caroppo C., Di Poi E., Karuza A., Rogelja M., Del Negro P.: Primary production and seasonal succession of different photoautotrophs (pico-, nano and microplankton) in the Mar Piccolo of Taranto

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# PRIMARY PRODUCTION AND SEASONAL SUCCESSION OF DIFFERENT PHOTOAUTOTROPHS (PICO-, NANO- AND MICROPLANKTON) IN THE MAR PICCOLO OF TARANTO

## *PRODUZIONE PRIMARIA E SUCCESSIONE STAGIONALE DEI DIVERSI ORGANISMI FOTOAUTOTROFI (PICO-, NANO- E MICROPLANCTON) NEL MAR PICCOLO DI TARANTO*

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## Abstract

In order to detect their seasonal dynamics, the abundance of planktonic phototrophs was investigated and associated with physical-chemical features of the water column and primary production. The lowest abundance of all planktonic photoautotrophs was detected in February 2014. In April 2014 the proliferation of the largest, micro-sized fraction, was observed. In June 2013, primary production (PP) rates were high, up to 10.14±1.45 µg C $l^{-1}$ $h^{-1}$ in the first inlet and 20.07±3.12 µg C $l^{-1}$ $h^{-1}$ in the centre of the second inlet. This major photosynthetic rate was likely ascribable to the nanoplanktonic fraction that reached its maximum abundance over the study period. In October the picophytoplankton peaked to $10^9$ cells $L^{-1}$, with higher abundances detected in the inner inlet. High densities of all photoautotrophic organisms caused phosphate depletion, especially in June 2013. Our results indicate a clear seasonal succession of plankton phototrophs in the very productive system of the Mar Piccolo.

**Key-words:** primary production, picophytoplankton, nanophytoplankton, microphytoplankton, Mar Piccolo.

## 1 Introduction

The Mar Piccolo is a shallow, nearly enclosed sea that consists of two basins, i.e. First and Second Inlet, with maximum depths of 13 and 10 m and surface areas of 8.28 km$^2$ and 12.43 km$^2$, respectively. The circulation is restricted as the water is exchanged with the Mar Grande only through two narrow channels. Salinity is influenced by the input of freshwater deriving from small tributary rivers, by water draining from the surrounding agricultural soils and by 34 freshwater springs (locally called ‘Citri’). Mussel farming is one of the main economic activities and the local mussels have been known for their quality since the early '900s (Caroppo *et al.*, 2012). As the photoautotrophs represent a high proportion of the mussels’ diet the aim of this study was to investigate their abundance, seasonal succession and primary production.

## 2 Materials and methods

Samplings were carried out in June and October 2013, and in February and March/April 2014. Surface and bottom water samples were collected using acid-rinsed 5 L Niskin bottles. Six stations (St.1G: 40.498 N, 17.263 E, St.1E: 40.496 N, 17.236 E, St.1B: 40.469 N, 17.249 E, St.1I: 40.476 N, 17.259 E, St.2B: 40.478 N 17.282 E, St.2C: 40.478 N, 17.299E) with two sampling depths were chosen, four located in the First and two in the Second Inlet. For the sample processing, analysis and determination of picophytoplankton, nanophytoplankton and microphytoplankton the methods described by Karuza *et al.* (2012), Di Poi *et al.* (2013), Zingone *et al.* (2009) respectively were followed. For the nanoplankton fraction, we used different shapes: circle, cone, ellipse, cylinder within six standard cell sizes (2-5 μm, 5-8 μm, 8-11 μm, 11-14 μm, 14-17 μm, 17-20 μm). Primary production (PP) was estimated *in situ* by the $^{14}$C technique as described by Fonda Umani *et al*. (2007). Nutrient analyses ($NH_4^+$, $NO_2^-$, $NO_3^-$, $PO_4^{3-}$ and $H_4SiO_4$) were performed on a four-channel, Quaatro SEAL Analytical, Continuous Flow Analyzer, according to Hansen *et al.* (1999).

## 3 Results

The minimum abundances for all phototrophic fractions were found in February 2014 at all stations due to low light availability. Over the study period, picophytoplankton varied from $5.5\pm0.3\times10^6$ cells L$^{-1}$ to $1.0\pm0.1\times10^9$ cells L$^{-1}$. Considerably higher abundances were detected in October 2013 especially in the Second Inlet. The abundance of nanophytoplankton ranged from $3.6\pm0.5\times10^5$ to $1.3\pm0.06\times10^7$ cells L$^{-1}$ with maxima in June 2013 when high abundance of *Chaetoceros* spp. (Ehrenberg, 1844) was found. In the First Inlet *Chaetoceros* spp. alone constituted up to 54% of the nanophytoplankton assemblage whereas in the Second Inlet this genus represented 25% of total nanophytoplankton. Microphytoplankton abundance widely ranged between $0.6\times10^4$ organisms L$^{-1}$ in February and $199.4\times10^4$ organisms L$^{-1}$ in April 2014 and was generally dominated by diatoms and dinoflagellates. The highest PP values were obtained in June, when hourly rates varied between 2.82±0.10 µg C $L^{-1}$ $h^{-1}$ in the First Inlet and 20.07±3.12 µg C $L^{-1}$ $h^{-1}$ in the Second Inlet. The Mar Piccolo was productive also in February, in lower light conditions, with values up to 3.22±0.14 µg C $L^{-1}$ $h^{-1}$ in the First Inlet.

## 4 Conclusions

During the four sampling campaigns a clear seasonal succession was observed in the Mar Piccolo: the largest phototrophs reached their maxima in April, followed by the nanoplankton fraction in June and the picophytoplankton in October. PP rates, that represent the first measurements for the Mar Piccolo, reflected the same pattern, with the highest values estimated in June and the lowest in February. High densities of all photoautotrophs caused phosphate depletion in the water column both in April and October. Our data indicate that the high abundance of phytoplankton throughout the year cannot be a limiting factor for the mussel farming performance despite its seasonal variability in composition that has been previously reported by Caroppo *et al.* (2012 and references therein).

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# CHAPTER 6

Cibic T., Bongiorni L., Borfecchia F., Di Leo A., Franzo A., Giandomenico S., Karuza A., Micheli C., Rogelja M., Spada L., Del Negro P: Ecosystem functioning approach applied to a large contaminated coastal site: the study case of the Mar Piccolo of Taranto (Ionian Sea)

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# Ecosystem functioning approach applied to a large contaminated coastal site: the study case of the Mar Piccolo of Taranto (Ionian Sea)

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## Abstract

Knowledge on ecosystem functioning can largely contribute to promote ecosystem-based management and its application. The Mar Piccolo of Taranto is a densely populated area at a high risk of environmental crisis. Here planktonic primary production (PP) and heterotrophic prokaryotic production (HPP) were measured as proxies of functioning in three sampling sites located in two inlets at different levels of industrial contamination, during three sampling surveys (June 2013, February and April 2014). To have a better overall view and provide some insights into the benthic-pelagic coupling, we integrated PP and HPP in the water column with those in the sediments and then discussed this with the origin of the organic matter pools based on analysis of stable isotopes. Heavy metals and Polychlorobiphenyls (PCBs) were also analysed in the surface (1 cm) sediment layer and related to the overall ecosystem functioning. MDS analysis, based on the main data, clearly separated the 2nd Inlet from the 1st one, more severely impacted by anthropogenic activities. The stable isotope mixing model suggested the prevalent terrestrial/ riverine origin of the particulate organic matter pools (mean 45.5%) in all sampling periods, whereas phytoplankton contributed up to 29% in February. Planktonic PP and HPP rates followed the same pattern over the entire study period and seemed to respond to phytoplankton dynamics confirming this community as the main driver for the C cycling in the water column. On the contrary, benthic PP rates were almost negligible while HPP rates were lower or comparable to those in the water column indicating that, although the Mar Piccolo is very shallow, the water column is much more productive than the surface sediments. The sediment resuspension is likely responsible for a pulsed input of contaminants into the water column. However, their interference with the proper functioning of the pelagic ecosystem seems to be limited to the bottom layers.

**Keywords:** Ecosystem functioning; Primary production; Heterotrophic prokaryotic production; C and N stable isotopes, Benthic-pelagic coupling; Contamination; Satellite imagery mapping

## 1 Introduction

Shallow coastal photic systems are among the most productive on the planet (Odum 1983). In these environments, light penetration to the bottom fuels multiple primary producers, including phytoplankton, benthic microalgae, macroalgae and seagrasses.

The coupling between planktonic primary production (PP) and prokaryotic utilization of the labile, i.e. rapidly decomposable, phytoplankton extracellular release has been demonstrated to be a key process in organic carbon cycling influencing the ecosystem functioning in aquatic ecosystems (Cole et al. 1988). Heterotrophic prokaryotes mainly consume and respire organic matter produced by photosynthesis, therefore heterotrophic prokaryotic production (HPP) is typically dependent on this organic matter supply. However, the coupling between PP and HPP may vary according to the ecosystem characteristics. For example, in estuarine and coastal systems the prokaryotic C demand could be supported not only by the degradation of phytoplankton exudates but also by local non-phytoplanktonic material and allochthonous organic matter supplies (Lee et al. 2001). This can be reflected in a loose coupling between PP and HPP and a shift to net heterotrophy of the planktonic system (Pugnetti et al. 2005; 2010).

Aside from light, temperature and nutrients, that are key parameters regulating system production, coastal processes are largely influenced by physical factors such as horizontal transport, sediment composition and resuspension. Rapid sinking of phytoplankton blooms, efficient filtration of the water column by benthic fauna and a tidal energy subsidy can determine a tight benthic pelagic coupling that leads to a high local benthic production (Kennish et al. 2014). Moreover, microbial mediated processes in sediments can enhance nutrient availability for primary production in benthic and pelagic habitats and become important in regulating the relative magnitude of benthic versus pelagic primary production (Kennish et al. 2014). Apart from phytoplankton and local benthic production, terrestrial matter carried by coastal rivers represents a non-negligible contributor to coastal organic matter pools.

The understanding of the nature and origin of the organic matter pools may provide interesting insights about the occurrence of natural processes and anthropogenic pressures in coastal environments (Hedges and Stern 1984). At this regard, analyses of stable isotopes of organic matter are useful tools to characterise nitrogen and carbon transport and transformation processes in continental margins (Sanchez-Vidal et al. 2009).

Shallow coastal photic systems rank among the most heavily impacted aquatic ecosystems, being affected by a wide range of anthropogenic activities. Multiple anthropogenic disturbances create both acute and insidious problems for many estuarine biotic communities and habitats that can compromise the stability and resiliency of these systems and their long-term integrity. Among other stressors, chemical contaminants can cause severe changes in ecosystem structure and function (Kennish et al. 2014).

Regarding organic pollutants and particularly polychlorinated biphenyls (PCBs), current sources of these compounds are landfills, open burning of products containing PCBs, waste incineration, accidental fires, and revolatilization from formerly exposed soils. Organic pollutants enter the sea and estuary via atmospheric deposition, river input and point source along the coast. Once delivered to the water column, the primary removal processes are sedimentation of atmospheric particles and partitioning of the gaseous/dissolved phase contaminants into organic carbon-rich particles with subsequent settling and accumulation in surface sediments (Di Leo et al. 2014).

