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Multiple stressors affect coastal marine ecosystems

Ph.D. thesis of:
Ettore Nepote

Tutor:
Prof. Roberto Danovaro

Co-tutor:
Prof. Antonio Dell'Anno

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RIASSUNTO

Gli ecosistemi marini sono sottoposti a diversi fattori di stress di origine antropica, i cui effetti non sono ancora del tutto noti. Questo gap conoscitivo rappresenta un punto cruciale, in quanto, la conoscenza degli effetti delle attività umane è essenziale per l’applicazione di piani di monitoraggio e di adeguate strategie di mitigazione. Di conseguenza, in questa tesi, sono stati selezionati e studiati quattro diversi tipi di pressioni antropiche; allo scopo di aumentare le conoscenze disponibili come richiesto dalla MSFD. La prima fonte di impatto selezionata sono gli impianti di desalinizzazione, per i quali è stato studiato l’impatto sulla comunità macrobentonica. I risultati ottenuti suggeriscono che non sono causa di alterazioni distinguibili dalla naturale variabilità spaziale della macrofauna. Ciò può essere messo in relazione alle piccole dimensioni degli impianti. Risultati simili sono stati ottenuti per la seconda pressione antropica studiata, cioè ripascimento e (ri)stenditura della sabbia sugli arenili, i quali, non hanno alterato la naturale variazione spaziale e temporale della macro e meiofauna. Anche in questo caso, la mancanza di un impatto significativo è da attribuire alla piccola scala dell’intervento. Il terzo caso di studio riguardava l’analisi dell’estensione (m^2) di una prateria di fanerogame nell’arco di 40 anni. I risultati indicano, come i due declini riscontrati fossero correlati a due diversi fattori, quali urbanizzazione e aumento della temperatura, i quali hanno agito non solo sull’estensione della prateria, ma anche sulla composizione tassonomica. Infine, è stato analizzato il potenziale impatto della pesca con draghe idrauliche, particolarmente intensa nel Mare Adriatico. Più specificatamente, è stato studiato il potenziale effetto della diminuzione dello sforzo di pesca (di circa il 50%) imposto dall’emergenza sanitaria del SARS-CoV-2 sulle comunità macrobentoniche. I risultati evidenziano un aumento significativo dei bivalvi, che porta ad una comunità macrobentonica significativamente diversa rispetto a quella presente prima del lockdown. In

conclusione, dall’analisi questi casi di studio è emerso come la “scala” della pressione antropica giochi un ruolo cruciale nel determinare l’eventuale impatto. Inoltre, è stato evidenziato come diversi ecosistemi mostrino una apprezzabile resilienza quando le pressioni antropiche diminuiscono in maniera significativa.

ABSTRACT

Multiple stressors affect coastal marine ecosystems, whose effects are often largely unknown. This represents a crucial point, since the knowledge about the impacts caused by human activities are essential to apply adequate monitoring plans and mitigation strategy. Consequently, in this thesis four different human pressures were selected and investigated, in order to increase the available information. The first impact source selected is represented by desalination plants, for which the impact on macrobenthic assemblage was investigated. The results suggest that they do not cause alterations superimposed to the natural spatial variability of the macrofauna assemblage. This has been related to the small sizes of the plants. Similar results were obtained for the second stressor investigated (i.e., beach nourishment and beach re-profiling), which did not alter the spatial-temporal trend of the macro-meiofauna assemblage. Again, the lack of a significant impact was associated to the small size of the interventions. The third case study investigated the long term (40 years) changes (in m^2) of seagrass meadow extension in the North Adriatic Sea. The results indicated that the two declines recorded were mainly correlated with two different drivers: urbanization and positive thermal anomalies respectively, which affected not only the meadow extension but also the species composition. Finally, the last pressure analyzed is the fishing with hydraulic dredging, which is extremely intensive in the Adriatic Sea. Specifically, here the effects of the decrease of fishing effort (of about 50%) imposed by the SARS-CoV-2 sanitary emergency was studied. From the results, it is evident a significant increase of bivalves, making the entire macrofauna assemblage significantly different from that present before the 2020. Overall, from these case studies, it is clear as a crucial role is played by the "size"

of the human pressure. In addition, it is clear that in different ecosystems, when there is a noticeably decrease of human pressures, an appreciable partial recovery is possible.

1. CHAPTER 1: INTRODUCTION

1.1 Multiple stressors affect marine ecosystems

Marine ecosystems are threatened by several local as well as global impacts, which can act in antagonist way or more frequently in additive (one after the other) or synergistically (at the same time) way resulting more incisive (Rossi 2013). Global impacts include all impacts related to global climate change, such as global warming and ocean acidification (Rossi 2013). Both are strictly related to the increase of CO_2 emission in the atmosphere and its cascade impact on marine ecosystems. Global warming caused the alteration of biogeochemical cycles, variation of oceanic water circulation and the increase of positive thermal anomalies. The latter can cause mass mortality events and shift in taxonomic composition from tropical to Mediterranean regions (McClanahan and Muthiga 2014; Di Camillo and Cerrano 2015; Bianchi et al., 2019). In addition, global warming can facilitate the spread of invasive species, which can determine important changes on different habitats such as seagrass meadows, marine caves and rocky reef (Pergent et al., 2008; Gerovasileiou et al., 2014; Gatti et al., 2015). Ocean acidification consists in a decrease of seawater pH with negative effects on calcifying organisms (e.g., reef-building corals and calcareous algae) (Milazzo et al., 2019). The decrease of building capacity can cause a significant habitat loss. Although global impacts are considered deleterious for the marine ecosystems, local impacts, due to direct human actions, are often considered superimposed to global change, with more evident effects (Barange et al., 2010). Local impacts include a high variety of impacts such as urban development, chemical pollution, overfishing and resource exploitation (Rossi 2013). Among them, urbanization is widely considered particularly intensive, especially when it is associated to coastal constructions, which cause a high sediment load with deleterious effects on marine habitats (Nepote et al., 2016,2017). Examples of coastal constructions are touristic and industrial harbors, road and defense structures such as seawalls and breakwaters (Colosio et al., 2007). Urbanization is often associated to a high tourism flow, which can promote habitat degradation (Danovaro et al., 2020). A common consequence of tourism flow and generally of high population density is the increase of organic enrichment, which cause the eutrophication of marine system (Rossi 2013). This can lead to anoxic/hypoxic conditions which compromise the metabolism of several organisms (Thomas et al., 2019). Another important stressor, often associated to urbanizations is the industrial plants development that showed a widespread distribution along the coastal zone, especially in the Mediterranean Sea (Bertocci et al., 2019). As they represent an important source of chemical compounds (i.e., hydrocarbons and heavy metals), they pose a serious risk for marine biodiversity (Chiarore et al., 2020). In particular, benthic organisms are particularly at risk, given that all pollutants present in the water column tend to settle on the seafloor (Bertocci et al., 2019). Marine sediment contamination can affect prokaryotic metabolism and turnover, with negative consequences on biogeochemical cycles (Dell'Anno et al., 2003). Similarly, the biodiversity of meiofauna assemblage decreases, with a consequent decrease of the ecosystem services associated (Mele et al., 2020). Industrial plants are not the unique source of chemical pollution. Indeed, touristic and industrial harbor represent a further source of chemical pollutants (Cecchi et al., 2019). Overall, coastal ecosystems are under the pressure of several stressors and the habitats present result dramatically compromised. In this view, it is necessary to improve our knowledge of all stressors acting on the coastal area and the environmental status of the different ecosystems to apply effective mitigation strategies and restoration plans. This is essential to make ecosystem goods and services again accessible (Danovaro et al., 2020).

1.2 European Union legislative framework

In order to counteract the negative consequences of the human pressures described above, the European Union applied legislative measures and policies to maintain a good status for marine waters, habitats and resources (Borja et al., 2010). The mains legislative frameworks for the marine environment developed are the *“Water Framework Directive”* (i.e., WFD) and *“Marine Strategy Framework Directive”* (MSFD). The former uses a “deconstructing structural approach”, where the ecosystem is divided in several Biological Quality Elements (BQE) that were compared individually, and only subsequently are combined to assess the status of the entire ecosystem. Conversely, the MSFD uses a *“holistic functional approach*” through the use of a set of 11 descriptors that together summarize the way in which the whole system function (Borja et al., 2010). The first evident difference, among the two directives, is the spatial application. Indeed, the WFD is applied from coast line to 1 nautical mile, while the MSFD reaching to 12 nautical miles. In addition, they use different terms, the former (i.e., WFD) aims at obtaining a Good Ecological Status (with 5 different levels such as high, good, moderate, poor and bad), which is “an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters”. However, the latter (i.e., MSFD) aims at obtaining a Good Environmental Status (GES) with only two levels: good and not good. MSFD defines GES area as the “marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive within their intrinsic conditions, and the use of the marine environment is at a level that is sustainable, thus safeguarding the potential for uses and activities by current and future generations" (Borja et al., 2010). The WFD includes only five Biological Quality Elements (BQE): i.e. besides physical chemical parameters, phytoplankton-macroalgae, Phanerogams, macroinvertebrates, fishes (only in transitional water). The MSFD is based on 11 descriptors: biodiversity, non indigenous species, exploited fish and shellfish, food webs, human induced eutrophication, sea floor integrity, hydrographical conditions, contaminants, contaminants in fish, marine litter and introduction of energy or noise. Furthermore, the two directives (especially MSFD) require to know in deep way the different pressures that act on marine ecosystems and their potential effects (Borja et al., 2010).

1.3 Objectives-Unstudied impacts on marine ecosystem- 4 case studies

In contrast to that required from the MSFD and WFD, most of the human impacts acting on coastal ecosystems are still poorly known. Here, we attempt to implement our knowledge about the effects of the human pressures on coastal ecosystems. To achieve the goal, we selected four different case studies, which represent common situations in coastal ecosystems, and likely to become more frequent in the next decades:

1) Effects of desalination plants on macrofauna assemblage.

2) Beach nourishment and sand redistribution (beach re-profiling) effects on meio- and macrofauna assemblage.

3) Multiple declines and recoveries of Adriatic seagrass meadows over forty years of investigation.

4) Effects of hydraulic-dredging reduction due to the SARS-CoV-2 pandemic situation on macrofauna assemblages in the Northern Adriatic Sea.

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CHAPTER 2. EFFECTS OF DESALINATION ON MACROFAUNA ASSEMBLAGE

ABSTRACT

Desalination plants are widely distributed, since they represent an effective way to obtain drinkable water in coastal area. However, they can represent a serious threat on the marine life, although their ecological impact is still debated. Therefore, understanding their consequences on marine ecosystems is of great importance especially in marine protected area or in area where they are planned. In this chapter, the potential impact of three desalination plants located in three islands (Linosa, Lipari and Ventotene) were investigated. To achieve the goal, we analyzed macrofaunal abundance and diversity (as number of the main taxa) along two transects of five stations located at increasing distance from the desalination plant outflow (0, 5, 10, 30 and 50 m). Findings reported in the present study revealed the lack of clear spatial patterns related to the distance from the brine outfall in any of the case study investigated. This is probably due to the small size of the plants and from other site characteristics which could mask or mitigate the potential effects of brine discharge on macrofaunal assemblages.

2.1 Introduction.

In the past decades, water resources were dramatically affected by domestic, industrial and agricultural users, especially in Mediterranean regions, where the associated low precipitation regime caused a noticeable scarcity of freshwater. Consequently, many countries started to search alternative methods to obtain drinkable water. As a result, desalination plants showed a widespread distribution. It has been estimated a worldwide production capacity of 24.5 million m^3/day of freshwater from the seawater desalination. In particular, in Mediterranean Sea the total production from seawater is about 4.2 million m^3/day (17% of the worldwide capacity) and the desalination plants are mainly distributed in the southern regions (Figure 1; Lattemann and Höpner 2008).

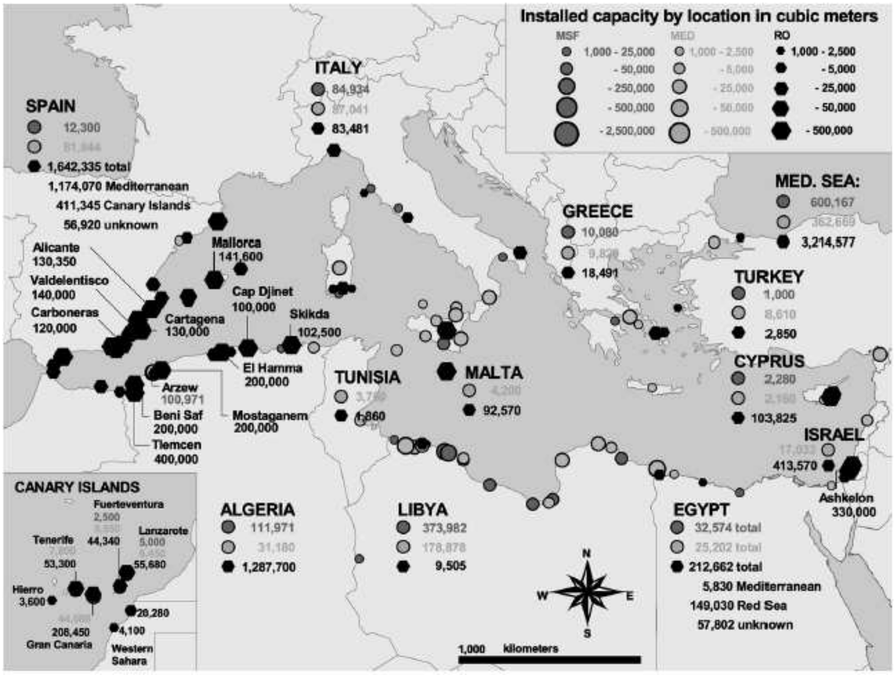

Figure 1. Desalination plants distribution in the Mediterranean Sea (from Lattemann and Höpner 2008).

From 1 m^3 of seawater can be obtained about 0.5 m^3 of freshwater, therefore for each m^3 of desalinated water about 0.5 m^3 of brine is produced and discharged in the seawater (Pistocchi et al., 2020 and references therein). The hypersaline brine discharged in seawater causes a bottom layer due to the higher density, which can alter the biological community living in the sediment. In addition, desalination plants release in the seawater chemical pollutants used in the desalination process such as: chlorine, halogenated organics, coagulants, coagulant aids, antiscalants, antifoaming agents, heavy metals (due to the corrosion), cleaning chemicals (i.e. alkaline (pH 11–12) or acidic (pH 2–3) solutions with additives such as: detergents, complexing agents, oxidants and biocides) (Lattemann and Höpner 2008). Overall, desalination plants represent a potential impact source for the marine life. Several studies investigated the effects on seagrass meadows that showed a shoots density decrease as well as a higher epiphyte growth (e.g,, Gacia et al., 2007; Ruíz et al., 2009). Previous studies reported that a desalination plant can maintain unchanged or increase the local fish abundance (Kelaher et al., 2019; Whitmarsh et al., 2020). Although, the studies about the effects of desalination plants on soft-benthic communities are still scarce, a decrease of meio-macrofauna abundance and diversity near the brine discharge has been reported (De-la-Ossa-Carretero et al., 2011; Riera et al., 2011; 2012). Moreover, brine discharge can negatively affects prokaryotic assemblage (Drami et al., 2011). In this study, the impact of three desalination plants on macrofaunal assemblage were investigated. Macrofauna assemblage was chosen as descriptor, since it responds well to human impacts and scarce information exists about its response to brine discharge (Riera et al., 2012). The importance of this study is also related to the presence of marine protected areas in 2 study sites (i.e., Linosa and Ventotene) that allow to obtain useful information for the MPA management.

2.2. Materials and methods

Study area and sampling strategy

The three desalination plants are located in three different islands. The first plant is located in the volcanic island of Linosa of “Area Marina Protetta Isole Pelagie” in Southern Italy (Fig. 2). The other plants are placed in the islands of Ventotene (Area Marina Protetta Isole di Ventotene e Santo Stefano) and Lipari (Fig. 2).

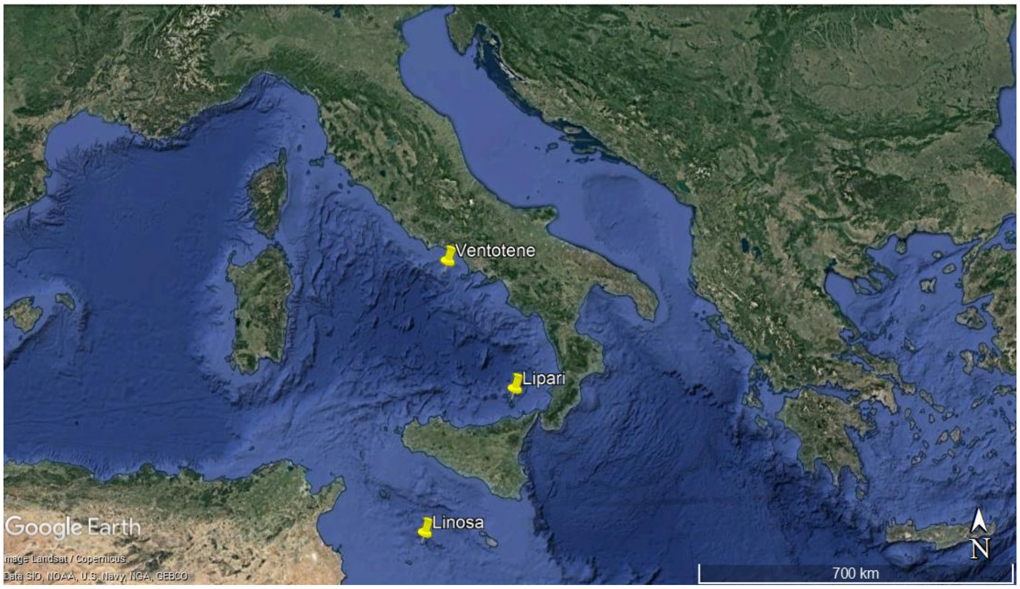

Figure 2. location of the three case studies in the Mediterranean Sea.

In each site, two transects (i.e., left and right for Linosa and Ventotene and North West and South East for Lipari) with 5 sampling stations located at 0, 5, 10, 30, 50 m distance from the outlet of brine discharge were sampled with scuba diving. In each station, three replicas of sediment were taken with a Plexiglas corer, and the samples were subsequently stored a -20°C. The outfalls of the three desalination plants are located in different habitats, the Ventotene plant is in a harbor, Lipari plants in a bay and Linosa in the open sea. The average desalination capacities are 15,500, 90,000 and 9,200 m^3/month respectively. Similarly, the outfall depths are different: 2, 6 and 36 m for Ventotene, Lipari and Linosa respectively, while, the main current directions are SE-NW, SW-NE and NW-SE in Ventotene, Lipari and Linosa, respectively.

Laboratory activity

In laboratory each sediment sample was sieved with a sieve of 500 µm to retain only the macrofaunal organisms. Which were subsequently sorted by taxa, under a stereomicroscope. The organisms of each taxon were preserved in a solution of alcohol 70%.

Data analyses

In order to assess the presence of differences among stations in the two transects, a PERMANOVA analysis, with three factors was performed. The first factor was the “location” (fixed with three levels: Lipari, Ventotene and Linosa), the second factor was the “transect” (random with two levels per each location), nested in the factor location and the third factor was the distance (fixed with 5 orthogonal levels). PERMANOVA analysis was used to analyze the variance of multivariate data explained by one or more explanatory factors and gives p-values calculated using all possible permutations (Clarke and Warwick, 1994). In addition, to assess in a visual way the presence of a gradient a CAP analysis has been carried out. Since human impacts affect benthic community at taxonomic levels higher than species, which are particularly susceptible to natural (‘nuisance’) variability (Warwick, 1988; Bevilacqua et al., 2018), we regrouped the organisms in 12 main taxa:

Nematoda, Bivalvia, Gastropoda, Oligochaeta, Polychaeta, Sipuncula, Amphipoda, Cumacea, Decapoda, Isopoda, Ostracoda and Tanaidacea.

2.3 Results

PERMANOVA analysis highlighted significant interactions between the transect(location) x distance(p<0.01; Tab 1), indicating that the differences among distances are not the same in the two transects in the different location.

Table 1. Output of PERMANOVA analysis performed on the macrofaunal assemblage of the three locations.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms	P(MC)
Location	2	7,7384E9	3,8692E9	1,5598	0,3382	9949	0,3342
Distance	4	1,1566E9	2,8916E8	0,54606	0,7381	9943	0,7125
transect(location)	3	7,4417E9	2,4806E9	11,74	0,0001	9950	0,0001
Locationxdistance	8	2,8907E9	3,6134E8	0,68238	0,7233	9938	0,7101
transect(location)xdistance	12	6,3544E9	5,2953E8	2,5061	0,006	9923	0,0081
Res	60	1,2678E10	2,113E8				
Total	89	3,826E10					

The CAP analysis indicated that the differences are not clearly evident among distances or locations (Fig. 3).

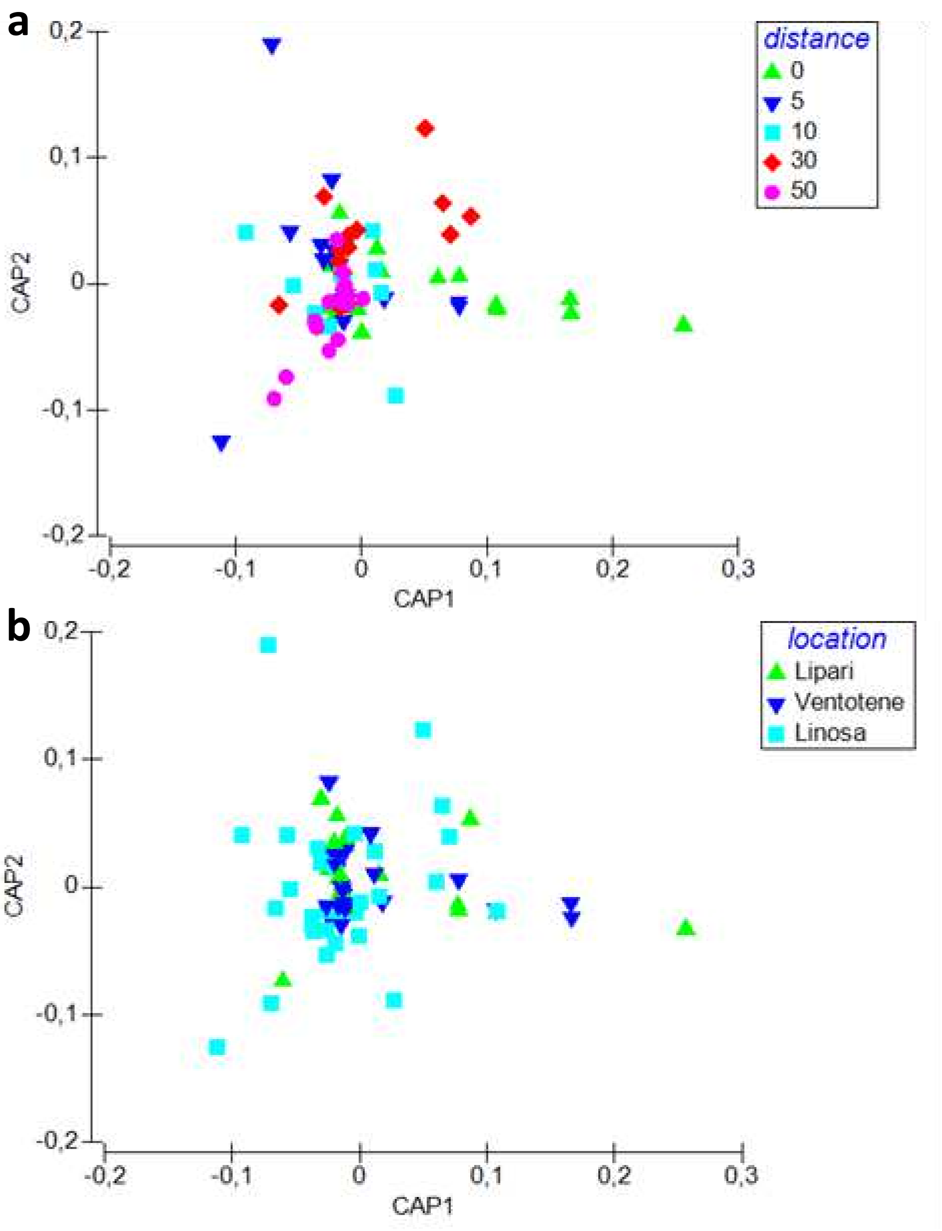

Figure 3. CAP analysis performed on macrofauna assemblage evidencing the factor distance (a) and location (b).

