COMPARATIVE STUDY OF MACROZOOBENTHIC COMMUNITIES FROM DIFFERENT SANDY BEACHES OF ADRIATIC SEA WITH DIFFERENT LEVELS OF HUMAN IMPACT

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In the name of those,

who have the courage to go against the winds.

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15 May, 2022 **Afghan**

Contents

Introduction

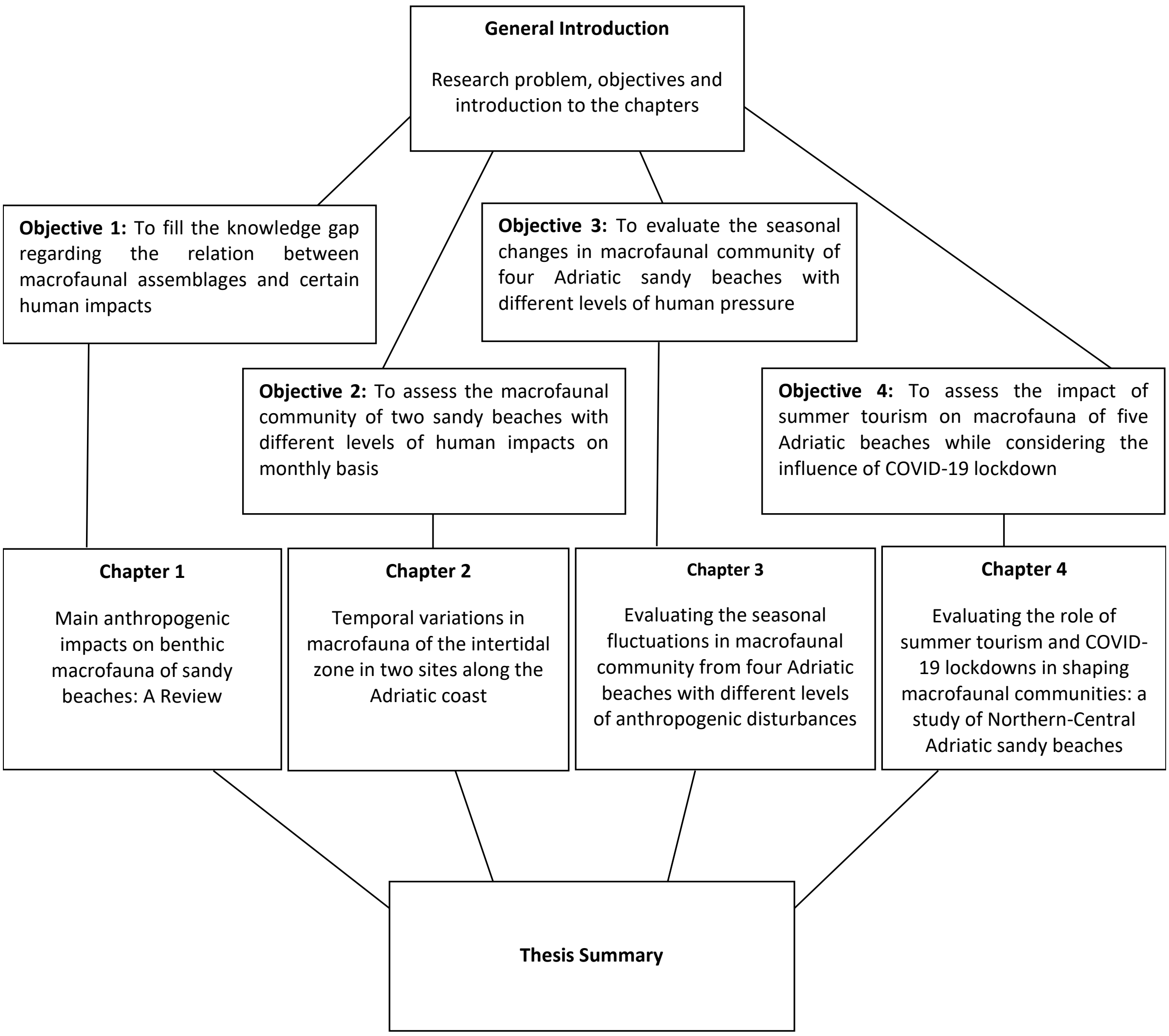
Sandy beaches are the ecosystems that are more frequently accessed by human for their recreational characteristics and ability to create economic opportunities globally (Amaral et al. 2016). Because of these features and advantages, disturbances related to human activities on sandy beaches are constantly increasing and making the ecosystem more susceptible and prone to anthropogenic pressure (Park and Kim, 2021). These cumulative impacts are in fact resulting from the demand for ocean space and resources as the human population and migration towards coastal areas increase (Halpernet al., 2015). The populations near the coasts are growing faster at a higher rate than anywhere where the pressure is constantly escalating on some vulnerable habitats like sandy beaches (Stelling-Wood et al., 2016).