The main process determining the distribution and concentration of contaminants in shallow systems is the exchange between the water column and the uppermost sediment layer that is repeatedly resuspended and settled again. In this way, contaminants are transferred to the water column, diluted and redistributed over the entire basin through water circulation. Usually, for monitoring purposes, analysis of contaminants is performed on a sediment layer up to 5 cm (Cardellicchio et al. 2007). However, focusing on the benthic-pelagic coupling, it is important to estimate the contaminants concentrations in the uppermost few millimetres of sediments that are often resuspended.

The Mar Piccolo is a shallow coastal basin located near the city of Taranto (southern Italy). Since 1960s Taranto and its coastline have been subjected to the industrialization process that has caused profound environmental changes. This industrial zone is characterized mainly by the presence of the largest steelworks in Europe and navy arsenal in Italy (Military area in Figure 1), 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, PCBs and dioxin. Previously collected data on organic and inorganic pollutants in the Mar Piccolo have shown high levels of contamination and stress conditions on different communities

(Cardellicchio et al. 2007; Spada et al. 2012). In 1998, this area has been declared Site of National Interest (SIN) i.e. a very large contaminated area, classified as the most dangerous by the Italian State and in need of remediation. According to the current legislation, the characterization plan of such polluted areas is based solely on the quantification of contaminants. In contrast, other pivotal ecological aspects, such as those focused on the C cycling through the production processes, have been completely neglected.

The principle 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 (Ottersen et al. 2011). 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). To study the ecosystem functioning of a particular environment, as much information as possible have to be gathered on that area. The simultaneous investigation of structural and functional parameters and their subsequent integration is needed in order to represent an overview of the C flow through the system. In the Mar Piccolo of Taranto there are no data available on either PP or HPP that are considered important proxies of ecosystem functioning. In this study, we have focused on the pelagic processes of carbon production. To gain more insights into the ecosystem functioning and the influence of pelagic-benthic and import/export processes we have: i) integrated the PP and HPP in the water column with those in the benthic domain; and ii) linked such information with the nature and origin of suspended and sedimentary organic matter (OM) pools based on analyses of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). Moreover, we discussed the high contamination levels in the very uppermost sediment layer, their transfer to the water column through bottom resuspension and the consequent potential effects on the overall ecosystem functioning of this basin.

## 2 Materials and methods

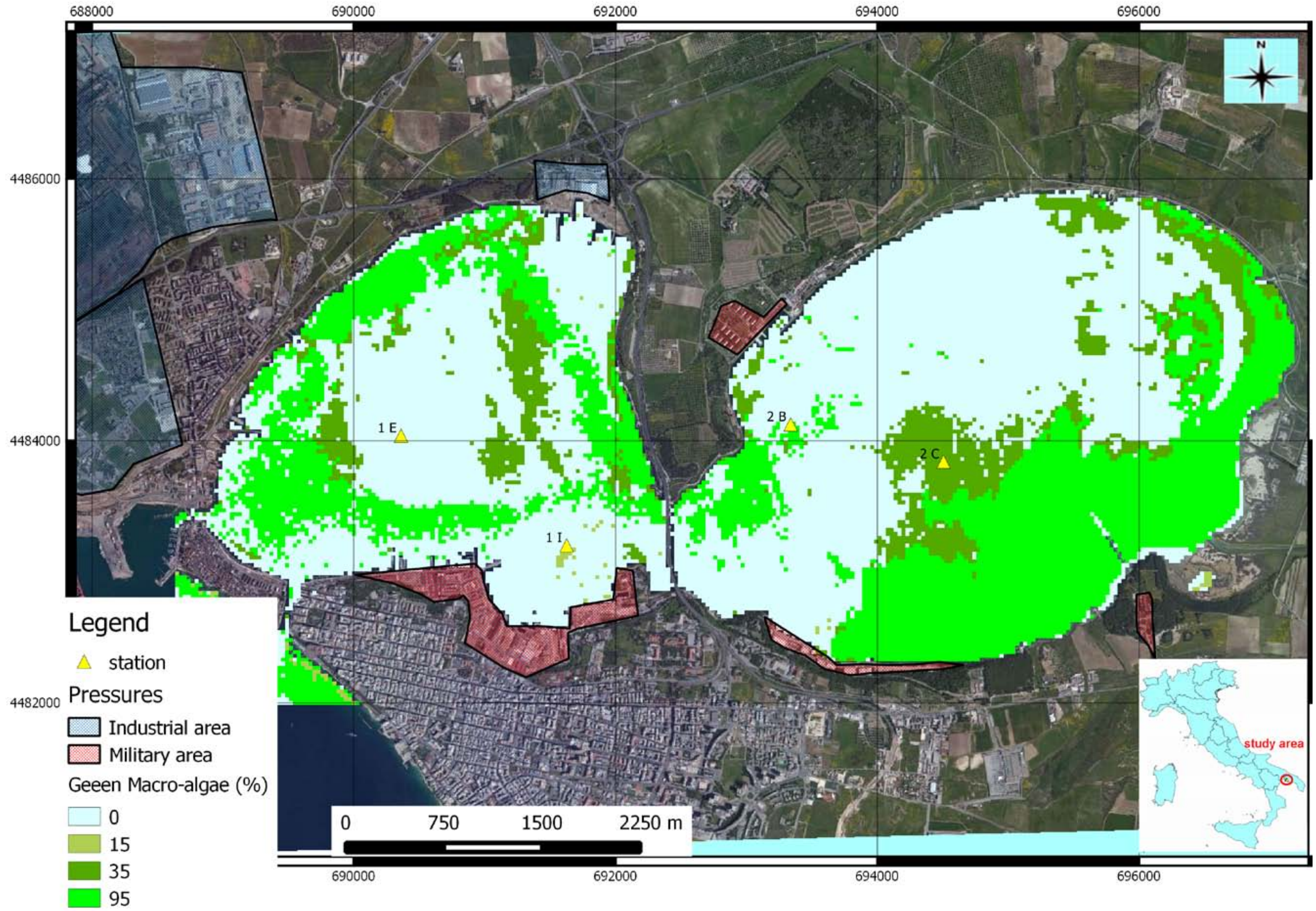
### *2.1 Study area*

The Mar Piccolo is a shallow, nearly enclosed basin connected through two narrow canals with the Mar Grande and widely with the Gulf of Taranto. It consists of two naturally divided basins, the 1st and 2nd Inlet, with maximum depths of 13 and 10 m and a surface area of 8.28 km$^2$ and 12.43 km$^2$, respectively. The sedimentation in this area is mainly influenced by land runoff, numerous submarine springs, small streams, sewage outfalls and industrial discharges rather than by marine currents. The sediment composition in both Inlets is clayey silt. The features of the Mar Piccolo in the Gulf of Taranto have been exhaustively described by Cardellicchio et al. (2015 this issue).

### *2.2 Sampling*

Sampling in the water column was carried out during 11th-17th June 2013, 3rd-5th February and 1st-8th April 2014 at three stations: St. 1E (depth = 11.2 m) was located in the middle of the 1st Inlet (40° 29' 01"N, 17° 14' 46"E), St. 1I (depth = 11.0 m) was located nearby the military arsenal (40° 28' 46"N, 17° 15' 38"E) and St. 2C (depth = 8.0 m) was positioned in the innermost part of the 2nd Inlet (40° 28' 57'N, 17° 17' 41"E) (Fig. 1).

**Figure 1** Location of the four sampling stations in the Mar Piccolo of Taranto (yellow triangles): 1I and 1E in the 1st Inlet and 2B and 2C in the 2nd Inlet. Superimposed are the industrial area and the military area (i.e. the navy arsenal in the 1st Inlet) and the green macroalgal coverage (%). Its distribution was assessed by means of atmospherically corrected Landsat 8 OLI multispectral data acquired on 13th June 2013 and suitably calibrated using *in situ* point measurements at the sampling stations.


688000
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1 E
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station
Pressures
Industrial area
Military area
Geeen Macro-algae (%)
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15
35
95
0
750
1500
2250 m
study area

Seawater temperature, dissolved oxygen and salinity were measured along the water column using a Seabird 19 Plus Seacat probe (June 2013 and April 2014) whereas in February 2014 seawater temperature was measured by a PNF-300 Profiling Natural Fluorometer and salinity by a CDM83 conductivity meter (Radiometer Copenhagen).

During each sampling, the Photosynthetic Available Radiation (PAR) was registered by a PNF-300 Profiling Natural Fluorometer (Biospherical Instruments Inc.). The PAR value at the sampling depth was expressed as the percentage of measured irradiance with respect to the surface irradiance (%PAR).

Water samples were collected at the surface layer (1 m below water surface) and bottom layer (1 m above bottom) by Niskin bottles. For isotopic analyses of suspended particulate organic matter (POM), water samples were collected from the bottom layer of the three stations listed above and at the additional St. 2B (depth = 7.0 m; 40° 28' 57"N, 17° 16' 42"E) located nearby the strait between the two Inlets. Replicated water samples (1-1.2 L) were filtered through precombusted (450°C for 4h) Whatman GF/F filters and stored at -20°C until analyses.

Sediment sampling was carried out in June 2013 and April 2014 at the four stations sampled for water analyses. At each station, at least three virtually undisturbed sediment cores were collected by scuba divers using polycarbonate sample tubes (12.7 cm I.D. with a sample area of 127 cm$^2$). The oxic sediment layer (0-1 cm ca.) of each core was collected, homogenised and used for the analysis of heavy metals and PCBs (see below) as well as primary and heterotrophic productions (as reported in Rubino et al. (2015 this issue) and Franzo et al. (2015 this issue), respectively).

Additional sediment samples (0-3 cm layer) were used to analyse the stable isotopic signatures of sedimentary organic matter (SOM) as described in Bongiorni et al. (2015 this issue). For this analysis, an additional sampling was carried out in February 2015. In order to characterize the contribution of different primary sources to POM and SOM pools, most common macroalgal species were also collected during June 2013 and April 2014 by hand or a van Veen grab (as reported in Bongiorni et al. (2015 this issue)).

### 2.2.1 Chlorophyll *a*

Water sub-samples for Chlorophyll *a* (chl *a*) analysis were collected on-board, stored in the dark and kept at 4°C until the filtration. In the laboratory the water was filtered immediately on 47 Ø mm Whatman GF/F filters and the filters were stored at -20 °C. Chl *a*, corrected for phaeopigments, was measured fluorometrically after extraction (90% acetone) and centrifugation of samples kept in the dark, according to Lorenzen and Jeffrey (1980), on a Perkin Elmer LS50B fluorometer.

### 2.2.2 Primary production (PP)

PP was estimated *in situ* by the $^{14}$C technique (Steemann-Nielsen 1952). Water samples were poured into 75 ml translucent and dark polycarbonate carboys (Nalgene) and kept in the darkness for 30 minutes to stop the residual photosynthetic activity. Subsequently, 6 μCi (0.22 MBq) of $NaH^{14}CO_3$ (DHI, Denmark) was added per bottle. Three light and one dark samples per depth were fixed on a rosette, lowered at the corresponding depth and incubated for 2 h around noon. At the end of the incubation, samples were transferred to 100-ml bottles and supplemented with 320 μl of 5 N HCl (Cibic and Virgilio 2011) to stop the photosynthetic activity and remove the residual labelled bicarbonate, not assimilated by the phototrophic plankton. From each sample 25 ml were filtered through polycarbonate 0.2 μm filters (Nuclepore) applying a low vacuum pressure (5 mmHg) in order to avoid cell damage. Filters were placed into 6 ml plastic scintillation vials (Perkin Elmer) and 5 ml of Filter Count scintillation cocktail (Perkin Elmer) was added. Disintegrations per minute (DPM) were measured by a QuantaSmart TRI-CARB 2900 TR Liquid Scintillation Analyzer (Packard BioScience, USA) including quenching correction, obtained using internal standards. Assimilation of carbon was calculated as described by Gargas (1975), assuming 5% isotope discrimination. Activity of the added $NaH^{14}CO_3$ and inorganic carbon concentration ($tCO_2$) were calculated on the basis of total alkalinity measured in the same samples.