Macrofauna assemblage in Ventotene was represented by Nematoda, Bivalvia, Gastropoda, Oligochaeta, Polychaeta, Sipuncula, Anphipoda, Cumacea, Decapoda, Isopoda, Ostracoda and Tanaidacea. Nematoda and Polychaeta were the dominant taxa and Oligochaeta was the only taxon that seems to decrease from the desalination outfall (Fig. 4).

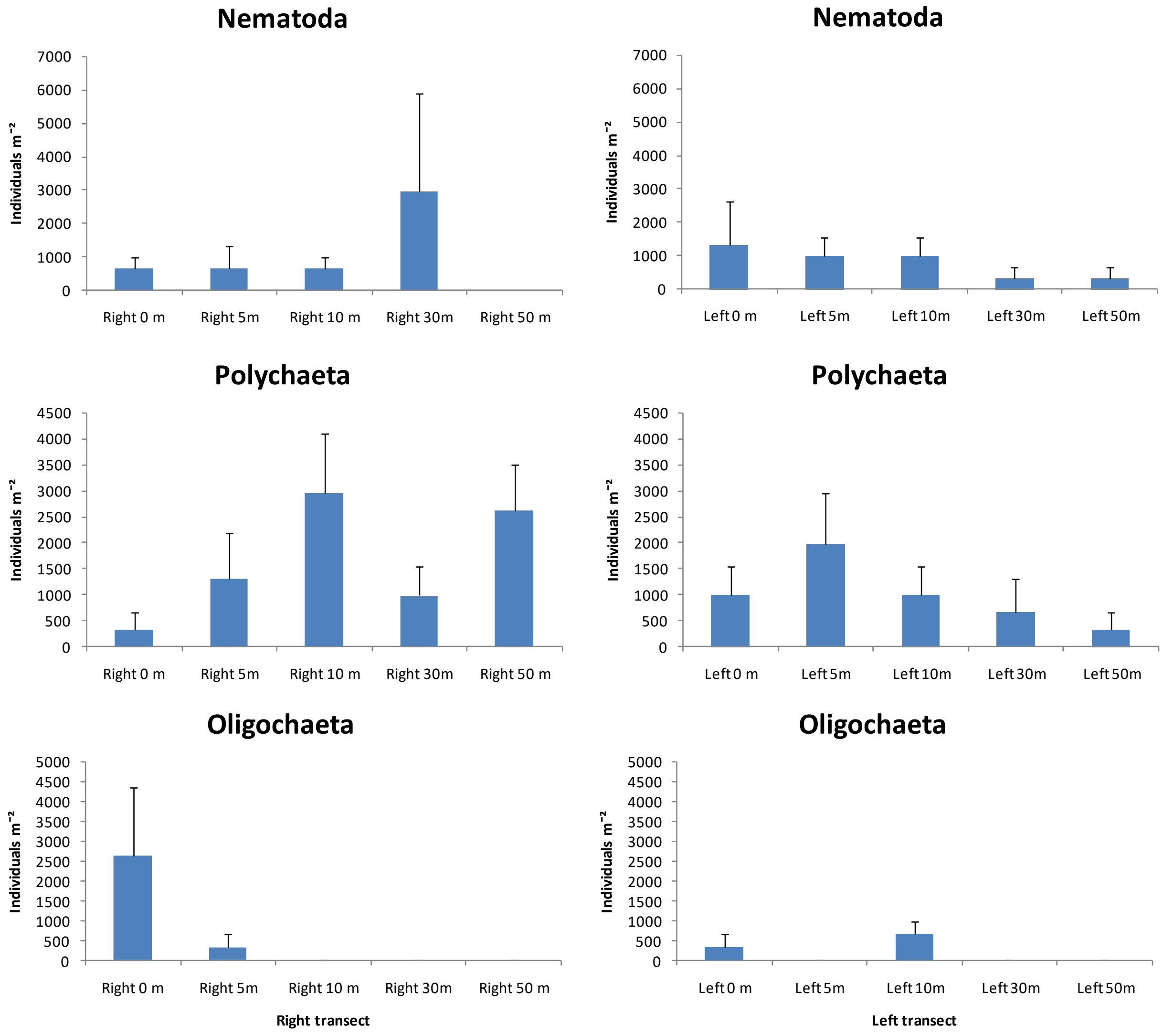

Figure 4. Abundance (average± standard error) of Nematoda, Oligochaeta and Polychaeta in the two transects of Ventotene.

In Linosa, 14 taxa of macrofauna were encountered Nematoda, Bivalvia, Gastropoda, Oligochaeta, Sipuncula, Amphipoda, Polychaeta, Copepoda, Cumacea, Decapoda, Isopoda, Tanaidacea, Acarina and Ophiuridea. Sediments of both transects were mainly dominated by Nematoda, Bivalvia, Sipuncula, Amphipoda and Polychaeta (Fig. 5), but no clear distance-patterns were identified.

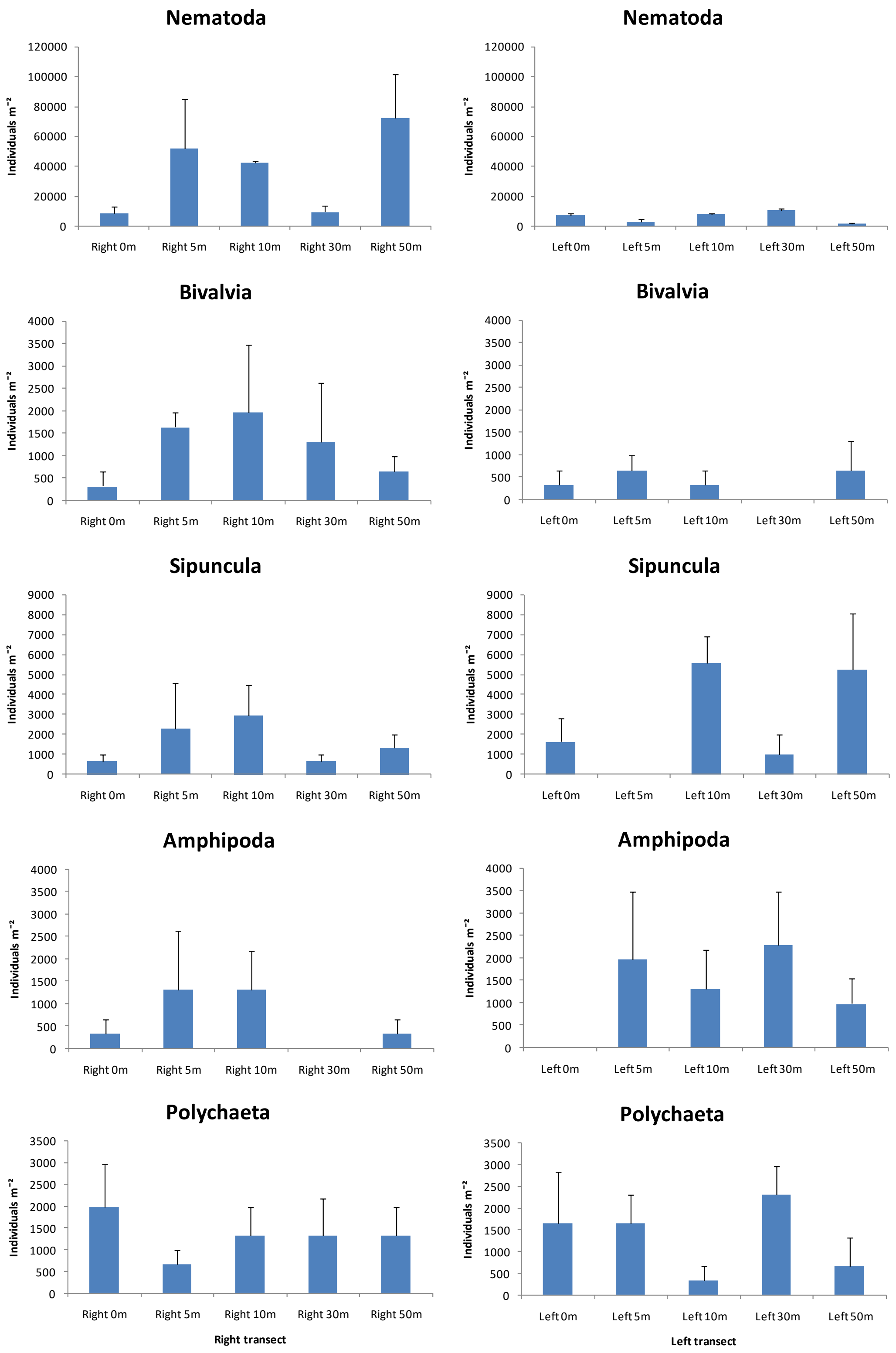

Figure 5. Abundance (average± standard error) of Nematoda, Bivalvia, Sipuncula, Amphipoda and Polychaeta in the two transects of Linosa.

In Lipari macrofauna assemblage were represented by 13 taxa: Nematoda, Bivalvia, Gastropoda, Oligochaeta, Sipuncula, Polychaeta, Copepoda, Cumacea, Decapoda, Isopoda, Tanaidacea, Echinoidea and Chordata. Sediments of the two transects were dominated by Nematoda and Bivalvia, but again no clear spatial patterns were observed (Fig. 6).

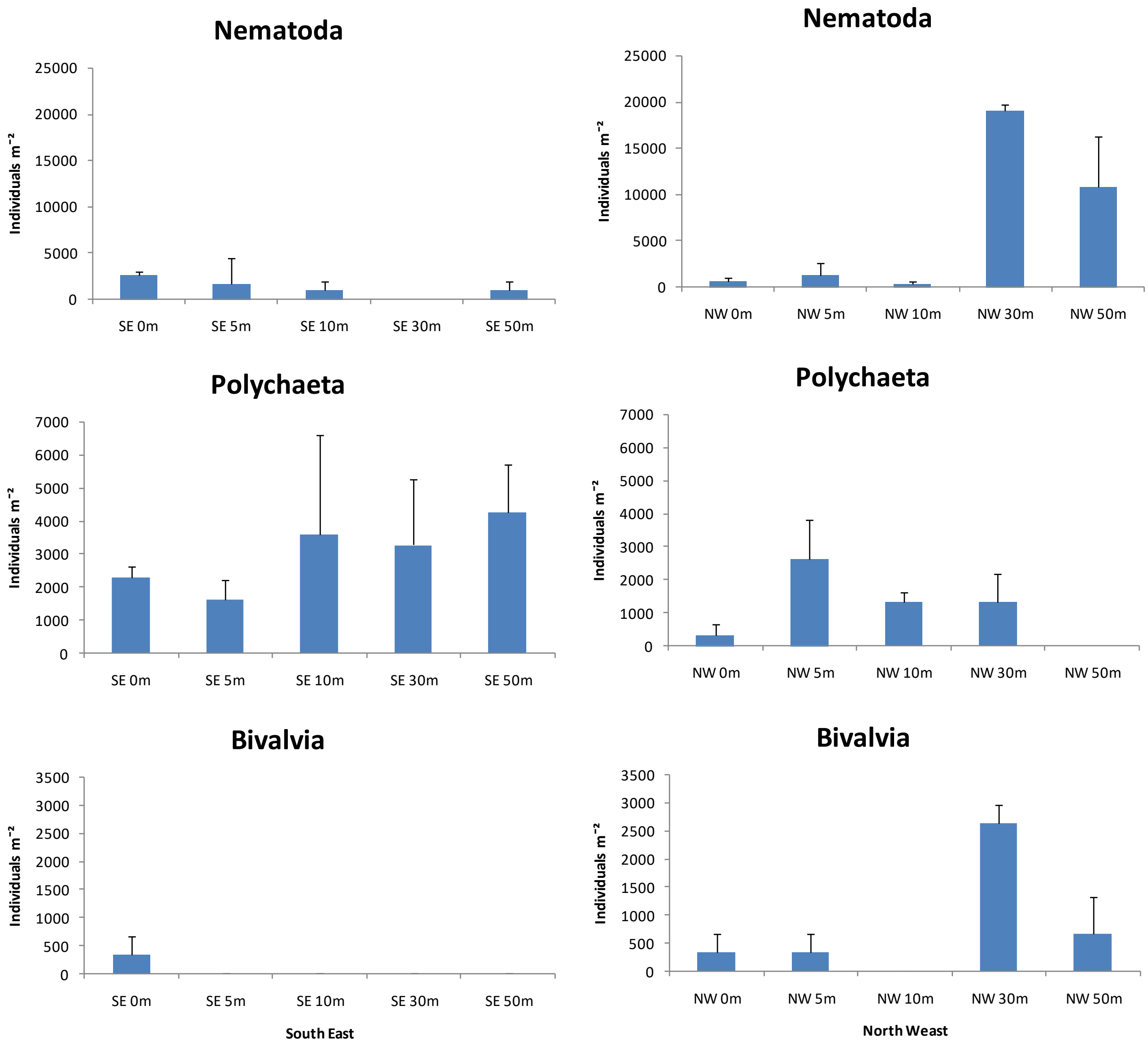

Figure 6. Abundance (average± standard error) of Nematoda, Polychaeta, and Bivalvia in the two transects of Lipari.

2.4 Discussion

Desalination plants represent a potential threat for the marine ecosystem (Del-Pilar-Ruso et al., 2008; Riera et al., 2012), therefore assessing their impact in the MPAs or area where are planned is essential (Heck et al., 2017). Furthermore, the knowledge about effects of desalination plants on coastal resident people is still scarce (Heck et al., 2016). Previous studies reported a shift of soft benthic community close to the desalination plant outflow with a replacement of macroinvertebrates such as Polychaetas, Crustaceans and Mollusks with Nematoda (Del Pilar-Ruso et al., 2007). In the present study a noticeable abundance of Nematoda was recorded, but they did not replace the other organisms. The high Nematoda abundance is commonly associated to increase in salinity (Broman et al., 2019). Interestingly, we found that polychaeta abundance did not display clear spatial trend and this is in contrast with a previous study performed by Nabavi et al., (2013). In the Ventotene, Oligochaeta was higher near the brine discharge. This can be explained by their resilience to perturbation, as previously described in a similar study (Riera et al., 2012 and references therein). Echinodermata were found only at 10 m distance in Linosa and 30 m distance in Lipari and no organisms were recorded at higher distances. Since they are sensible to salinity, this is a further confirmation of the limited brine discharge impact (Fernández-Torquemada et al., 2013). Accordingly, another study performed by Sola et al., (2020); did not find a significant impact of desalination plant on echinoderms. Overall, no significant changes related to plants was found. A possible explanation of the absence of significant impact is the small size on the three plants when compared to impacts documented in other studies (Riera et al., 2012). In addition, this type of habitat is characterized by high variability, which can make difficult to discriminate the effects of the plants (Raventos et al., 2006). Moreover, the Ventotene plant is located in a harbor, therefore it is reasonably to hypothesize that the area is already characterized by a stressed community (Dimitriou et al., 2020). Conversely, the Linosa desalination plant has an outfall in the open sea, therefore it is possible that it is subjected to a major "dilution process" due to current. Furthermore, it is also possible that the study area is not enough big to detect spatial change (Benedetti-Cecchi 2001). Overall, results of this study indicate that the three desalination plants can have a very limited impact on macrofaunal assemblages and suggests that small size desalination plants can be an eco-sustainable solution to provide drinkable water.

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CHAPTER 3. IMPACT OF BEACH NOURISHMENT ON MEIO AND MACROFAUNAL ASSEMBLAGE

Abstract

Beach nourishments are widely used as effective tool to counteract the coastal erosion. However, their ecological effects are still debate. Indeed, some studies defined them as “eco-sustainable soft engineering technique”, while, other studies suggest a significant impact on different biological components. In this study, we assessed the effects of a beach nourishment as well as coastal works (i.e., beach re-profiling) over four years. To achieve the goal, we sampled both macro and meiofauna assemblage in control and impact stations at different depths (3, 6 and 10 m). The results indicate that both macro and meiofaunal assemblage are mainly driven by natural patterns. While, beach nourishment and re-profiling works do not seem affect the natural trend of the area. This can be mainly attributed to the small scale of the nourishment, both in terms of work days as well as the quantity and typology of the material used. Furthermore, the study area is characterized by a community well adapted to environmental stress. Overall, this study suggests that in certain conditions, beach nourishment can effectively be considered an eco-sustainable tool to contrast coastal erosion.

3.1 Introduction

Intensive urbanization associated with sea level rise and frequent storm events are dramatically increasing the coastal erosion (Amarouche and Akpınar 2021). Consequently, there is need to find solutions to counteract the progressive regression of coastal shoreline. Several hard structures such as seawall and breakwaters have built up (Colosio et al., 2007), especially in the Adriatic Sea. However, they are associated to biodiversity loss and the potential spread of non indigenous species (Fabi et al. 2002, Bulleri and Airoldi 2005, Bulleri et al., 2006).

Beach nourishment is usually considered a more eco-sustainable approach (Speybroeck et al., 2006; Danovaro et al., 2018), but its sustainability depends on nourishment technique, season, quantity and quality of sand used and characteristic of the receiving site (Speybroeck et al., 2006; Staudt et al., 2021). In fact, they can cause an increase of sedimentation rate, with consequences for many components of marine food-webs, including autotrophic organisms due to the decrease of light penetration as well as for heterotrophic animals, which can be impacted by sediment redeposition (Nelson 1989; Afghan et al., 2020). Therefore, the sandy beach community can show a reduction of prey availability even for seabirds (McLachlan and Brown, 2006). Beach nourishments can also cause a decrease of macrofauna assemblage (Leewis et al., 2012; Peterson et al., 2006;2014; Targusi et al., 2019). In addition, important beach nourishments can affect also nearby beach not directly subjected to nourishment activity (Witmer et al., 2019). To date, most of the studies about the effects of beach nourishment focused on macrofaunal assemblage and scarce information exist about its effects on meiofauna. This represents an important knowledge gap, since meiofauna is a food source for higher trophic levels and play a crucial role in nutrient cycles (Schratzberger et al., 2018). Furthermore, meiofauna assemblage responds quickly to environmental changes and human impacts due to the small size, short life cycles, limited mobility and high abundance (Gambi et al., 2020). The aim of this chapter is to assess the effects of beach re-profiling and a small-scale nourishment on both meiofauna and macrofauna assemblage in a coastal area of the Central Adriatic Sea often subjected to beach nourishments and annual beach re-profiling (Danovaro et al., 2018). To achieve the goal, we sampled a control and an impact area four times.

3.2 Materials and methods

Study area

The study area is located in the “Portonovo Bay” along the Italian Adriatic coast in the Central Adriatic Sea (Fig. 1). The area hosts two different precious habitats of particular interest at European community level (European Union Habitat Directive 92/43/EEC; site of community importance IT5320005 and IT5320006): “Sandbanks slightly covered by sea water” (Habitat type 1110), which hosts the typical “Biocoenosis of fine sands” (SFBC, identification code RAC/SPA III.2.2) and “Reef” (Habitat type 1170) hosting the “Biocoenosis of infralittoral algae” (identification code RAC/SPA III.6.1).

The Conero Promontory represents the main hard bottom habitat of the Adriatic coast, and hosts facies dominated by important habitat forming species as the mollusk bivalve *Mytilus galloprovincialis*, the polychaete worm *Sabellaria spinulosa*. While, light-exposed reefs have assemblages dominated by algae and other photophilous organisms as the sea anemone *Anemonia viridis*, hosting a rich associated fauna (Danovaro et al., 2018). In addition, the biogenic and calcareous nature of the reef allow the presence of boring fauna such as the sponge *Cliona* *adriatica* and the mollusks *Lithophaga lithophaga* and *Pholas dactylus*. The sampling strategy for the meiofauna assemblage study included two transects (i.e., A and C, Fig. 1), one defined as "impact" (C) located in the area directly subjected to the nourishment activity and a second transect considered as "control" (A) located 1500 m away from the former. Each transect included three different sampling depth: 3, 6 and 10 meters. In each station, three replicas were taken with a core of 3.6 cm diameters and the first 2 cm were collected and subsequently stored at -20°C for both meiofauna and grain size analysis. For the assessment of the potential impact on macrofaunal assemblage, the experimental design included five transects and two depths (i.e., 6 and 10 m). The sediment was collected with a Van veen grab and sieved on board at 500 µm. The remaining material was preserved in 70% ethanol. During the study period, beach re-profiling was carried out in all years of sampling, while a nourishment was performed only in July 2018 for three days of works. Where a total of 2800 m^3 of sand were distributed along 310 m of littoral. It was done only in night hours, in order to reduce the impact on terrestrial fauna. Therefore, the pressure of the intervention was not constant over time. The works were performed with wheel loaders, and the material used had the same grain size of the sand already present in the area. In addition to the beach nourishment, the submerged part of the beach was also dredged and the sediment redistributed along the beach.

The sampling was performed in June and December 2017 (before and after the beach re-profiling) respectively), in November 2018 (after nourishment) and July 2019 (only for macrofauna organisms; after beach re-profiling). The four sampling times were named T_0, T_1, T_2 and T_3 respectively. It is important to note that this area is strongly subjected to intensive tourism flow (Danovaro et al., 2020). Therefore, the study area was already subjected to other beach nourishments in the previous years (2006, 2008, 2009, 2010, 2011 and 2013) to guarantee the utility of the beach. While, in 2012, 2014, 2015 and 2016 beach re-profiling (through material re-distribution) was performed. Overall, the area was subjected to cumulative impacts.

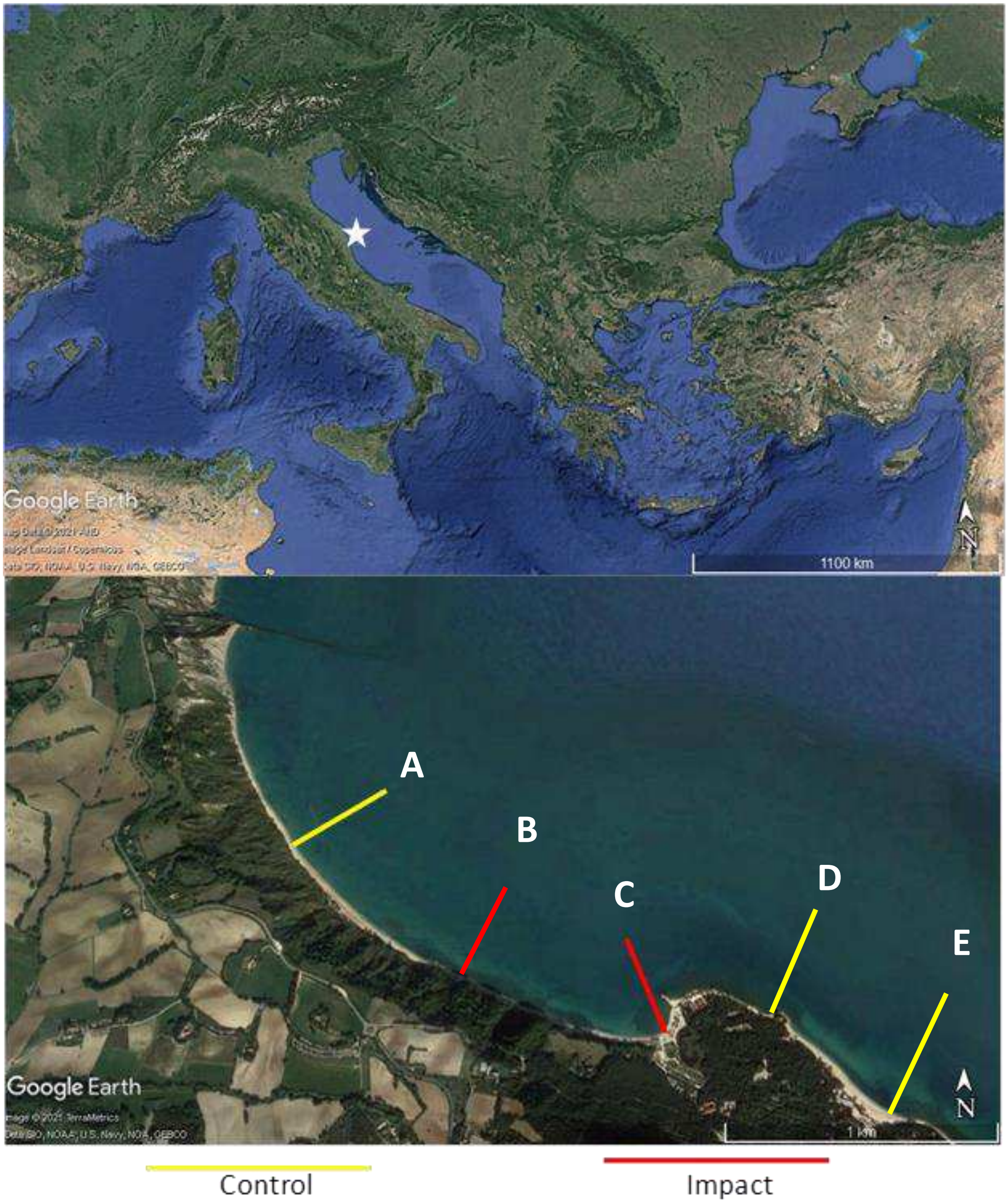

Figure 1. Location of the study area and transects sampled.