Characterized by their sand, wave and tidal regimes, sandy beaches also provide habitat to the diversity of different fauna especially burrowing invertebrates, molluscs, crustaceans, polychaete worms, encompassing scavengers, predators and filter-feeders (Defeo et al., 2009). The biodiversity, habitats and other ecosystem services are disturbed by impacts such as dredging activities (Žilinskas et al., 2020), artificial barriers (Munari et al., 2011), trampling (Reyes-Martínez et al., 2018), mechanical beach cleaning (Griffin et al., 2018), playing games on the beach, release of chemicals into the sea, offroad vehicles on the beach etc. All these factors cause modifications in the sandy coastal ecosystems one way or the other, affecting their resilience. Beaches in urbanized setup are actually trapped between human impacts on terrestrial side, and climate change impacts on the ocean side (Schlacher et al, 2007). The beaches covered in this study are mainly impacted by trampling, mechanical beach cleaning, presence of artificial breakwater barriers, touristic infrastructure such as restaurants and playing areas. In general, there are numerous studies on the impacts of man-made disturbances in different regions globally, however, there are less studies available focusing on these impacts in the Adriatic Sea.

At the same time, certain recent events such as the COVID-19 lockdowns induced a break in the exploitation of the beach by visitors which possibly could influence the abundances of different organisms. In several regions around the world, an increase in abundance of coastal organisms has been reported resulting from COVID-19 lockdowns (Ormaza-Gonzaìlez et al., 2021). It is worth mentioning here that a certain period with restrictions on ecosystem exploitation at global and regional scale is very important to investigate. Although such events could occur rarely, they possibly have the potential to induce changes in the ecosystem. To fill all the mentioned knowledge gaps regarding the influence of benthic macrofauna, the following objectives were set for the project.

1. To contribute filling the knowledge gap regarding the relation between macrofaunal assemblages and certain human impacts in the Adriatic Sea
2. To assess the macrofaunal community of two sandy beaches with different levels of human impacts on monthly basis
3. To evaluate the seasonal changes in macrofaunal community of four Adriatic sandy beaches with different levels of human pressure
4. To assess the impact of summer tourism on macrofauna of five Adriatic beaches while considering the influence of COVID-19 lockdown

In order to achieve the objectives mentioned above, the thesis was structured as below.



The first part of the project was to write a systematic review about the major impacts on sandy beaches (Chapter 1). From literature, three main impacts were selected which were less studied for their influence on macrofaunal taxa. The three impacts were trampling, mechanical beach cleaning and the presence of breakwater barriers. Certain gaps were identified at this stage and the project was developed to do a comparative analysis of macrozoobenthic communities of different sandy beaches with different levels of human impacts. The first phase (Chapter 2) of the project was conducted on monthly basis at two sites (Palombina and Torre Cerrano) for one year to understand the response of the taxa to disturbances as well as the intensity of beach use through different months. To explore the topic further, the scope of the study was extended as this phase included four beaches (Palombina, Montemarciano, Senigallia and Torre Cerrano) with different infrastructure and surrounding neighbourhood. Instead of months, four seasons were considered to portray the seasonal changes in macrofaunal community responding to the beach activities (Chapter 3). The third phase of the study (Chapter 4) included five beaches (Palombina, Montemarciano, Senigallia, Senigallia 2 and Torre Cerrano) but the study focused on different stages of summer season i.e., before-summer, mid-summer and after-summer, since summer is the period when the beaches receive maximum pressure. Senigallia and Senigallia 2 are the sites in the same locality but the later is without breakwater barriers. Meanwhile, the period before the summer season was also involving COVID-19 related restrictions and their role in shaping the macrofaunal community was considered.

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Chapter 1: Main Anthropogenic Impacts on Benthic Macrofauna of Sandy Beaches: A Review

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Abstract: Sandy beaches provide several ecosystem services such as coastal protection and resilience, water filtration and nutrient mineralization. Beaches also represent a hub for social, cultural and economic relationships as well as educational activities. Increasing urbanization, recreational activities and mechanical beach cleaning represent major anthropogenic disturbances on sandy beaches leading to loss of biodiversity as well as good and services. Information about the impacts of anthropogenic pressures on benthic macrofaunal communities could be useful to assess the environmental status of sandy beaches and to promote a sustainable use of beach ecosystem. Here, scientific articles about three major anthropogenic impacts on sandy beach macrofauna were reviewed to provide the state of knowledge about these impacts, to highlight gaps, to supply considerations about the methodologies and the used indicators and to give insights for future studies. The stressors considered in our review are: 1) trampling, 2) breakwater barriers, 3) mechanical beach cleaning. This review underlined that there are few studies regarding individual human disturbances on sandy beach macrofauna and specifically, there is a lack of sufficient indicator species for the assessment of such stressors. Similarly, the researches have covered specific regions, highlighting the need for such studies in other parts of the world. In particular, the impacts of breakwater barriers on surrounding communities have been found to be given less attention in the literature and there is enough that could be explored.

