UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea Magistrale in BIOLOGIA MARINA

"A COMPARATIVE STUDY OF BENTHIC MACROFAUNA FROM FOUR ADRIATIC SANDY BEACHES WITH DIFFERENT LEVELS OF MAN-MADE PRESSURE"

"UNO STUDIO COMPARATIVO DELLA MACROFAUNA BENTONICA DA QUATTRO SPIAGGE SABBIOSE DELL'ADRIATICO CON DIVERSI LIVELLI DI PRESSIONE ANTROPICA"

Tesi di Laurea Magistrale di Luciano Di Florio

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Riassunto

Le spiagge sabbiose sono ambienti molto dinamici che ospitano una grande varietà di forme viventi e che provvedono a molti servizi ecosistemici come la protezione e resilienza delle coste, la filtrazione delle acque e la formazione di flussi di energia tra componenti biotiche e abiotiche.

In particolare, la zona intertidale di una spiaggia sabbiosa naturale è un ambiente di transizione tra la spiaggia emersa ed il sandbank perennemente sommerso, dove si svolgono importanti scambi trofici tra organismi terrestri e specie marine. In questo habitat, la macrofauna svolge un ruolo rilevante, non solo per l’attività di bioturbazione dell’habitat, ma anche perché rappresenta una fonte trofica per altri animali, come insetti o uccelli marini.

Le spiagge sono anche luoghi turistici dove sono svolte numerose attività ricreative e dunque rappresentano un settore fondamentale per l'economia di molti Paesi, tra cui l’Italia. A causa della crescente urbanizzazione e dello sfruttamento non sostenibile degli habitat costieri, le spiagge naturali lungo il versante Italiano dell’Adriatico sono quasi del tutto scomparse, con conseguente perdita di biodiversità e di servizi ecosistemici.

Le comunità macrobentoniche della zona intertidale sono particolarmente soggette agli effetti degli impatti antropici sulle spiagge, come la costruzione di stabilimenti balneari, la movimentazione di sedimenti, il trampling e la presenza di barriere frangiflutti.

L’obiettivo principale di questo studio è quello di verificare se spiagge sabbiose sottoposte a diversi livelli di impatto antropico presentino differenti comunità macrobentoniche. Per testare questa prima ipotesi, sono state scelte quattro spiagge della costa adriatica centro-settentrionale: Senigallia e Palombina, entrambe con molte strutture turistiche e barriere frangiflutti, Montemarciano, con barriere frangiflutti ma priva di stabilimenti balneari ed infine la spiaggia

della zona B dell'area marina protetta Torre del Cerrano, priva sia di infrastrutture che di barriere frangiflutti.
Inoltre, al fine di verificare possibili variazioni temporali nella composizione in specie e nell'abbondanza della macrofauna di fondo mobile, i rilievi sono stati condotti in quattro periodi diversi: maggio 2020, settembre 2020, gennaio 2021 ed aprile 2021.
Per ogni sito e periodo sono stati analizzati i nutrienti; inoltre, per valutare il tasso di trampling, sono state contate le persone che transitavano sulla battigia e/o nella zona intertidale nell'intervallo di tempo di un'ora.
I bivalvi rappresentano il taxon maggiormente rappresentato in tutti i siti, seguito dai taxa Amphipoda, Gastropoda, Cumacea, Isopoda e Polichaeta.
L'analisi dei dati mostra che le differenze nei valori di abbondanza sono significative sia per il fattore 'sito' che per il fattore 'periodo'; il confronto a coppie (pairwise) mostra che il sito Torre Cerrano è diverso da tutte le altre spiagge considerate, mentre non ci sono differenze significative tra gli altri tre siti. Tali discrepanze potrebbero essere dovute alle maggiori pressioni antropiche presenti nelle spiagge di Palombina, Senigallia e Montemarciano.
Il primo dei campionamenti coincideva col termine del lungo periodo di lockdown (69 giorni) del 2020 imposto per via della pandemia di Covid19. L'interruzione forzata di tante attività ludiche ed economiche ha avuto conseguenze positive sulla qualità dell'acqua, come riportato nella relazione del Sistema Nazionale per la Protezione dell'Ambiente (SNPA, 2020), e probabilmente anche sull'ambiente di spiaggia.
I risultati di questo studio potranno essere utilizzati per futuri confronti con ricerche effettuate al termine della pandemia.

1. Introduction

1.1 Definition, importance and ecosystem services of sandy beaches

Sandy beaches represent very dynamic environments inhabited by a wide variety of life forms (**Escrivà et al., 2020**; **Defeo and McLachlan, 2005**; **McLachlan, 1983, 2001**; **McLachlan and Defeo, 2018**). Natural sandy beaches provide several ecosystem services, such as balancing transport, storage of sand and increasing coastal protection and resilience **(Short, 1996; Nel et al., 2014; Parlagreco et al., 2019**). Additionally, sandy beaches are very active zones for remineralization resulting from water exchange in the porous and permeable media providing organic matter and electron acceptors, mostly oxygen from seawater (**Mouret et al., 2020; Anschutz et al., 2009; Billerbeck et al., 2006; Charbonnier et al., 2013; Rocha et al., 2009; Santoro, 2010**). Sandy beaches also offer water filtration (**Huettel & Rusch, 2000**), shape energy fluxes between biotic and abiotic components (**Pacheco et al., 2011**), modulate benthic-pelagic exchange into sediments (**Volkenborn et al., 2007**) and allow the establishment of trophic relationships among marine and dune ecosystems (**Defeo et al., 2009**).

Besides their ecological value, beaches represent a hub for social, cultural and economic relationships **(Lozoya et al., 2011; Sardá et al., 2015)**, as well as educational activities **(Fanini et al., 2019; Lucrezi et al., 2019)**. In particular, sandy beaches are used for tourism and recreational activities **(Reis & Rizzo, 2019)**. Consequently, these coastal areas generate revenue and support the economic system by entertaining millions of visitors **(Zielinski et al., 2019)**.

This rapid and intense anthropogenic development has been causing degradation of coastal habitats and loss of ecosystem services **(Defeo et al., 2009)**.

1.2 Macrobenthic communities of sandy bottoms

Marine macrobenthic communities in soft sediments are important providers of ecosystem functioning **(Lam-Gordillo et al., 2020)**. They not only contribute in regulating the fluxes of energy and matter but are also bioindicators of ecosystem health due to their sensitivity to natural and anthropogenic disturbance (**Borja et al., 2000; Reiss et al., 2009; Snelgrove et al., 2014; de Juan et al., 2015; Lefcheck and Duffy, 2015; Lam-Gordillo et al., 2020**). They also play an important role in the phenomenon of bioturbation (**Kristensen et al. 2012).**

Bioturbation is a set of physical effects including mixing, ventilation, oxygenation and irrigation of sediments **(Aller, 1988; Meysman et al., 2005; Huhta, 2007; Kristensen et al. 2012).** The bioturbation activity has positive effects on nutrient cycling **(Aller, 1988; D'Andrea et al., 2009),** substrate permeability **(Huettel & Rusch, 2000),** redistribution of food resources **(Kristensen et al. 2012),** buffering against nutrient enrichment **(Lloret & Marín, 2011)** and benthic-pelagic coupling **(Rhoads, 1973; Aller, 1978; Graf, 1992).** The chemical reactions in general (eg. redox) are positively influenced **(Aller, 1988)** and the depth of the oxic layer is extended over the anoxic one **(Koike & Mukai, 1983; Aller, 1988**): this fact is significant since near-coastal sediments are usually anoxic, except for the upper layer **(Glud, 2008),** and this could be problematic for most aerobic organisms**.**

Macrofauna can indeed act as “ecosystem engineer” **(Jones et al., 1994; Meysman et al., 2006; Kristensen et al. 2012; Passarelli et al., 2014)**, for their role in modification, creation and maintenance of the habitats and their complexity, being able to model the sediment structure (eg. constructing tubes, digging channels and burrows) **(De Smet et al., 2015).**

All these benefits can contribute to the increase or the maintenance of biodiversity, with the additional input from the moderate disturbance resulting from bioturbation **(Widdicombe et al., 2000),** and shaping of the complex environment by macrofaunal communities **(Aller, 1988)**.