Laboratory analysis

According to the method used by Bianchelli et al., (2016) for the meiofauna assemblage, samples were sieved with a 500-µm mesh, and a 30-µm mesh was used to retain the smallest organisms. The fraction remaining on the latter sieve was re-suspended and centrifuged three times with Ludox HS40 (final density of 1.18 g cm^{-3}). All animals remaining in the surnatant were passed again through a 30 µm mesh net, washed with tap water and, after staining with Rose Bengal, sorted, counted and identified under a stereomicroscope (×40 magnification). Similarly, macrofaunal samples were firstly filtered to remove the alcohol and subsequently sorted and divided in four main taxa crustacea, annelida, mollusca and echinodermata. Four main taxa were voluntary chosen since macrofauna organisms respond better to human impact at high taxa level respect to low taxonomic levels, which are particularly susceptible to natural (nuisance) variability (Warwick, 1988; Bevilacqua et al., 2018).

Sediment grain size was determined by the sieving technique. Where samples were treated with a 10% H_2O_2 solution to remove organic matter, and the total and non-biogenic grain size distribution determined with the subdivision in the categories indicated by the scale of Wentworth: Granule, Very coarse sand, Coarse sand, Medium sand, Fine sand grain, Very fine sand grain and Pelite (Gambi et al., 2020 and references therein).

Statistical analysis

To test the significance of the differences among control and impact stations, a PERMANOVA analysis (based on Bray-Curtis similarity index) was performed on both meio and macrofauna data, using all possible permutations. The statistical design for meiofauna assemblage included three orthogonal factors: status (fixed, two levels: control and impact), depth (fixed with three levels: 3, 6 and 10 m) and time (fixed with three levels T_0 T_1, and T_2). When significant differences were encountered, a post-hoc comparison test was performed. The interaction "status x depth x time" indicates if the difference among control and impact in a certain depth varies with the time. Furthermore, to assess in visual way the differences among controls and impacts, a non metric MDS was performed. In addition, for meiofauna data analysis, a PERMANOVA with the same statistical design was performed on "rare taxa", which are the taxa with an abundance less than 1% and can be more sensible to environmental changes (Bianchelli et al., 2010). The PERMANOVA analysis on the rare taxa can help to identify changes that could be less detectable considering the entire dataset due to the high dominance of Nematoda and Copepoda, which can mask minor changes (Bianchelli et al., 2010). For the macrofauna assemblage, the experimental design included four factors: status (fixed with two levels, control and impact), transect (random and nested in status with 5 levels), time (fixed and orthogonal with four levels T_0, T_1, T_2 and T_3) and depth (fixed with two levels (6 and 10 m). Finally, to assess in visual way the differences in sediment grain size, a PCA (i.e., Principal Canonical Analysis) was carried out.

3.3 Results

Meiofauna assemblage

A total of 13 meiofaunal axa were encountered, including: nematoda, copepoda and their nauplii, polychaeta, ostracoda, kinorhyncha, gastrotrica, cumacea, amphipoda, tanaidacea, acarina, cladocera, rotifera, priapulida. At 3 m depth both impact and control stations showed an increase of total abundance from T_0 to T_2 (from 360 ±108 to 1495 ±384 individuals for 10 cm^2 and from 618 ±31 to 1195 ±46 individuals for 10 cm^2 in control and impact station respectively; Fig. 2).

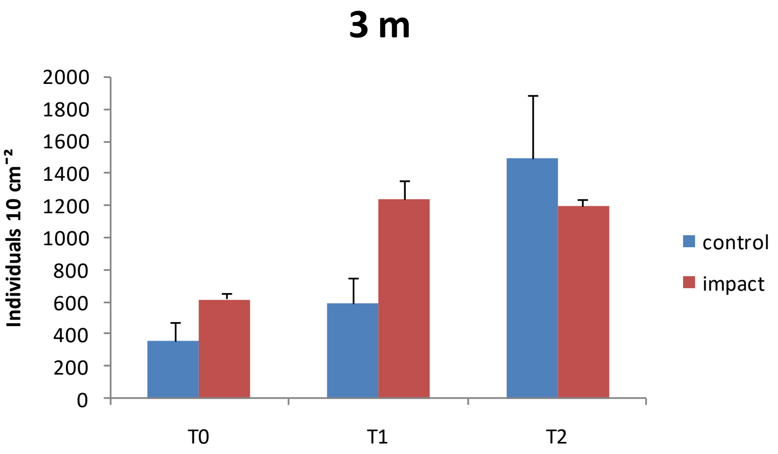

Figure 2. Average (± standard deviation) of meiofaunal abundance in control and impact stations in the three sampling periods at 3 m depth.

However, at 6 m depth, the total abundance increased from 508 ±30 to 1005 ±565 individuals for 10 cm^2 in control station, while in impact station, the total abundance increased from 544 ±217 to 1124 ±275 individuals for 10 cm^2 from T_0 to T_2 (Fig. 3).

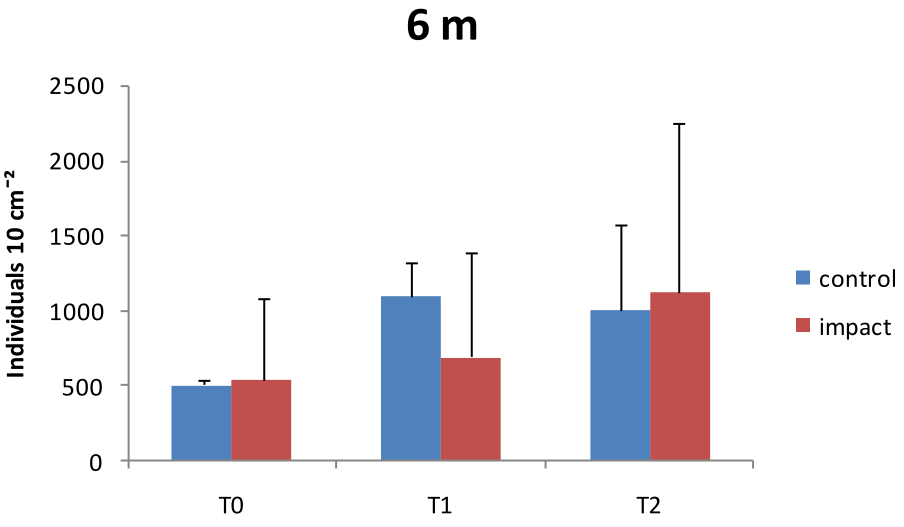

Figure 3. Average (± standard deviation) of meiofaunal abundance in control and impact stations in the three sampling periods at 6 m depth.

Finally, at 10 m depth, control station showed an increase from T_0 to T_1, from 596 ±170 to 1374 ±189 and then decrease until 628 ±31 individuals for 10 cm^2 at T_2. Similarly, impact station displayed an increase of meiofaunal abundance from T_0 to T_1, (1093 ±32 and 1808 ± 339 individuals for 10 cm^2, respectively). While it decreased in T_2 reaching 845 ±177 individuals for 10 cm^2 (Fig. 4).

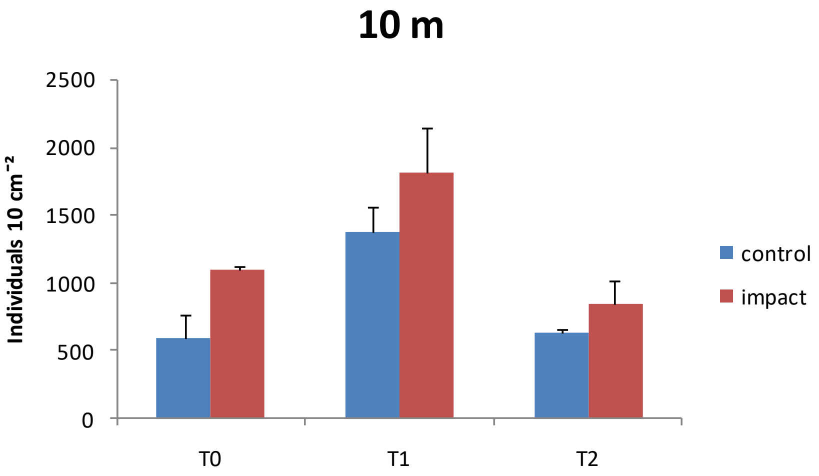

Figure 4. Average (± standard deviation) of meiofaunal abundance in control and impact stations in the three sampling periods at 10 m depth.

In all sampling periods nematodes were the dominant taxon (always higher than 70% of the total number of individuals), followed by copepoda and their nauplii (Fig. 5, 6, 7).

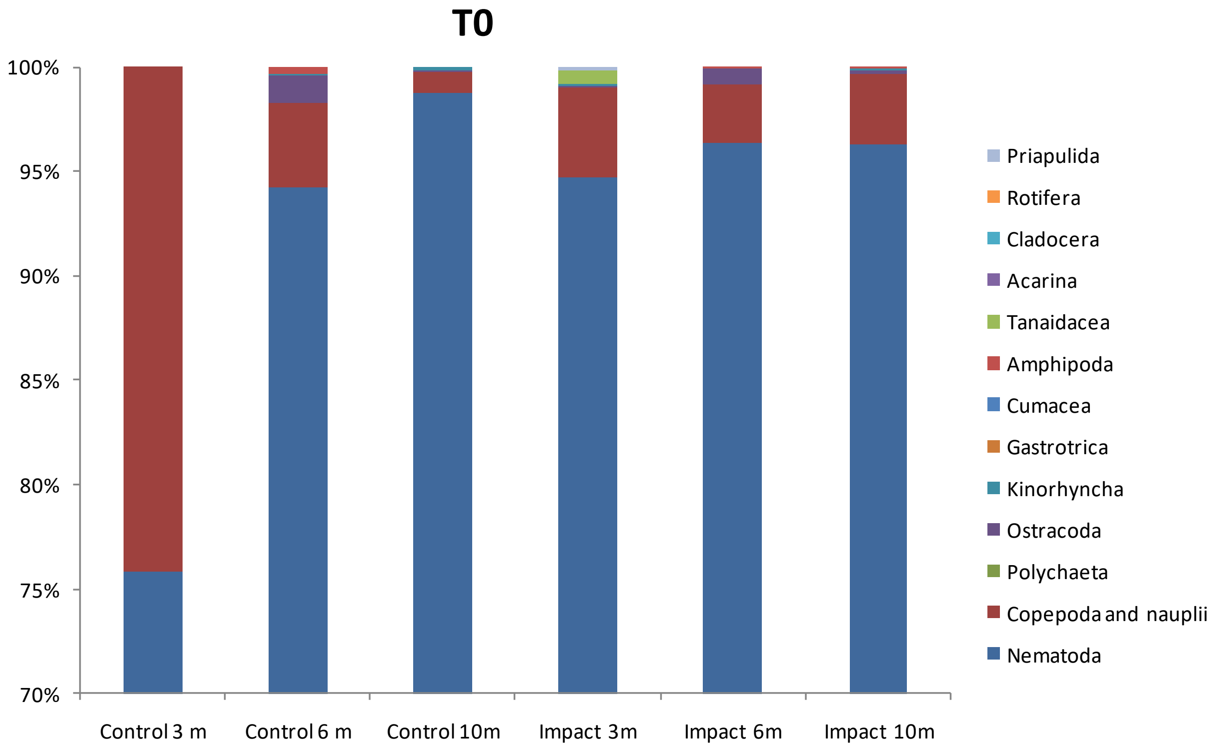

Figure 5. Meiofauna assemblage composition (%) the control and impact stations at 3,6 and 10 m depth at T_0.

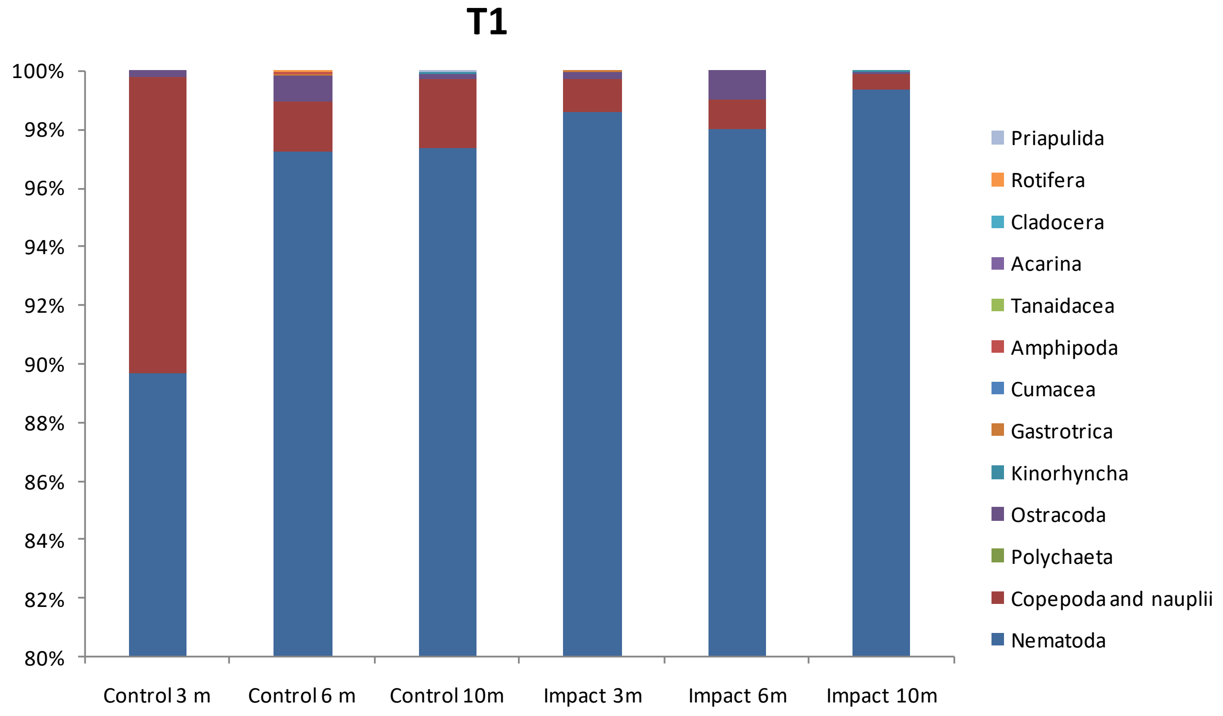

Figure 6. Meiofauna assemblage composition (%) the control and impact stations at 3,6 and 10 m depth at T_1.

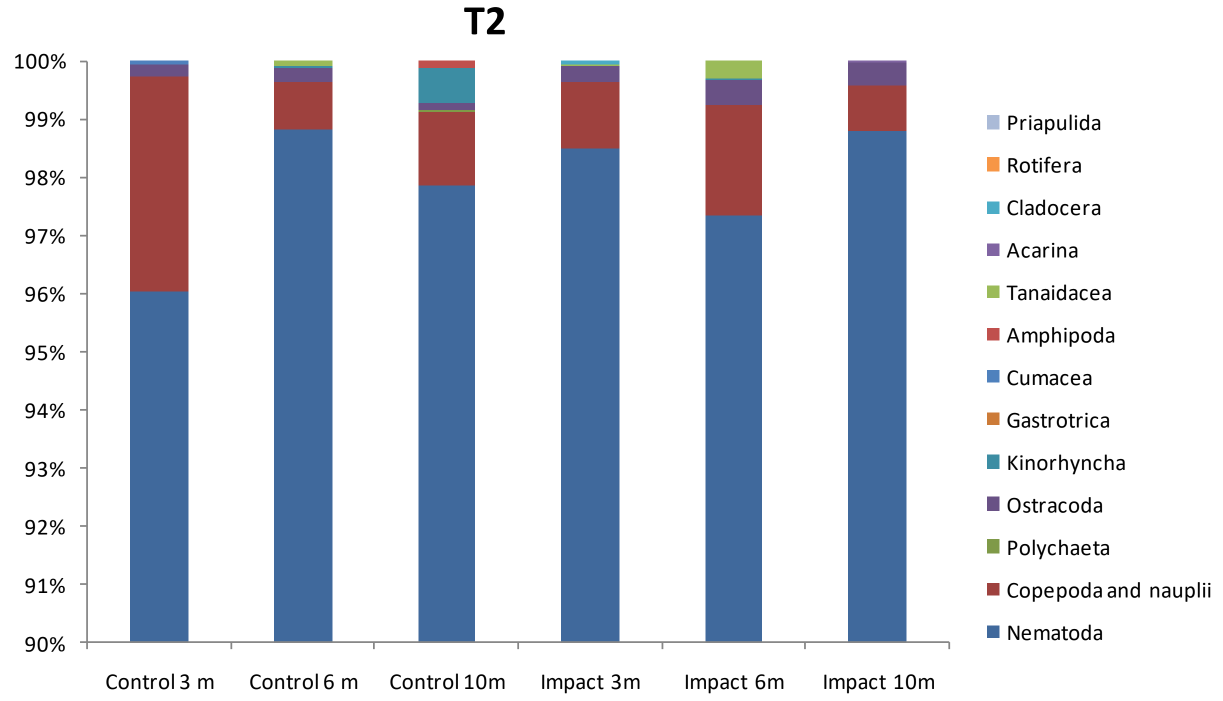

Figure 7. Meiofauna assemblage composition (%) the control and impact stations at 3,6 and 10 m depth at T_2.

The nMDS performed on the entire data set did not reveal differences among replicates of control and impact stations (Fig. 8).

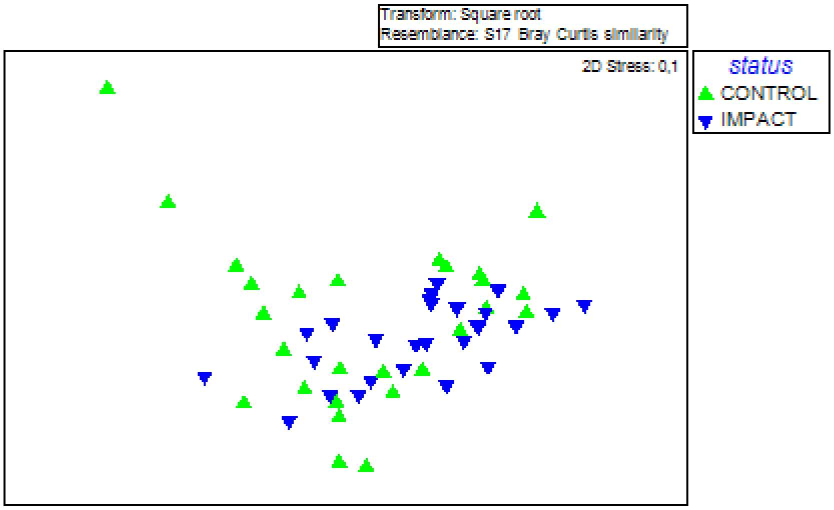

Fig. 8. Non metric MDS performed on meiofauna assemblage of control and impact stations.

The PERMANOVA analysis highlighted as significant the interaction “status x depth x time” ($p<0.001$) (Table 1.), which indicates that the differences among control and impact station among depth are not the same in the three times. From pairwise test, it is emerged as at 3 m depth the control and impact station were significantly different at T_0 ($p<0.05$) and T_1 ($p<0.01$), while no significant differences were found at T_2. Furthermore, at 6 m depth, the only significant differences present were encountered at T_1($p<0.05$). Conversely, at 10m depth, the only significant difference was found at T_0 ($p<0.01$).

Table 1. PERMANOVA Output performed on meiofauna assemblage.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
status	1	822,71	822,71	13,184	0,0001	9958	0,0001
depth	2	1659,8	829,88	13,299	0,0001	9937	0,0001
time	2	2687,3	1343,6	21,532	0,0001	9952	0,0001
statusxdepth	2	1207	603,52	9,6716	0,0001	9949	0,0001
statusxtime	2	220,04	110,02	1,7631	0,1254	9937	0,1347
depthxtime	4	2087,8	521,95	8,3645	0,0001	9944	0,0001
statusxdepthxtime	4	1205,3	301,32	4,8287	0,0001	9938	0,0004
Res	36	2246,4	62,401				
Total	53	12136					

Among all taxa observed, the "rare taxa" resulted to be: polychaeta, ostracoda, kinorhyncha, gastrorica, cumacea, amphipoda, tanaidacea, acarina, cladocera, rotifera and priapulida. The PERMANOVA analysis indicate the interaction "depth x time" as significant (Table 2). This indicates that different depths showed different temporal trends. However, the factor "status" did not result significant. From the pairwise test, it is emerged as the 3 m depth T_0 was significantly different from T_1 and T_2 (p<0.05), which were not significantly different among them. While at 6 m depth the only significant difference was found between T_1 and T_2 (p<0.05). Finally, at 10 m depth no significant temporal change was observed.

Table 2. PERMANOVA Output performed on meiofauna rare taxa.

Source	**df**	**SS**	**MS**	**Pseudo-F**	**P(perm)**	**Unique perms**	**P(MC)**
Status	1	11,596	11,596	1,4668	0,2228	9955	0,2269
Depth	2	214,87	107,44	13,59	0,0001	9946	0,0001
Time	2	43,6	21,8	2,7576	0,0262	9937	0,0303
Statusxdepth	2	28,021	14,01	1,7722	0,1182	9938	0,123
Statusxtime	2	27,114	13,557	1,7148	0,1288	9950	0,1366
Depthxtime	4	106,25	26,562	3,36	0,0008	9936	0,0017
statusxdepthxtime	4	57,642	14,41	1,8228	0,0608	9921	0,0698
Res	36	284,6	7,9055				
Total	53	773,69					

At 3 m depth the rare taxa mostly changed are ostracoda and tanaidacea. While, at 6 m, the amphipods disappeared at T_2 and tanaidacea increased their relative abundance. Finally, at 10 m depth, the dominant rare taxa remain ostracoda and kinorhyncha, whereas priapulida increase their importance in T_1 (Fig. 9- 11).

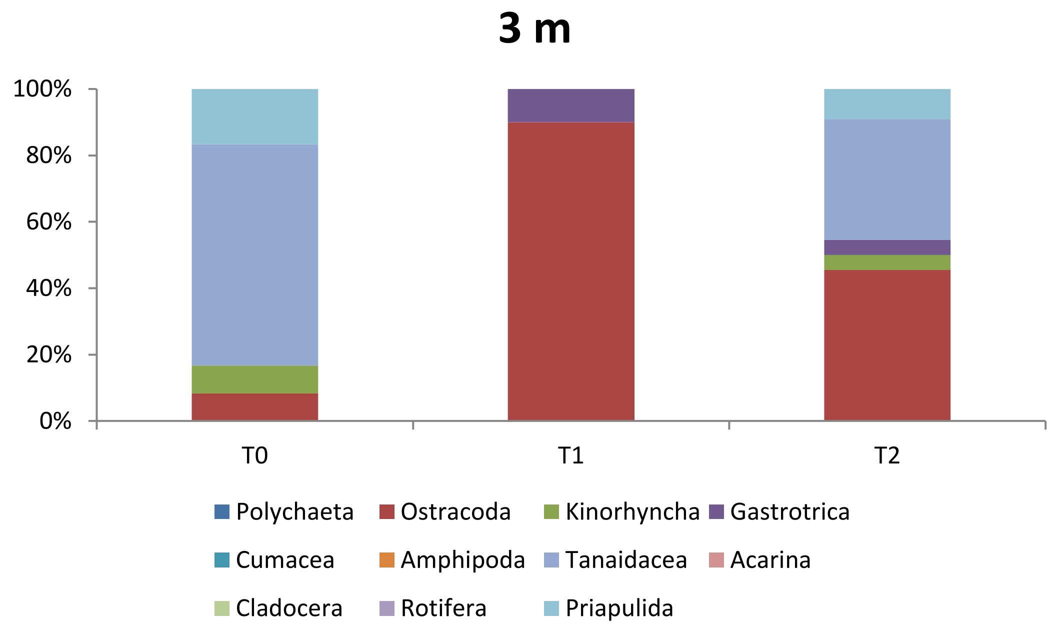

Figure 9. Percentage abundance of rare taxa in the three times at 3 m depth.