Keywords: sandy beach; macrofauna; macrobenthos; benthic; human impacts; beach cleaning; trampling; breakwaters

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1. Introduction

Natural sandy beaches provide key ecosystem services such as balancing transport, storage of sand, increasing coastal protection and resilience [1–3]. Sandy beaches also o er water filtration [4], shape energy fluxes between biotic and abiotic components [5], modulate bentho-pelagic exchange into sediments [6] and allow the establishment of trophic relationships among marine and dune ecosystems [7]. Besides their ecological value, beaches represent a hub for social, cultural and economic relationships [8,9] as well as educational activities [10,11].

Due to valuable goods and services of beaches and their vulnerability to erosion, flooding and overexploitation of natural capital, the European Commission launched a proposal for a Directive for maritime spatial planning and integrated coastal management [12], in order to promote "the sustainable growth of maritime and coastal activities and the sustainable use of coastal and marine resources". Sandy beaches are used mainly for tourism and recreational activities [13]. These coastal areas generate revenue and support the economic system by entertaining millions of visitors [14]; however, a rapid and intense anthropogenic development has been causing degradation of coastal habitats and loss of ecosystem services [7].

There is an increasing pressure on shoreline due to coastal engineering [15–17], and several other anthropogenic activities like trampling, mechanical beach cleaning and motor vehicle tra c that impact the sandy beach environments at different spatial and temporal scale [18–21]. Besides the vital role of sandy beaches in modern society, the ecological and socioeconomic impacts are not investigated appropriately [22]. Anthropogenic changes in sandy beaches had been there since long time and are projected to become even more intense in coming decades [7]. These activities could potentially alter habitat features and macrofaunal community structure resulting in the loss of biodiversity [23], loss of ecosystem services and difficulties in facing climate crisis. Benthic macrofauna living in soft substrates plays a pivotal role in particles reworking [24,25], nutrient cycling [24,26] and serving as food for other organisms [27,28].

Ecological status of submerged sandy beaches can be assessed by analyzing the composition and abundance of macrofauna and through the elaboration of biotic indexes [29,30]; however, these indexes put in evidence the sensitivity to organic enrichment and they do not consider effects of other forms of impact. Macrofauna living in the intertidal zone can be particularly vulnerable to beach activities [13,31–33]. In recent decades, there are some studies based on understanding the response of macrofaunal communities and populations towards physical disturbances [34–36].

Community structure of macro-, meio- and microfauna hosted in beach environments is influenced by the interaction of several physico-chemical factors such as sand granulometry, mineralogy and tides or beach exposure [7,37–39], suggesting that alteration of natural beach dynamics due to human pressure could affect ecological traits of these organisms and overall functioning of beach ecosystem [40].

The main objective of our study is to review researches on three kinds of stressors affecting the integrity of sandy beach macrofaunal communities: 1) trampling, 2) breakwaters, 2) mechanical beach cleaning. Moreover, we showed temporal trends of these studies and their geographical distribution. We have focused on those relevant impacts on beach macrofauna which are scarcely considered by scientific community. In particular, we did not evaluate the studies regarding impact of beach nourishment on beach macrofaunal communities because this topic has been the subject of numerous studies and reviews [41–45].

2. Materials and Methods

An extensive bibliographic search was conducted on Elsevier's Scopus database using keywords in the search engine selecting the option "Article title, Abstract, Keywords" limiting it to the cut-o date of 1 September 2019 (Figure 1). The document type chosen in search strategy included 'article', 'congress paper', and 'review'. In the first step, the keywords searched were, namely: 'trampling', 'breakwater or coastal defense structure' and 'mechanical beach cleaning'. In order to look for studies on breakwaters, we also added the keyword 'coastal defense structure' as initial studies had addressed the breakwaters as 'coastal defence structures'. Since coastal defense structures also include other structures such as seawalls and jetties etc., we chose the articles that were based on breakwaters only.