Similarly to the water column, benthic PP was estimated using $^{14}C$ as radiotracer. $^{14}C$-incubation of sediment slurries was performed *in situ*, then samples were treated and analysed as described in detail by Cibic et al. (2008).

### *2.2.3 Heterotrophic prokaryotic production (HPP)*

HPP was measured by the incorporation of $^{3}H$-leucine (Leu) (Kirchman et al. 1985). Triplicate seawater aliquots (1.7 ml) and two controls killed by the addition of 90 μl 100% trichloracetic acid (TCA), were amended with a 20-nM radiotracer and incubated *in situ* in the dark. Incubations were stopped with TCA (5% final concentration) after 2 h. The extraction with 5% TCA and 80% ethanol was carried out using the microcentrifugation method (Smith and Azam 1992). Activity in the samples was determined by a QuantaSmart TRI-CARB 2900 TR Liquid Scintillation Analyzer (Packard BioScience, USA) after the addition of 1 ml scintillation cocktail (Ultima Gold MV; Perkin Elmer).

Benthic heterotrophic production was measured by the incorporation of $^{3}H$-leucine following the method by Manini et al. (2004). $^{3}H$-incubation of sediment slurries was performed *in situ*, and then samples were treated and analysed as exhaustively described by Cibic et al. (2012).

### *2.2.4 Stable isotope analysis*

Analyses of C and N stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of POM and SOM, and primary producers were conducted as detailed in Bongiorni et al. (2015 this issue). Briefly, $\delta^{13}C$ was analysed after acidification with HCl (1 N) to remove carbonates which present a higher $\delta^{13}C$ than organic carbon (Hedges and Stern 1984) while $\delta^{15}N$ was analysed without any prior treatment. The $\delta^{13}C$ and $\delta^{15}N$ ratios in the samples were determined using a Delta Plus XP isotope ratio mass spectrometer (ThermoFinnigan) equipped with a Flash EA 1112 elemental analyser (ThermoFinnigan). Ratios were expressed as parts per thousands (‰) differences from a standard reference material VPDB (Vienna Pee Dee Belemnite) for $\delta^{13}C$ and AIR for $\delta^{15}N$. The Uncertainty of methods was 0.3 ‰ for $\delta^{13}C$ and $\delta^{15}N$.

In order to evaluate the contribution of different primary sources to the POM and SOM mix pools we applied a Bayeasian mixing model by using the package SIAR V4 (Stable Isotope Analysis in R, (Parnell et al. 2010)). The greatest advantage of this procedure is the incorporation of uncertainty linked to sources, consumers and trophic enrichment factors within the model (Dubois et al. 2012; Parnell et al. 2010; Phillips et al. 2014). The model was run for POM and SOM separately and for each of the four sampling stations/seasons assuming a zero fractionation factor for each isotopes.

Both models included two variables ($\delta^{15}$N an d $\delta^{13}$C) and up to five end-members (macroalgae, terrestrial/ riverine POM, phytoplankton, treated sewage for POM and SOM models and autochthonous POM for SOM model). Due to similar signature of different macroalgal species, only the average isotopic value was used in the model (-16.83 ± 2.49 and 10.45 ± 1.27 for $\delta^{13}$C and $\delta^{15}$N, respectively). The isotopic values of the end-members that were not directly measured during our surveys, were extracted from the literature: phytoplankton (Harmelin-Vivien et al. 2008, -19.95 ± 0.93 and 4.50 ± 0.80 for $\delta^{13}$C and $\delta^{15}$N, respectively), riverine/terrestrial POM (Berto et al. 2013 and Carlier et al. 2007, -25.58 ± 0.67 and 2.92 ± 0.45 for $\delta^{13}$C and $\delta^{15}$N, respectively), and treated sewage (Berto et al. 2013 and Berto unpubl. data, -24.76 ± 0.88 and 19.11 ± 3.92 for $\delta^{13}$C and $\delta^{15}$N, respectively).

### *2.2.5 Satellite imagery mapping of the seabottom and primary productivity of green algae*

The green macroalgal distribution in the Mar Piccolo of Taranto was assessed by means of the high resolution multispectral satellite remote sensing technique based on the data acquired on 13 June 2013 by the new NASA polar Landsat 8 OLI (Operational Land Imager) sensor. The OLI multispectral data were previously corrected for atmospheric noise (Borfecchia et al. 2013a) (scattering/attenuation from image derived AOD and adjacency effects) and then they were classified into three classes using a supervised ML (Maximum Likelihood) parametric algorithm. Different *in situ* cover values (%) of the dominant green algae *Caulerpa* sp., recorded at the four stations in the same period, allowed us to properly calibrate the remotely sensed data. The improved spectral and radiometric features of the OLI multispectral sensor (five acquisition bands in the VIS and NIR ranges including the new deep blue coastal one, at 30 m resolution with increased SNR) allowed us to obtain these preliminary results in terms of green algae effective mapping (Borfecchia et al. 2013b) in the optically complex shallow waters of the Mar Piccolo. The primary productivity of green macroalgae (such as *Caulerpa prolifera*, *Caulerpa* sp.) in the Mar Piccolo was evaluated through an integrated method including satellite remote sensing technique and *in situ* observations. In order to obtain an estimate of the overall primary productivity of the Mar Piccolo, a maximum hourly rate of productivity (Pmax) of *Caulerpa* sp., in relation to depth and seasonal variation, was used according to Terrados and Ros (1992) and Bernardeau-Esteller et al. (2011) and converted from oxygen to carbon to allow an integration with primary production data in the water column.

### *2.2.6 Analysis of polychlorobiphenyls (PCBs) and heavy metals*

PCBs congener IUPAC No PCB 18, 31, 28 (Tri-CBs), 44, 52 (Tetra-CBs ), 95, 101, 99, 110, 123, 118, 114, 105 (Penta- CBs) 151, 149, 146, 153, 138, 128, 167, 156, 157 (Hexa-CBs) and 187, 183, 177, 180, 170, 189 (Hepta-CBs) (Ultra Scientific, Co.) in lyophilized sediments were extracted with n- exane/acetone (1:1 v/v) by Microwave Extraction (EPA Method 3546), purified with Florisil (EPA Method 3620C) and analysed by GC-MS (EPA method 8082A).

Metals (As, Cd, Cr, Cu, Fe, Ni, Zn, Pb, Al, V, Mn, Sn, Hg) in lyophilized sediments were digested with 9 mL of nitric acid, 2 mL of hydrochloric acid, and 3 mL of hydrofluoric acid (SW-846 EPA Method 3052, 1995) using a MARSX microwave oven and analysed by ICP-MS. For a more detailed explanation of the protocols for PCBs and metals analyses see Di Leo et al. (2015 this issue).

### *2.3 Statistical analysis*

Data normality was checked by Shapiro-Wilk's test. Mean HPP and PP data and values of surface and bottom layers were compared by Mann-Whitney U-test. In order to highlight differences in PP, HPP and stable isotopes among sampling months and stations One-way and Two-way ANOVA were applied. Prior to analyses, the heterogeneity of variance was tested using Cochran's C test, and when the assumption was not reached, data were appropriately transformed. When significant differences were observed, means were compared using a Tukey's HSD test. To highlight interactions between structural and functional variables the non parametric Spearman's rank correlation analysis (R) was applied. All analyses including the Bayesian Stable Isotope mixing model (SIAR V 4 package), were performed using the R software (R Development Core Team 2005).

Multidimensional Scaling Analysis (MDS) was performed using PRIMER software v.5 on bottom and surface data separately. The two data matrices were constructed with three replicate samples of PP and HPP rates, and were implemented with structural biological parameters such as the abundances of picoplankton (both heterotrophic and autotrophic fractions) and nanophytoplankton (data from Karuza et al. 2015 this issue). Stable isotopes data ($\delta^{15}$N and $\delta^{13}$C signature of POM) were included only in the bottom layer dataset. Normalized Euclidean distance was applied. Variation in trends among sampling periods, inlets and stations was subsequently tested for significance with an analysis of similarity (ANOSIM ) using the same software. ANOSIM tests *a priori*-defined groups (subgroupings based on the above mentioned factors) against random groups in ordinate space. A zero (0) indicates that there is no difference among groups, while a one (1) indicates that all samples within groups are more similar to one another than any samples from different groups. Only statistically significant data are presented.

## 3 Results

### *3.1 Physical-chemical parameters in the water column*

At the three sampling stations temperature varied between 20.9 and 23.5°C in June and between 15.5 and 17.0 °C in April, whereas no thermocline was registered in February (Table 1). The water column displayed oxygen saturation conditions both in June and April, reaching 124.6% at the bottom layer of the 2nd Inlet in April. A strong halocline was observed in all sampling periods, with differences of up to 3.7 between the surface and the bottom layers (measured in the center of the 1st Inlet during February), probably due to surface freshwater inputs. During all sampling periods, PAR irradiance was never a limiting factor for the phototrophic organisms; the lowest values did not drop below 22.6 $\mu Em^{-2}s^{-1}$ at the bottom layer in February, representing 4.9% of the surface PAR. Phaeo /chl *a* ratio showed low values in June (with the exception of the surface layer at St. 2C) and in April indicating the presence of fresh produced organic matter of phytoplankton origin.