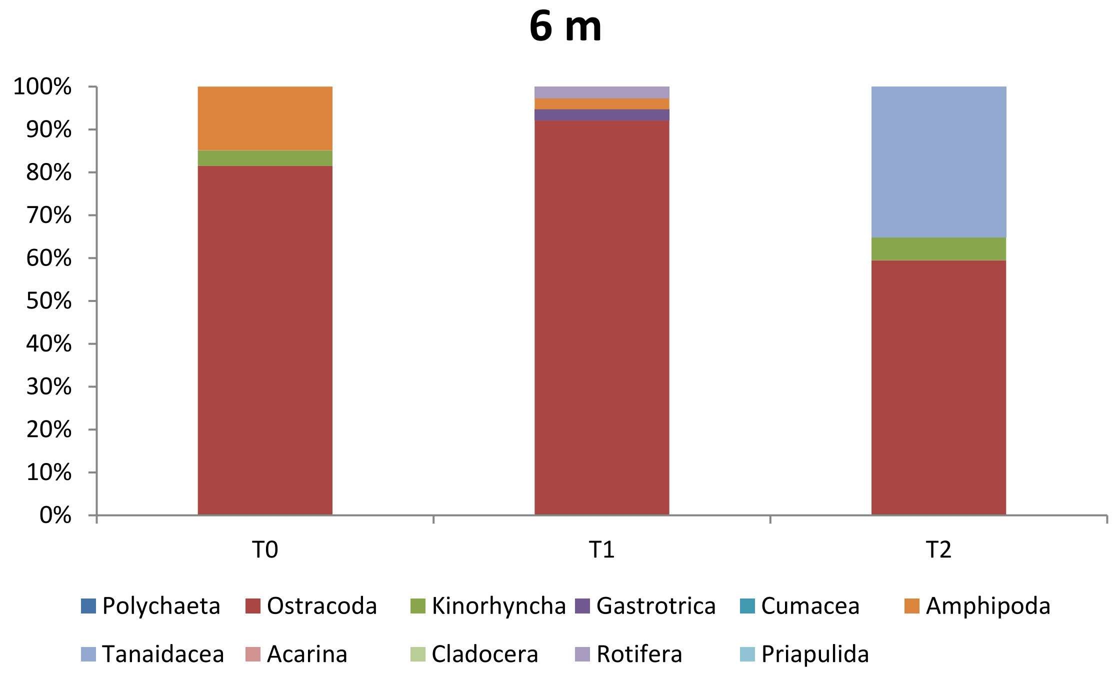

Figure 10. Percentage abundance of rare taxa in the three times at 6 m depth.

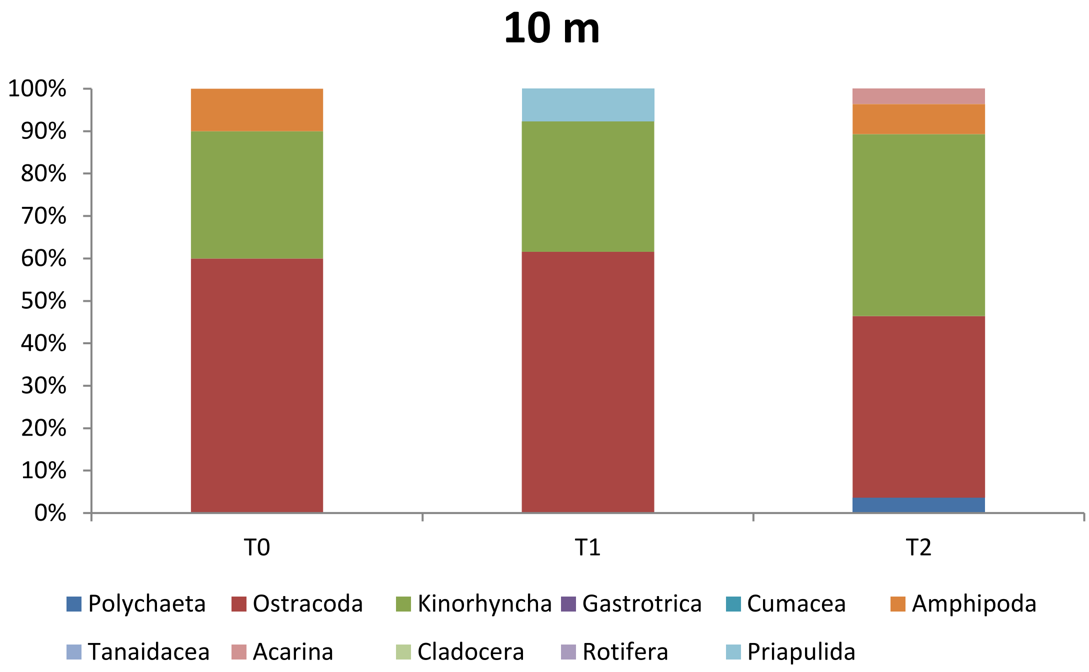

Figure 11. Percentage abundance of rare taxa in the three times at 10 m depth.

Macrofaunal assemblage

The total macrofauna abundance at 6 m depth increased noticeable from T_0 to T_1 in both control and impact transects (from 337± 137 to 1587± 231 individuals for m^2 and 393± 33 to 5203± 810 individuals for m^2 respectively; Fig. 12). Both impacts and controls showed values of about 2000 individuals for m^2 at T_2 and T_3. The same temporal trend was observed in both control and impact transects, where the only evident difference was found at T_1.

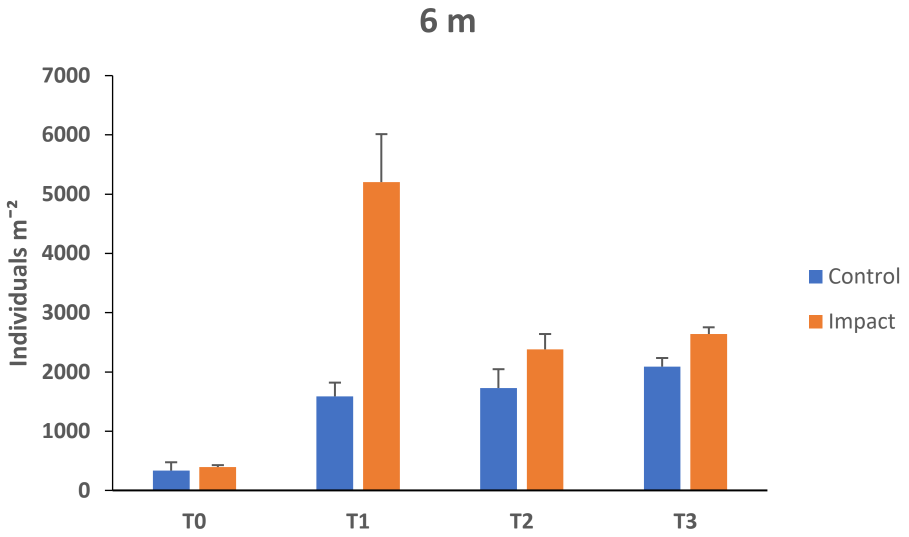

Figure 12. Average (± standard error) of macrofaunal abundance in control and impact transect in the four times at 6 m depth.

At 10 m depth both control and impact transect showed a dramatic increase of the total abundance from T_2 to T_3 passing from 733± 139 to 2375± 595 individuals for m^2 and from 1009± 216 to 2303± 540 individuals for m^2 respectively (Fig. 13).

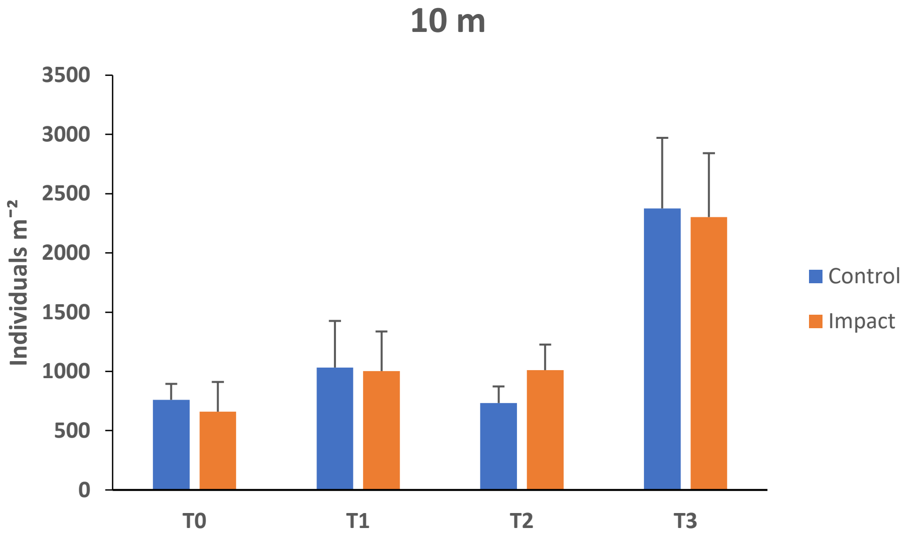

Figure 13. Average (± standard error) of macrofaunal abundance in control and impact transect in the four times at 10 m depth.

Both control and impact transects were mainly dominated by mollusks (mostly Bivalvia), crustaceans and annelids and the most evident change was observed between T_0 to T_1, when a dramatic increase of mollusks in both control and impact transects was encountered (Fig. 14).

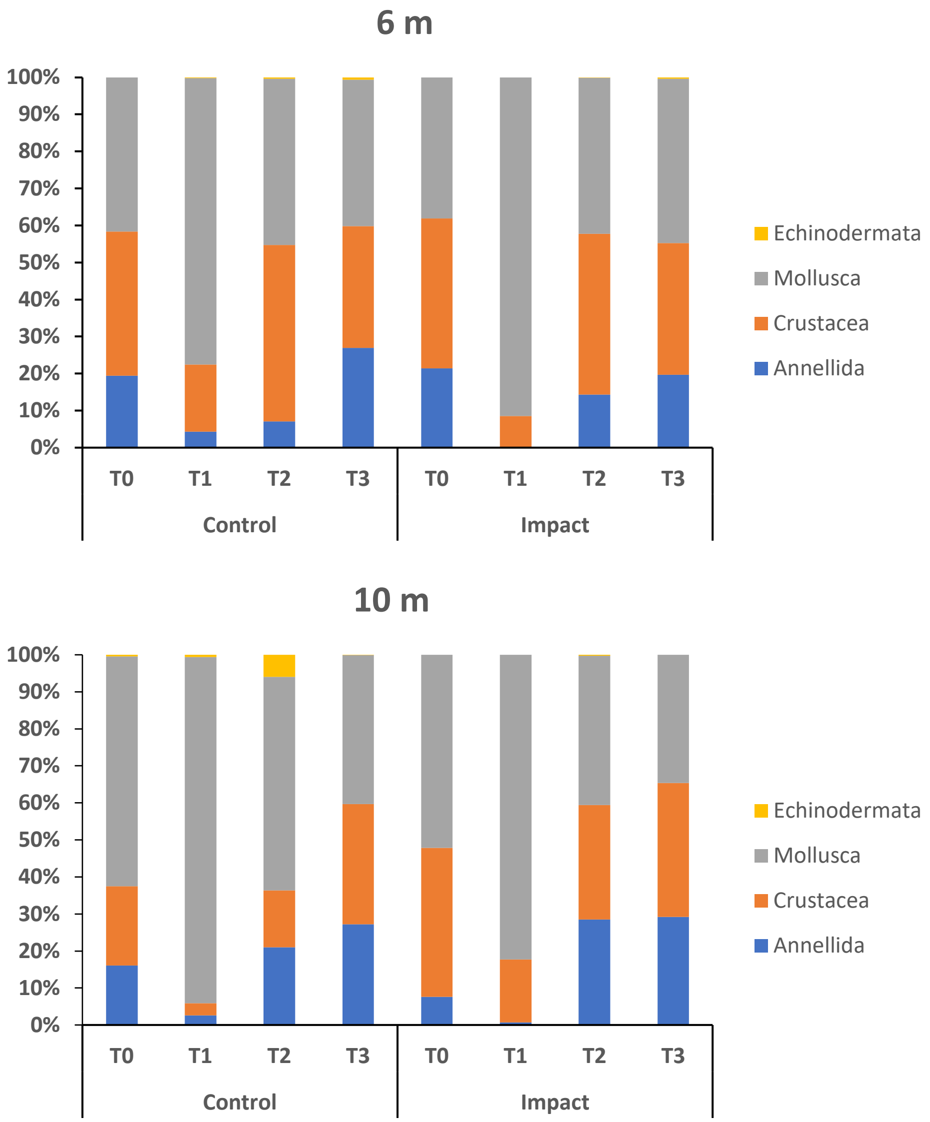

Figure 14. Macrofauna assemblage composition (%) in the control and impact stations at T_0, T_1 T_2, T_3 at 6 and 10m.

The nMDS performed did not highlight differences among replicates of control and impact stations (Fig. 15).

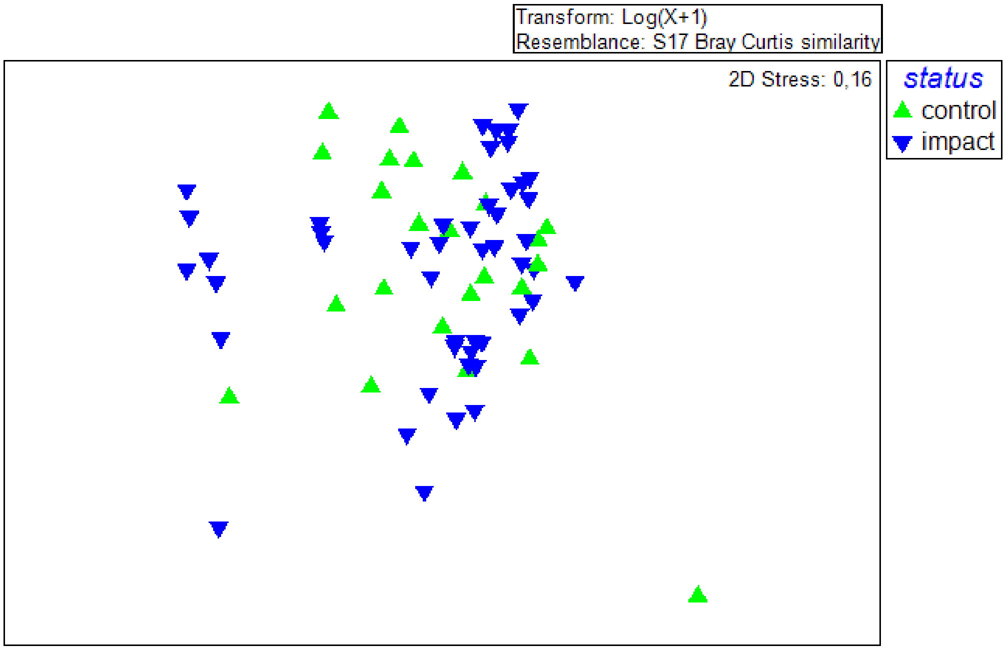

Fig. 15. Non metric MDS performed on macrofauna assemblage of control and impact stations.

The PERMANOVA analysis highlighted the transect(status)xdepthxtime interaction as significant (*p*<0.001; Tab. 3). This indicates that the differences among transects (in a certain status) depend on water depth and change occurring over time.

Table 3. PERMANOVA Output performed on macrofaunal assemblage.

Source	Df	SS	MS	Pseudo-F	P(perm)	Unique perms
status	1	314,73	314,73	-6,3975	0,9471	9983
depth	1	413,41	413,41	2,4777	0,1912	9969
time	3	4408,1	1469,4	12,163	0,0092	9961
transect(status)	1	-48,391	-48,391	-0,81197	0,9825	9978
statusxdepth	1	567,65	567,65	3,3993	0,1379	9984
statusxtime	3	877,51	292,5	2,4288	0,1751	9964
depthxtime	3	801,98	267,33	0,82173	0,5492	9970
transect(status)xdepth	1	163,97	163,97	2,7513	0,086	9963
transect(status)xtime	3	355,93	118,64	1,9908	0,0972	9948
statusxdepthxtime	3	394,66	131,55	0,40614	0,7762	9967
transect(status)xdepthxtime	3	961,81	320,6	5,3795	0,0002	9950
Res	47	2801,1	59,597			
Total	70	13231				

Grain size

PCA analysis carried out on grain size indicated a clear segregation of the samples according to water depth (Fig. 16). Samples collected at 10 m depth were dominated by very fine sand grain, while samples at 6 m depth were characterized by an increase of the fine sand grain fraction. Finally, samples at 3 m depth displayed an increase of medium and coarse sand.

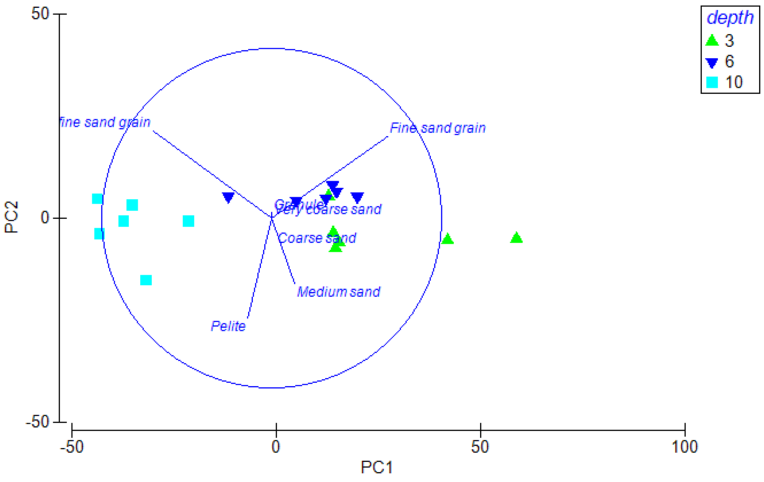

Figure 16. PCA performed on grain size data set.

Accordingly, the graphs below (Fig. 17, 18, 19) clearly show as the replicas taken at 10 m depth are mainly dominated by very fine grain size, while at 6 m depth there is an increase of fine grain size and a decrease of very fine grain size. Furthermore, the only depth where a significant percentage of high dimension class (i.e., medium and coarse sand) is 3 m. The most evident difference among control and impact stations was observed in 2017 at 3 m depth, where the control was characterized by a higher percentage of fine sand (Fig. 20) as well as in 2018, where the impact station showed a higher percentage of very fine sand (Fig. 20). However, no particular temporal trends were observed.

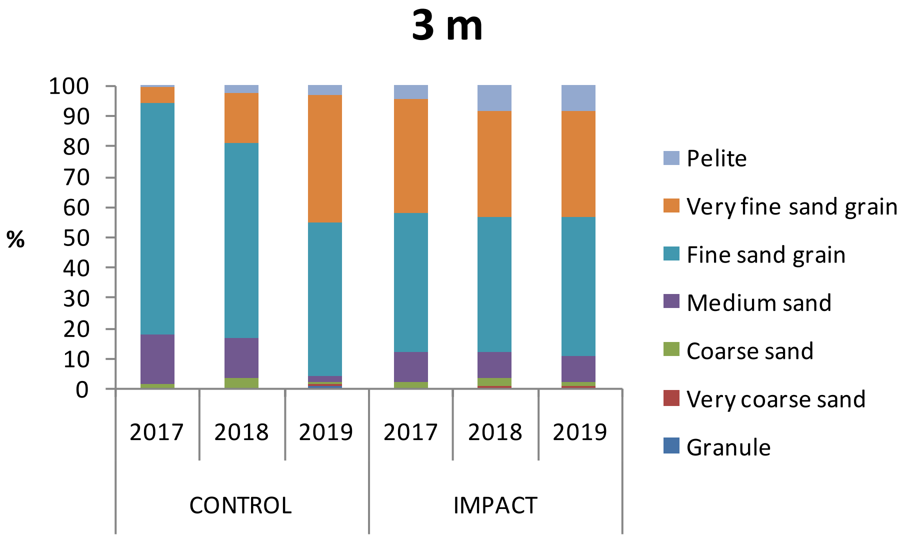

Figure 17. Grain size distribution (%) in control and impact replicas over time at 3 m depth.

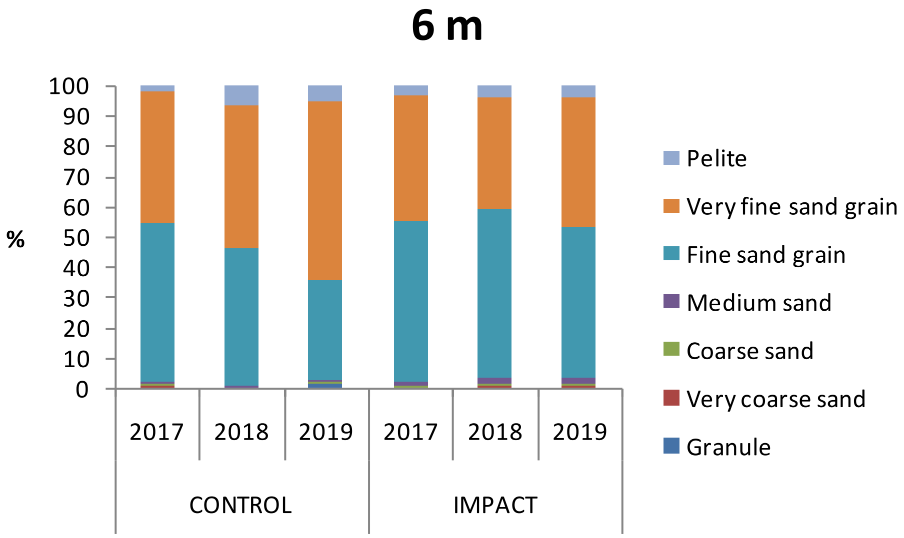

Figure 18. Grain size distribution (%) in control and impact replicas over time at 6 m depth.

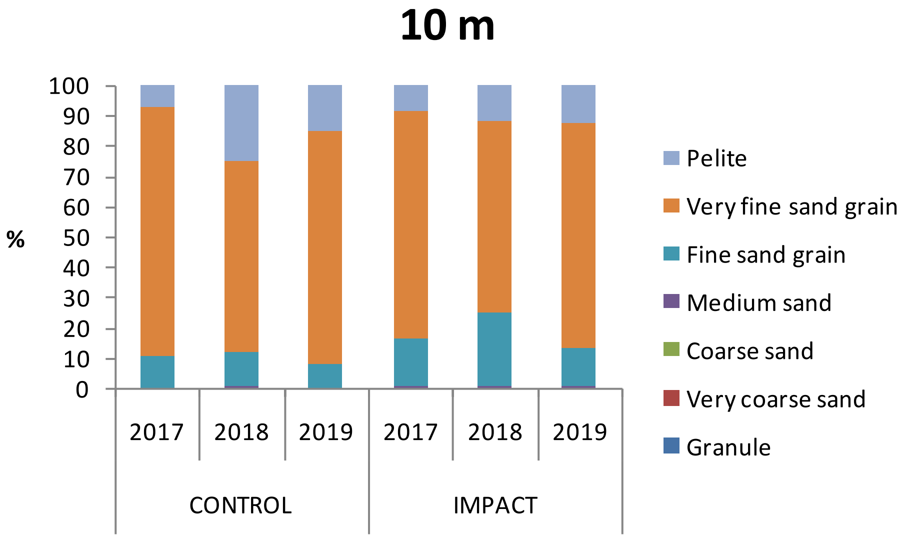

Figure 19. Grain size distribution (%) in control and impact replicas over time at 10 m depth.