In the second step, the results obtained were refined by searching 'macrofauna OR macrobenthos' within the obtained articles. The higher number of articles in the first step was because the articles were not restricted to biology or ecology and rather, related to all disciplines. In the third step, the articles were further screened by reading the titles and abstracts followed by the exclusion of the articles that were irrelevant to our objective. In the fourth and last step, the remaining articles were thoroughly screened and the ones eligible for our study were considered. Important criteria for considering articles was that the articles must be in English language, accessible through online databases and the article or part of article must be clearly addressing the impacts of at least one of the three mentioned stressors on macrofaunal communities (i.e., trampling, breakwater barriers and mechanical beach cleaning). Moreover, it was made sure that all the scientific articles we have considered, were focused on sandy beaches only. The articles relating to rocky beaches or reefs were excluded. Additionally, the references in the selected papers were used to find articles that we could have missed in our searching strategy. A stepwise sketch of the searching strategy is given as Figure 1.

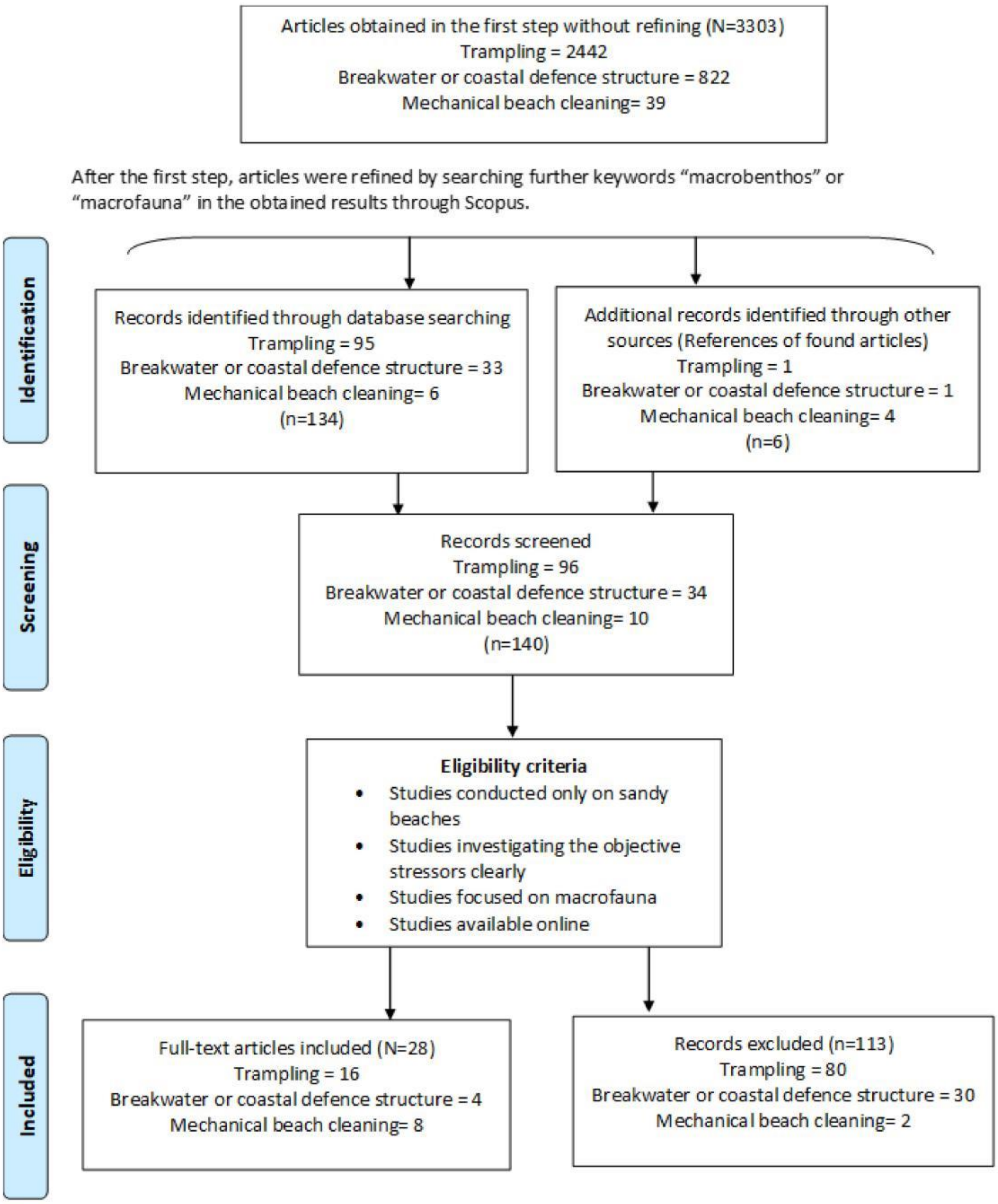

Figure 1. A flow chart illustrating steps for obtaining scientific articles in English language about impacts on sandy beach communities from Scopus search engine.

The studies considered were assessed based on the type of methods utilized, the use of indicators, geographical location, sampling equipment and number of beaches covered by each study. Additionally, temporal distribution of studies as well as period of the year in which the studies were conducted were considered in the assessment process.