Generally, in sandy beaches molluscs, crustaceans and polychaetes are found as dominant groups of macrofauna, often constituting more than 90% of the species and biomass (**Gray and Elliott, 2009; McLachlan and Defeo, 2018; Escrivà et al., 2020)**.

1.3 Biotic interactions of macrobenthic communities

In addition to the phenomenon of bioturbation (**Kristensen et al. 2012)**, macrofaunal communities are also involved in several biotic interactions with other categories of organisms and can regulate the fluxes of energy and of organic matter **(Reiss et al., 2009)**. These relationships can be trophic (predation or parasitism), competitive or cooperative.

A very important relationship is the one that involves macrofauna and meiofauna: macrobenthos usually preys on meiofauna, but meiofauna can in turn affect the structure and abundance of macrofaunal communities (**Giere, 2009**), preying selectively on various groups, eg. larvae or juveniles (**Watzin, 1983**). Also meiofauna play an important role in bioturbation (**Cullen, 1973**) and can influence the settling and recruitment of macrofauna larvae (**Watzin,**

1983), together with competition for food and space, until they occupy the uppermost layers of sediment (**Bell & Coull, 1978**).

Macrofauna can also interact with smaller organisms (like phytoplankton or microorganisms), detritus, particulate organic matter (POM) and dissolved organic matter (DOM): stranded natural detritus or beach wrack (eg. macrophytes and/or wood) can provide a food source and refuge from desiccation to many macrofauna organisms (eg. isopods, mysids, amphipods and crabs) (**Brown & McLachlan, 2006; Defeo et al., 2009; Morton et al., 2015**); amphipods can feed on the film of microorganisms, scraping the substrate surface (**Guerra-García et al., 2014**). Also bivalves consume phytoplankton (eg. diatoms and dinoflagellates) (**Shumway et al., 1987; Burge et al., 2016**), together with bacteria, viruses, protozoans and POM, filtered with seawater (**Gosling, 2003**).

The relationship between macrofauna and megafauna is also important: Macrofauna can be a food source to the organisms belonging to higher trophic levels (**Carvalho et al., 2018**), eg. fishes (**Constable, 1999**) or shorebirds (**Morton et al., 2015; Schlacher et al., 2016**). The biotic interactions of macrobenthic communities of sandy beaches are summarized in **figure 1**.

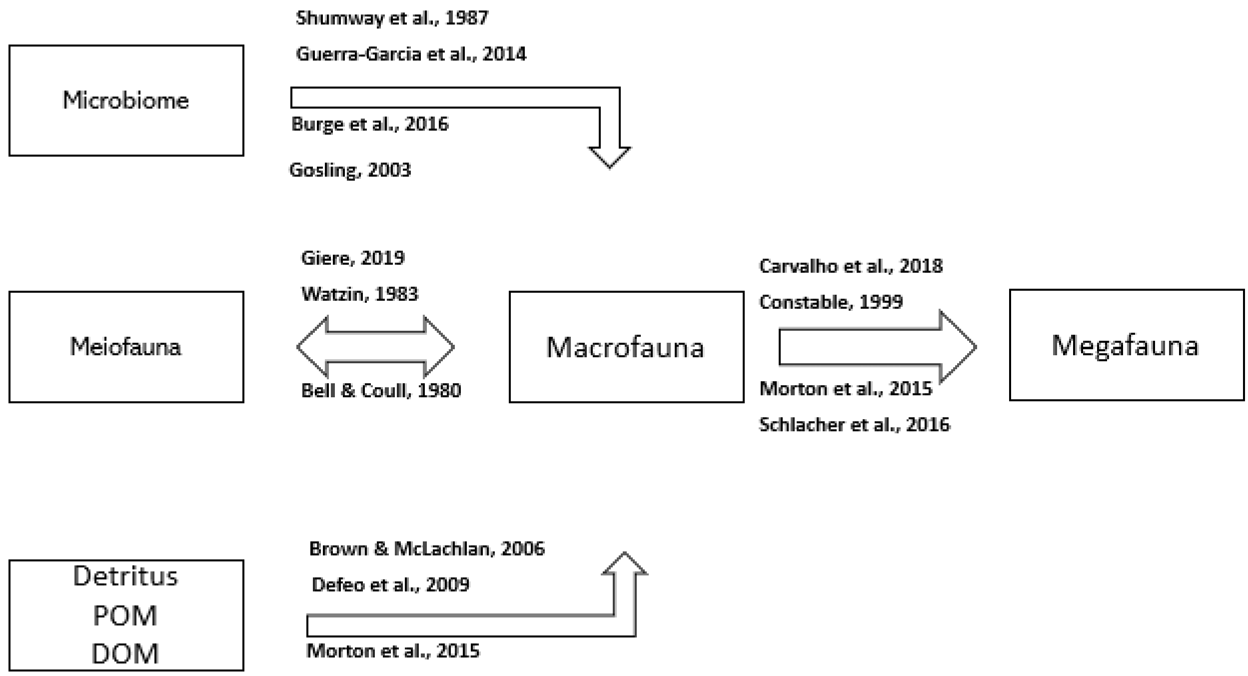


Fig. 1: Trophic relationships of macrobenthic communities of sandy beaches

1.4 Physical and biological factors shaping the biotic component in sandy beaches

Community structure of macrofauna hosted in beach environments, in addition to several biotic interactions with other organisms, is also influenced by various physical and biological factors, acting synergistically or independently, such as sand granulometry, tides, beach exposure (**Wright & Short, 1984; Dexter, 1992; Defeo et al., 2009, Barboza & Defeo, 2015**); nutrients and food supply (**Pearson & Rosenberg, 1987**), organic matter content (**Pearson & Rosenberg, 1978**), hypoxia (**Josefson & Widbom, 1988**), local hydrodynamic conditions (**Schückel et al., 2010**), sediments texture and heterogeneity (**Jayaraj et al., 2008**); competition for limited resources, predation and physical disturbance (**Ólaffson et al., 1994**). The **figure 2** shows two beaches with different physical factors.

Macrofauna living in the intertidal zone (like polychaetes, molluscs and crustaceans) can be particularly vulnerable to beach activities (**Vieira et al., 2012; Bessa et al., 2013; Bessa et al., 2014; Reis & Rizzo, 2019**). However, different species have different sensitivity to disturbance, since certain species have been reported to be more vulnerable than others (**Veloso et al., 2006**).

Fig. 2: **a**, **b**) two beaches a few km away from each other in the same day and time; the beach **a** is very exposed to hydrodynamic conditions and has rough sea while the beach **b** is very protected and has a calm sea

1.5 Anthropogenic factors

As mentioned in the first paragraph, a rapid and intense anthropogenic development has been causing degradation of coastal habitats and loss of ecosystem services **(Defeo et al., 2009**). Besides the vital role of sandy beaches in modern society, the ecological and socio-economic impacts are not addressed properly **(Cardoso et al., 2016**). Human-induced changes in sandy beaches had started nearly two centuries ago and are projected to become even more intense in the coming decades (**Defeo et al., 2009**). These activities have a high potential to modify the habitat features and influence changes in the macrofaunal community structure, leading to loss of biodiversity **(Reyes-Martinez et al., 2015)**

There is an increasing pressure on shoreline due to coastal engineering **(Dafforn et al., 2015; Pioch et al., 2018; Morris et al., 2019)** and several other anthropogenic activities like trampling, mechanical beach cleaning and motor vehicle traffic that impacts the sandy beach environments at different spatial and temporal scale **(Davenport & Davenport, 2006; Schlacher et al., 2007; McLachlan et al., 2013; Machado et al., 2017**). These impacts could affect also ecological traits of these organisms living in these habitats and overall functioning of beach ecosystem (**Thrush et al., 2017**). Breakwater barriers also cause a huge pressure on shoreline **(Martin et al., 2005; Jackson et al., 2015**). These barriers are artificial structures aimed to increase coastal protection and minimize shoreline erosion by waves and currents **(Bertasi et al. 2007)**.