**Table 1** Physical-chemical parameters measured in the water column at the three stations and three sampling periods: temperature (Temp), oxygen saturation (%O2), salinity, PAR irradiance measured in the water column (E0), surface PAR irradiance measured in the air (Eref), the percentage of measured PAR irradiance in the water column with respect to surface PAR irradiance (%PAR), ratio between pheopigments and chlorophyll *a* (phaeo/chl *a*).

| **Sampling date** | **Station** | **Depth** m | **Temp** °C | **%O2** | **Salinity** | **E0** $\mu Em^{-2}s^{-1}$ | **Eref** $\mu Em^{-2}s^{-1}$ | **%PAR** | **phaeo/chl *a*** |
|---|---|---|---|---|---|---|---|---|---|
| 11/06/2013 | **St. 1E** | 1 | 22.8 | 114.8 | 36.1 | 1477.3 | 2486.4 | 59.4 | 0.72 |
| | | 10 | 20.9 | 101.0 | 38.4 | 206.6 | 2486.4 | 8.3 | 0.31 |
| 13/06/2013 | **St. 1I** | 1 | 21.4 | 102.0 | 36.3 | 1451.3 | 2443.3 | 59.4 | 0.49 |
| | | 10 | 20.9 | 105.1 | 38.3 | 218.6 | 2453.7 | 8.9 | 0.44 |
| 15/06/2013 | **St. 2C** | 1 | 23.5 | 113.6 | 36.2 | 1734.7 | 2343.8 | 74.0 | 2.44 |
| | | 7 | 21.3 | 115.6 | 37.7 | 368.6 | 2347.2 | 15.7 | 0.56 |
| 05/02/2014 | **St. 1E** | 1 | 12.0 | NA | 34.3 | 126.7 | 716.4 | 17.7 | 1.94 |
| | | 10 | 13.2 | NA | 38.0 | 38.5 | 797.4 | 4.8 | 2.12 |
| 04/02/2014 | **St. 1I** | 1 | 12.1 | NA | 36.3 | 134.3 | 466.1 | 28.8 | 1.48 |
| | | 10 | 13.2 | NA | 38.3 | 22.6 | 460.1 | 4.9 | 3.71 |
| 03/02/2014 | **St. 2C** | 1 | 12.2 | NA | 34.9 | 112.6 | 407.6 | 27.6 | 1.58 |
| | | 7 | 12.8 | NA | 37.4 | 26.9 | 406.5 | 6.6 | 3.45 |
| 01/04/2014 | **St. 1E** | 1 | 16.5 | 106.4 | 35.6 | 755.8 | 2108.9 | 35.8 | 1.04 |
| | | 10 | 15.5 | 105.7 | 37.4 | 161.8 | 1971.1 | 8.2 | 1.19 |
| 03/04/2014 | **St. 1I** | 1 | 17.0 | 105.3 | 35.6 | 396.4 | 2006.6 | 19.8 | 1.01 |
| | | 10 | 15.7 | 110.4 | 37.5 | 146.9 | 1906.2 | 15.3 | 1.02 |
| 07/04/2014 | **St. 2C** | 1 | 16.6 | 111.9 | 35.7 | 301.9 | 1576.6 | 19.2 | 0.54 |
| | | 7 | 16.6 | 124.6 | 36.8 | 91.3 | 1318.0 | 6.9 | 0.99 |

### *3.2 Primary (PP) and heterotrophic prokaryotic production (HPP)*

Planktonic PP was always significantly higher at the surface than at the bottom water layer (Mann-Whitney U test, U = 587, $p < 0.001$). The only exception occurred in April in the 2nd Inlet, where not significant differences between the two layers were found. The Two-way ANOVA highlighted differences in PP of the surface layer between seasons and sampling stations ($F_{2,18}$=200.70 and $F_{2,18}$=20.3, $p < 0.001$, respectively). Similar results were obtained for PP at the bottom layer ($F_{2,18}$=841.83 and $F_{2,18}$=141.47, $p < 0.001$). At both surface and bottom layers, with the only exception of February, higher values were consistently recorded at St. 2C (Tukey's HSD, $p < 0.01$). The highest PP values were obtained in June, when hourly rates varied between 2.82 ± 0.10 µgC $L^{-1}$ $h^{-1}$ at the bottom of St. 1I and 20.07 ± 3.12 µg C $L^{-1}$ $h^{-1}$ at the surface of St. 2C (Fig. 2).

The Mar Piccolo was productive also in February, i.e. in lower light conditions, with values up to 3.22 ± 0.14 µg C $L^{-1}$ $h^{-1}$ at the surface layer of the 1st Inlet. Intermediate rates were estimated in April, varying between 1.20 ± 0.14 µg C $L^{-1}$ $h^{-1}$ at the surface of St. 1I and 6.71 ± 0.91 µg C $L^{-1}$ $h^{-1}$ at the bottom of the 2nd Inlet. In June and April, chl *a* – normalized photosynthetic rates displayed quite a different pattern compared to the not normalized rates. At the bottom layers of the three stations the two rates were rather comparable, whereas at the surface layers PP $chl^{-1}$ values were about half of the not normalized rates (Table 2).

In February, except for the surface layer of St. 1I, where a higher PP $chl^{-1}$ rate was calculated, comparable PP normalized and not normalized rates were obtained. Mean PP values were calculated from data collected at the two depths along the water column to obtain the areal phytoplankton production (PPa). At the shallowest St. 2C, PPa rates displayed the highest variability, ranging from 1.20 mg C $m^{-2}$ $h^{-1}$ in February to 12.62 mg C $m^{-2}$ $h^{-1}$ in June and representing the absolute minimum and maximum of the study period. With the exception of February, higher PP areal rates were obtained in the 2nd Inlet.

**Table 2** Primary production measured at the sampling stations in the three study periods. PP $chl^{-1}$ = chl *a* – normalized photosynthetic rates; PPa = phytoplankton production areal rates.

| **Sampling date** | **Station** | **Depth** | **PP $chl^{-1}$** µg C µg chl $a^{-1}$ $h^{-1}$ | **PPa** mg C $m^{-2}$ $h^{-1}$ |
|---|---|---|---|---|
| 11/06/2013 | **St. 1E** | 1 | 5.65 | 6.59 |
| | | 10 | 2.03 | |
| 13/06/2013 | **St. 1I** | 1 | 4.27 | 4.32 |
| | | 10 | 1.95 | |
| 15/06/2013 | **St. 2C** | 1 | 10.92 | 12.62 |
| | | 7 | 4.93 | |
| 05/02/2014 | **St. 1E** | 1 | 3.50 | 1.87 |
| | | 10 | 0.82 | |
| 04/02/2014 | **St. 1I** | 1 | 2.86 | 1.44 |
| | | 10 | 1.15 | |
| 03/02/2014 | **St. 2C** | 1 | 2.05 | 1.20 |
| | | 7 | 0.24 | |
| 01/04/2014 | **St. 2E** | 1 | 2.13 | 2.58 |
| | | 10 | 1.11 | |
| 03/04/2014 | **St. 2I** | 1 | 2.46 | 3.24 |
| | | 10 | 0.98 | |
| 07/04/2014 | **St. 2C** | 1 | 2.21 | 6.09 |
| | | 7 | 1.81 | |

PP was always higher than HPP ($U = 324$, $p < 0.001$). The HPP rates in the water column displayed quite similar patterns to those observed for the PP (Fig. 2). HPP rates resulted regularly higher at the surface than at the bottom depth ($U = 554$, $p < 0.01$); only at St. 2C in April no difference was observed between the sampling depths.

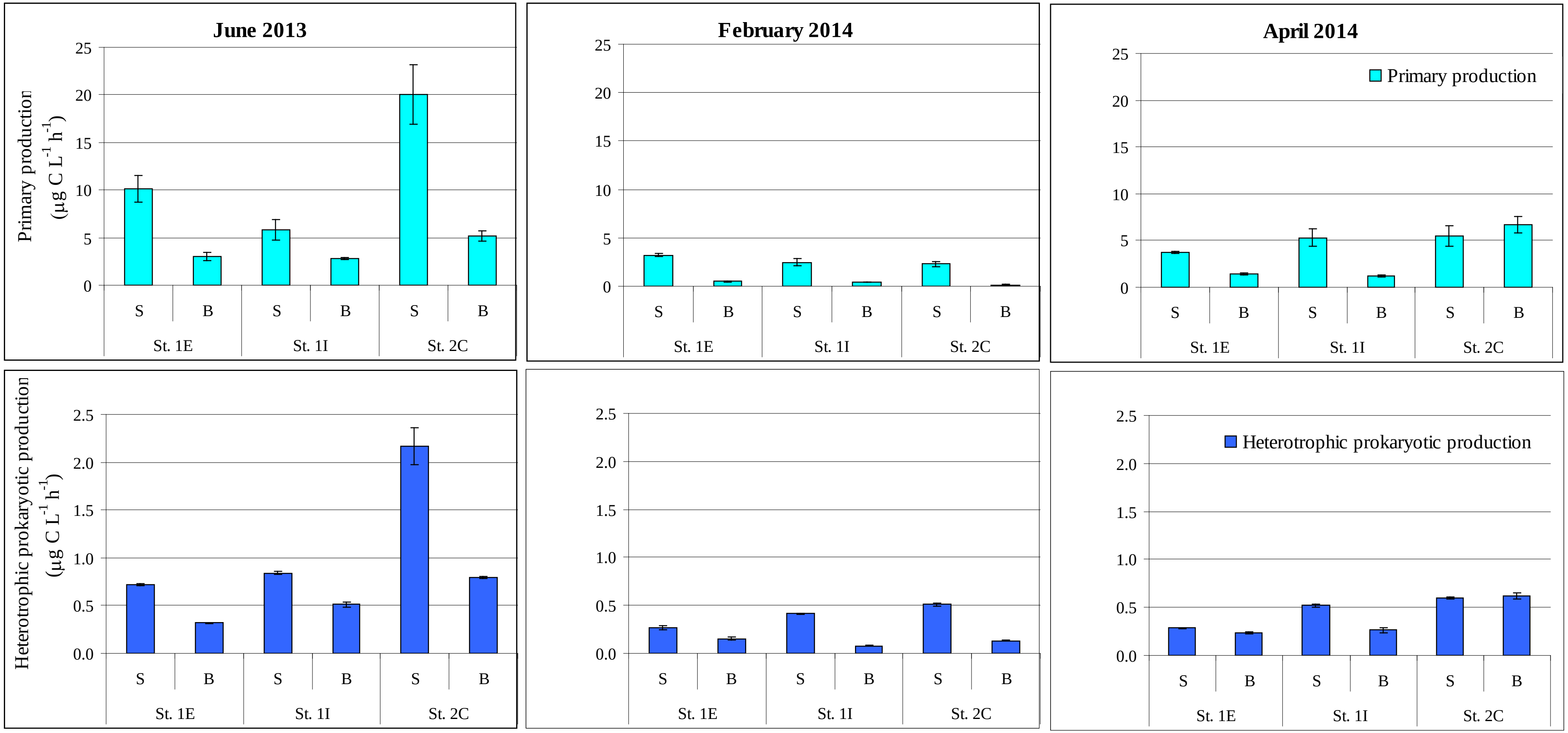

**Figure 2** Primary production and heterotrophic prokaryotic production at the three sampling stations in the three study periods.

The Two-way ANOVA highlighted differences in the HPP rates of the surface and bottom layers between seasons and sampling stations ($F_{2,18}$=491.46 and $F_{2,18}$=252.91, $p< 0.001$ for surface layers and $F_{2,18}$=695.02 and $F_{2,18}$=131.14 $p< 0.001$ for bottom layers, respectively). The highest HPP rates were detected in June (Tukey's HSD, $p< 0.01$), always exceeding 0.72 ± 0.01 µgC $L^{-1}$ $h^{-1}$ at the surface and 0.32 ± 0.01 µgC $L^{-1}$ $h^{-1}$ at the bottom. At St. 2C the HPP rates were always higher than at the other sampling stations (Tukey's HSD, $p< 0.01$), and reached the maximum value (2.17 ± 0.19 µgC $L^{-1}$ $h^{-1}$) in June.