3.4 Discussion

Beach nourishments represent a common strategy to counteract the coastal erosion, yet there is a widespread disagreement about their ecological consequences (Danovaro et al., 2018 and references therein). In the present study at 3 m depth, a noticeable increase in total meiofauna abundance was found over time in both control and impact station. This suggests that the beach re-profiling and the beach nourishment did not alter to a major extent the natural trend of the area. Interestingly, PERMANOVA analysis indicated that the control and impact stations was significantly different only at T_0 and T_1. Consequently, the entire area apparently underwent a certain homogenization, where the natural trend was superimposed to the effects of the nourishment. Conversely, at 6 m depth, no particular trend or differences among stations emerged in terms of meiofaunal abundance. However, PERMANOVA analysis indicated significant differences among control and impact station at T_1. This is due to the higher abundance of ostracoda in the control station. Yet, since this difference disappeared at T_2 after no-nourishment, it is more reasonable to attribute such an effect to a "natural" change. Similarly to 3 m depth, at 10 m depth, the total abundance increased in both control and impact station from T_0 to T_1 and then decreased in T_2. Overall, also at 10 m depth the changes seem to be more related to natural variation respect to the nourishment. Although the increase of meiofauna abundance often found in T_1 and T_2 (i.e., after the finish of summer) can appear strange, it was already reported in the area (Moodley et al., 1998). The general increase of meiofauna abundance could be easily attributed to the continuous reproduction strategy, which allows a rapid colonization of the habitats, making them less vulnerable to environmental stress (Schratzberger and Somerfield 2020). The decrease of meiofauna abundance at 10 m at T_2 cannot be attributed to the nourishment pressure, since it is present also in the control station. Accordingly, the analysis on rare taxa confirms the apparent lack of impacts related to human activity, and highlights seasonal changes as indicated by the significance of the "depth x time" interaction. These results should be viewed with caution since the experimental design was based on a sampling strategy spatially and temporally pseudo-replicated. Further investigations including a set of control and impact transects as well as different sampling intervals before and after each human intervention are needed to draw robust conclusions. Despite that, a natural trend of the meiofauna abundance superimposed to a human pressure is quite expected for the Adriatic Sea (Danovaro et al., 2004; Greco et al., 2009). According to our results, a previous study focused on the effects of a bigger nourishment (159,000 m^3 on 2 km of coast line) on meiofauna assemblage did not reveal any effects (Menn et al., 2003). However, literature also reported negative effects for repeated nourishments (Menn et al., 2003; Fegley et al., 2020). Furthermore, the negative effects of beach nourishments on meiofauna was confined to copepoda (Menn et al., 2003 and references therein), while nematodes (i.e., the dominant taxa in our study) seem to be less sensitive.

The temporal trend of macrofauna abundance seems to follow the season variability, where the highest abundance detected in T_3 can be explained by recruitment period of most of the Mediterranean species which occurs in summer and can continue until autumn depending on the local conditions (Forni and Occhipinti Ambrogi 2005; Guerra-Garcia and Garcia Gomez 2006; Targusi et al., 2018). This is also confirmed by the high presence of mollusca, which are mostly constituted by juvenile bivalves that have the recruitment periods in summer season (Targusi et al., 2018). In addition, in all sampling periods (except for T_1 at 6 m) both controls and impacts showed very similar values of total abundance, indicating the absence of significant effects of human interventions. Furthermore, control and impact transects denoted a similar assemblage composition. PERMANOVA analysis confirmed this conclusion, indeed the factor status or its interactions with transect and depth never resulted significant. However, significant effect was encountered for the interaction transect(status) x depth x time. This indicates that the transects of a certain status showed significant differences that depend on depth and change over time. Therefore, this is a further confirmation of the very high natural variability typical of soft bottom community that make very difficult discriminate the changes induced by human interventions (Danovaro et al., 2018 and references therein; Targusi et al., 2018).

Overall, these findings suggest that the differences found are more related to natural spatial and temporal (seasonal) changes. Yet, these results can be partially influence by the temporal pseudo-replication of the sampling design, in fact more sampling times before and after each human intervention could help disentangling the human intervention effects from the high natural variability. However, the very similar assemblage composition in control and impact sites in most cases induces to retain sufficiently robust the conclusion obtained. Although the differences encountered in terms of grain size are little, it is probably that they can influence the infauna assemblage (Targusi et al., 2018). Yet, the absence of evident changes of grain size distribution in impact site suggests that the various human interventions (beach re-profiling and nourishment) did not affect the grain size of the area. This is probably due to the fact that the nourishment was carried out with a material having the same grain size of the receiving area. The lack of impacts can be related to different factors. The most important is probably the small size of the nourishment, in terms of material added. In addition, the days of works of the nourishment were very limited. Furthermore, the area is already subjected to a natural high sedimentation rate with a consequent significant turbidity (Danovaro et al., 2018). Moreover, the benthic assemblage of the North Adriatic Sea is composed by a conspicuous number of opportunistic species, able to tolerate environmental stress (Simonini et al., 2009). Typically, beach environment is a highly dynamic system, with significant spatial and temporal natural changes in physical and morphological features, with a benthic community well adapted to stress and environmental changes (Targusi et al., 2018). Therefore, discriminating the effects of human interventions from the natural trends is not easy to address, and can be resulted in limited impacts as previously reported for macrofauna assemblage in other studies (Gorzelany and Nelson, 1987; Menn et al., 2003; Danovaro et al., 2018). Another important factor certainly contributed in limiting the nourishment effects is grain size (Speybroeck et al., 2006). In fact, the sand utilized was comparable with that previously present in the receiving area. Furthermore, in order to reduce the turbidity, it was firstly cleaned to remove the fine fraction. In conclusion, it is clear that the natural trend of the area is superimposed on the effects of beach nourishment. Therefore, this study reinforces the concept that a small-scale nourishment can be considered an eco-sustainable tool to contrast the coastal erosion.

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CHAPTER 4: MULTIPLE DECLINES AND RECOVERIES OF ADRIATIC SEAGRASS MEADOWS OVER FORTY YEARS OF INVESTIGATION

Ref: Danovaro, R., Nepote, E., Martire, M. L., Carugati, L., Da Ros, Z., Torsani, F., Dell'Anno, A., & Corinaldesi, C. (2020). Multiple declines and recoveries of Adriatic seagrass meadows over forty years of investigation. Marine Pollution Bulletin, 161, 111804.

Abstract

This chapter investigated the long-term changes (from 1973 to 2013) of the seagrass meadows of *Zostera marina, Zostera noltei* and *Cymodocea nodosa* in the Adriatic Sea subjected to multiple pressures. We examined the changes of the meadows by means of field data collection, observations and analysis of aerial photography to identify the most important drivers of habitat loss. The major decline of seagrass extension observed from 1973 to 1989, was primarily driven by urban development, and by the increase of the blue tourism. From 1989 to 2007 seagrass habitats progressively recovered due to the decrease of urbanization, but from 2007 to 2013 a further significant loss of seagrass meadows was apparently driven by thermal anomalies coupled with an increasing anthropogenic pressure. Our long-term analysis provides evidence that the rates of seagrass loss are faster than the recovery rates (i.e., ⍰ 4.5 loss rate vs +2.5% recovery rate per year).

4.1 Introduction

Seagrass meadows are key habitats that provide important ecosystem goods and services such as primary productivity, sediment stabilization, nutrient cycling, carbon capture and storage, and habitat for fisheries resources (Costanza et al., 1997). Their economic value has been estimated at US$ 19,002 ha-1y^{-1}(Costanza et al., 1997). Among the ecosystem services provided by seagrass meadows C sequestration (i.e. Blue Carbon) has a great importance since it mitigates the effects of global change (Lavery et al., 2013). These habitats, protected by the EU Habitat Directive 92/43/EEC, are threatened by multiple stressors, spanning from local-scale impacts (i.e. urbanization, fisheries) to global climate change, which are causing their widespread decline (Cullen-Unsworth et al., 2014).

In the Mediterranean Sea, six species of seagrass are present: *Zostera marina*, *Zostera noltei*, *Cymodocea nodosa*, *Posidonia oceanica*, *Halophila stipulacea* and *Ruppia marittima* (Pergentet al., 2014). Among them, *Zostera marina* is a species colonizing sheltered areas (e.g., coastal lagoons and sheltered bay), which for its affinity for cold temperatures, is particularly threatened by seawater warming (Pergent et al., 2014). Although *Zostera noltei*, which forms only sub tidal meadows in sheltered areas, shows a high resilience in terms of coverage, others seagrass, such as *Posidonia oceanica*, can have a much lower resilience (Pergent et al., 2014). Most of the studies conducted in the Mediterranean Sea on *Posidonia oceanica* reported a significant decline of its meadows (Molinier and Picard 1952; Marbà et al., 2014; Holon et al., 2015; Montefalcone et al., 2015; Burgos et al., 2017), and the progressive replacement with macroalgae. Other seagrass species such as *Zostera marina*, *Zostera noltei* and *Cymodocea nodosa* received comparatively less attention (Boudouresque et al., 2009; Tuya et al., 2014a,b), but evidences of their progressive decline are increasing in Mediterranean Sea and oceanic regions (Bernard et al., 2007; Tuya et al., 2014a,b; Lefcheck et al., 2017). Yet, they have a significant economic value due to the ecosystems goods and services that supply. For example, the economic values of *Cymodocea nodosa* is estimated to be 8.6 E+2 €·ha^{-1} as "fishery" grounds and a value as 'nursery' grounds equal to 9.6 E+1 €·ha^{-1}·a^{-1} using fish market values (Burgos et al., 2017 and references therein). In addition, Lefcheck et al., (2017) quantified a loss of 1.51 billion of dollars for 2175 ha of *Zostera marina* meadow loss.

The cause of seagrass meadows' decline is generally attributed to coastal development, increase of the maritime traffic, eutrophication and chemical contamination (Pergentet al., 2014), which can act synergistically with global change. Among the global change impacts, heat waves such as those observed in 1999, 2003 and 2006 (Mayot et al., 2005; Marbà and Duarte 2010; Telesca et al., 2015), along with the spread of fast-growing invasive species of macroalgae, such as *Caulerpa cylindracea*, can cause the rapid loss of shoot density, an increase of the energy needed for reproduction and production of defense compounds (Pergent et al., 2008).

In recent years it has been also documented a reversal of declining trend in European seagrass meadows (De Los Santos et al., 2019). It becomes thus crucial to better understand the factors determining seagrass decline and recovery.

The Adriatic Sea is one of the most severely threatened regions of the Mediterranean Sea strongly affected by coastal infrastructures (including breakwaters barriers), hydraulic dredging and trawling (Colosio et al., 2007). In particular, since 1988 a significant increase of sea-surface temperature has been detected (on average +1.6°C in the last 3 decades), with three exceptional positive thermal anomalies in 2003, 2009 and 2011 (Di Camillo and Cerrano 2015). Which are associated with large changes in salinity (Russo et al., 2002), often accompanied by hypoxic phenomena, and the appearance of mucilage events (i.e. large marine amorphous aggregates) events and toxic algal blooms. The northern Adriatic Sea also experienced a progressive increase of the eutrophication from 70'till mid-80's, followed by a steady decrease of the nutrient concentration in the last decades (Giani et al., 2012). Therefore, it is clear the need to apply adequate protection measurements and monitoring plans (Micheli et al., 2013; Bastari et al., 2016). Nevertheless, our data on the long-term changes in seagrass extension are extremely scant (Calleja et al., 2017), while information about the causes and rates of decline and resilience are of primary relevance for planning adequate measures against habitat degradation and define the conditions for its restoration (Van Katwijk et al., 2009).

In the present study, we investigated the long-term change of seagrass extension in an area of the Northern Adriatic Sea over 40 years, from 1973 to 2013, using a combination of aerial photography and field surveys. The investigated meadows are composed by *Zostera marina*, *Zostera noltei* and *Cymodocea nodosa* and have been exposed to multiple pressures, (including increased urbanization, tourism, habitat degradation due to different kinds of fishery, heat waves) since historical times. The decadal-scale (40 years) analysis of the present study allowed us to provide, for the first time, insights into the rates of seagrass decline and recovery in the Northern Adriatic Sea and the main drivers of the changes of the seagrass meadows. Data presented here also provide novel information on the conditions allowing optimization of active and passive restoration of degraded seagrass meadows.

4.2 Materials and Methods

Study area and identification of local drivers

The study area is located along the coast of the Northern Adriatic Sea at Gabicce Mare (Marche Region, Italy; see Fig 1). The beach extends for 1800 m (with 165 m from the coastline and the breakwaters) made of 6% of pebbles and 94% of sand (Danovaro unpublished data). It is bordered to the North by a touristic harbor and a stream channel reaching the sea (Tavollo) to the north, and by the rocky coast of “Monte San Bartolo” (Colantoni et al., 2004) to the south. Along the coast of Marche Region, in the 1960s and 1970s, important extractions of sediments were carried out from the riverbeds, and several harbors were built. As a result, a decrease of sediment supply to the coasts was reported, and several breakwater barriers and seawalls were placed to protect the beaches from erosion process (Colantoni et al., 2004). Moreover, the area is often subjected to beach nourishment (Hanson et al., 2002). In the investigated area, as far as the sediment transport along the coast is directed towards North, the sediment is accumulated near the touristic harbor. The amount of material redistributed for beach nourishment was estimated through official data cross-checked with the webcam images of the Gabicce Mare Municipality, which resumes in real time the beach.

To quantify local human pressures, the following data were acquired: (i) resident population, (ii) number of houses, (iii) number of accommodation facilities (e.g., hotels, restaurants, bar, campgrounds) tourist presences (iv). Accommodation facilities are considered linked to the demand and thus are used to assess the touristic flow (Lorenzini et al., 2011). All data were obtained from the website of Italian Institute of Statistical Science (formally ISTAT see www.istat.it) with the exception of the number of tourists presence that was obtained from in the website of “Osservatorio Regionale Turismo” (see http://statistica.turismo.marche.it/DatiTurismo/ tabid/100/language/it-IT/Default.aspx).

Data on long-term changes nutrient concentrations (phosphates, nitrates, nitrites and ammonium) salinity and temperature were obtained from the ABCD4 (Adriatic BiogeoChemical Data set version 4) and integrated from 1978 to 1981 to additional measures (Zavatarelli et al., 2000).

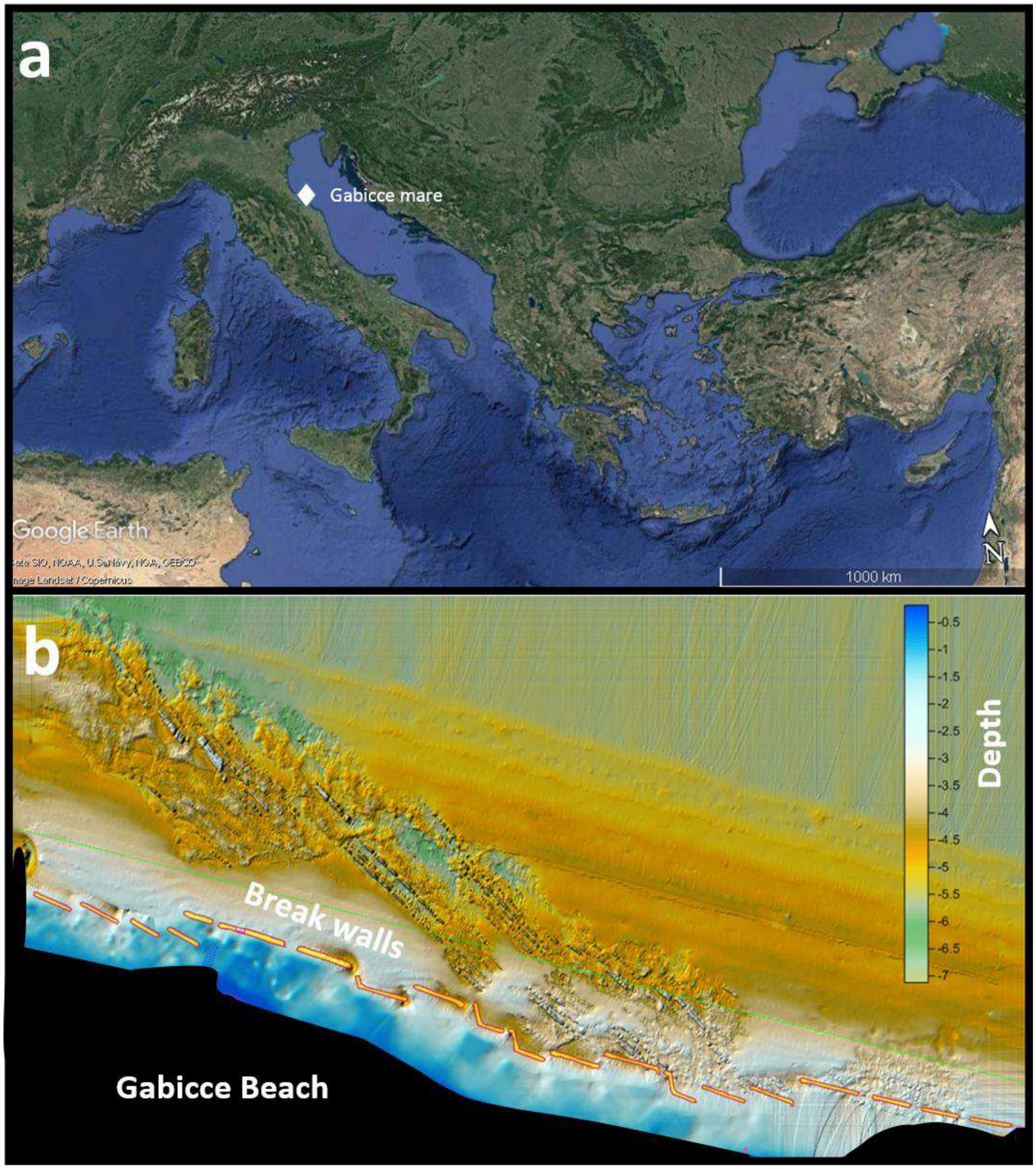

Fig. 1. Location of the study area. Reported are: (a) Aerial imagery (data Google Earth Pro 7.3.2) and(B) bathymetric map of the study area.

Analysis of seagrass extension by aerial photography and field surveys

The map of seagrass meadows was determined by aerial photography (Fig 2). This tool enabled the analysis of the distribution of the seagrass at shallow depths (i.e.<5 m depth) for the following years: 1973, 1982, 1983, 1989, 1991, 2004, 2007, 2008, 2010, 2011 and 2013.

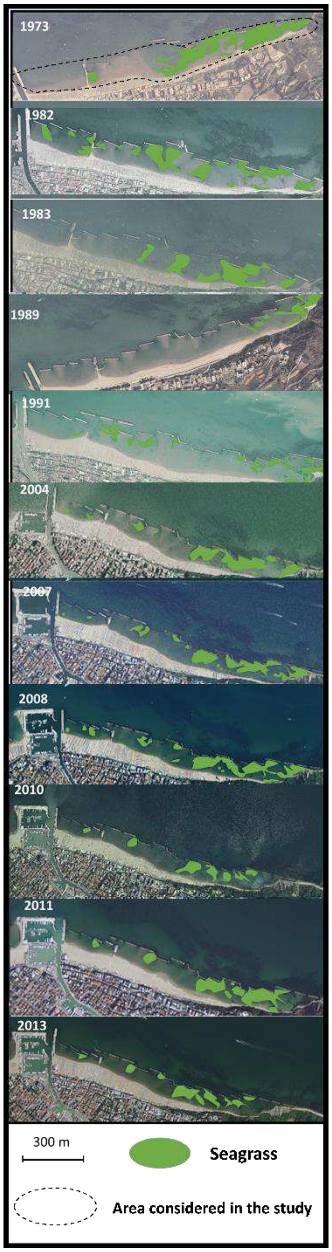

Fig. 2. Aerial photos of the study area in each investigated year (source Terra- Italy; https://www.terraitaly.it/).

Among the different methods used to map seagrass, aerial photography has been demonstrated to be one of the best methods to map shallow meadows (Lericheet al., 2006; Pace et al., 2017), and it has been frequently used in several studies to assess the temporal change over time of the total area covered by seagrass meadows (Ward et al., 2003; Bernard et al., 2007; Orth et al., 2017; Lefchecket al., 2017) as well as local impacts such as boat propeller scarring.

The aerial color photographs were taken at different times of the year from an altitude of 900-8300 m through a vertical photography (Table 1). Analogic photographs were digitized (1800 dpi) with a PhotoScann 2000scanner (Zeiss) using Photoscan and Autoscan software.

Table 1. Pixel resolution, fly elevation and camera used to collect the images utilized to calculate the coverage by seagrass meadows.

Date	Scale/pixel resolution	Height	Type of camera
22/07/1973	1:10.000 cm	1550 m	Wild RC8a
04/06/1982	1:10.000 cm	1550 m	Wild RC10
21/08/1983	1:10.000 cm	1550 m	Wild RC8a
04/08/1986	1:17.000 cm	2600 m	Wild RC8a
06/03/1989	1:15.000 cm	2300 m	Wild RC20
30/05/1991	1:10.000 cm	1550 m	Wild RC8a
12/02/2001	1:17.000 cm	2700 m	Wild RC30
04/06/2004	1:40.000 cm	6100 m	Wild RC30
10/05/2007	1 pixel: 50 cm	4800 m	Leica ADS40
06/07/2008	1 pixel: 10 cm	900 m	Canon EOS 1D
25/05/2010	1 pixel: 50 cm	5500 m	Zeiss DMC

31/03/2011	1 pixel: 50 cm	8300 m	UltracamVexcel XP
27/07/2012	1 pixel: 10 cm	900 m	Canon EOS 1D
08/06/2013	1 pixel: 50 cm	4800 m	Leica ADS80

The aerial images were georeferenced using UTM WGS 84. Patches of seagrass were delimited by means of ImageJ, which utilizes automated image analysis to record area surface (Crawford and Mortensen 2009). Since photo aerial analysis does not allow species identification, field surveys were also conducted in order to collect data on shoot density of each species.

The fine scale distribution of the seagrass meadows was determined by SCUBA-diving activities, and their areal extension was calculated through Qgis of QGIS Development Team (2012). Seagrass distribution from aerial photography was compared with field data (collected through underwater surveys conducted in 2011 and 2017). The results of the aerial photography matched consistently the field data (average error <5%).

In addition, in 2002 and 2017, shoot density (i.e., the mean number of shoots per surface area unit) of *Zostera marina* and *Zostera noltei* (and *Cymodocea nodosa* in 2017) was assessed by counting the shoots within deployments of 1 m^2 frames (Mazzella et al., 1998) for a total of 25 replicates random distributed along the entire meadow. Additional random replicated samples were collected for calculating the leaf area index determined by multiplying the mean leaf surface per shoot by the meadow shoot density (Mazzella et al., 1998). Finally, biomass was sampled by removing a clod within a quadrat 40 x 40cm for 25 random replicates and the epigean and hypogean seagrass biomass (i.e., shoot and rhizomes) were determined drying pre-cleaned seagrass samples at 60°C to constant weight, according to standard methods epiphytes were not removed (Duarte and Chiscano 1999).

Statistical analyses

The presence of significant differences between 2002 and 2017 was tested with a PERMANOVA analysis based on Euclidean distance for each investigated variable during the field survey. Moreover, in order to provide evidence of the linkage between temperature increase, urbanization and the seagrass decline a GAM model (carried out with a poisson distribution) was performed with R (R Core Team 2018) using the data of the Max time interval (days) with $T \geq 23°C$ (Table 2) and the number of tourists as predictor variables and the seagrass cover as explanatory variable, for the year 2004, 2007, 2008, 2010, 2011 and 2013:

$$\text{Seagrass cover} = \alpha + f\,(\text{days}) + f\,(\text{tourist presence}) + \epsilon$$

Where α is the “intercept”, f (days) and f (tourist presence) are the smoothing functions, which do not provide a direct mathematical expression, indeed a curve is obtained, and ϵ is the error. After that, the dispersion of the model is tested and considered acceptable if it is close to 1 (>1 is over dispersed and <1 is under dispersed). Finally, the model validation is performed plotting the “Pearson residual versus Fitted values” and versus “Max time interval (days) with $T \geq 23°C$ as well as “tourist presence” to test the homogeneity and independence respectively. If there are no clear patterns in the residual distribution the GAM has passed the model validation.

Table 2. Data of water temperature from 2000 to 2013 daily collected from the transducer T020 TTA placed on the sea surface in the Ancona's NT station (43° 37' 29.16''; 13° 30' 23.46'').