Especially the organisms that live buried in the sand, such as polychaetes, molluscs and crustaceans are directly damaged by beach activities (**Reis & Rizzo, 2019**).

1.6 Aim of the study

In recent decades, there are in general more studies based on understanding the response of macrofaunal communities and populations towards physical disturbances (see for eg. **Defeo & de Alava, 1995; Veloso et al., 2006, 2008, 2010; Lucrezi et al., 2010; Reyes-Martínez et al., 2015; Schlacher et al., 2016; Machado et al., 2017**). Some of the studies are based on single indicator species, while others are focused on the overall macrofaunal community. On the contrary, the studies on the intertidal macrozoobenthic communities in the

Adriatic Sea are few and the comparative assessment of these communities in line with the anthropogenic factors is limited (see **Afghan et al., 2020**). Furthermore, the covid19 pandemic has caused restrictions in the last year that could alter the situation and influence unexpected and unpredictable changes in the community structure. Some studies on the fisheries and on pollution during covid19 pandemic have been conducted in the Adriatic Sea (**Depellegrin et al., 2020**; **Braga et al., 2020**) but none of them is on intertidal macrozoobenthic communities.

The aim of the current study is to characterize and compare the macrozoobenthic communities of 4 Adriatic beaches with different levels of anthropogenic activities and tourism pressure. Additionally, this work may be a starting point to address future researches to assess the possible impact of movement restrictions related to covid19 pandemic on the community structure.

Going further into details, the objectives of the current study are:

O1) To characterize the structure and the dynamics of macrozoobenthic communities of sandy beaches in the intertidal zone of the North-Central Adriatic Sea

O2) To test the hypothesis that beaches subjected to different anthropogenic pressure may have a different macrofaunal assemblage, in terms of abundance and species composition.

O3) To analyse possible over time variations in the macrofaunal assemblage from the chosen sites.

2 Materials and methods

2.1 A brief explanation

To test our hypotheses of Table 1, four sites along the North-Central Adriatic Sea have been chosen: two touristic beaches with breakwater barriers and many facilities (Palombina (P) and Senigallia (S)), a small pebble beach with breakwater barriers but no touristic infrastructure (Montemarciano (M)), although surround by industrial and urban area. The fourth one, is inside the B Zone of the Torre del Cerrano MPA (T); it is a low-impacted beach with a backshore and without breakwater barriers or touristic facilities. Seasonal samplings have been carried out from May 2020 to April 2021, setting up 3 transects during the low tide level in the intertidal zone. Three falcon tube have been taken for seawater and brought to the lab for nutrient analyses.

2.2 Study areas

The selected sites are four and are distributed along the North-Central Adriatic Sea. Three of them (Senigallia, Montemarciano and Palombina) are situated in Marche region and the fourth one (Torre Cerrano) is in Abruzzo region. All the beaches are dissipative (**Defeo and McLachlan, 2005; McLachlan, 1983, 2001; McLachlan and Defeo, 2018; Escrivà et al., 2020**), exhibiting different features and are exposed to different levels of anthropogenic pressure (**Fig. 3**).

Fig. 3: Location of the sites with their pressure

Senigallia's beach (**Fig. 4**) is the site with the highest latitude. It is a sandy beach (**Escrivà et al., 2020**) characterized by tourist infrastructure, urbanized neighbourhood and has a huge flux of people during the summer season. There are more bars, restaurants and playing areas than the other beaches according to our observations, which portray Senigallia as the beach facing maximum anthropogenic pressure. The beach is divided in two parts from the Misa river close to which the harbor is located. The samplings were carried out on the beach located to the north of the river which is characterized by breakwater barriers. The beach located south of the river has no breakwater barriers. The beach is neighboured by residential areas and some commercial buildings.

Fig. 4: Senigallia’s beach; **a**) beach with the breakwater barriers; **b**, **c**) facilities and residential areas; **d**) tourist port

Palombina’s beach (**Fig. 5**) is the northernmost beach of Ancona. It is a sandy beach (**Escrivà et al., 2020**) and has a lot of facilities nearby, including the railway line. This site is also a touristic attraction with lots of people coming especially in summer like Senigallia. The beach has also breakwater barriers installed to counter erosion by dissipating the intensity of waves. An important aspect of this place is the presence of artificial sand barriers to protect the backshore and to prevent beach erosion (in non-swimming season); the sand is accumulated by bulldozers out of swimming season and then it is re-distributed on the beach just before the summer period.

Fig. 5: Palombina's beach; **a**) beach with breakwater barriers; **b**) facilities and residential areas; **c**, **d**) beach with and without artificial sand barriers

Montemarciano's beach (**Fig. 6**) is located a few kilometers to the north of Palombina and it is without any restrictions but no commercial activities and tourist infrastructure is present here. The beach is a small strip of pebbly sand surrounded by a large amount of artificial reefs that make it difficult to reach. Montemarciano is also neighbored by a railway track with a small station and residential area. In addition to this, the site is not a touristic zone therefore it is pretty quiet even in summer. Compared to Palombina, there is more plastic waste, branches and pieces of wood in Montemarciano in our observation but usually it's not because it is generated more here; in fact, there is no beach cleaning activity here like in Palombina. The breakwater barriers are present here as well.

Fig. 6: Montemarciano's beach; a) beach with breakwater barriers; **b**) artificial reefs which surround all the beach; **c**, **d**) trash and pieces of wood all around the artificial reefs

Torre Cerrano's beach (**Fig. 7**) is very different from the other sites, not only because it is the most distant (the other three are at most half an hour away from each other) and with the lowest latitude but also because it is the only beach among the four considered that is a Marine Protected Area. The protected area was established in 2010 having an extension of 7 kilometers along the shore and 3 NM into the sea. The area is divided into B, C and D zones according to the protection level (**Fig. 8**). The samples were taken from B zone next to the main tower which is the area with highest level of protection since no reserve zona A is present here. Torre Cerrano is situated between the municipalities of Silvi and Pineto and it is characterized by the presence of sand dunes and a pinewood. Although there are no commercial touristic facilities, there is a bike trail and a railway line in the backshore. Being a Marine Protected Area, there are no breakwater barriers. Torre Cerrano is the only site with the backshore characterized by agricultural lands, while the other three sites have the backshore comprised of urbanized setup.

Fig. 7: Torre Cerrano's beach; **a**) natural beach with sand dunes; **b**) pinewood surrounds the beach

Fig. 8: Location and zonation of Torre Cerrano MPA

2.3 Sampling design and fieldwork

The sampling activities have been carried out in the various sites in the period of May 2020, September 2020, January 2021 and April 2021. Sampling was conducted in good weather conditions and low tide situation, where the samples were taken from the intertidal zone. Before starting the sampling, the area was observed and photos were taken to record the visual data (presence on the beach of shells, branches, waste and more). Three replicates were taken each time from each sampling site (**Fig. 9**) parallel to the shore at an average distance of 45 meters from a fixed point, where each sample consisted of a 5 meter strip taken through a sampler having a width of 35cm connected to a net. The distance between two sampling points was kept as 5 meters (**Fig. 10**).