3. Results

Initially, 2442 articles were found for 'trampling', 822 for 'breakwater or coastal defense structure', and 39 for 'mechanical beach cleaning'. After refining the articles by inducting further keywords 'macrofauna OR macrobenthos' in the obtained results, the number of articles was 95 for trampling, 33 for breakwaters and 4 for mechanical beach cleaning. The articles were further refined by reading at least the title and abstract. The articles that were not in relevance with our objective were removed. Additionally, 1 article was found for trampling, 1 for breakwater and 4 for mechanical beach cleaning by searching through references of obtained articles. In the end, 28 articles were included where 16 were on trampling, 4 on breakwater and 8 on mechanical beach cleaning. The following paragraphs will illustrate temporal and geographical distributions of the considered papers; all articles details (publication year, Authors, title, DOI, applied methodologies, indicator species and sampling frequency) are available in Table S1.

3.1. Temporal Distribution of the Selected Studies

Figure 2 shows the studies on considered three forms of impact on macrofauna through the years. The oldest two articles found on the trampling impacts on sandy beach macrofauna were published before 2000 (1997 and 1998, citations in Table S1). Afterwards, no articles could be found until 2006, while six articles were published between 2006 and 2010, four between 2011–15 and further four between 2016 and 2019.

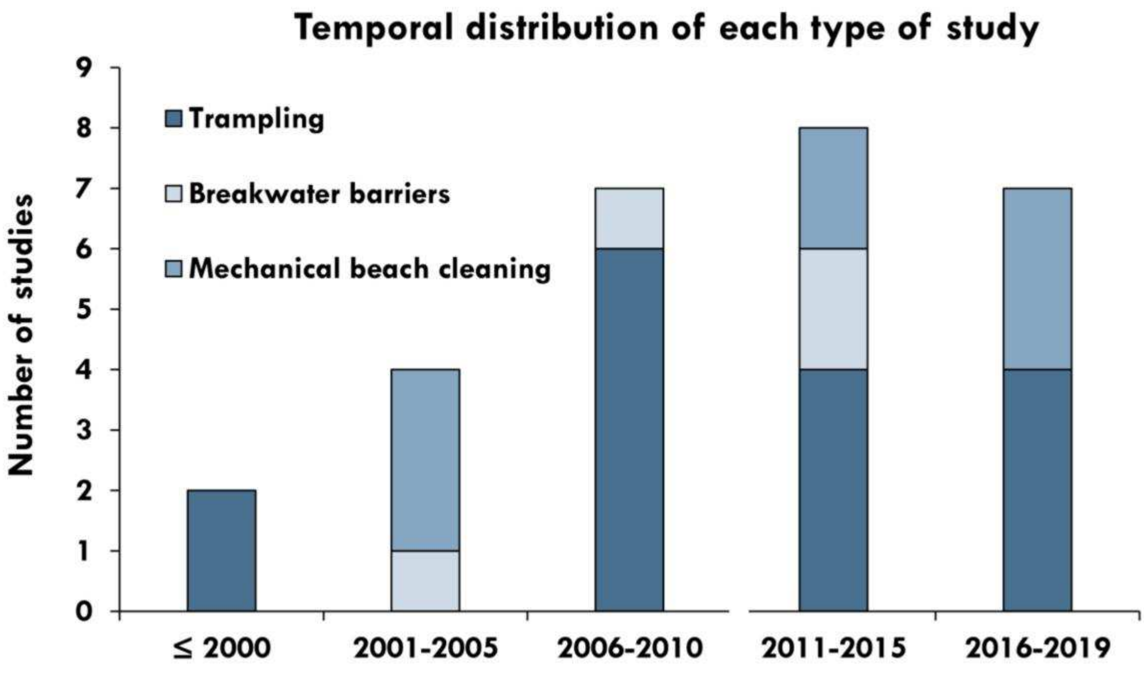

Figure 2. Histogram showing temporal distribution of studies about trampling, breakwater barriers, mechanical beach cleaning impacts.

Out of the four studies on the impacts of breakwaters, the first one was published in 2001–2005, the second in 2006–2010 and the other two in 2011–2015. The third anthropogenic impact considered was ‘mechanical beach cleaning’, for which eight articles were found in relevance with the objective. The first three articles were found between 2001 and 2005, two in 2011–2015, and three in 2016–2019.

3.2. Geographical Distribution of Studies

Articles covering more than one country are counted for all the countries that were included in the study. Out of 28 studies, most of the papers regarding all the three stressors were conducted in four countries i.e., Italy (6), Australia (5), Brazil (5) and Spain (4), while the remaining studies were performed in South Africa, Scotland, Sweden (2), England, Greece, South Korea, the Netherlands and the USA (1) (Figure 3A).