	2000	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**
Max time interval (days) with T ≥ 23°C	93	77	91	99	86	-	-	72	64	121	77	82	94	79
Max time interval (days) with T ≥ 27°C	3	5	3	86	1	-	12	1	2	6	11	5	4	5
Highest summer temperature (°C)	27.8	28	28.4	32	28.4	-	28.4	27.3	27.9	27.7	28.2	27.9	27.8	27.5

4.3 Results

Local drivers

The urbanization in the investigated area increased notably from 1971 to 1981, when the urbanization expanded (i.e., number of houses increased from 1,929 to 2,911 and the accommodation facilities increased from 191 to 406 units; Fig 3a,c). Conversely, from 1981 to 2001 a decrease of accommodation facilities (Fig 3c) occurred in the area, and the tourist presences in Gabicce Mare declined from 760,350 in 1994 to 611,020 in 2011 always showing a very high tourist presence (Fig. 3d). After that, the tourist flow increased until 2013 reaching 750,703 presences per year (Fig. 3d) despite of the number of the residents remained relatively constant between 1973 and 2013 (5,364 and 5,713 respectively; see Fig 3b). Sand redistribution was carried out every year along the beach in late winter-spring (webcam images or personal observation). From the analysis of aerial photographs, it is emerged that between 2004 and 2007 the touristic harbor expanded for about 35,000 m^2. In 2008, waste-water treatment was complete thanks to the presence of a brand-new depurator (data from the archive of Marche Region Administration).

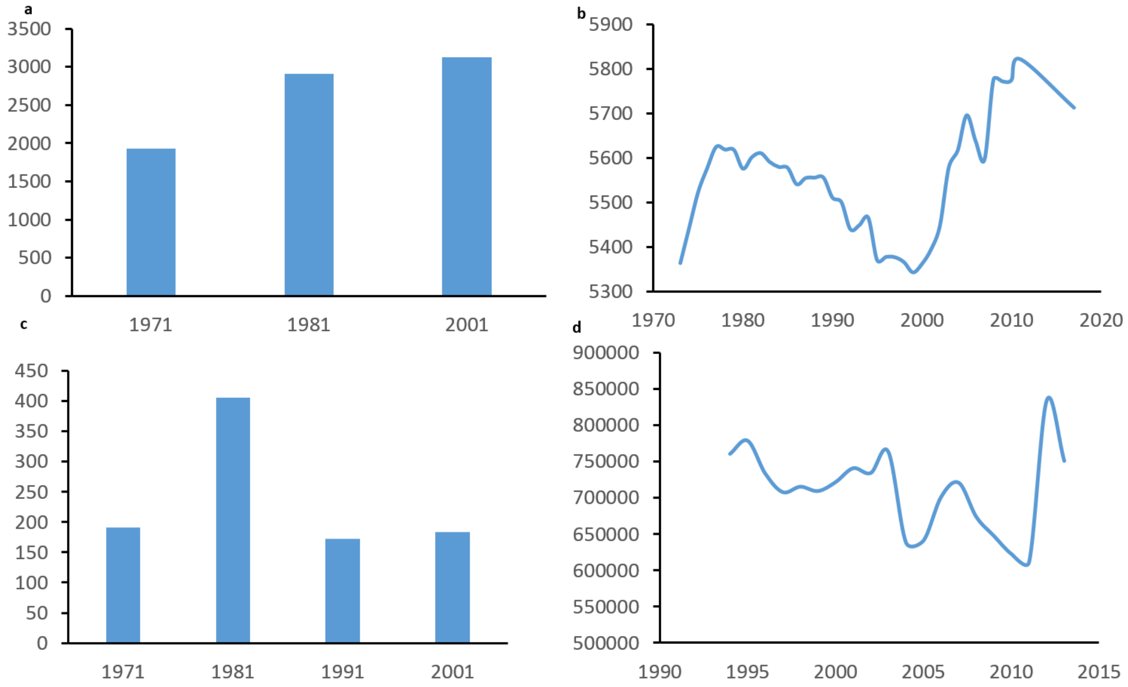

Figure 3. Temporal trend of coastal development. Reported are: a) Number of houses, b) resident people, c) accommodation facilities, d) number of tourists.

The analysis of nutrient and other oceanographic data showed that the phosphate concentration increased from 1978 to 1981 (0.07 and 0.48 μmol dm^{-3} respectively), whilst nitrates and nitrites showed a high variability, without any specific trend (data from ABCD4; as Figure 4). The sea temperature showed an increase from 1978 to 1980 (22.76 and 23.75°C respectively). Similarly, salinity increased, on average of summer values, from 33.36 to 35.03 (in 1978 and 1980 respectively). The data of these variables for 1981 (7.65°C and 36.78 for salinity) cannot be compared with those of the previous years since they were taken in a different season.

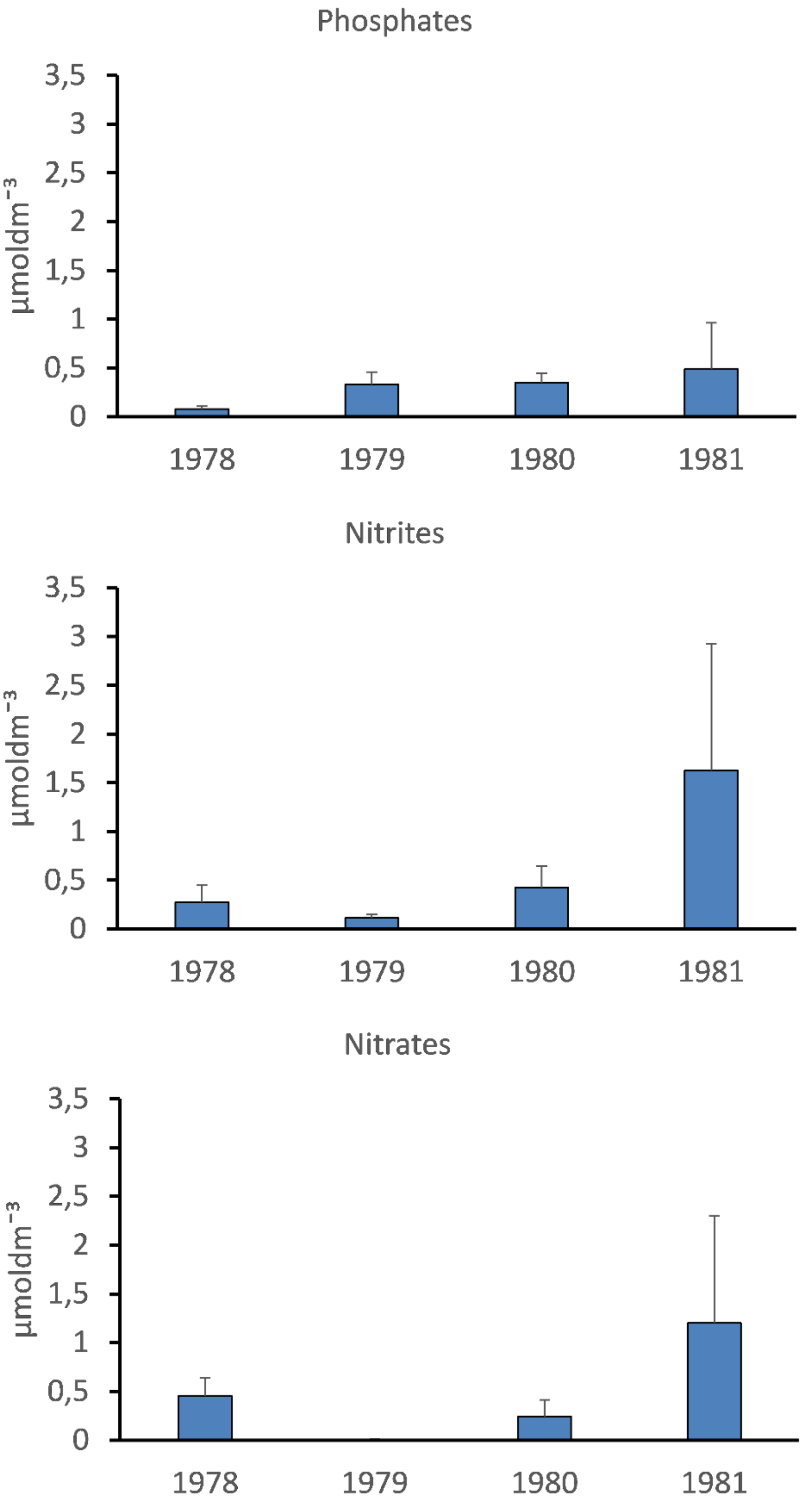

Fig. 4. Temporal trends of nutrient concentrations from 1978 to 1981 (reported are average concentrations ± SE) in front of Gabicce Mare.

Seagrass meadows

During the investigated period three main phases can be recognized: i) from 1973 to 1989, a period characterized by a drastic decrease of seagrass coverage (54,934 and 15,167 m², respectively) with a 72%loss of seagrass extension, corresponding to a decrease of the meadow surface by 4.5% per year; ii) from 1989 to 2007 the seagrass coverage showed a noticeable expansion, reaching an overall extension of 40,175 m². During this period a recovery of 63% of the loss of the seagrass meadow extension (equivalent to 2.5% per year) was observed; iii) from 2007 to 2013, the seagrass extension decreased again to 27,305 m^2, equivalent to ca 50% of the area covered in 1973. The overall loss calculated with respect to the surface covered in 2007 was ca. 32%, with a decrease of 5.3% of the surface per year (Fig. 5).

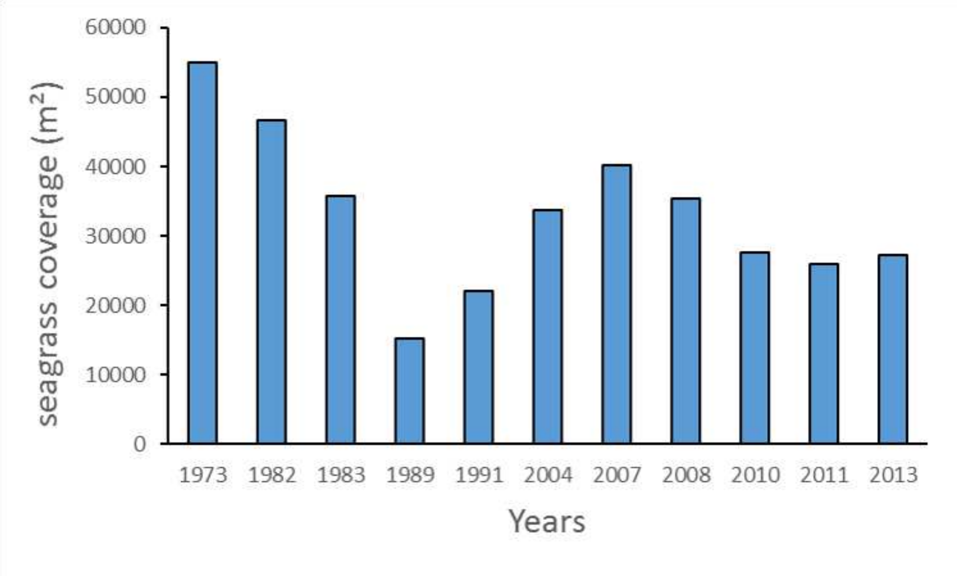

Figure 5. Temporal trend analysis is Seagrass coverage in the investigated area (Gabicce Mare).

In 2002, the field survey revealed that *Zostera marina* showed a lower shoot density compared to that of *Zostera noltei* (460 and 4,414 shoots for m^2 respectively Fig. 6). However, *Zostera noltei* was found only along the boundaries of the meadows. According to Qgis analysis, the seagrass extension was 24,748 m^2, and a similar value was obtained from the aerial photo analysis, which indicated an area of ca 26,000 m^2 and 25,724 m^2 in 2011 and 2017, respectively. The results from the field activity carried out in 2017 found an average density of 1,366 ± 140 shoots for m^2 of *Cymodocea nodosa*, 1,878± 84 of *Zostera noltei* and 798± 164 shoots for m^2 of *Zostera marina* (Fig.6). Both *Zostera noltei* and *Zostera marina* densities are significantly different among the two years ($p < 0.01$ and $p<0.05$ respectively). The epigean biomass of *Zostera marina* is significantly higher in 2017 ($p<0.05$). While, both *Zostera noltei* and *Zostera marina* hypogean biomass are significantly lower in 2017 ($p<0.01$). Finally, the leaf area index of *Zostera marina* is significantly higher in 2017 ($p<0.05$). The area outside of the breakwater barriers was also investigated and no seagrass meadows were observed, while a significant presence of macroalgae was reported in both years. Therefore, it has been excluded by the photo analysis.

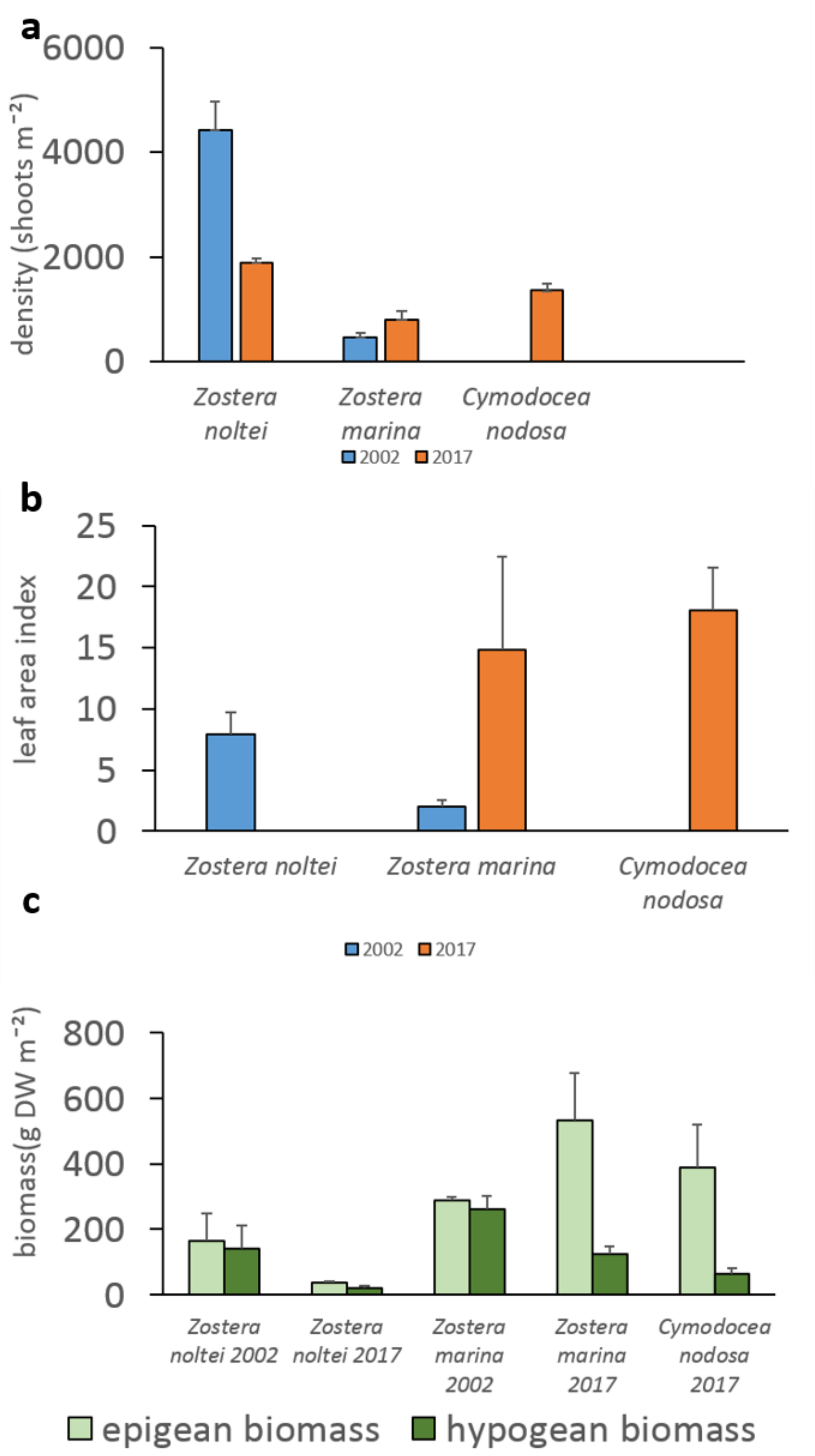

Figure 6. Changes in seagrass variables from 2002 to 2017. Reported are: A) shoot density of seagrasses (B) leaf area index (LAI); (C) biomass (data are reported as average ± SE; DW: dry weight).

The GAM model obtained is:

Seagrass cover=10.351748 + f (days)+ f (tourist presence) + ϵ

All terms of the Model are significant ($p<0.01$), which indicates a strong non-linear relationship (adjusted R^2 = 0.66, explaining 93% of the deviance; Figure 7). Moreover, since the Pearson residual are randomly distributed in the plots representing the “Pearson residual versus Fitted values”, versus “Max time interval (days) with T ≥ 23°C “and versus “tourist presence” the model validation was successful (see Supplementary information Fig. S2, S3, S4). However, the model is slightly overdispersed (2.7). Overall, this indicates that the changes observed from 2004 to 2013 is driven both by temperature anomalies and tourist presence.

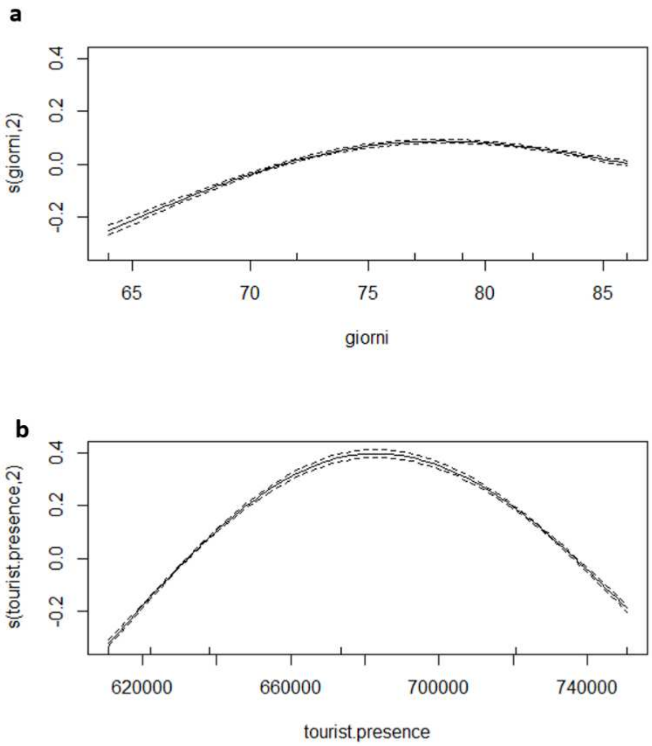

Fig. 7. Smoothed functions of Max time interval (days) with T ≥ 23 ◦C (a) and tourism presence (b)R version 3.5.2. The values in the x-axis are observed data. The y-axis represents the spiline function. Dashed lines indicate 95% confidence bounds.

4.4 Discussion

In the present study, we investigated the change over time of the seagrass meadows extension by using a combination of in situ analyses and aerial photography, which provided crucial information for assessing the ecological changes and shift over a long-time monitoring (Gatti et al., 2015). At the same time, an in-situ analysis of the current seagrass coverage is fundamental for assessing the accuracy of the photographic results, as well as for determining the changes in species composition. The negligible differences found comparing the seagrass coverage in the investigated area through the aerial image analysis with the coverage obtained from the field assessment indicated a very good consistency between the results, in terms of surface coverage, of the two methodologies. Despite the quality and resolution of the pictures have improved over time, this did not affect the comparison among the aerial photography results.

Since the beginning of the ecological research on seagrass meadows in the early 70's, a consistent decline of their distribution has been documented at global scale (Cunha et al., 2005; Lefcheck et al., 2017; Calleja et al., 2017; York et al., 2017). In the Mediterranean Sea, several studies reported a clear decline of the extension of the seagrass meadows, associated with a corresponding decrease in the related ecosystem goods and services (Montefalcone et al., 2009; Montefalcone et al., 2015; Burgos et al., 2017). However, recent studies documented a significant recovery of seagrass meadows in several European regions (De Los Santos et al., 2019).

In 2002 and 2017, field (in situ) investigations revealed that shoot density and leaf area index of *Zostera marina* were higher than those reported in literature for the Mediterranean Sea (Mazzella et al., 1998). Instead, the shoot density of *Zostera noltei* showed different values in 2002 and 2017 but similar to those reported in a previous study (Curielet al., 1996). These data suggest that the seagrass meadows were characterized by a good health status (Procaccini et al., 2003). In 2017, the increase in shoot densities of *Cymodocea nodosa* (rarely found in 2002) and the decrease of *Zostera noltei* were reported to be driven by the increase of sea temperature, considering that they are warm and cold affinity species, respectively (Pergent et al., 2014).

These results represent the first attempt to assess the long-term changes of the seagrass meadows in the northern Adriatic Sea, and revealed a clear decrease of the meadows' extension from 1973 to1989. Which was associated to the explosion of coastal urbanization and the intensification of tourism flow as indicated by the rise of the number of accommodation facilities from 1971 to 1981. The urbanization of the area was documented also by the significant increase in the number of houses and hosting facilities, without a noticeable increase of resident habitants. At the same time, during these years, the North Adriatic Sea was characterized by a significant increase of eutrophication due to the Po river flow, with the appearance of strong eutrophication and episodic events of anoxia at the water-sediment interface (Giani et al., 2012). Indeed, we observed an increase of nutrients concentration from 1978 to 1981. Although the concentrations of phosphates and nitrates reported in the present study are lower than the values considered harmful for seagrasses (i.e., ca 10 µmol dm^{-3} for phosphates and 55 µmol dm^{-3} for nitrates)(La Nafie et al., 2012), the effects of the progressive eutrophication were likely exacerbated by concomitant presence of fringing reefs, which reduced local circulation.

Overall, data reported here allowed us to conclude that the years from 1971 to 1981 were characterized by an evident increase of the anthropogenic impacts, with deleterious effects on the investigated seagrass meadows. These results are consistent with those reported from a long-term analysis conducted from 1869 to 2016 in European Seas that showed a peak of seagrass losses in the 1970s and 1980s due to disease, deteriorated water quality, and coastal development (De Los Santos et al., 2019).

Conversely, from 1981 to 2001 the number of accommodation facilities and of tourist presences decreased. Furthermore, during this period, a decrease of primary productivity is reported for the North Adriatic Sea (Giani et al., 2012). The decrease in the direct and indirect human pressures on the investigated area promoted a significant recovery of the extension of the seagrass meadows till 2007. These results are consistent with previous studies conducted in different oceanic regions (Cardoso et al., 2008; Tuya et al., 2014; Lefcheck et al., 2017). Although coastal constructions and infrastructures can have a significant impact on marine habitats (Nepote et al., 2017), the extension of the local harbor, apparently, did not cause a loss of the seagrass habitat. This could be explained by the fact that the distance between the meadows and the harbor is 800 m, which is higher than what is considered a “safety distance” for the impact of infrastructures at sea over the seagrasses (i.e. 500 m)(Tuya et al., 2014). However, other studies suggest that harbor development can damage seagrass meadows up to 5 km distance (Holon et al., 2018). The result can be explained by the sediment transport, which are transported in the opposite direction respect to the seagrass meadows (Personal observation). Again, consistently with results reported here, the long-term analysis conducted on seagrass meadows in Europe, revealed that loss rates slowed down for most of the species and fast-growing species recovered in some locations, making the net rate of change in seagrass area experience a reversal in the 2000s, while density metrics improved or remained stable in most sites.

Based on the GAM model, the second decline observed after 2007 was associated with the rise of the temperatures, with peaks in 2009 and 2011 (Di Camillo and Cerrano 2015), which showed

121 and 82 days with a temperature ≥ 23°C (Table 1), which has been reported as the threshold temperature for the survival of *Zostera noltei* (Davison and Hughes 1998). Seawater warming can also favor the replacement of *Zostera noltei*, with *Cymodocea nodosa*, which displays a higher affinity for higher temperatures due to the ability of this species to balance the heat-enhanced photosynthetic with respiratory rates (Pergent et al., 2014). The other driving factor identified for the decline of the seagrass was the number of tourists, which are typically associated to a direct increase of the local impacts. In fact, an higher tourist number imply an increase of urban and domestic pollution (Bernard et al., 2007) and intertidal trampling along the beach, which can damage the benthic community (Davenport and Davenport 2006). The results of the present study indicate that the main drivers of change of seagrass meadows are local urbanization and thermal anomalies.