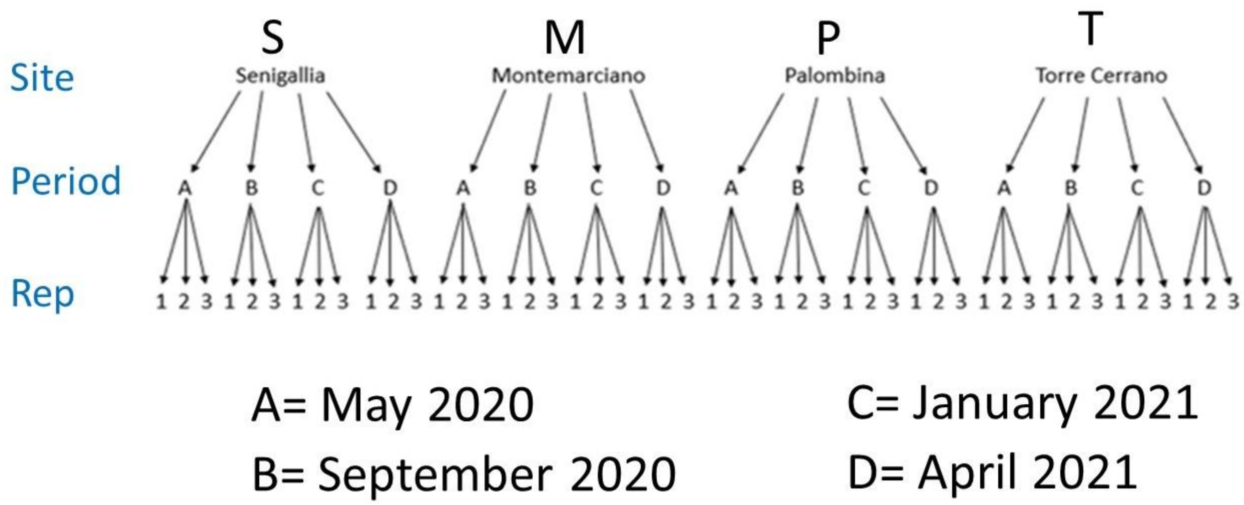
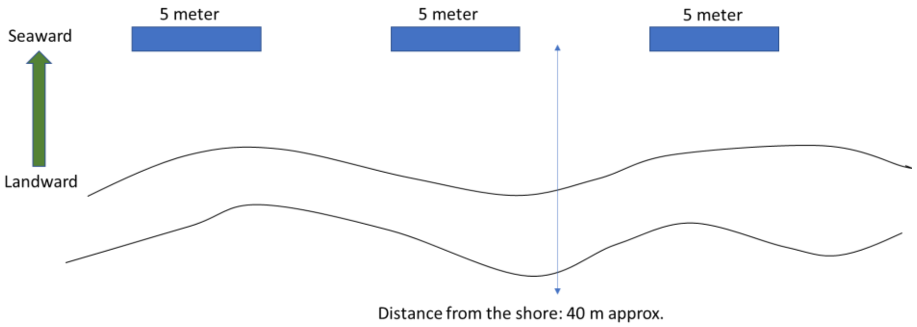


Fig. 9: Schematic representation of the samplings This scheme is valid for both macrofauna and nutrient sample collection.

Fig. 10: Sampling design for all the sites

The sampler was pushed into the intertidal surface for 8 cm (the height of the sampler); it had two different size meshes to hold different things: the mesh with the largest size (10 mm) was used for hold the shell fragments while the one with the smallest size (500 micrometer) was used to hold the organisms (**Fig. 11**).

Figure 11a: Sampling design



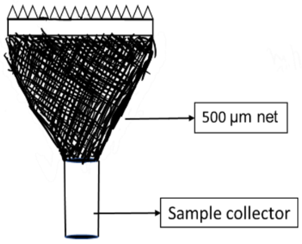


Figure 11b: The sampling gear

Fig.11: **a**) sampling design; **b**) the sampling gear

The shells were placed in closed sachets while the organisms in plastic jars with seawater and alcohol 95% for preservation. At the same time, from each of the sites, three falcon tubes were taken for seawater from 45m away from the shore and immediately placed in a cooler and brought to the lab for nutrient analyses. Additionally, sediment samples were also taken in January and in April from 45m away from the shore. People were counted on hourly basis on each of the sampling day at all the sites. Counting was considered for both the people trampling/running on the beach as well as people having certain activities in the intertidal zone. Activities in the intertidal zone included bathing and playing games mainly.

2.4 Laboratory work

The samples were observed with a stereomicroscope, where the organisms were counted and their densities were calculated (number of items/m3). The organisms have been identified to the lowest possible taxonomic level using a stereomicroscope, an optical microscope and the available literature. The research has been focused especially on living organisms: crustaceans (amphipods, cumaceans, tanaidaceans, isopods and mysids), clams (bivalves and gastropods) and polychaetes. The shell fragments have been weighted and their density has been estimated (g/m3). The water samples were analyzed to find nutrient concentrations (NH3, NO2, NO3, DIN, Ptot, DIP, DOP, silicates/SiO2).

2.5 Environmental data collection

In Addition to the biotic data, abiotic data was also taken including wave heights and periods, maximum wave height and air temperature were taken from different sources (www.meteopesca.com; www.ilmeteo.it/portale/meteo-mare) each day and a monthly average was made for each of them.

2.6 Statistical Analyses.

We have used PRIMER-E, version 7, to process our data and check how much of significance could be found not only among sites but also among seasons. PRIMER; Plymouth Routines In Multivariate Ecological Research, is a statistical package which is a collection of univariate, multivariate and graphical routines for analyzing species data for community ecology. We have tested the significance of the data by running two way ANOSIM considering both the seasons and the sites.

3. Results

Obtained results are shown below in **Figure 12-22**.

The first histogram (Figure 12) shows temporal variations in macrofauna density in the four sites. The macrofauna assemblage is dominated by juveniles of bivalves in all the considered periods. In particular, the sample collected on May 2021 in Palombina is mainly composed of small bivalves (5-10 mm) of the genus *Lentidium* and their quantity is much higher respect the values of other taxa. The bivalves from other sites included *Donax* spp. and *Chamelea gallina*.

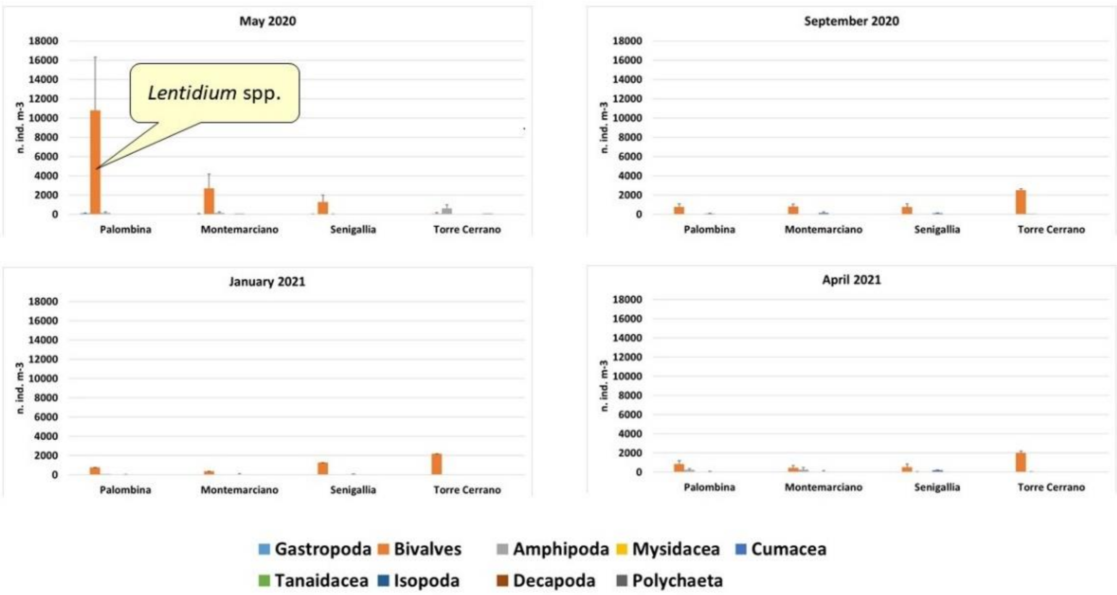


Figure 12. Temporal variations in macrofauna abundance in the considered sites.

Excluding the taxon ‘Bivalves’ from the graphs (Figure 13), it was possible to highlight a trend of less abundant taxa, i. e., Gastropods, Amphipoda, Tanaidacea, Isopoda, Decapoda, Mysidiacea, Cumacea and Polychaeta. There are no substantial differences among the considered periods except for January, when the values of abundance are lower, and for the taxon ‘Amphipoda’, that is particularly abundant at Torre Cerrano in May 2020. Amphipods were mainly represented by mainly by *Bathyporeia guilliamsoniana*, other amphipods present are *Pontocrates altamarinus* and *Echinogammarus stocki* (Figure 18); where the latter is present only in spring in Montemarciano and Torre Cerrano.