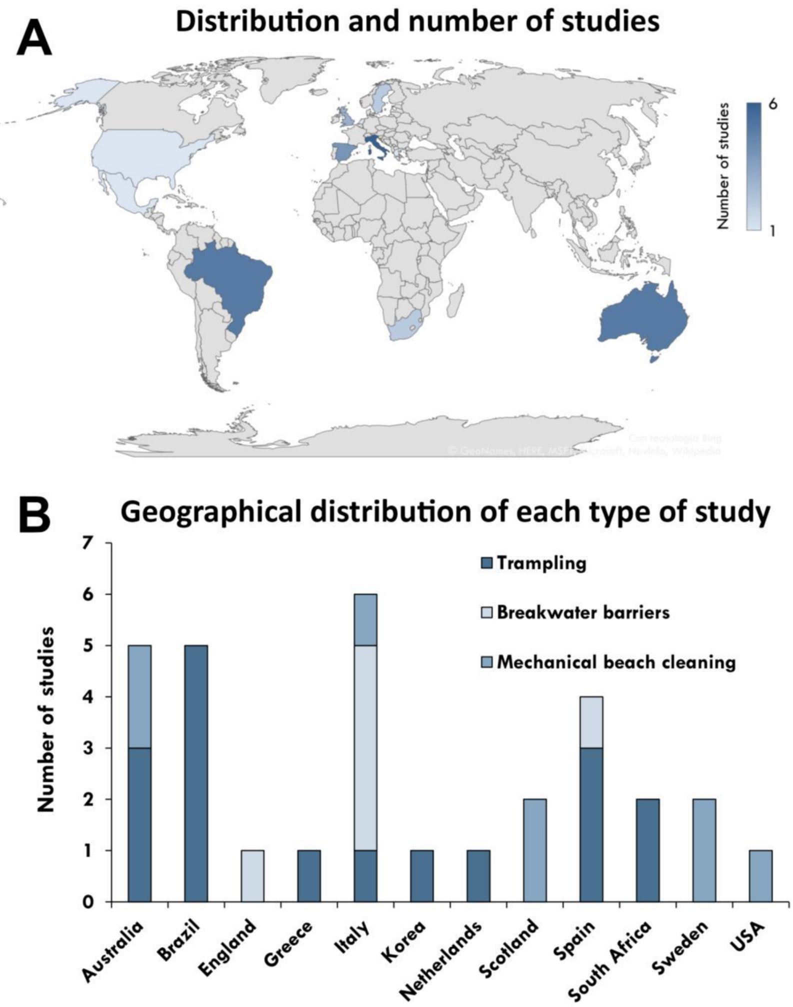

Figure 3: (A) Map of distribution and volume of all studies on the three considered forms of impact (B) Geographical distribution of studies for each impact typology.

Concerning the typology of the gathered studies (Figure 3B), studies on trampling impacts were carried out in Brazil (5/5), Australia (3/5) and Spain (3/4); one of these studies (Table S1) was jointly conducted in Brazil and Spain. Articles about breakwaters were published in Italy (4), Spain (1) and England (1) only. Concerning the mechanical beach cleaning impacts, there are two articles from Australia, Scotland and Sweden and one from Italy. Italy was the only country were all the three kinds of impact were studied.

In total, seven articles could be found covering the Mediterranean basin, where four of the articles focused on the impacts of breakwater barriers. In the remaining, two articles were about trampling, while only one was about mechanical beach cleaning impacts on macrofauna. Regarding trampling impacts, one study was carried out along the Italian side of the Tyrrhenian coast (Western Mediterranean), while the one in Greece was performed in the South Aegean Sea (Eastern Mediterranean). Among the studies on breakwater barriers' impacts, one was found on Tuscan coast (Tyrrhenian Sea, Italy), two on the Italian Adriatic coast and one covering together England, Spain and Italy. The only study about mechanical beach cleaning was conducted in Italy (Tuscany) on the Tyrrhenian coast.

3.3. Methods of Study and Considered Taxa

In most of the considered studies, the sampling activities were conducted two to six times where the sampling was either seasonal or following Before-After impact design. Additionally, one study had 13 sampling sessions while another had 15. Five of the studies had used experimental stressors before sampling, which could explain the sudden response of target species towards the impacts, but for long-term impacts, seasonal or other long-term sampling could be more appropriate. In case of before and after impact sampling, the time period of the year could also be potentially crucial. While other sampling gears used have more reliability, methods such as burrow counting for crabs, pitfall traps or arthropod traps may not be the most appropriate tools to evaluate the aforementioned impacts.