Since seagrass meadows experience either losses and recoveries becomes crucially important investigate the rates of these processes. The first seagrass decline (from 1973 to 1989) showed a loss equivalent to 72% of the initial area, while the second decline (from 2007 to 2013) showed a further loss of the 32% of the surface present in 2007. Similarly, another study conducted on *Zostera marina* in the Atlantic Ocean provided evidence of an 80% decrease in shallow waters, at a rate of loss of about 3% per year (Lefcheck et al., 2017). A study conducted on *Zostera noltei* along the Spanish coasts of the Atlantic Ocean showed an overall decline of the extension ranging from 33 to 88% from 1984 to 1989, with a decline rate ranging from 7 to 18% per year (Calleja et al., 2017). Conversely, a study conducted in the Western Pacific region found seven highly variable decline rates from 0.4 to 78% (Short et al., 2014). In our study, we calculated that the loss rates was 4.5% y-1 (during the first decline phase) and 5.3% yr^{-1} (during the second decline phase), which fall within the range of values previously reported in other regions.

After the observed decline, the meadows showed a clear resilience. Indeed, seagrass meadows, composed by *Cymodocea spp*. and *Zostera spp*., typically show a relatively high resilience (Curiel et al., 2004; Bertelli et al., 2017; Calleja et al., 2017). Positive interactions among eelgrass shoots may have contributed in the early phase of re-colonization process (Worm and Reusch 2000). Indeed, from 1989 to 2007 we found a recovery rate of 3.5% per year. Information from literature on the recovery rates of *Zostera spp*. indicates that these are very variable and potentially dependent on the specific environmental drivers and multiple stressors. Indeed, previous other studies on decadal temporal scale (12-64 years) from Puerto Rico and Canadian coastal areas showed an increase in seagrass extension by ca. 1.3% to 24-39% per year (Hernández-Cruz et al., 2006; Warren et al., 2010). At the same time, other investigations conducted along Florida coastal ecosystems indicated a seagrass meadow recovery rate of ca 9% per year (Robbins 1997). Finally, a study conducted on *Zostera noltei* meadows in Wales (Bertelli et al., 2017) reported a recovery rate ranging from 0.4 to 271% per year.

4.5 Conclusions

Our long-term analysis provides evidence of the multiple declines and recovery patterns in the seagrass meadows of the Northern Adriatic Sea. We show that the main driver of seagrass declines changed over time: till the end of the 80’s, the seagrass decline was associated with the local urbanization, whereas from 2004 to 2013 the main drivers of habitat loss were both the rising of water temperatures and tourist presence as explained by GAM model. Our data also showed that the rates of seagrass recovery documented here are approximately half of the loss rates (on average approximately 4.5% for loss rates vs 2.5% of recovery per year). These data provide evidence of the need to prevent any form of impact on the *Zostera* and *Cymodocea seagrass* meadows, in the northern Adriatic Sea, especially in the light of the expected negative effects due to the rising surface temperatures linked to climate change. The decrease of hypogean biomass in both *Zostera spp.* coupled with the increase of epigean biomass in *Zostera marina* suggest that the changes of seagrass meadow over time are not limited to seagrass extension and composition. Giving the low recovery rates these results highlight the need of protecting the natural capital and of planning active restoration actions to contrast the loss of seagrass meadows predicted as a result of the climate change in the Adriatic Sea.

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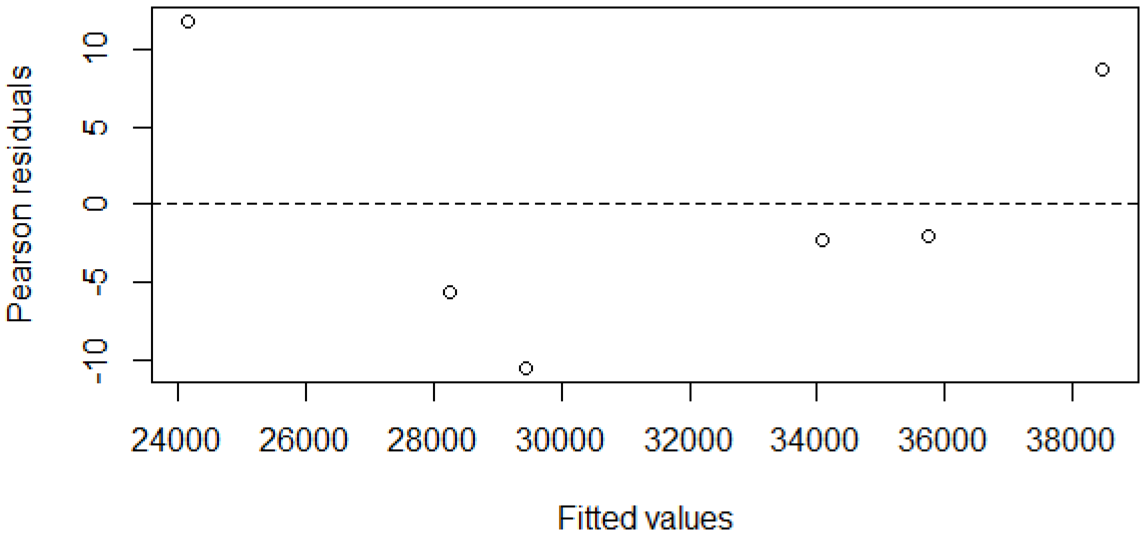
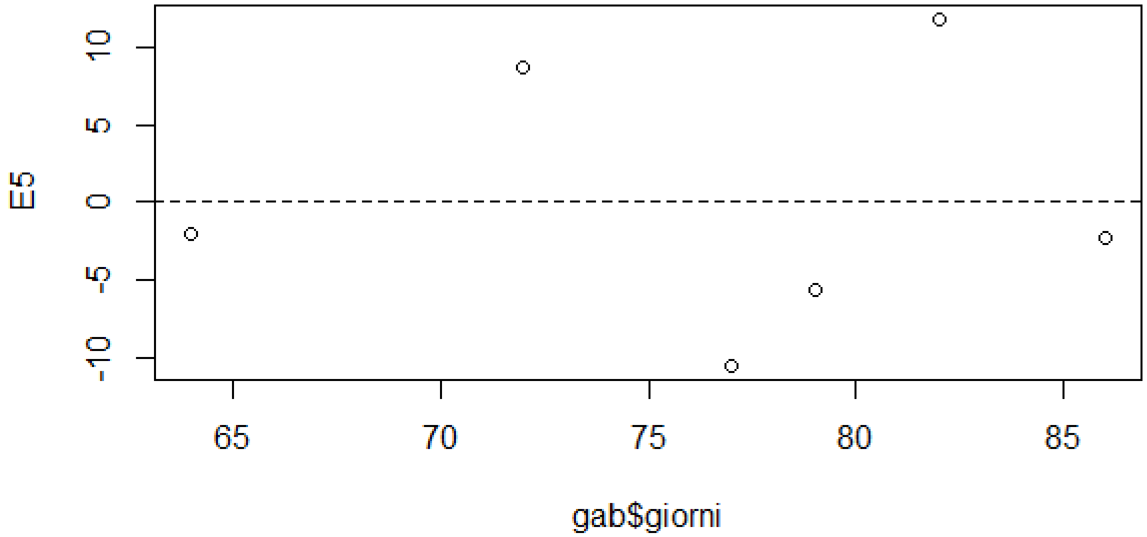
Supplementary information

Supplementary information R code and output S1:

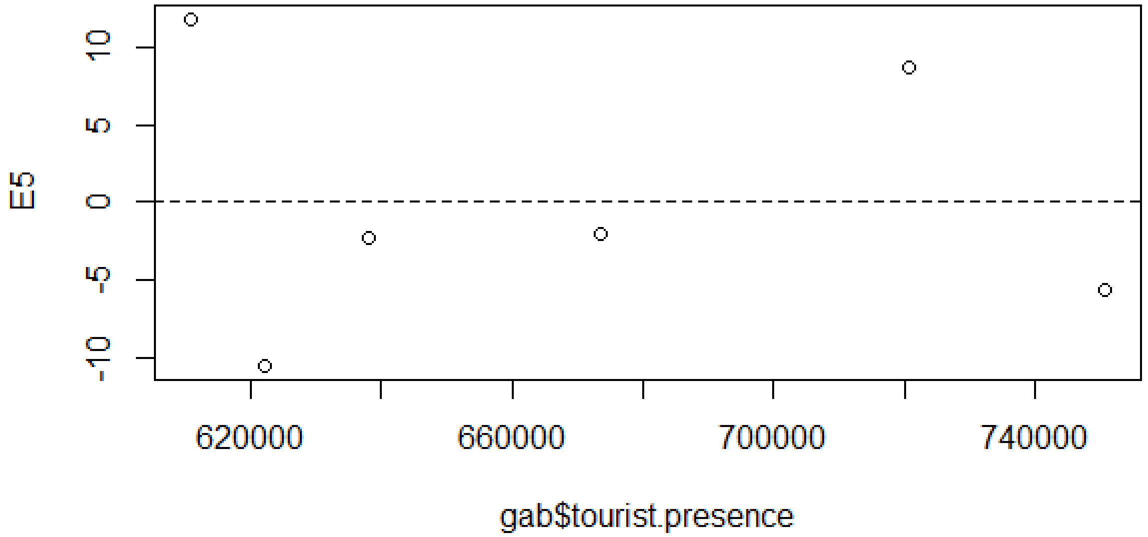
```
 M5 <- gam(seagrass ~ s(giorni, fx = TRUE, k = 3) + s(tourist.presence, fx = TRUE, k = 3), family = poisson(), data = gab)

Family: poisson

Link function: log


Formula:  seagrass ~ s(giorni, fx = TRUE, k = 3) + s(tourist.presence,

fx = TRUE, k = 3)


Parametric coefficients:

Estimate Std. Error z value Pr(>|z|)

(Intercept) 10.351748   0.002321    4459   <2e-16 ***

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:  edfRef.dfChi.sq p-value

S (giorni) 2      2  716.7  <2e-16 ***

s(tourist.presence)   2      2 3088.9  <2e-16 ***

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


R-sq.(adj) =  0.658   Deviance explained = 92.5%

UBRE = 61.499  Scale est. = 1        n = 6

> E5 <- resid(M5, type = "pearson")

> Overdispersion5 <- sum(E5^2) / M1$df.res

> Overdispersion5

[1] 2.71544
```



Supplementary Figure S2. Pearson residual vs Fitted values to test the heterogeneity of the data, R version 3.5.2.



Supplementary Figure S3. Pearson residual vs the covariate “Max time interval (days) with T ≥ 23°C” to test the independence of the data, R version 3.5.2.



Supplementary Figure S4. Pearson residual vs the covariate "tourist presence" to test the independence of the data, R version 3.5.2.

CHAPTER 5. EFFECTS OF HYDRAULIC-DREDGING REDUCTION DUE TO THE SARS-CoV-2 PANDEMIC ON MACROFAUNA ASSEMBLAGES IN THE NORTHERN ADRIATIC SEA

Abstract

The Northern Adriatic Sea is historically subjected to intensive bottom trawling and extensive use of hydraulic dredging for the harvesting of infaunal bivalves: such practice is known to exert deleterious effects on benthic habitats. In order to gather information on this destructive fishing practice, we investigated the abundance and assemblage composition of soft-bottom macrofauna in a coastal area of the Northern Adriatic Sea (Mediterranean Sea), by comparing the year before and the year after the fishing reduction imposed by the SARS-CoV-2 pandemic. Our results indicate that macrofaunal assemblages changed significantly in abundance and composition. While bivalves doubled their abundance, other opportunistic scavenger taxa decreased or disappeared. Despite fishery intensity decreased by ca 50% in 2020, the harvesting of *Chamelea gallina* fished increased from 18,706 tons in 2019 to 19,092 tons in 2020. Thus, the fishing reduction imposed by the pandemic can result in an increase of the yearly clam harvesting. Our results indicate that soft-bottom macrofaunal assemblages show appreciable recovery when the fishing effort decrease noticeable. Therefore, a long-term stop or decrease of clam harvesting and the establishment of fishery restricted areas or marine protected areas could lead to a more sustainable fishery and probably increase the clam fish yields.

5.1 Introduction

The Mediterranean Sea and, in particular, the Northern Adriatic Sea are historically subjected to a significant fishing pressure, and most of the species of commercial interest are now exploited beyond maximum sustainable yields (Amoroso et al., 2018). The most significant impacts on benthic habitats are due to the bottom contact of the fishing gears. In particular, the Adriatic Sea is now one of the most trawled area in the world (Romanelli et al., 2009; Amoroso et al., 2018), and severely impacted by hydraulic dredging for harvesting bivalves (especially *Chamelea gallina*) living within the soft sediments. This fishing practice appears to be particularly destructive for soft-bottom ecosystems (Morello et al., 2005; Petetta et al., 2021), as the dredge is deployed on the seafloor and high-pressure water injections are used to harvest the bivalves from the sediments. The heavy dredge trawled by the fishing vessel along with the "water gun" have a significant impact on the sediment stratification and integrity where a high number of macro and megabenthic species are present (Gilkinson et al. 2003). The effects are nearly instantaneous, with plume of resuspended sediments and visible furrows on the seafloor. The extension of these physical impacts depends largely on the sediment type and gear's characteristics (the width of the dredge, the length of the tooth, the cutting depth of the blade and/or the pressure of the water jets) and the fishing effort (as number of vessels and number of days at work; Gaspar and Chícharo2007). The temporal persistence of the dredge furrows is strictly correlated with local sedimentation rates and sediment type, with a persistence from a few hours to several days for tows performed in sandy and sandy-mud sediments respectively (Gaspar and Chícharo 2007). As a consequence of the disturbance induced by dredging, bioturbation activity significantly declines with consequent effects on benthic biodiversity, assemblage composition, nutrient fluxes and food webs (Olsgard et al., 2008). Moreover, hydraulic dredging alters biogeochemical cycles, either causing the sediment resuspension, and by changing the biochemical composition of the sediment organic matter (e.g., the protein to carbohydrate ratio) and reducing the bioavailable fraction of organic carbon (Pusceddu et al., 2005). In addition, at the end of the fishing, the cage is hauled up and the whole catch is dumped into a collecting box and then sorted and the under-size individuals are discharged back at sea (Moschino et al., 2003; Çolakoğlu 2020). The consequence is that the survivors are generally partly damaged or stressed and thus subjected to an increased risk of predation (Urra et al., 2021). For this reason, the abundance of the commercial bivalves (especially *Chamelea gallina*) showed a progressive decrease over time in the Adriatic Sea (Romanelli et al., 2009; Froglia 2000; Carducci et al., 2020). In order to favor the restoking, the European Union adopted the Regulation 1967/2006, for which the use of hydraulic dredging is strictly forbidden within 0.3 nautical miles from the coast. However, despite restrictions, often the illegal fishery prevails. Another measure to protect the bivalve stocks is the temporary suspension of the fishing activity in summer (duration: typically 30 days).

A study performed using a BACI design revealed a significant impact of hydraulic dredging on bivalve abundance (Morello et al., 2005), with a consequent reduction of energy transfer efficiency to higher trophic levels (Libralato et al. 2010). While different studies were carried out on the effects of fishing on bivalve abundance and macrofauna assemblage (Gallmetzer et al., 2017; Vasapollo et al., 2020), limited information is available on the effects of a strong and continued decrease of fishing efforts.

Here, we investigated the effects on the macrofauna assemblage in the Adriatic Sea, of the long lockdown (i.e., three months) imposed by SARS-CoV-2 emergency in 2020, when a reduction of more than 50% of the fishing efforts happened and continued after the lockdown (plus 30 days of complete stop in August) instead of the usual 30 days requested by national regulations to preserve the clam stock (Russo et al., 2021). The importance of this study is also due to the presence of a strong debate about the possibility to establish a Marine Protected Area in that area.

5.2 Material and Methods

Study area and sampling design

The study area is located in the “Portonovo Bay” along the Italian Adriatic coast (Fig. 1). The seafloor of the bay is characterized by a mix of sandy and rock substrate, where the prevalent habitats depend on site (Fig. 1). The area hosts two precious habitats protected by European Union (European Union Habitat Directive 92/43/EEC): “Sandbanks slightly covered by sea water” (Habitat type 1110), which hosts the typical “Biocoenosis of fine sands” (SFBC, identification code RAC/SPA III.2.2) and “Reef” (Habitat type 1170) hosting the “Biocoenosis of infralittoral algae” (identification code RAC/SPA III.6.1). Therefore, two Sites of Community Importance are present (IT5320005 and IT5320006). In addition, a strong debate is present about the possibility to establish a Marine Protected Area. To assess the changes in the macrofauna assemblage induced by the fishing stop, we performed a BA design (i.e., Before vs After, 2019 and 2021 summer respectively). In the two sampling times, 4 transects (i.e., A, B, C and D) with three stations at three depths (i.e., 3, 6 and 10 m) were sampled, with a maximum distance from the coast of about 1300 m (depending on the transect), with, the exception of D transect, where the sampling at 3 m depth has been impossible due to the higher grain size of the sediment. Since the European Union does not allow the use of hydraulic dredging until a coast distance of 0.3 nm (i.e., about 450 m, European Regulation 1967/2006), our sample activity was performed both in trawled and protected area. In each station, three replicas were collected with a Vaan-veen grab, and successively the sediment was sieved at 500 µm to retain only the macrofaunal organisms, which were conserved in 70% ethanol.

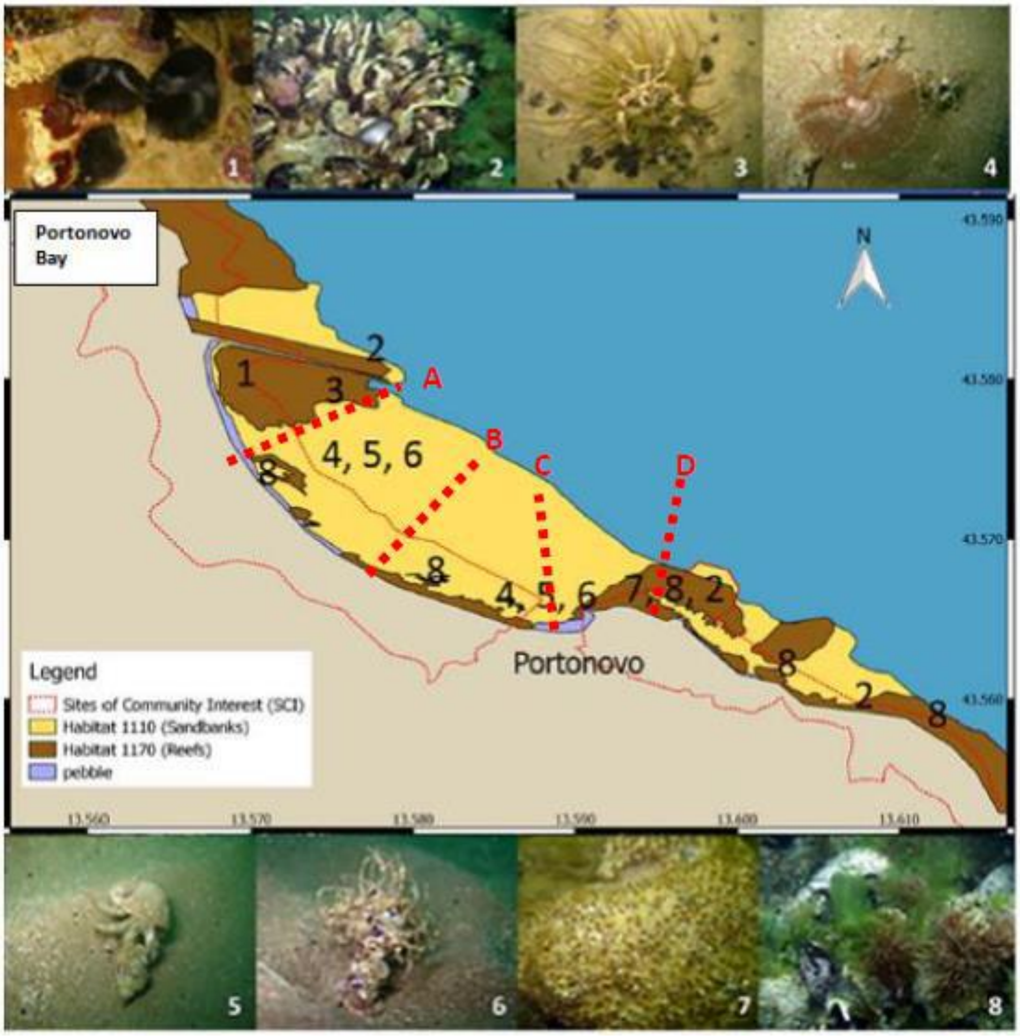


Figure 1. Study area and extension of the different habitats with the locations of the four transects.

Laboratory analysis

The samples were sorted under stereomicroscope and organisms were identified at the following levels: phoronida, polychaeta, bivalvia, gastropoda, nematoda, ostracoda, copepoda, cumacea, tanaidacea, decapoda, amphipoda caprellidea, amphipoda gammaridea, isopoda, nemertea*,* *Echinocardium sp*., ophiuroidea, actiniidae, holothuria, platelminta, oligochaeta and astereoidea.

Statistical analysis

To assess in visual way the differences between before and after the lockdown, a nMDS (based on Bray-Curtis similarity index on log(x+1) transformed data) was performed. In addition, to test the significance of the difference eventually encountered, a PERMANOVA analysis was performed. This method analyses the variance of multivariate data explained by one or more explanatory factors and gives p-values calculated using all possible permutations (Clarke and Warwick 1994). The statistical design included two fixed orthogonal factors: “before vs after” (two levels before and after) and “station” (with 11 stations). The stations were named combining the letter of the transect (A, B, C and D) and the depth, which were named 1, 2 and 3 corresponding to 3, 6 and 10 m depth, respectively (e.g., A1, A2 and A3). The significance of the interaction “before vs after x station” indicates that the change is not the same in the different stations. The absence of the station D1 does not allow to include the factor “transect” in the statistical analysis. In addition, when necessary, a univariate PERMANOVA analysis was performed to test the significance of difference between before and after lockdown of some taxa.

5.3 Results

The nMDS analysis carried out on macrofaunal assemblage composition revealed a clear separation between replicate samples collected before and after the 2020 (i.e., the year of the lockdown; Fig. 2). The statistical significance of such difference is confirmed by PERMANOVA analysis (Table 1). The significant effect of the interaction "before vs after x station" (*p*<0.001) highlighted that the change was not equal among stations.

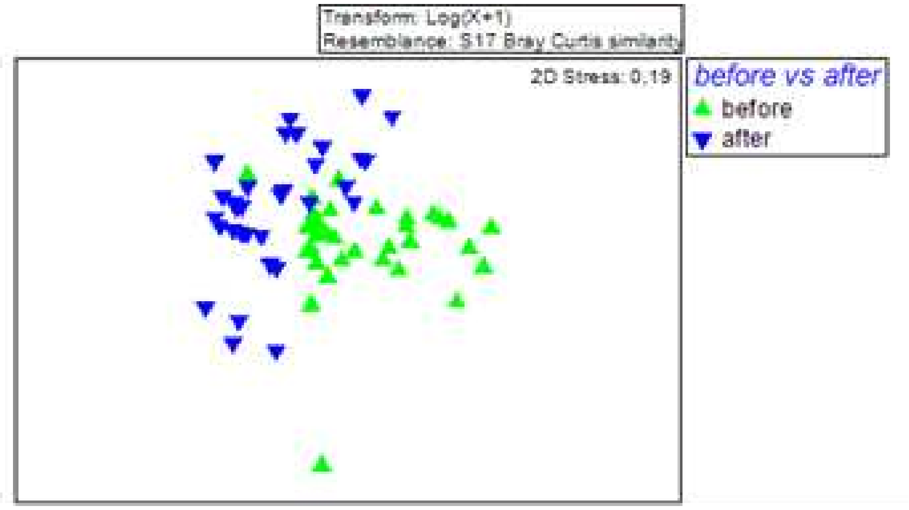


Figure 2. nMDS performed on macrofauna assemblage composition.