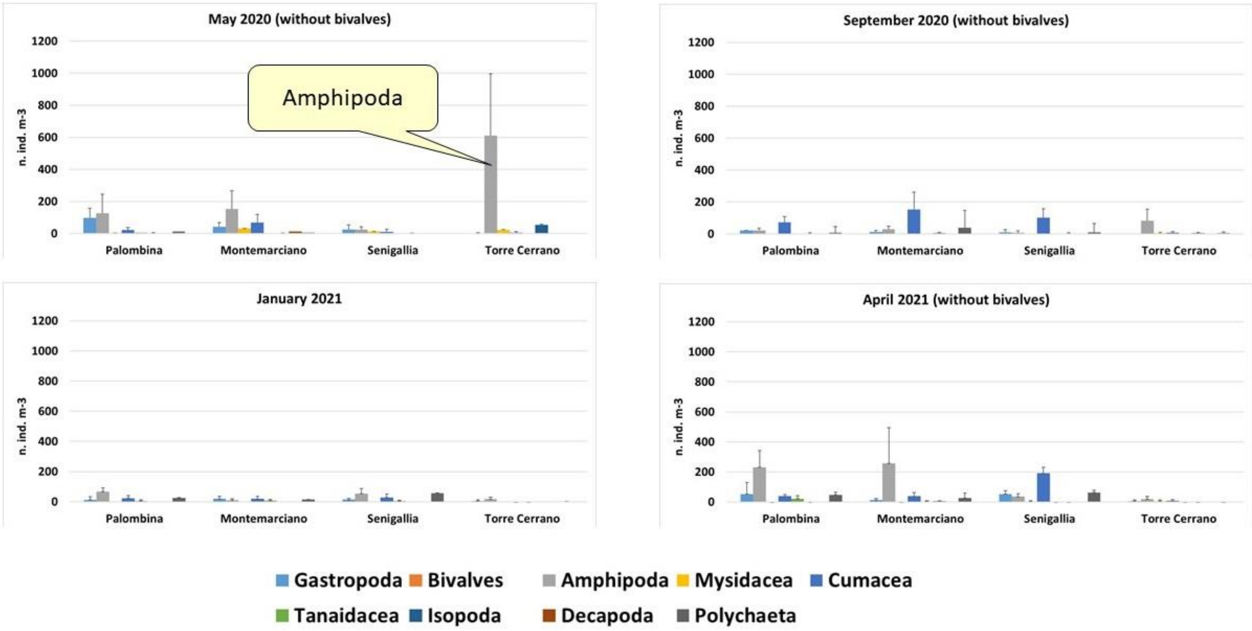


Figure 13. Temporal variations in macrofauna abundance (bivalves excluded) in the considered sites.

Considering the abundance expressed as percent composition of the several taxa (Figures 14, 15), we can clearly see the dominance of bivalves.

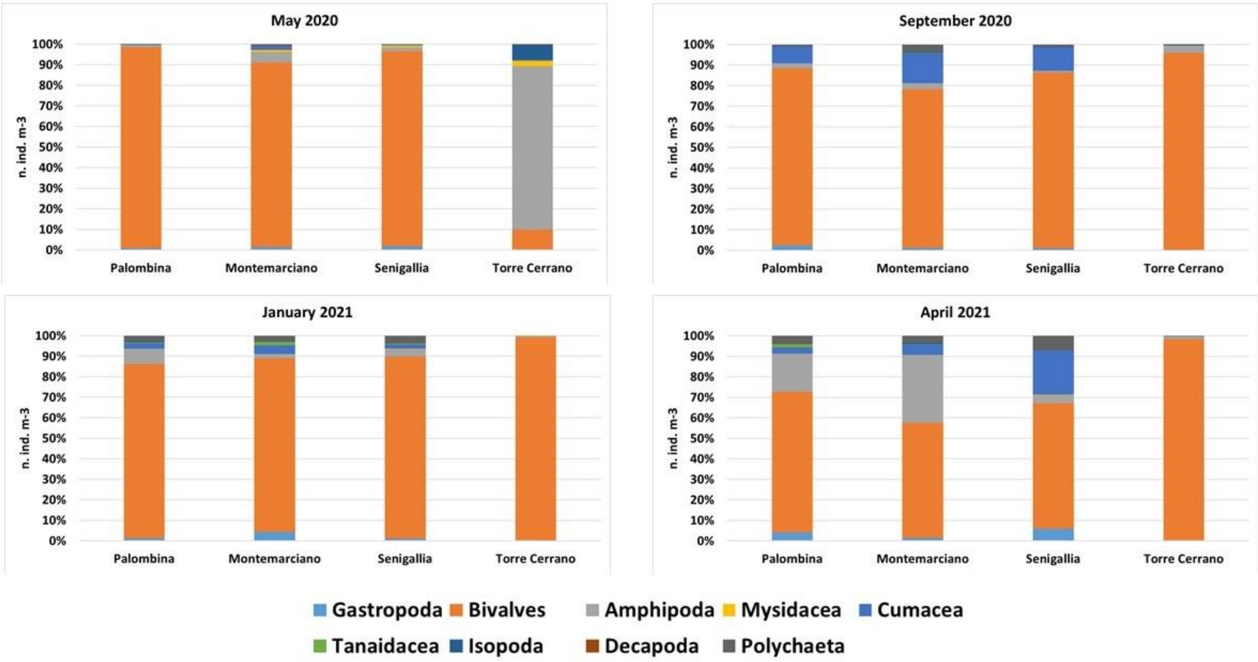
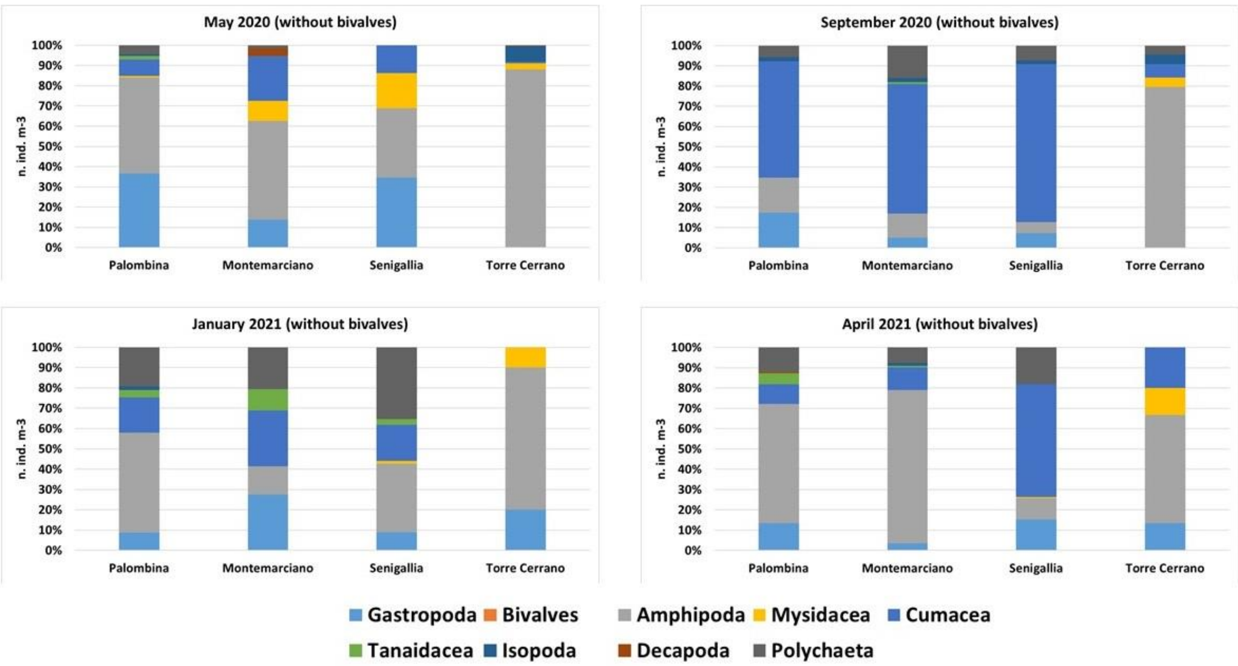


Figure 14. Percent composition of the considered taxa in the four sites.