The methods used by studies regarding trampling impacts on macrofauna mainly included i) trapping crustaceans (3), ii) direct on-site counting of organisms/burrows (3) and iii) collection of sediments with macrofauna from a delimited squared area (5) or by using cylindrical corers (5). While most of the studies (9) covered the overall macrofaunal community, crustaceans (amphipods, crabs) were found to be used as indicator species in six studies; only one study considered a gastropod species (Tritia neritea).

In case of breakwater barriers (four studies, Table S1), all the researches employed Van Veen grabs as the main equipment for collection of benthic macrofauna. In one study, PVC corers were used as well at some sites. The Van Veen grabs used were ranging between 270 cm^2 to 600 cm^2, while all the studies used overall macrofaunal community as indicator. The criteria considered by different studies to evaluate the impact of breakwaters were the distance from breakwater, landward/seaward side of the breakwater or the position of sampling site from breakwater.

In case of mechanical beach cleaning impacts (eight studies, Table S1), the sampling materials and methods used by researchers were corers (three articles), counting burrows/organisms (3) and an aspirator (1). The corers used in all studies were having a height from 10 to 20 cm, while one article [14] among the total eight was a review paper. Two studies had used a single indicator species i.e., ghost crab (Ocypode cordimanus) and sand hopper (Talitrus saltator), while the rest of the studies were based on overall macrofaunal community (Table S1). Among the organisms most affected by mentioned forms of impact (Table S1), the highest number of species belonged to polychaetes, bivalves (in particular, the Cardida order), amphipods and isopods (31, 21, 17, and 7, respectively; Figure 4A,B).

Overall, 42 (46%) species considered in the reviewed studies were found to be affected by trampling, 37 (41%) by breakwaters and 12 (13%) by mechanical beach cleaning (Table S1). While amphipods were especially used for assessing the impact of trampling, bivalves were mainly considered for the beach cleaning impact. Only one species has been used for assessing all the three forms of impact (the polychaete Hediste diversicolor); while, the bivalve Limecola baltica (order Cardida) and the amphipod Talitrus saltator were considered both for trampling and mechanical beach cleaning.