Table 1. Output of PERMANOVA performed on the macrofauna assemblage.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
before vs after	1	3355.3	3355.3	33.585	0.0001	9942
station	10	8082.7	808.27	8.0904	0.0001	9873
before vs afterxstation	10	4633.2	463.32	4.6377	0.0001	9868
Res	44	4395.8	99.904			
Total	65	20467				

Pair wise test indicated that all the stations at 3 m depth were significantly different from before to after the lockdown (*p*<0.05). However, at 6 m depth all stations were significantly different from before to after (*p*<0.05), with the exception of station D2. Finally, at 10 m depth all stations were significantly different before to after lockdown (*p*<0.05), with the exception of A3 (*p*= 0.05). The most evident change was the dramatic change of bivalvia (*p*<0.001), which increased from 801 ±95 to 2076± 303 (Fig. 3) in the entire area.

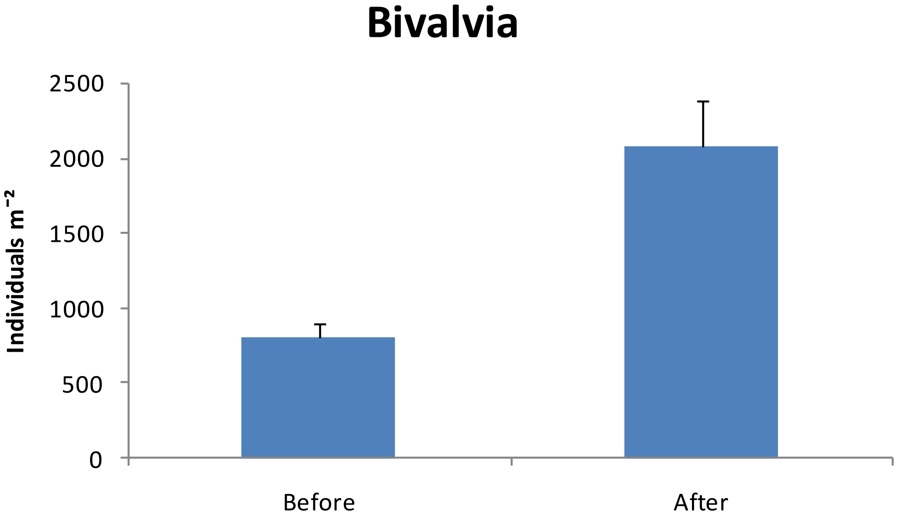


Figure 3. Bivalvia abundance before and after lockdown (average ± standard error).

Conversely, phoronida showed a significant decrease (*p*<0.001; from 1148± 244 to 160± 52 individuals m^{-2}; Fig. 4). Similar trend was detected for polychaeta that decreased from 648± 67 to 246± 21 individuals m^{-2} (*p*<0.001; Fig. 5). Furthermore, copepoda decreased from 46 ± 8 to 10± 2 individuals m^{-2} (*p*<0.001; Fig. 6). Finally, tanaidacea decreased significantly (*p*<0.05) from 112± 13 to 73± 8 individuals m^{-2} (Fig. 7).

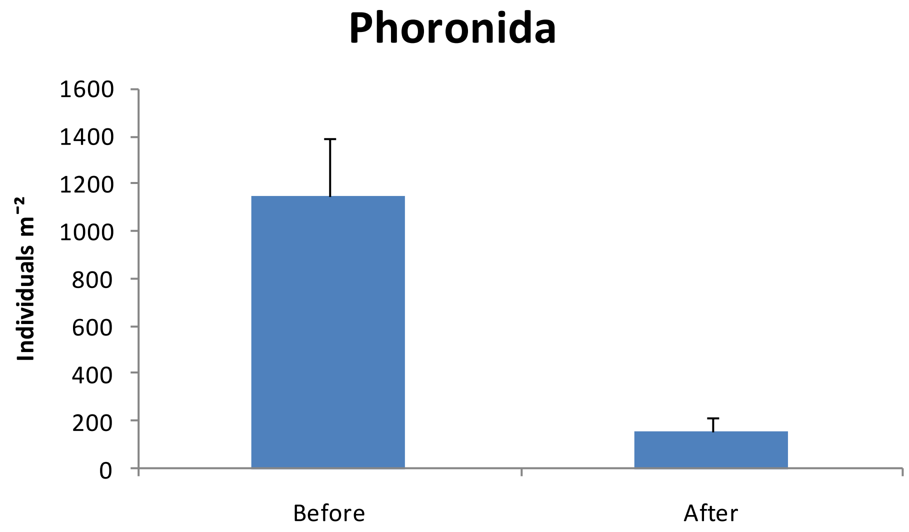


Figure 4. Phoronida abundance before and after lockdown (average ± standard error).

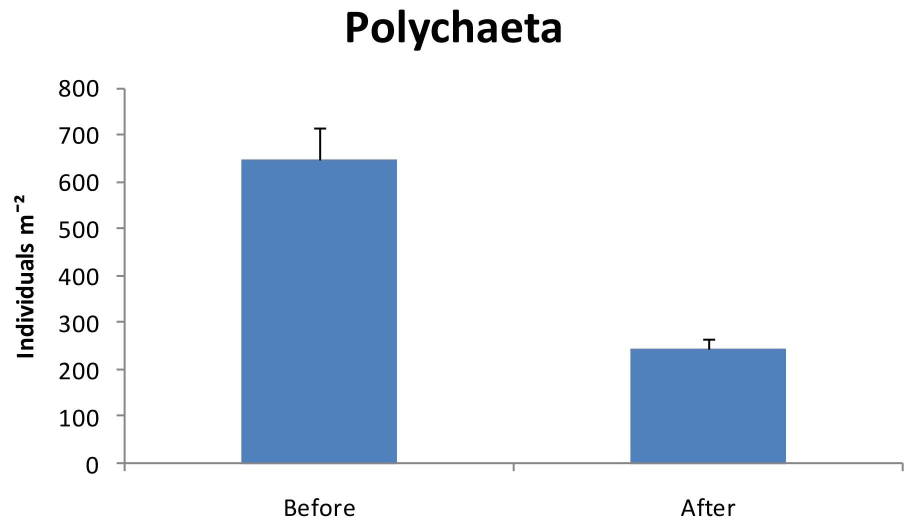


Figure 5. Polychaeta abundance before and after lockdown (average ± standard error).

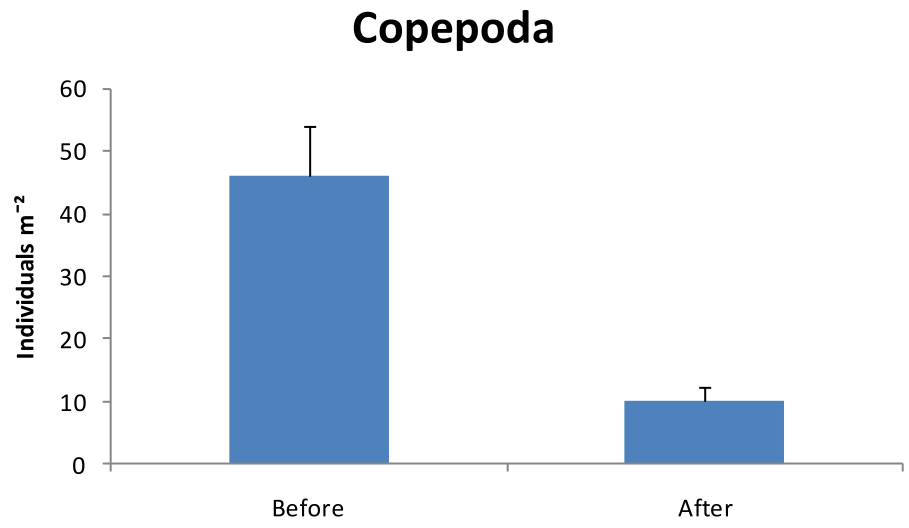


Figure 6. Copepoda abundance before and after lockdown (average ± standard error).

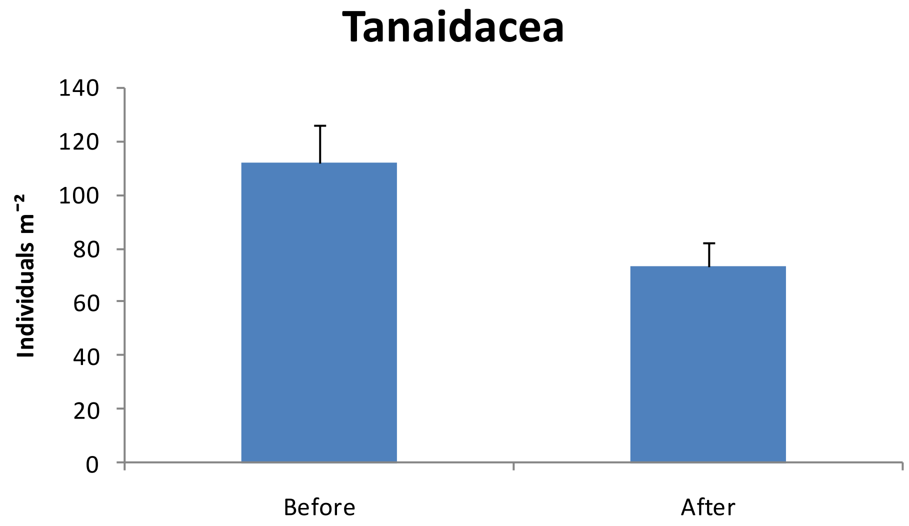


Figure 7. Tanaidacea abundance before and after lockdown (average ± standard error).

In contrast, ostracoda (Fig. 8) increased from 200± 35 to 400± 45 individuals m^{-2} (p<0.01). Similarly, amphipoda (Fig. 9) increased significantly (p<0.001) from 185± 20 to 792± 144 individuals m^{-2}.

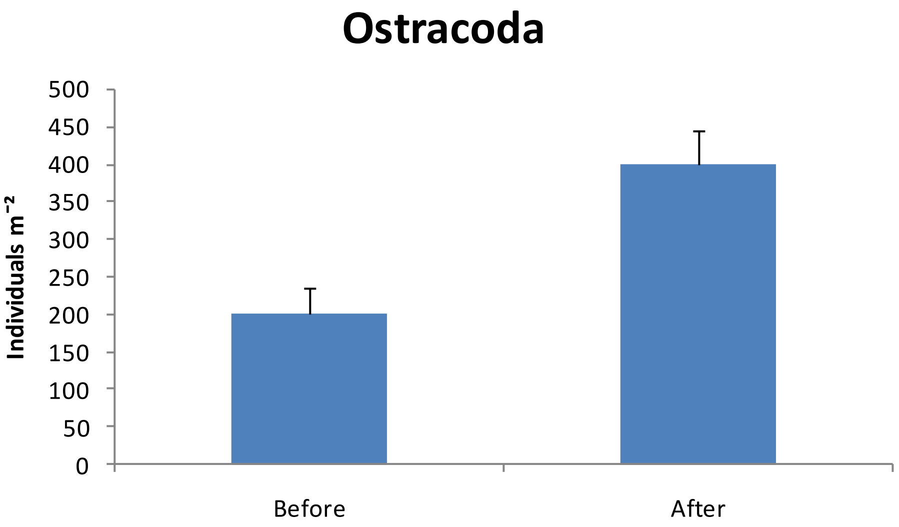


Figure 8. Ostracoda abundance before and after lockdown (average ± standard error).

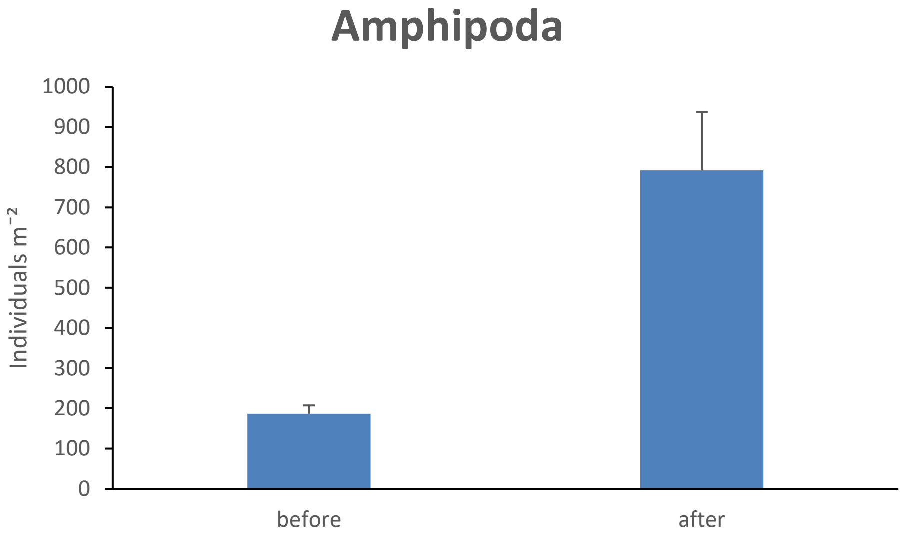


Figure 9. Amphipoda abundance before and after lockdown (average ± standard error).

Finally, cumacea, did not show significant temporal change (Fig. 10). Individuals belonging to other taxa such as Echinocardium, holoturidea, platelminta, oligochaeta and asteroidea were present in very low abundance (i.e., on average ca. 2 individuals m^{-2}). Yet, holoturoidea and ophiuroidea were not detected after the lockdown.

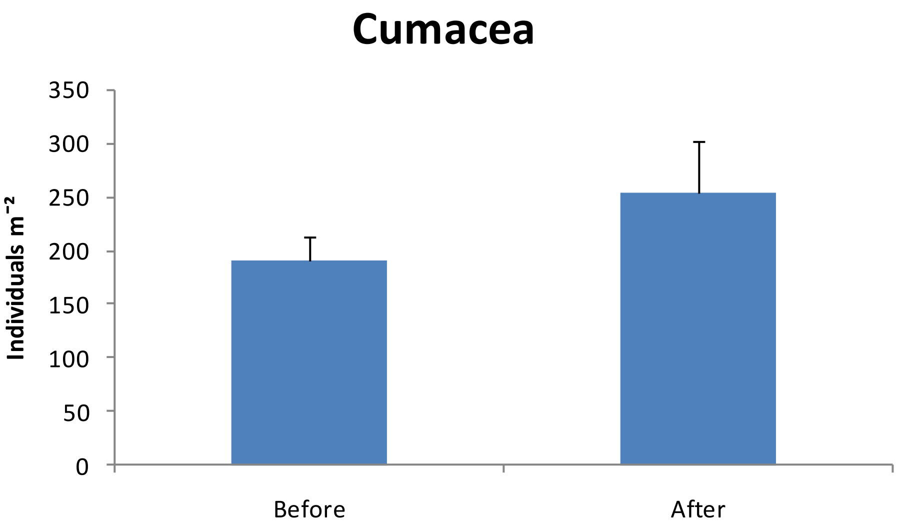


Figure 10. Cumacea abundance before and after lockdown (average ± standard error).

As the picture below explained (Fig. 11) the macrofaunal relative composition changed from before to after the lockdown, with a noticeable increase of the importance of Bivalvia.

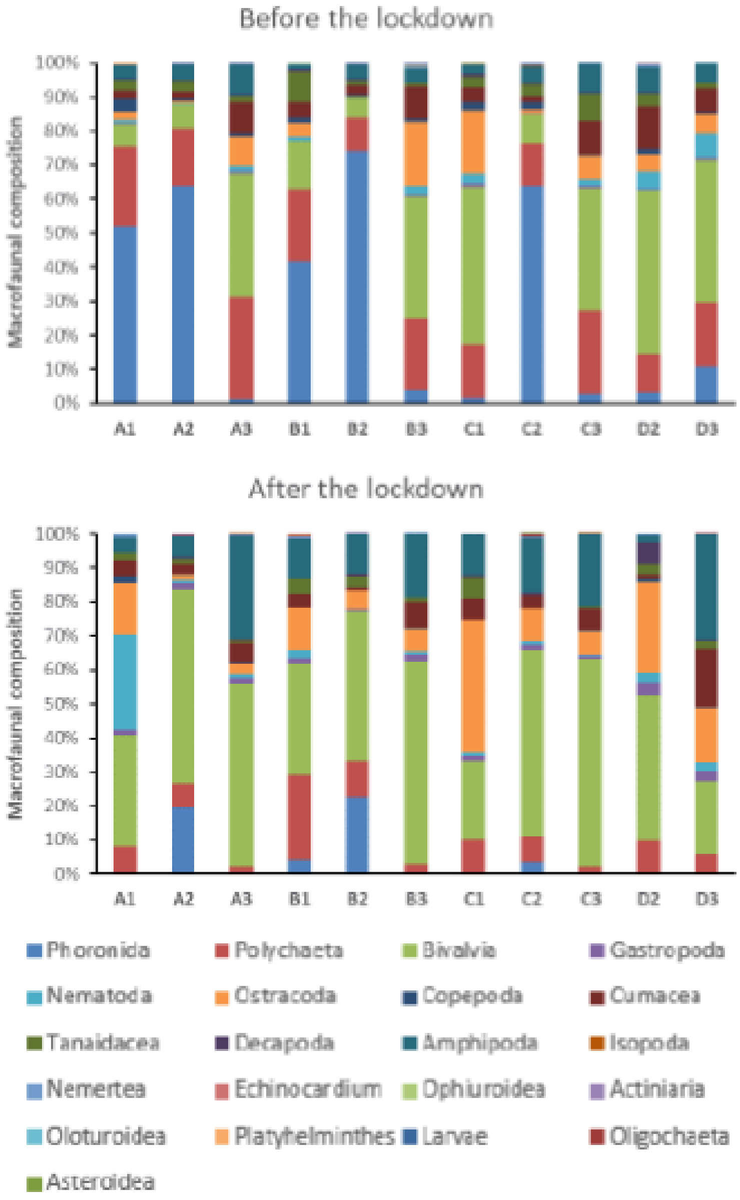


Figure 11. Relative abundance of the macrofaunal taxa before and after the dredge lockdown.

5.4 Discussion

The lockdown imposed by the COVID19 emergency in spring 2020 caused a prolonged stop of several human activities and related pressures (approximately 2 months of complete stop). This stop has been reported to have positive effects on air and water quality, noise pollution (Braga et al., 2020; Lamprecht et al., 2021; Čurović et al., 2021) and resulted in an overall increase of the environmental quality (Lal et al., 2021; Abubakar et al., 2021), but information on its effects on benthic marine assemblages is no existent to the best of our knowledge.

Here, we report that the assemblage composition of macrofauna displayed remarkable differences before and after the 2020, with a clear shift of the most abundant taxa (i.e., bivalves, phoronids and polychaetes).

Comparing the before vs after macrofaunal assemblages, the most evident result is the significant increase of bivalve abundance, which was associated to a shift in macrofaunal assemblage composition. Our results, pointed out that after the reduction of the hydraulic dredging effort, due to the COVID pandemics, bivalve abundance displayed a clear resilience. However, three months without any clam harvesting have been previously reported to be insufficient for the recovery of bivalve abundance (Dimitriadis et al., 2014). This suggests that a noticeable decrease of the fishing effort over the entire year could be a more effective measure respect a unique short complete fishing stop. The effects of the reduction of dredging were apparent also for several other components that are typically favored by sediment disturbance, such as Ophiuroidea and polychaetes (Vasapollo et al., 2020). Other observed changes could be associated to the interannual variability in the recruitment or other environmental factors (Targusi et al., 2018). Assessing the impact on benthic assemblages of hydraulic dredging on soft bottoms has been always difficult due to the lack of appropriate controls (Morello et al., 2005; Vasapollo et al., 2020). However, despite the (temporal) pseudo-replication of our experimental design due to the lack of more sampling times before and after the 2020, our findings suggest that the reduction of clam harvesting can result in an evident and measurable resilience of macrofaunal assemblage, thought non homogeneous across station as shown by the analysis of the "Before vs. After x station" interaction. The reliable of our results is also due to the use of high taxa level, which have largely minimized the natural variability that can be mostly detected at low taxonomic resolution (Warwick, 1988; Bevilacqua et al., 2018). Nevertheless, the influence of other factors related to lockdown and/or natural variations (e.g., improve of water quality) can be not excluded (Braga et al., 2020). Interestingly, the positive effects of the fishing effort reduction on bivalve abundance lasted, also after the restart of normal fishing activities (as evident from the results obtained in 2021). Since the fishing reduction was widespread distributed in the whole Adriatic coastline the recovery of the bivalve stocks observed in the Portonovo bay can be an effect at the whole basin scale (Haselmair et al., 2021).

The Annual Fishing Relations of 2019 and 2020 (data from the Italian Ministry of Agricultural and Forestry Policies) pointed out that, due to the pandemic, the number of fishing days decreased from about 1,300,000 in 2019 to 1,050,000 in 2020 (i.e., by ca 19,2%). During the entire 2020, 58% of Italian Fishing Vessels was underused, 25% moderately used and only 15% fully used (Vessel Use Indicator i.e., VUI). Cumulatively, the lower number of working days during the lockdown, coupled with the use of reduced intensity of dredging in the remaining part of the year resulted in a decrease of fishery intensity of ca 50% in 2020 (Russo et al., 2021). At the same time, available data reported that the harvesting of *Chamelea gallina* fished increased from 18,706 tons in 2019 to 19,092 tons in 2020. Overall, it can be concluded that the reduction by ca 50% of fishing intensity resulted in an increase, although of limited extent, of clam harvesting in 2020.

The results obtained in the present study from an exceptional situation (i.e., the lockdown imposed by the SARS-CoV-2) offered a unique opportunity to test the possible effects of a (future) strict protection measures (e.g., the zone A of marine protected areas or a fishery restricted area). Our findings, indeed, let us to hypothesize that the reduction of clam harvesting can have positive effect on the bivalve abundance and assemblage composition. Since the Adriatic Sea is one of the most intensively dredged area of the world (Amoroso et al., 2018), the enforcement of the fishery stops, the creation of the MPAs and/or the creation of fishery restricted areas in this region could be a suitable solution for protecting and restocking the bivalve abundance, while maintaining (or even increasing) the clam harvesting. Similar management plan should be a priority for developing a sustainable clam harvesting in this region (Bastari et al., 2016).

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CHAPTER 6. GENERAL CONCLUSIONS

Marine ecosystems are subjected to multiple stressors that include local (e.g., overfishing, coastal construction, sewage) and wide scale (e.g., temperature increase and ocean acidification) impact, which often act synergistically (Rossi 2013). In this thesis four “case studies” were analyzed. Although these case studies are related to different sources of impact (desalination, urbanization, positive thermal anomalies, beach nourishment, hydraulic dredging), all of them are frequent in marine ecosystems. For both desalination plant and beach nourishment case studies, the impact on soft bottom assemblage was rather undetectable likely due to the high natural variability of the assemblage (Afghan et al., 2020; Sola et al., 2020) and the relatively small size of the source of impact. Therefore, an adequate modulation of such pressure can result in a limited ecological impact. This represents a crucial information necessary to reach the “Good Environmental Status” imposed by the MSFD. Results from the study about the potential effects of the lockdown and relative fishing stop suggest an appreciable resilience of the soft bottom assemblage (Morello et al., 2005; Vasapollo et al., 2020), and this should be also taken into account for assessing the potential benefits deriving from creation of MPAs and/or fishery restricted areas in the Adriatic Sea. However, the analyses of the long-term changes of seagrass meadows highlighted the need to include not only local stressors, but also drivers related to global change. The identification and quantification of the pressures (both local and global) should be received more attention respect to that usually given. In fact, both WFWD and MSFD (Borja et al., 2010) do not consider in sufficient way the “size” of the pressure (e.g., plant size, tourist number, time of action and eventual suspension time of the pressure), but a more detailed assessment of the scale of the pressures can be useful to predict their impacts and identify adequate mitigation measurements.

Interestingly, both studies on the seagrass meadow of Gabicce Mare as well as the stop of the fishing with hydraulic dredging highlight that diverse ecosystems (i.e., seagrass meadow and soft bottom assemblages) can show an appreciable (but not total) resilience. Overall, this thesis provides new insights to address MSFD initiatives, which require the study of the pressures as well as the identification of mitigation and conservation measures (e.g., decrease of pressure-size and creation of fishery restricted area) to be applied for a sustainable management of marine ecosystems and the goods and services they provide for human wellbeing. This is the case of the use of small size desalination plants as well as the decrease of hydraulic dredging fishing effort. In fact, small size desalination plants can be able to supply drinkable water without a significant environmental impact, while the reduction of hydraulic dredging fishing can promote the sustainable maintenance of clam harvesting over time. In conclusion, the sustainable management of marine ecosystems could be achieved, but requires robust scientific investigations, the selection of adequate ecological descriptors, sounding strategies for the mitigation of the human impacts and appropriate conservation tools.

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