Excluding bivalves (Figure 15), it is possible to see that on May 2020, Amphipods were present in all the sites and in all the periods; both Amphipoda and Mysidiacea decreased in September 2020, while, Cumacea (represented by *Cumopsis* sp. and *Pseudocuma* sp.) were more common in late summer. Polychaetes are rare at Torre Cerrano, while, the tanaidaceans have not been found in this MPA. The most common polychaete's genus is *Glycera*.



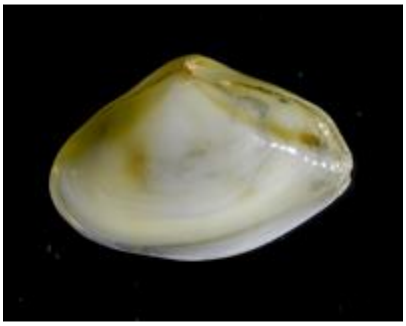


Fig 17: *Lentidium mediterraneum*

Tritia neritea is the most frequently found species of gastropod. This group has been found above all in the Palombina site.

Fig. 18: Some crustaceans found during the samplings

All the taxa are listed in Table 2.

Table 2: List of total taxa

Species/group	Taxon	Senigallia	Montemarciano	Palombina	Torre Cerrano
Apseudopsis sp.	tanaid	x	x	x	
Apseudopsis latreillii	tanaid	x	x		
Bathyporeia guilliamsoniana	amphipod	x	x	x	x
Bittium sp.	gastropod	x	x	x	
Chamelea gallina	bivalve	x	x	x	x
Cumopsis sp.	cumacean	x	x	x	x
Diogenes pugilator	hermit crab		x	x	
Donax trunculus	bivalve	x	x	x	x
Echinogammarus stocki	amphipod		x		x
Eriphia verrucosa	crab		x		
Eurydice sp.	isopod			x	x
Gammaroidea	amphipod	x	x	x	x
Glycera sp.	polychaete	x	x	x	
Idotea sp.	isopod		x	x	
Lentidium mediterraneum	bivalve	x	x	x	x
Mactra stultorum	bivalve			x	
Metapenaeus monoceros	shrimp	x			
Mysinae	mysid	x	x	x	x
Nassariidae	gastropod	x	x	x	x
Ophiuroidea	brittle star	x			
Peringia ulvae	gastropod	x	x	x	
Pontocrates altamarinus	amphipod	x	x	x	x
Pseudocuma sp.	cumacean	x	x	x	x
Sabellidae	polychaete	x			
Scolelepis sp.	polychaete		x		
Sphaeroma serratum	isopod				x
Spionidae	polychaete		x	x	
Tritia neritea	gastropod	x	x	x	
Unknown fragmented polychaete	polychaete	x	x	x	x
Veneridae	bivalve	x	x	x	x

3.2 Shells

The amount of shells per site over the seasons is shown in **Fig. 19.**

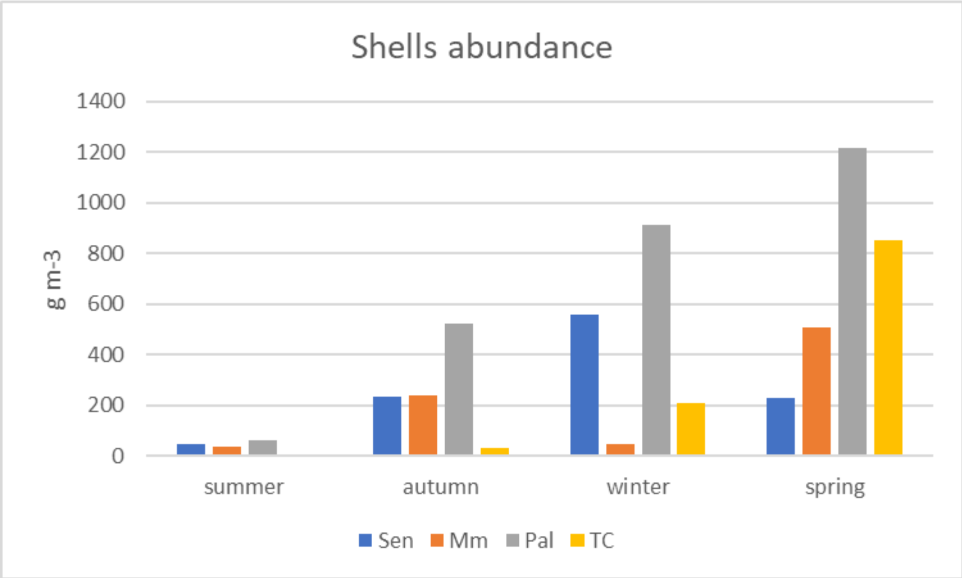


Fig. 19: Abundance of shells per site

The graph represents the total abundance of the shells founded in every site. By shells we mean whole shells or even fragments larger than 1 cm. The most of shells belonged to the Veneridae and Donacidae family in all the sites. The amount of shells could be observed to be very low in may 2020. In the later months, the amount of shells could be seen to be increased in September and onwards. The increase of shells in April 2021 was the maximum however it was mixed in January 2021. In January 2021, Torre Cerrano and Senigallia experienced an increase in the amount of shells while Montemarciano experienced a decrease. Palombina was the only site to have a constant increase in the abundance of shells from one period to another, i.e. June 2020 to April 2021.

3.3 Nutrients

The level of the nutrients is shown in the Figure 20. Nutrients' level was assessed at all the sites through all the four time periods except for Torre Cerrano where the samples couldn't be taken for April 2020 for some technical issue. Additionally the data for April 2021 could also be not presented since the analyses hasn't been completed yet. However, overall Montemarciano could be seen as having the maximum values for Si-SiO2 through almost all the seasons. DIN could be observed to be more or less constant through almost all periods and sites excepts Montemarciano where it has a peak in May 2020. In Torre Cerrano, total phosphorus was found to be high compared to the other sites where the levels were roughly maintained through all the seasons and sites. Dissolved organic phosphorus was found to be high in Torre Cerrano while very less in other sites compared to Torre Cerrano. N-NO3 was found to be fluctuating through periods for all sites except for the period of January 2021 where it was observed to be higher than other months while being roughly consistent.

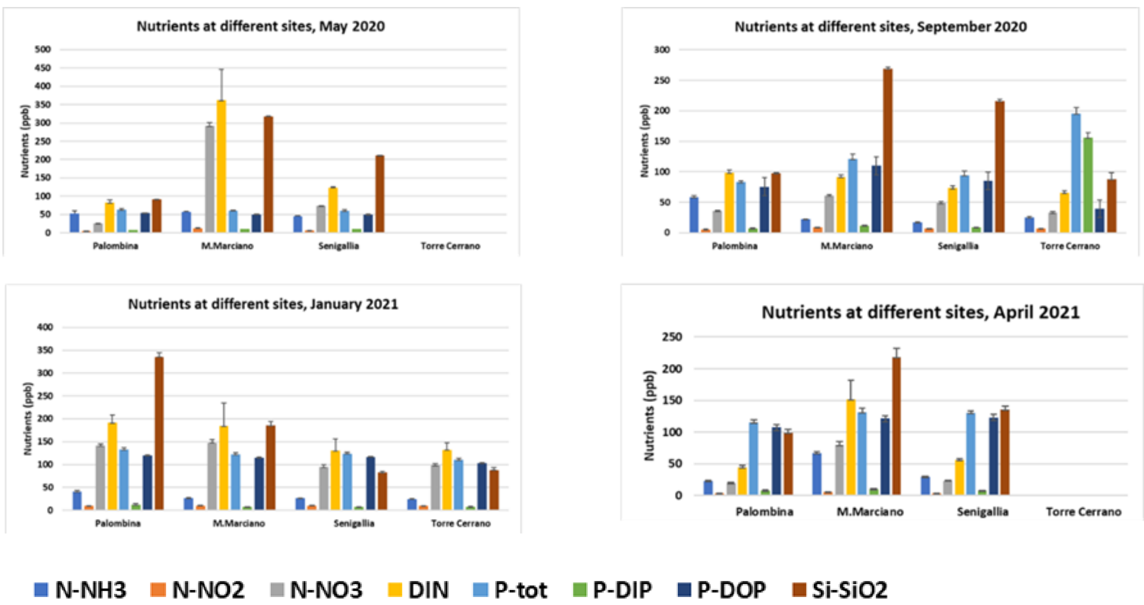


Fig. 20: Nutrients of the four sites during the seasons

3.4 Other data

Other data such as trampling and environmental data were also taken during the samplings. The presence of the people in the sites is shown in **Figure 21**.