### *3.3 Particulate and sedimentary C and N stable isotopic ratios and contribution of organic matter sources to POM and SOM*

The $\delta^{13}$C signature of POM ranged between -26.49‰ (St. 2C, April) and -21.75‰ (St. 1I, June, Fig. 3a, Table S1) and values were distinct among sampling seasons and stations (Two–ways ANOVA, $F_{2,24}$=17.58, $p< 0.001$; $F_{3,24}$=3.30, $p< 0.05$, respectively). $\delta^{13}C_{POM}$ was higher in June compared to April (Tukey's HSD test, $p<0.01$) and at St. 1I than at St. 2C ($p< 0.05$). In the whole basin, $\delta^{15}N_{POM}$ ranged between 4.92‰ (St. 1E, June) and 9.81‰ (St. 2B, June) and values changed among seasons ($F_{2,24}$=7.16, $p< 0.005$) and stations ($F_{3,24}$=12.64, $p< 0.001$, Fig. 3b, Table S1). $\delta^{15}$N data were higher in June and April compared to February (Tukey's HSD test, $p< 0.01$) and higher at St. 2B than at the other stations ($p<0.01$, Fig. 3b, Table S1). The average C:N ratio of POM was 6.90 ± 0.41 and did not change among seasons and stations (Table S1). $\delta^{13}$C values in POM were generally lower than in SOM (t-test, t=4.58, df=11, $p< 0.001$) while no differences were observed for $\delta^{15}$N (Fig. 3 and Table S1).

The $\delta^{13}$C isotopic ratio of SOM ranged between -22.98‰ (April, St. 1I) and -20.09‰ (April, St. 2B) and did not show appreciable seasonal variations (Fig. 3a, Table S1). However, differences were observed among stations (One-way ANOVA, $F_{3,8}$= 14.47; $p< 0.01$). $\delta^{13}C_{SOM}$ at St. 2B, was higher than at the other stations (Tukey's HSD test, $p< 0.01$). The $\delta^{15}$N values of SOM ranged between 6.12‰ (St. 1E, February) and 9.00‰ (St. 2B, June, Fig. 3b). $\delta^{15}$N isotopic ratio did not show a seasonal trend but changed among stations ($F_{3,8}$=23.67, $p< 0.001$). St. 2B and 2C displayed higher values than St. 1E and St. 1I (Tukey's HSD test, $p< 0.01$, Fig. 3b and Table S1).

The C:N ratio in SOM ranged between 6.55 (St. 1E, February ) and 17.13 (St. 1E, April) and did not change among seasons and stations (Table S1). The C:N ratio of SOM (12.14 ± 2.85) was higher than C:N $_{POM}$ (t=2.43, df = 10, $p < 0.05$, Table S1).

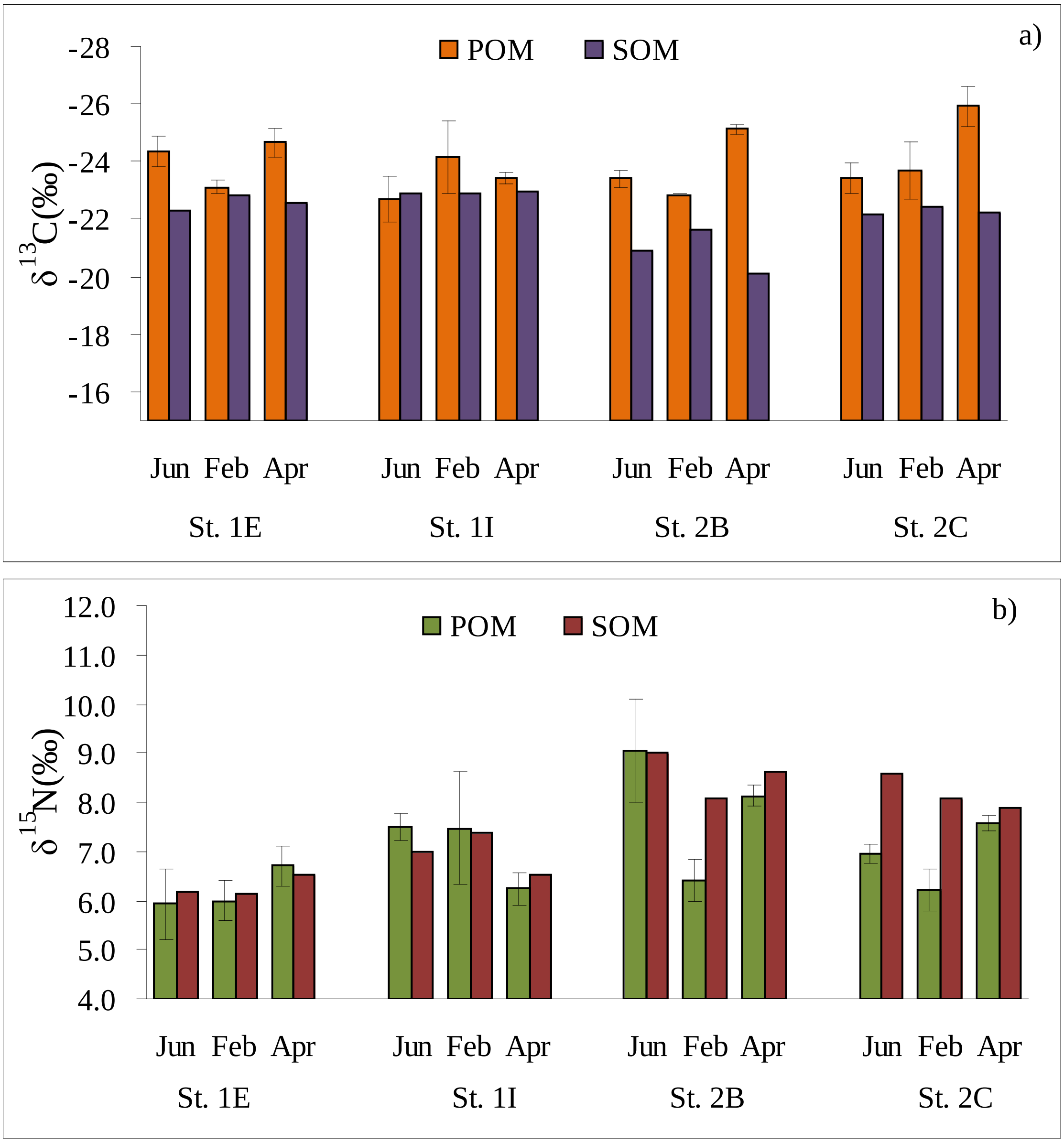

**Figure 3** Average values of $\delta^{13}$C (a) and $\delta^{15}$N (b) of POM and SOM at each sampling station and season. The y-axis begins with a value of -16 in a) and 4 in b) to better highlight variations among stations and months. Values are shown in Table S1, supplementary material.

Results of the stable isotope mixing model (SIAR) suggested that terrestrial/ riverine POM mainly contributed (45.5%) to POM pools at all sampling stations and months (Fig. 4a, Table S2).

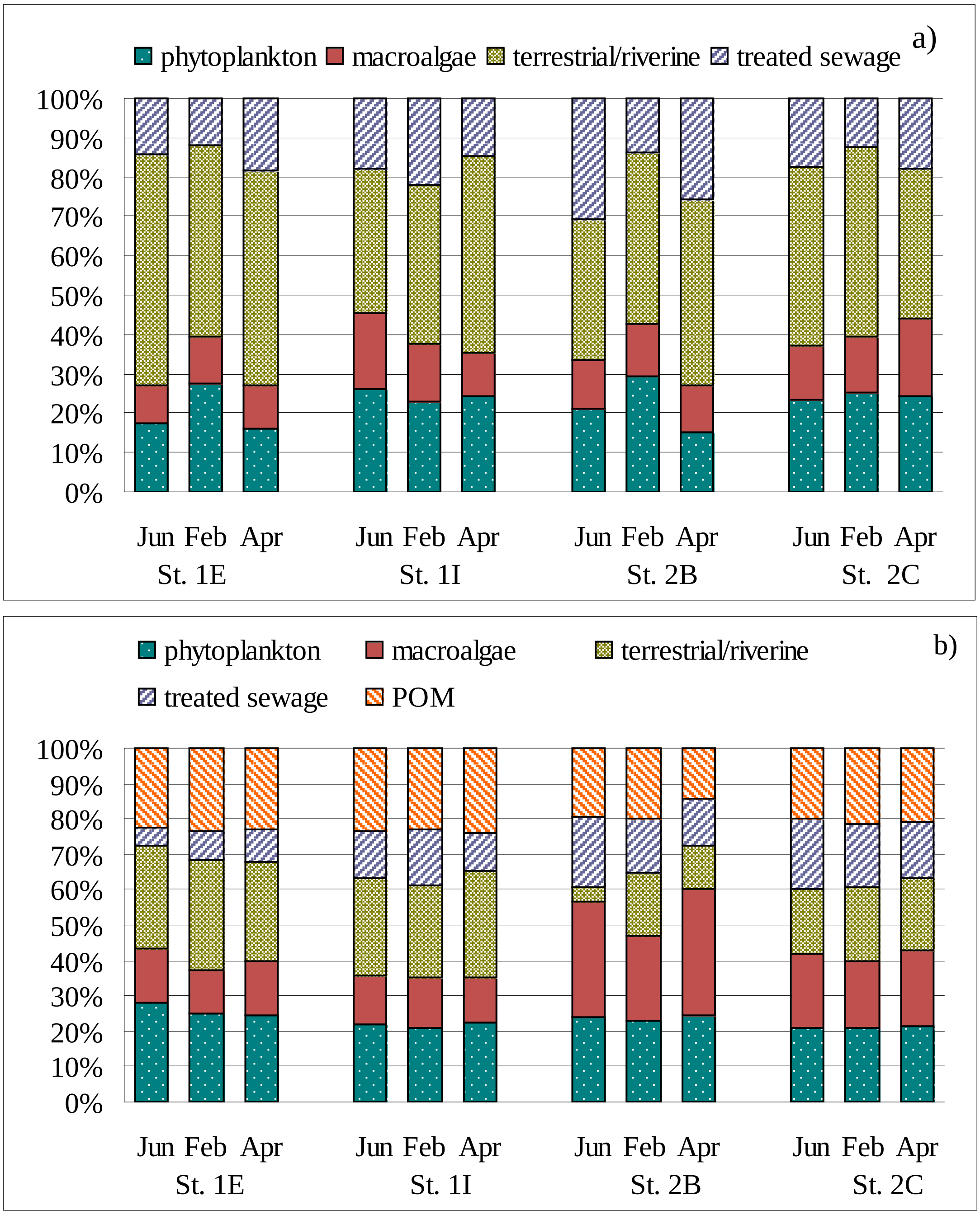

**Figure 4** Output of the mixing model (SIAR) showing a) the mean percentage of contribution of potential organic matter sources to the particulate organic matter (POM) pool and b) to the sediment organic matter (SOM) pool. Values (mean, and 95% confidence intervals) for each source are shown in Table S2 in the supplementary material.

Such contribution was slightly higher during April and reached its maximum at St. 1E (mean contribution 54%). Phytoplankton only contributed up to 29% at St. 2B in February. Contribution from sewage sources peaked at St. 2B in June and April (Fig. 4a, Table S2). As expected, SOM resulted a more homogeneous mix of organic inputs (Fig. 4b). Comparatively a higher contribution of terrestrial/riverine POM was evident in the sediments of the 1st Inlet while the influence of sewage matter was higher in the 2nd one. Notably the contribution of macroalgae to the sediment pool increased up to 30-35% in June and April at St. 2B.

### *3.4 Macroalgal coverage*

According to the map obtained by high resolution multispectral satellite remote sensing, the south-eastern area of the 2nd Inlet was the most densely colonised by macroalgae, while in the 1st Inlet a much lower areal coverage was observed. Different green macroalgae were observed by divers in the 2nd Inlet, mostly *Caulerpa prolifera* and *Caulerpa racemosa* but also *Chaetomorpha* sp. that were occasionally trapped in a van Veen grab or occurred in our sediment cores. Coverage percentages resulted to be 0% at St. 1E; 10-15% at St. 1I; 30-40% at St. 2C and 90-100% at St. 2B with a very patchy distribution at this latter station (Fig. 1). Pmax of *Caulerpa* sp. was 0.22 mg C $m^{-2}$ $h^{-1}$ at St. 1I, 0.52 mg C $m^{-2}$ $h^{-1}$ at St. 2C, whereas reached 1.42 mg C $m^{-2}$ $h^{-1}$ at the patchy colonized St. 2B.

### *3.5 Contaminants in surface sediments*

In June total PCBs concentrations ranged from about 46.0 ng $g^{-1}$ d.w. (St. 2B and 2C) to 1159.7 ng $g^{-1}$ d.w. (St. 1I) and in April from 39.0 ng $g^{-1}$ d.w. (St. 2C) to 1067.6 ng $g^{-1}$ d.w. (St. 1I) (Table 3). PCB 153 resulted the most abundant congener that accounted for approximately 15-30% of the total concentration (Table 3). PCB patterns were always dominated by hexa- (ranging from 39.3% at St. 1I in June to 58.5% of the total load at St. 1E in April), penta- (from 21.0% at St. 1E to 38.6% at St. 2B, both in June) and heptachlorinated biphenyls (up to 30.1% at St 1I in June), while the lower chlorinated congeners (tri- and tetrachlorobiphenyls) generally represented a small (<5%) contribution to the total concentration.

**Table 3** PCBs concentrations analysed at the four stations in June 2013 and April 2014, expressed as the sum of 28 PCB congeners, the sum of 7 target PCBs (∑PCB 28-52-101-118-153-138 and 180) and of the congener PCB153 that is commonly used as a proxy of the PCB concentration.

| **PCBs (ng $g^{-1}$ d.w.)** | **Sampling date** | **Stations** | | | |
|---|---|---|---|---|---|
| | | **St. 1E** | **St. 1I** | **St. 2B** | **St. 2C** |
| **PCB ∑28** | **June 2013** | 551.8 | 1159.7 | 45.3 | 46.0 |
| **PCB ∑7target** | | 280 | 590.7 | 24.3 | 22.5 |
| **PCB 153** | | 84.1 | 167.3 | 9.0 | 8.5 |
| **PCB ∑28** | **April 2014** | 164.9 | 1067.6 | 164.8 | 39.0 |
| **PCB ∑7target** | | 89.1 | 521.1 | 82.7 | 19.1 |
| **PCB 153** | | 50.9 | 189.3 | 32.4 | 7.9 |

Overall, metal concentrations measured in surface sediments were higher in April than in June, except for Fe, Al and Cr (Table 4). Higher concentrations were obtained in sediments from the 1st Inlet than those from the 2nd one. In particular, As, Cu, Zn, Pb, Mn, Sn and Hg, consistently displayed higher concentrations at St. 1E and St. 1I compared to those at St. 2B and St. 2C.

**Table 4** Concentrations of the 13 heavy metals analysed at the four stations in June 2013 and April 2014.

| **Stations** | **Sampling date** | **As** | **Cd** | **Cr** | **Cu** | **Fe** | **Ni** | **Zn** | **Pb** | **Al** | **V** | **Mn** | **Sn** | **Hg** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | mg kg$^{-1}$ d.w. | | | | | | | | |
| **St. 1E** | **June 2013** | 15.05 | 0.87 | 35.50 | 34.86 | 16590 | 26.90 | 135 | 50 | 27774 | 47 | 189.08 | 6.91 | 0.90 |
| **St. 1I** | | 17.16 | 0.90 | 26.44 | 56.69 | 22570 | 27.40 | 189 | 100 | 21499 | 51 | 163.39 | 7.86 | 1.62 |
| **St. 2B** | | 11.90 | 0.90 | 26.76 | 25.80 | 17894 | 25.67 | 99 | 23 | 26923 | 49 | 136.36 | 2.48 | 0.60 |
| **St. 2C** | | 11.03 | 0.99 | 32.09 | 25.66 | 21800 | 27.02 | 108 | 21 | 32942 | 55 | 125.89 | 1.99 | 0.30 |
| **St. 1E** | **April 2014** | 19.66 | 1.06 | 13.03 | 180.35 | 16136 | 43.59 | 231 | 80 | 18231 | 74 | 382.55 | 10.57 | 1.34 |
| **St. 1I** | | 26.45 | 1.40 | 25.99 | 100.48 | 33840 | 59.28 | 319 | 152 | 16838 | 109 | 375.74 | 14.84 | 5.74 |
| **St. 2B** | | 17.40 | 1.60 | 59.70 | 64.91 | 40760 | 63.22 | 222 | 74 | 25705 | 128 | 320.66 | 6.36 | 0.49 |
| **St. 2C** | | 17.44 | 2.64 | 48.52 | 55.19 | 26944 | 63.94 | 180 | 51 | 25168 | 122 | 335.65 | 4.02 | 0.10 |

### *3.6 Multidimensional scaling (MDS) and Analysis of Similarity (ANOSIM)*

A good spatial separation of samples was obtained in the MDS both for surface (stress = 0.05) and bottom data (stress = 0.07) (Fig. 5a, b).

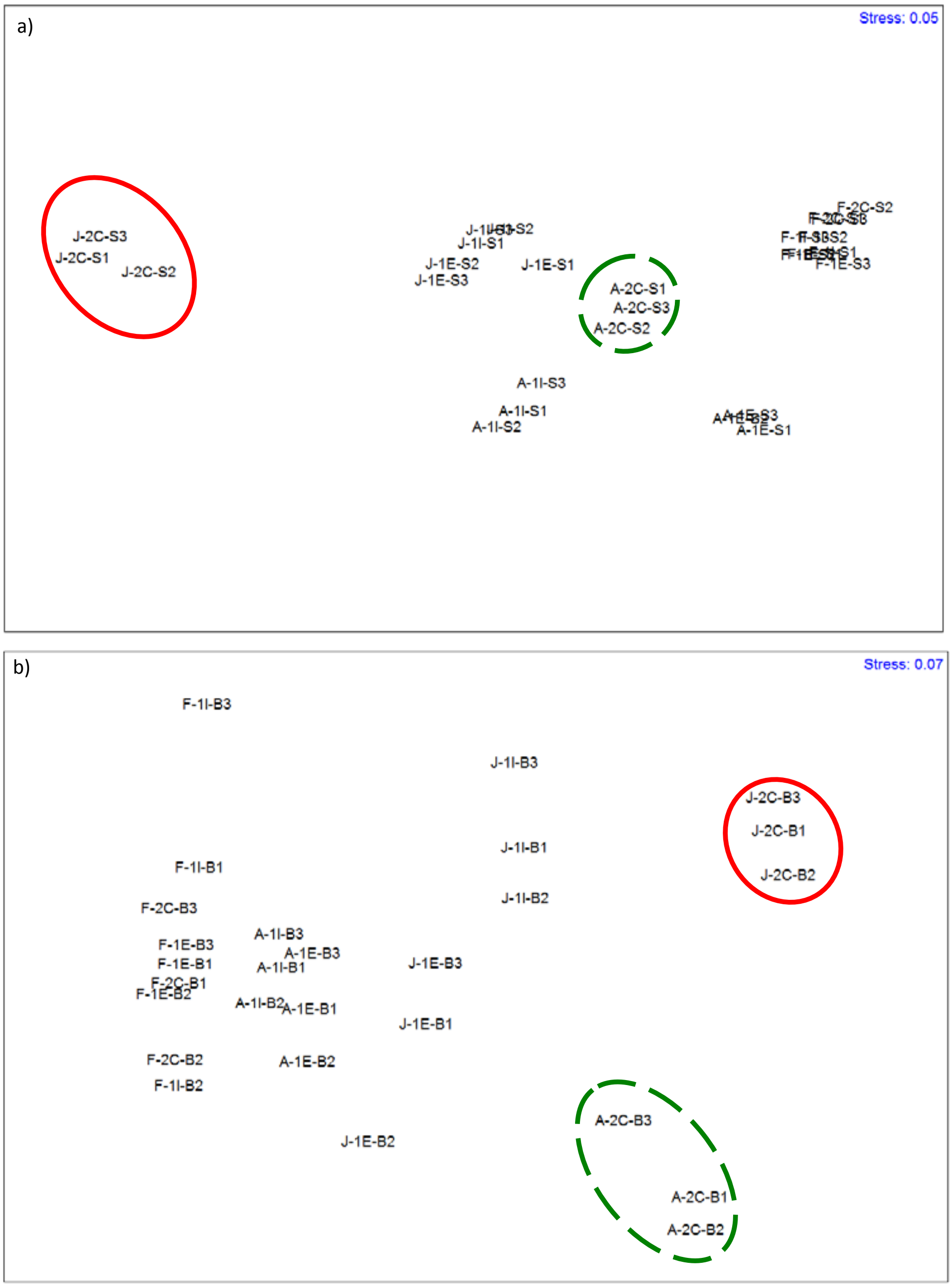

**Figure 5** Multidimensional scaling analysis (MDS) based on PP and HPP, abundances of autotrophic and heterotrophic picoplankton and autotrophic nanophytoplankton (a) at the surface (S) and (b) at the bottom layer (B). In b) also the $\delta^{15}N$ and $\delta^{13}C$ signatures of POM are included in the analysis. Replicated (n = 3) samples are indicated per station (1E, 1I and 2C) and samplings month (F-February, A-April, J-June). Green dashed and red full circles enclose April and June samples from the 2nd Inlet, respectively. February data are not clearly separated between Inlets and thus are not enclosed in circles.

According to ANOSIM, significant differences were obtained among sampling periods both for surface ($R_{ANOSIM} = 0.658$, p = 0.1%) and bottom samples ($R_{ANOSIM} = 0.456$, p = 0.1%), between the two Inlets ($R_{ANOSIM} = 0.195$, p = 2.6% and $R_{ANOSIM} = 0.408$, p = 0.5% for surface and bottom data, respectively) as well as among stations ($R_{ANOSIM} = 0.118$, p = 3.3% and $R_{ANOSIM} = 0.173$, p = 0.9% for surface and bottom data, respectively). The pairwise ANOSIM test performed on stations did not result significantly different for surface data, whereas the bottom of St. 2C was significantly different from both St. 1E ($R_{ANOSIM} = 0.224$, p = 2.1%) and St. 1I ($R_{ANOSIM} = 0.181$, p = 3.8%).

## 4 Discussion

### *4.1 Primary production and heterotrophic prokaryotic production in the Mar Piccolo*

In this study, for the first time, we measured primary production (PP) and heterotrophic prokaryotic production (HPP) in the Mar Piccolo of Taranto. PP values were well above 2 µg C $L^{-1}$ $h^{-1}$ also during winter indicating that the Mar Piccolo is a quite productive basin. Values were strongly influenced by light availability in the water column, as demonstrated by the highly significant correlation with the PAR irradiance (R = 0.76, $p<0.001$, Table 1). Due to the high phytoplankton production, the basin was oversaturated in oxygen, as confirmed by the oxygen profiles registered in the water column in two sampling periods (Table 1).