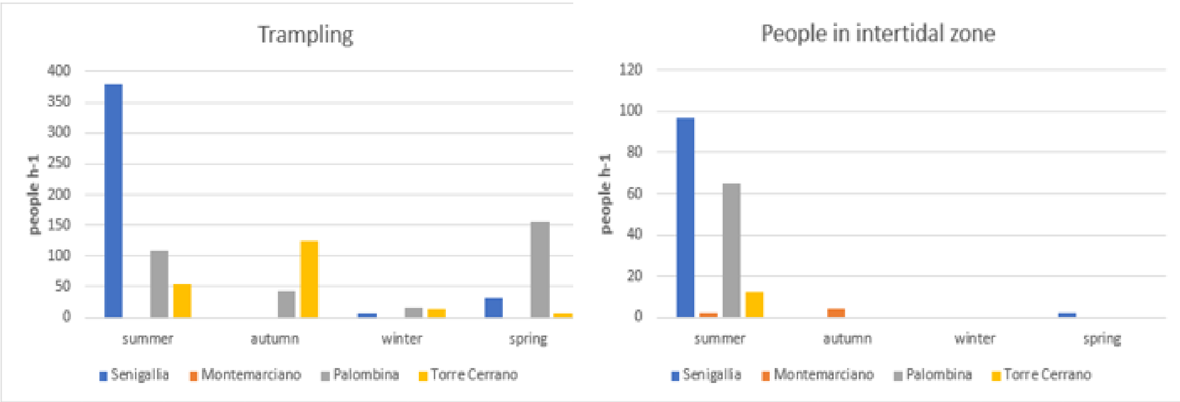


Figure 21: people walking on the shoreline and in the intertidal zone

It can be noted that trampling is very high in Senigallia during the summer season while it is totally absent in Montemarciano in all the year; the minimum trampling for all four sites are in winter. In the intertidal zone there are more people in the summer especially at the sites of Senigallia and Palombina.

Other data that could be interesting are reported in the appendix except for the average sea temperature and the average significant height of the waves which are reported in **Figure 22** (the **Figure 22** represent only Senigallia because the results of the other sites are similar).

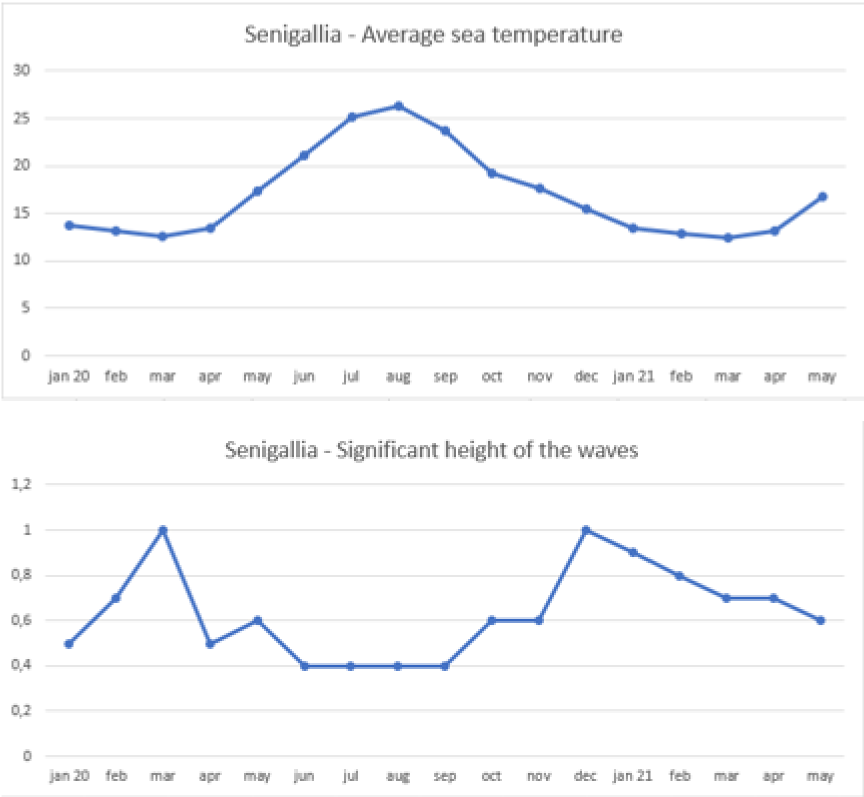


Fig. 22: average sea temperature and the average significant height of Senigallia

The above data is taken from daily checks on meteorological sites (www.meteopesca.com; www.ilmeteo.it/portale/meteo-mare).

Beach facilities and play areas were counted on the beach using google earth and personal visual observations. The beaches of Palombina and Montemarciano were found to be hosting more beach infrastructure. Overall a length of 500 meters along the beach was considered for counting the structures. In Palombina about 51 restaurants and 18 playing areas (including mostly volleyball courts) were found, in Senigallia about 48 restaurants and 25 playing areas were found. The term “about” is used because the number could be one up of one down since at certain points it was difficult to differentiate one facility from another. In Montemarciano and Torre Cerrano no commercial touristic structures and facilities could be seen. However, in Montemarciano there are some private beach cabins which are separated from the beach by a road where frequently cars do park. We are also taking in account the number of visitors at the beaches and that could be somehow related to the beach infrastructure and facilities. A visual representation is given below in **Figure 23**.

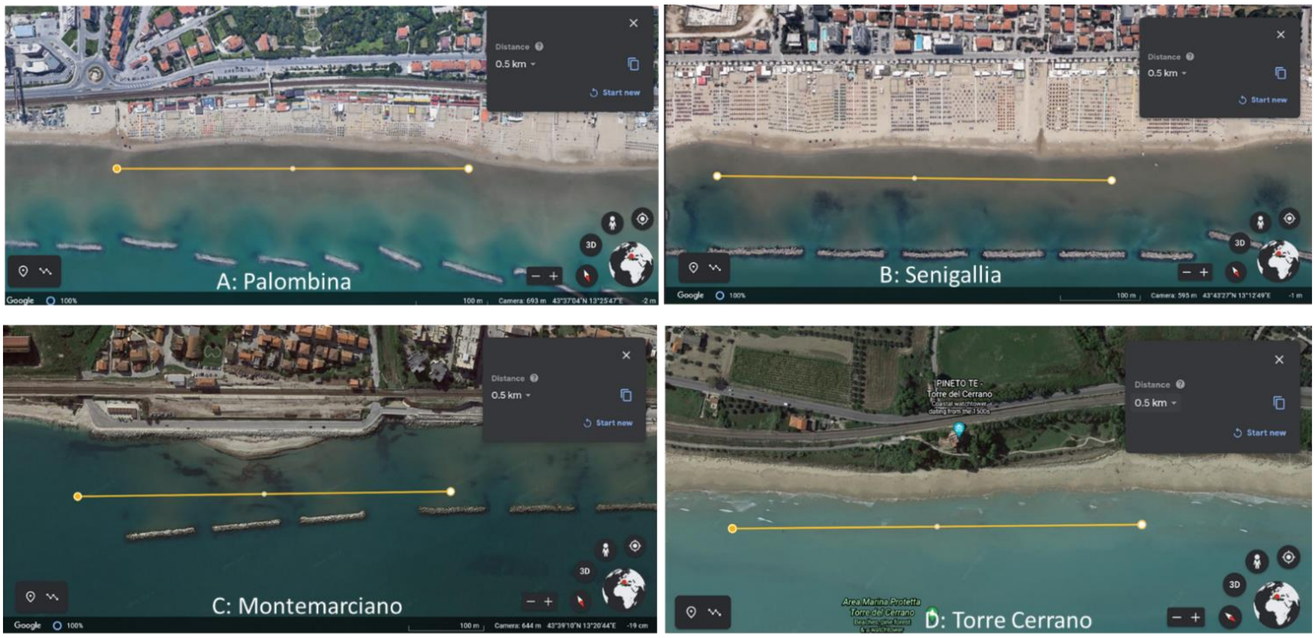
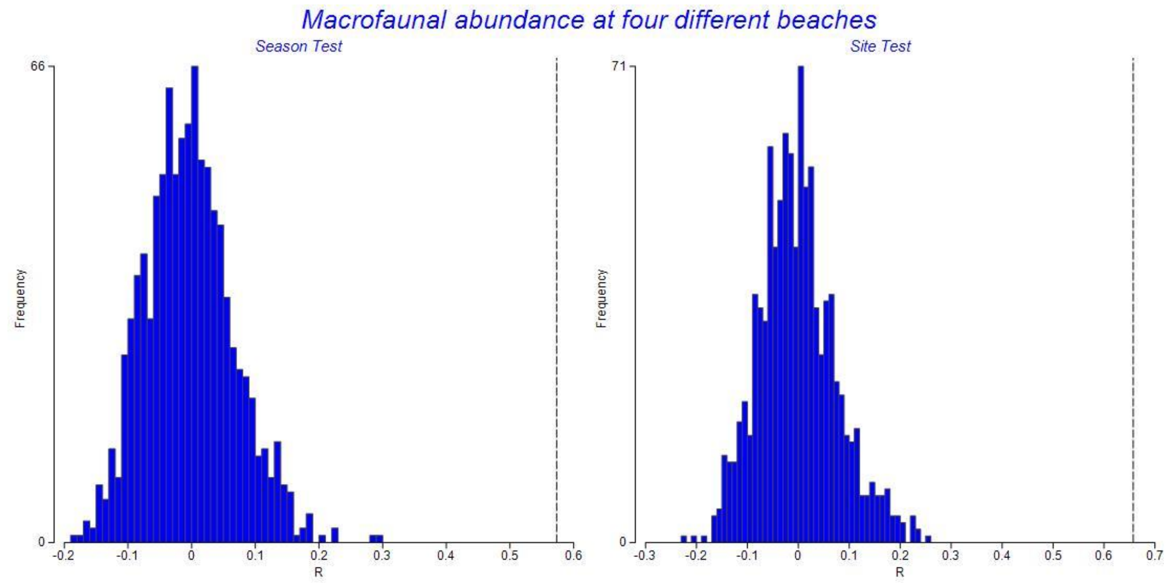


Fig. 23: Facilities present in 500 m strip along the sites.

3.5 Significance of the differences among the data

Significance of the data was tested via using PRIMER-E and running ANOSIM. A two-way ANOSIM test was run on the data which considered both the seasons and the sites.



The season test as shown on the left side of the plot, shows a significant but moderate differences of macrofaunal abindance among the seasons as indicated by the R value. On the right side, the graph shows the macrofaunal differences based on the sites which are slightly stronger than the differences based on seasons. In both the cases, the differences are quite significant.

Table :Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
01-May-20, 01-Sep-20	0.87	0.1	10000	999	0
01-May-20, 01-Jan-21	0.704	0.2	10000	999	1
01-May-20, 01-Apr-21	0.981	0.1	10000	999	0
01-Sep-20, 01-Jan-21	0.407	0.1	10000	999	0

01-Sep-20, 01-Apr-21	0.537	0.4	10000	999	3
01-Jan-21, 01-Apr-21	0.213	9	10000	999	89

The table above shows the pairwise comparison among different seasons. The highest differences could be found between the summer samples and the spring samples i.e. May 2020 and April 2021 respectively. The weakest differences could be found between the samples belonging to January 2021 and April 2021. The table also displays that these differences between January and April 2021 are not significant.

Table : Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
P, M	0.204	5.7	10000	999	56
P, S	0.481	0.8	10000	999	7
P, T	1	0.1	10000	999	0
M, S	0.324	1	10000	999	9
M, T	0.861	0.1	10000	999	0
S, T	0.954	0.1	10000	999	0

The table above shows the pairwise comparison of the sites. The differences among sites could be seen significantly highy from the histogram given earlier but here more interesting comparisons could be observed as overall Montemarciano and Palombina are not significantly different. In another combination, Palombina and Senigallia are also not significantly different regarding macrofaunal abundance but when it comes to Torre Cerrano, it turns out to be the most significantly different site of all. The highest differences could be found between Senigallia and Torre Cerrano which also makes sense as Torre Cerrano is a protected area which Senigallia is the beach with the highest number of restaurants and volleyball courts among the studied sites. On the second ranking, the differences found in Montemarciano, and Torre Cerrano are also significantly high with the differences being quite strong. Interestingly, the differences between Palombina vs Montemarciano, Palombina vs Senigallia and Palombina vs Torre Cerrano were not significantly high. The overall picture portrays that considering the macrofaunal abundance, the protected area Torre Cerrano tends to be the most different one.

Discussion and conclusions

Overall, it could be easily said based on the statistical analyses that all the four sites we studied are significantly different from each other as far as macrofaunal abundance is concerned. In June 2020, Palombina was dominated completely by bivalves, specifically *Lentidium mediterraneum.* the second most abundant beach with macrofauna was Torre Cerrano. Usually, Palombina is one of the beaches with maximum visitors, but the higher number of bivalves could be related to the restrictions due to covid 19 pandemic. The data from Montemarciano was quite interesting since this beach is next to industrial infrastructure and is faced by very limited tourism pressure. The macrofaunal abundance found in Montemarciano was less than that of Palombina and Torre Cerrano but visual observation suggests that this site could potentially host more abundance compared to Palombina and Senigallia. The in Torre Cerrano, the site was dominated by crustaceans, mostly cumaceans and amphipods in June 2020. However, in the following months, the change in trend could be seen as the bivalves prevail. Through all time period studied, it was a very common observation that Torre Cerrano was mostly hosting the Donax family of bivalves, while Palombina was inhibited by Lentidium family. At the same time, depending on periods, but Senigallia and Montemarciano were found to be hosting both the types of bivalves but not like Palombina and Torre Cerrano. However, still the bivalves of Montemarciano comprised most of Donax which gives and impression regarding their sensitivity toward tourism and human activities (since Montemarciano doesn't have commercial tourism activities and is visited by very few people). The gastropods were mostly found in Palombina which mainly belong to *Tritia Neritea*, especially in summer. Polychaetes were not found that commonly in May 2020 samples, however they could be observed in the later months. Shells could be found more frequently in the months after summer 2020, but not that abundant in May 2020. This could also be related to the limited or no dredging activity due to covid 19 restrictions as in literature, its reported that the dredging activity is one of the important reasons for increased amount of shells in beaches. To assess the tourism pressure on the beaches, people were counted on hourly bases which suggests that Senigallia is the most frequented beach of all the considered beaches, followed by Palombina. Trampling and bathing/playing in the intertidal zone were main activities observed in intertidal zone. Montemarciano was the least visited beach where the trampling activity was found to be very rare since it has a road nearby, which is used for walks and jogging instead of the beach. Additionally, Senigallia and Palombina were found to have more tourism infrastructures and playing areas such as basketball and tennis courts. In Montemarciano and Torre Cerrano, no commercial beach infrastructure could be found except the beach umbrellas for personal use. The statistical analyses suggests that the areas are significantly different than each other with the difference being moderate on seasonal bases and strong on site-to-site bases. The Torre Cerrano was found to be the beach with more different characteristics as compared to the other three. The data we found to compare the sites is really interesting and portray a very good picture, however it cant be said as the last word since data could potentially change in normal years since this past year was hit by lock downs time to time which had kept a limit on public to visit the beaches. Further work could be done to explore the research problem further and possibly, the future data could be compared to ours or other work during lock down to portray the macrofaunal abundance of these sites in different scenarios.

Fig. 24: some waste found at various sites; **a**) Montemarciano; **b**) Palombina; **c**) Senigallia; **d**) Torre Cerrano

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