PP rates were highly correlated with chl *a* concentrations (R = 0.80, $p<0.001$). The relatively high phaeo/chl *a* ratios (1.5 at the surface layer and up to 3.7 at the bottom) observed in February suggested that in that month the phytoplankton assemblage was in the senescent phase. Interestingly, the phytoplankton assemblage responsible for the highest PP rate estimated in June in the 2nd Inlet was presumably also in a senescent phase, as indicated by a phaeo/chl *a* ratio of 2.44.

The sinking degrading phytoplankton represent highly palatable substrata for heterotrophic prokaryotes and could have fueled HPP rates that peaked in June 2013. Moreover, the high abundance of small diatoms observed in this period (Karuza et al. 2015 this issue) could have been responsible for a major availability of labile exudates (Hoagland et al. 1993), which stimulate the prokaryotic growth. HPP rates were further tightly related to the concentrations of both the Particulate and Dissolved Organic matter (POC and DOC) in the water column (R = 0.81, $p<0.001$ with POC, and R = 0.73, $p<0.01$ with DOC). In particular, in June 2013 DOC varied between 1.273 mg $L^{-1}$ and 1.587 mg $L^{-1}$ at the bottom of St. 2C and St. 1E, respectively. On the other hand, in April 2014 it ranged from 0.902 mg $L^{-1}$ at the bottom of St. 1E to 1.963 mg $L^{-1}$ at the surface of St. 2C (Kralj et al. 2015 this issue). The extracellular release of recently fixed photosynthate contributes to the production of DOC in marine ecosystems and is particularly important for the trophic ecology of the plankton. The released compounds are susceptible to rapid uptake by heterotrophic bacteria, giving way to a linkage between primary and bacterial production that is essential for the cycling of matter through the microbial loop and the microbial food web (Marañón et al. 2004). Indeed, considering the entire study period, our PP and HPP hourly rates exhibited exactly the same pattern (Fig. 2) and their correlation was highly significant ($R = 0.90$, $p<0.001$). Our mean PP/HPP ratio was 20.96 and, excluding one outlier, it varied between 7.09 and 29.89. Previously, Puddu et al. (1998) demonstrated that in the northern Adriatic the bacterial mediated processes are tightly coupled with the phytoplankton production. They reported that a very high percentage (40-80%) of the carbon fixed by the phytoplankton has been requested for bacterial metabolism. Data of PP and HPP in the Mar Piccolo suggested that, especially in June, these two processes were clearly in phase. Therefore, in this basin the phototrophic plankton appear to be a main driving force in C-cycling, independently from their dimensional range and seasonal succession. The flow of C through the system seemed clearly shaped by two essential steps, i.e. the processes of primary production and OM degradation, quite active and efficient in the water column.

Higher PP and HPP rates in the 2nd Inlet are likely due to a major biomass of both autotrophic and heterotrophic organisms observed in the 2nd Inlet during our surveys (Karuza et al. 2015 this issue). Indeed, from 2002 onwards, higher plankton biomass in the 2nd Inlet has been well documented (Caroppo et al. 2012), mostly ascribable to its more lagoonal features. This Inlet is shallower and characterised by a higher freshwater input and lower salinity compared to the 1st one (Cardellicchio et al. 2015 this issue) favouring the development of the phototrophic organisms in the water column. Although mussels that cover 2/3 of the 2nd Inlet sequestrate a good part of the available biomass (Caroppo et al. 2012), the remaining fraction is still higher compared to that of the 1st Inlet. We infer that a possible co-factor responsible for both the overall lower phytoplankton biomass and PP and HPP rates in the 1st Inlet may be the higher concentration of contaminants in its surface sediments. Through resuspension these contaminants could be temporarily transferred to the water column, entering the pelagic trophic web and interfering with the proper functioning of the pelagic ecosystem. Several studies have reported detrimental effects of contaminants on phytoplankton (Caroppo et al. 2006, Lafabrie et al. 2013a, Tiano et al. 2014) and photosynthesis (Pérez et al. 2006) in association with increased respiration rates (Lafabrie et al. 2013b).

In order to have an overview of the PP and HPP in the entire basin, the rates measured in the water column were integrated to those obtained in surface sediments (Table 5) (Franzo et al. 2015 this issue; Rubino et al. 2015 this issue). Since we estimated benthic processes only in June 2013 and April 2014, an integration was possible only in these two sampling periods. Primary and heterotrophic prokaryotic production rates obtained in the water column (PPw, HPPw) were converted from $\mu g\ C\ L^{-1}\ h^{-1}$ to $mg\ C\ m^{-2}\ h^{-1}$ and added to those estimated in surface sediments (PPs, HPPs) to have an evaluation of the total rates (PPi, HPPi) at the three sampled stations in the Mar Piccolo (Table 5).

**Table 5** Primary and heterotrophic prokaryotic production rates in the water column (PPw, HPPw), in surface sediments (PPs, HPPs) and as integrated rates (PPi, HPPi) at the three sampled stations and during the two sampling periods.

| | June 2013 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | PPw | PPs | PPi | PPs/PPw | HPPw | HPPs | HPPi | HPPs/HPPw |
| | mg C m$^{-2}$ h$^{-1}$ | | | | mg C m$^{-2}$ h$^{-1}$ | | | |
| St. 1E | 6.59 | 0.69 | 7.29 | 0.11 | 0.52 | 0.57 | 1.09 | 1.10 |
| St. 1I | 4.32 | 0.06 | 4.38 | 0.01 | 0.68 | 0.33 | 1.01 | 0.49 |
| St. 2C | 12.62 | 0.25 | 12.86 | 0.02 | 1.48 | 0.55 | 2.03 | 0.37 |
| | April 2014 | | | | | | | |
| | PPw | PPs | PPi | PPs/PPw | HPPw | HPPs | HPPi | HPPs/HPPw |
| | mg C m$^{-2}$ h$^{-1}$ | | | | mg C m$^{-2}$ h$^{-1}$ | | | |
| St. 1E | 2.58 | 0.27 | 2.85 | 0.10 | 0.26 | 0.10 | 0.36 | 0.41 |
| St. 1I | 3.24 | 0.13 | 3.37 | 0.04 | 0.39 | 0.05 | 0.44 | 0.14 |
| St. 2C | 6.09 | 0.50 | 6.59 | 0.08 | 0.61 | 0.16 | 0.77 | 0.27 |

PPw values were always much higher than PPs ones. The highest contribution of PPs to PPi was around 10.5% and was calculated in the center of the 1st Inlet in both sampling months. The lower PPs contribution was found at St. 1I close to the navy base. These total primary production rates (up to 12.86 mg C m$^{-2}$ h$^{-1}$) are quite low for an enclosed shallow basin, such as the Mar Piccolo. In fact, considering the PP both in the water column and in the sediments, the microphytobenthos alone may contribute up to 50% of the total PP in shallow coastal systems (Perissinotto et al. 2002; Montani et al. 2003). We suggest that low PPs rates in the Mar Piccolo, especially at St. 1I, are likely ascribable to the high concentration of PCBs, heavy metals (Tables 3, 4) and other contaminants accumulated in the sediments that can inhibit benthic PP and therefore interfere with the proper functioning of this ecosystem.

Yet, these estimates do not consider the contribution of macroalgae. At St. 1E the bottom was unvegetated, as confirmed by the satellite map (Figure 1), and therefore no additional contribution had to be considered. At St. 1I, *Caulerpa* sp. with a $P_{max}$ of 0.22 mg C m$^{-2}$ h$^{-1}$ could lead to an integrated value up to 4.60 mg C m$^{-2}$ h$^{-1}$ in June. At St. 2C, with a macroalgal contribution of about 0.52 mg C m$^{-2}$ h$^{-1}$, the estimated total production of the system could reach 12.91 mg C m$^{-2}$h$^{-1}$. Overall, at the investigated stations the phytoplankton is confirmed to be by far the most important primary producer in the Mar Piccolo.

The contribution of HPPs to HPPi was much higher in June than in April (Table 5). In June, the calculated rates in the water column and in the surface sediments were comparable, particularly at St. 1E, whereas HPPs contributed approximately for one third to HPPi at the other two stations. The low phaeo/chl *a* ratios observed in June at St. 1E re-enforce the idea that the higher rates could be boosted by the input of fresh organic matter from the water column as further indicated by the mixing model in which the plankton contribution to the SOM pool was slightly higher compared to the other months and stations. Conversely, an overall lower HPPs contribution to HPPi was obtained in April, especially at St. 1I (ca. 12%).

### *4.2 Isotopic signature of POM and SOM and contribution of organic matter sources*

Mean $\delta^{13}C$ (-23.90‰) of particulate organic matter (POM) in the Mar Piccolo resulted comparable to values reported in the Lapalme and Venice lagoons (Berto et al. 2013; Carlier et al. 2007) and in the bay of Marseille (Cresson et al. 2012) and lower than those reported for other Mediterranean coastal surface waters and lagoons (Mazzola et al. 1999; Vizzini and Mazzola 2003). POM was highly $^{13}C$ depleted when compared to the typical signature of marine phytoplankton indicating the predominance of allochthonous sources of terrestrial origin (Cresson et al. 2012; Harmelin-Vivien et al. 2008). This result was confirmed by the SIAR model which showed a contribution (up to 54%) of terrestrial/riverine POM to the entire pool. The $^{13}C$ depleted signal was more pronounced during April and could reflect high late-winter land runoff or freshwater intrusion which is one of the main features of the this basin (Cardellicchio et al. 2015 this issue). $\delta^{15}N_{POM}$ values in the Mar Piccolo were comparable to those reported by Cresson et al. (2012) in the bay of Marseille. Both $\delta^{15}N$ values and the SIAR model clearly reflected a major nutrient enrichment of the 2nd Inlet (higher $\delta^{15}N$ values detected during June and April at St. 2B for POM and at St. 2B and 2C for SOM) compared to the 1st Inlet. This enrichment could be due, as suggested by the mixing model, to the input of wastewater nutrients or alternatively to the organic matter derived from animal waste from the nearby mussel farming area (Owens 1985; Vander Zanden and Rasmussen 2001; Vizzini and Mazzola 2004) which covers 2/3 of the surface of the 2nd Inlet. The measurement of specific end-members for the mussel farm activities (faecal material, biodeposits) will help to resolve this issue.

### *4.3 Impact of contaminated-sediment resuspension on the ecosystem functioning*

At the four investigated sites, PCBs displayed dissimilar seasonal patterns. Particularly in April, PCBs concentrations increased at St. 2B while they decreased at St. 1E. This could be attributed to natural and anthropogenic disturbance events that cause episodic sediment resuspension present in these areas of the basins and that lead to changes in the chemical properties of sediments. High levels of PCBs measured in the sediments of St. 1I obtained in this study are in agreement with previous reports in which this area is described as a major source of PCBs for the Mar Piccolo of Taranto (Cardellicchio et al. 2007). The general uniformity in the PCB pattern found in the surface sediments of the Mar Piccolo suggests that the contamination source was probably the same in all the investigated sites, likely related to the navy arsenal activities. In particular, considering the sum of the 7 target PCBs, PCB 153 alone accounted for 57% of their concentration, followed by PCB 138 and PCB 180, a pattern that is in agreement with previously reported data (Gómez-Lavín et al. 2011; Okay et al. 2009; Secco et al. 2005).

We obtained higher concentrations of most of the analysed metals in sediments of the 1st Inlet compared to those of the 2nd one. Our findings are in agreement with the results of Calace et al. (2005) and Cardellicchio et al. (2009), who reported that the 1st Inlet of the Mar Piccolo is more contaminated by metals than the 2nd one. These derive partially from the seawater coming from the Mar Grande through the two channels (in turn influenced by industrial wastewaters) but, to a much greater extent, from the presence of shipbuilding activities of the main Italian navy base, located in the 1st Inlet. The frequent passage of navy ships and submarines in the centre of the 1st Inlet, where the maximum depth reaches 13 m, likely resuspend the first centimetres of sediments that from the deeper layers reach the surface. In these subsurface layers, due to anoxic conditions and the presence of hydrogen sulphide, metals are present in the form of insoluble sulphurs, tightly linked to the organic matter (Caroppo and Cardellicchio 1995). Once at the surface, under oxic conditions a fraction of metals could change the oxidation state becoming more bioavailable and facilitating, therefore, their entry into the pelagic food web.

Overall, higher concentrations of As, Cu, Zn, Pb, Mn, Sn and Hg were observed in April compared to June data. Also considering a certain degree of spatial variability that may have occurred during sampling, metal concentrations were consistently higher at the four stations in April. These great differences between the two sampling periods (up to more than twice) may be attributable to phytoplankton dynamics (Heimbürger et al. 2010). According to these authors, higher metal concentrations in the water column coincide with blooms of nano- and picophytoplankton because they accumulate more efficiently particle-reactive trace metals, especially Hg, due to their greater surface/volume ratio compared to microphytoplankton. Therefore, we can infer that our lower metal concentrations in surface sediments in June may be attributed to a partial sequestration of these compounds by nanophytoplankton that reached up to $1.3\times10^{7} \pm 5.9\times10^{5}$ cells $L^{-1}$ in that month (Karuza et al. 2015 this issue).

To date, the effects of resuspension of contaminated sediments on pelagic organisms and ecosystem functioning remain underresearched (Lafabrie et al. 2013a). For instance, the processes of PCBs transfer at the lowest trophic levels are poorly understood and the mechanisms of PCBs uptake by plankton are still a matter of scientific discussion (Tiano et al. 2014). Our results provide new insights into these open questions as summarised in the MDS outputs. The pelagic system functioning seems to be variably influenced by resuspension events according to the season and the depth. In Figure 5b, in June St. 2C was clearly separated from the other sites suggesting a different environmental situation than that in the 1st Inlet. The stratification of the water column that began in early summer probably slowed down the water exchange between the two Inlets and enhanced the confinement characteristics of the 2nd one. In contrast, February samples were not well separated, following the winter mixing of the water column while the intermediate position of April samples seems to corroborate the hypothesis of the gradual decrease of naturally-induced sediment resuspension from winter towards early summer. On the other hand, the MDS performed on surface data (Figure 5a) suggests that the effects of resuspended contaminants on the pelagic system could vary according to the distance from their main source, i.e. the sediments. Surface samples, in fact, did not show a clear separation among stations and/or inlets suggesting that, at the surface, even the most impacted site was comparable to the others. This hypothesis is also confirmed by the pairwise ANOSIM test performed on stations separating surface from bottom samples. While at the surface the stations were not significantly different among each other, the bottom of St. 2C was significantly different from both St. 1E ($R_{ANOSIM}$ = 0.224, p = 2.1%) and St. 1I ($R_{ANOSIM}$ = 0.181, p = 3.8%).

## 5 Conclusion

In this study, for the first time, phytoplankton primary production (PP) and heterotrophic prokaryotic production (HPP) were measured in the Mar Piccolo of Taranto and used as proxies of the ecosystem functioning. This semi-enclosed basin resulted quite productive over the study period. Considering the three major primary producers (phytoplankton, microphytobenthos and macroalgae), the phytoplankton resulted by far the most important primary producer at the investigated sites. This was also confirmed by the SIAR model that, although indicating an overall major contribution of allochthonous material of terrestrial origin to the POM pool, pointed to a more pronounced contribution of phytoplankton compared to that of macroalgae. The contribution of macroalgae to the sediment pool was higher in one site of the 2nd Inlet densely colonized by macroalgae, as confirmed by the satellite map of the seabottom.

Over the study period, PP and HPP data exhibited exactly the same pattern, indicating that the heterotrophic prokaryotes were boosted by the extracellular release of recently fixed photosynthates and degrading phytoplankton cells. In spring and early summer, significantly lower PP and HPP rates were found in the 1st Inlet compared to the 2nd one, suggesting a detrimental effect of contaminants which could be resuspended from the heavily polluted sediments of the navy arsenal and spread over the whole 1st Inlet. However, their interference with the proper functioning of the pelagic ecosystem seems to be limited to the bottom layers.

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# CONCLUSION

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 the structural variables can often lead to misleading conclusions and solely by combining them with the functional processes it is possible to have a more detailed and correct insight into the benthic ecosystem functioning. This is also confirmed by the findings that emerge from my thesis. Regarding the microphytobenthos, this particular phototrophic community has shown to be less sensitive to stressors compared to its heterotrophic counterparts since it may adapt to and survive in more demanding environments, even at the extreme conditions of the thermal vents. That renders the microphytobenthos even more suitable as a bioindicator and extends its applicability to a wider range of conditions.

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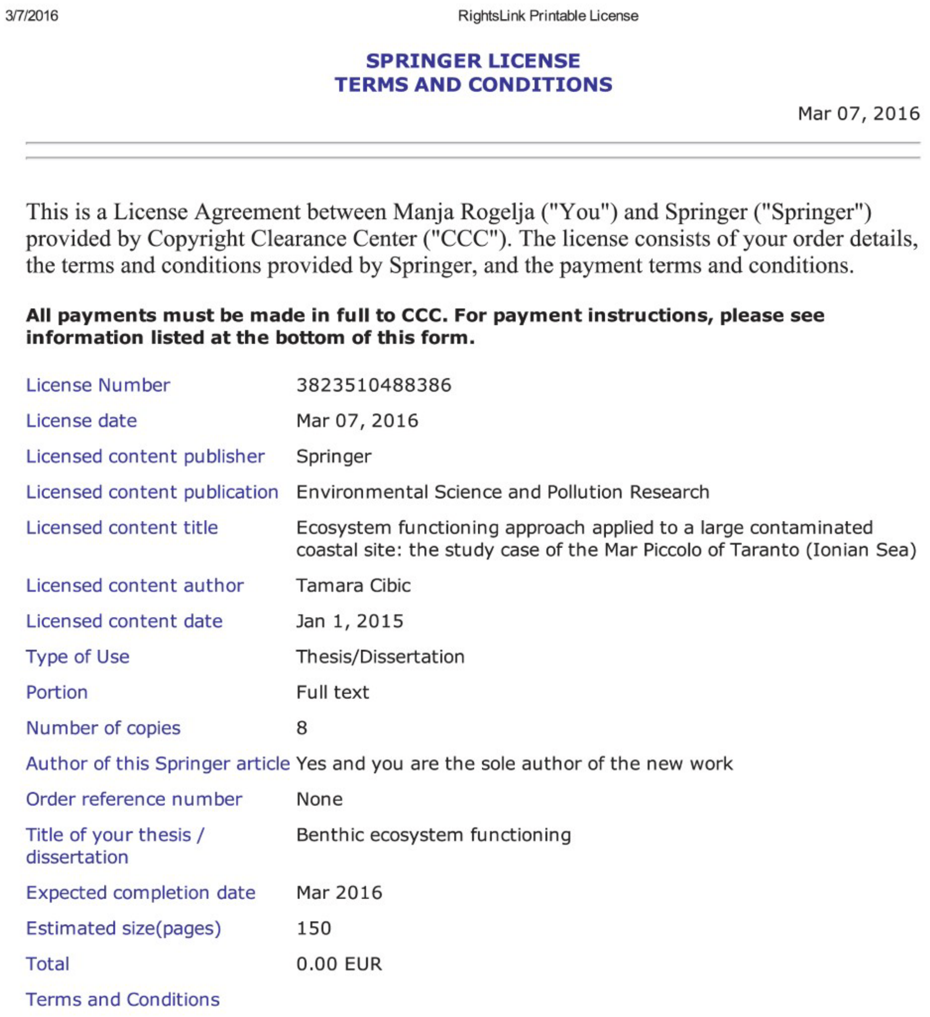
# ANNEXES

## Annex A



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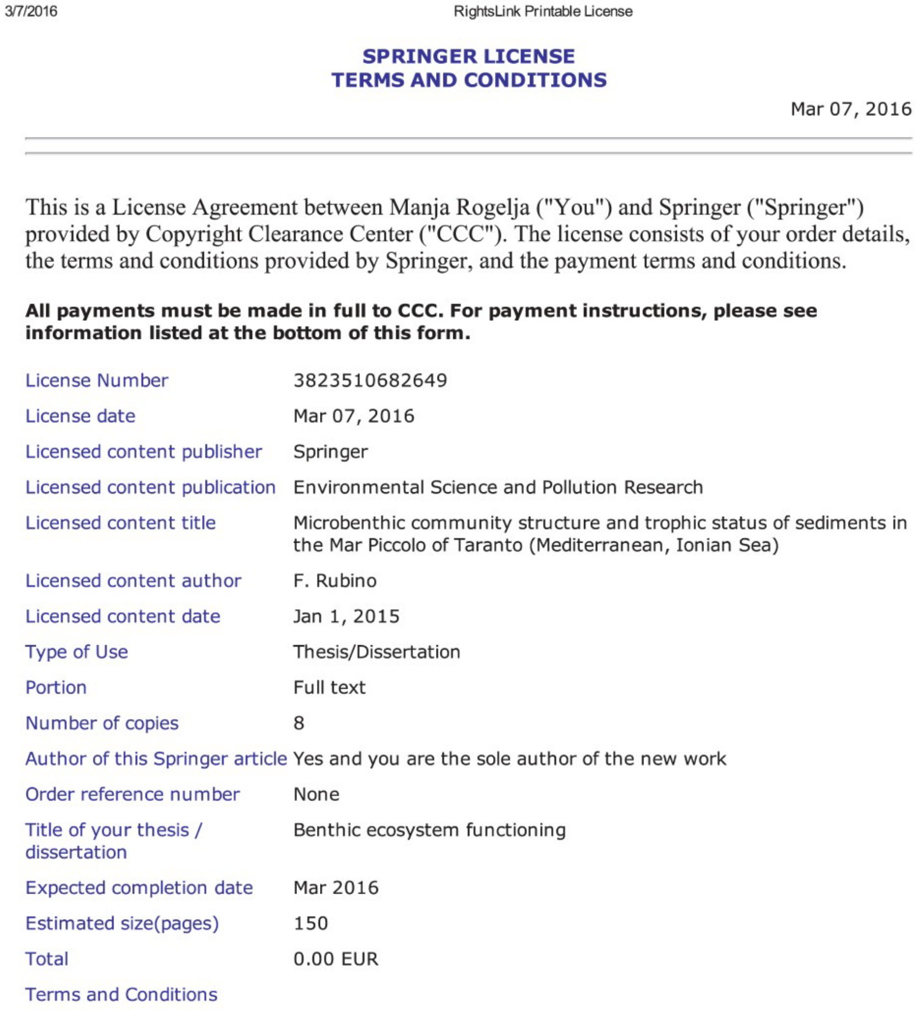

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