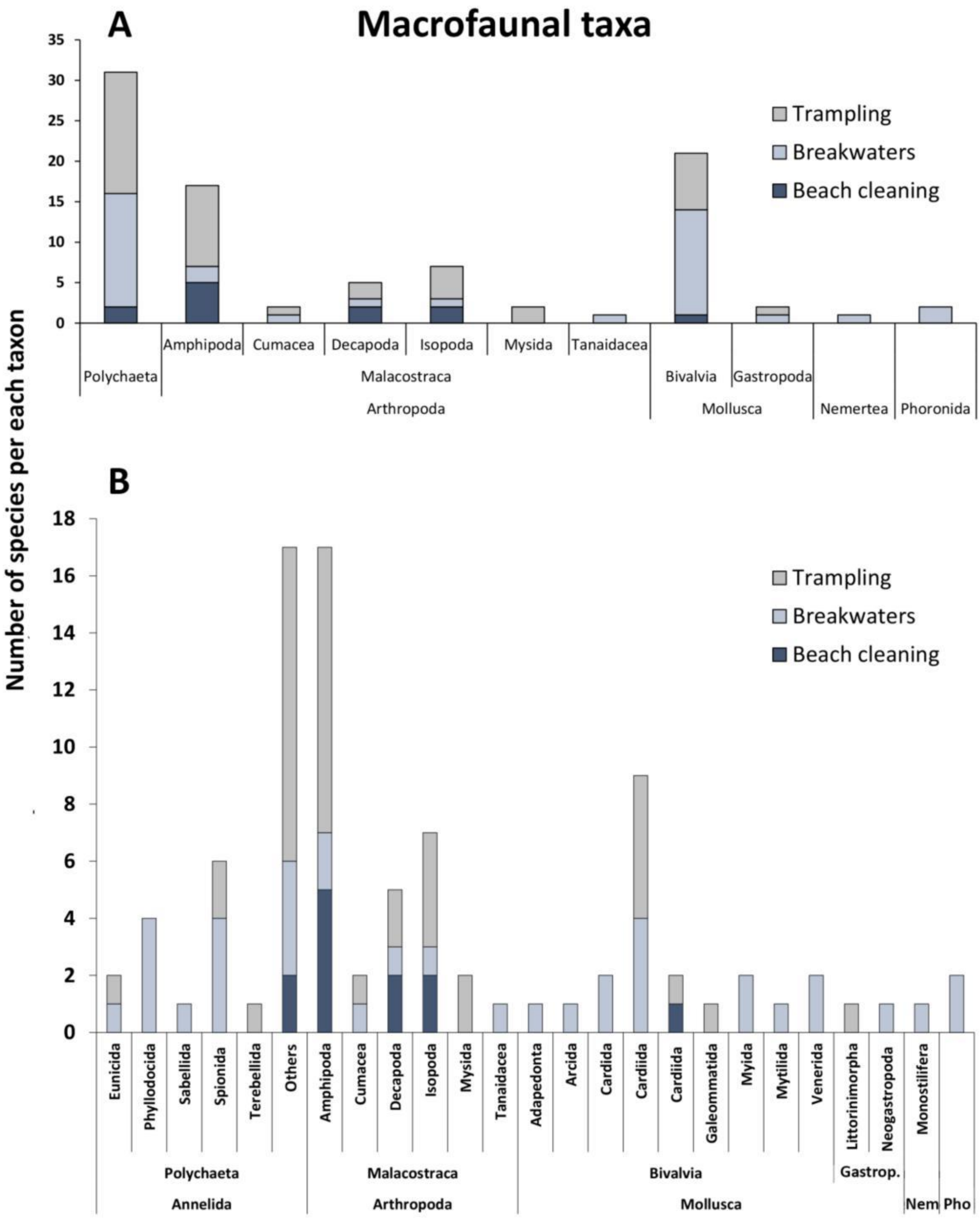

Figure 4. Main taxa found to be affected by trampling, breakwater barriers and mechanical beach cleaning. (A) Most commonly found taxa are polychaetes, bivalves and crustaceans. (B) Orders including the highest number of vulnerable species are Spionida (class Polychaeta), Amphipoda and Isopoda (Malacostraca) and Cardida (Bivalvia).

4. Discussion

4.1. Studies on Trampling

Trampling related to the recreational activities is one of the main threats faced by macroinvertebrates and their predators [46]. Most articles regarding trampling impacts on macrofauna (Table S1) have reported a decrease in abundance of macrofauna, even if not all species were affected at the same level, since certain species are more vulnerable than others [34]. Out of the studies we found, only one concluded that trampling did not have a significant impact on indicator species, attributing it to the sustainable use of the beach without violating the carrying capacity [47]. The first study that included impacts of beach activities on macrofaunal communities was conducted in Chile by [48], but it did not separate trampling from other recreational activities.

Both the first studies on trampling impacts, [49] and [50] were conducted in South Africa. Wynberg and Branch [49] found that the trampling influence—resulting from bait collection for sand-prawns (Callianassa kraussi)—lasted not less than six weeks and the prawn population could be seen fully recovered by 32 weeks. Similarly, Moffet et al. [50] reported a different response of different species to changing intensity of trampling (see Table S1). Certain species have the ability to apply peculiar behavior to limit the impact: for example, the bivalve *Donax serra* is able to move to a depth where the effects of trampling due to walking cannot cause damages; however, it was reported to be significantly affected (i.e., shells were damaged) by vigorous trampling during games like volleyball. Another bivalve, *Donax sordidus* and the isopod *Eurydice longicornis* were found to be more resistant since they were affected only at high intensities of trampling [50]. Veloso et al. [34] supported the same idea of different responses from different species and, in their study conducted on different beaches with almost the same morphodynamics, they reported that both decapods and amphipods were vulnerable taxa to trampling.

In the Netherlands, different responses of macrofauna to trampling were documented when temporal stages of life cycles were considered [51]. The population dynamics of the bivalves *Limecola balthica* and *Cerastoderma edule* were altered and experienced a negative impact in adult specimens, potentially because they were killed or buried through footsteps. In contrast, the recruitment of juveniles of *L. balthica* got enhanced probably because larval recruitment was always high in the considered period, while no effect was reported for larvae/juveniles of *C. edule*. According to the authors, in the long terms, *L. balthica* could replace *C. edule* with relevant changes in ecosystem functioning.

The impact of trampling was demonstrated by Schlacher and Thompson et al. [52] on macrofaunal communities in a sandy beach in Queensland, Australia. Significant differences among the impacted (i.e., accesses to beach) and control plots were found on lower shore (i.e., the shore at the lower limit of the tide) as out of nine common species, eight were found to be less abundant at the impacted sites with a decline in mean abundance from -90% (*Urohaustorius halei*) to -12% (*Glycera sp. 1*), contributing to an 84% dissimilarity among impacted and controlled sites on SIMPER analysis. On the other hand, the difference in total abundance at impacted and controlled sites at upper shore (i.e., the shore at the upper limit of the tide) was not significant. The variation on lower shore was attributed to the different sensitivity levels of species occupying two portions of the beach.

A study in Cadiz bay, Spain reported different responses of different organisms towards human trampling, assessed before and after the tourist period [23]. The study investigated macrofaunal assemblages at three sectors with different trampling intensity in three beaches. In all the sectors, during the tourist period, the contribution of crustaceans faced a decline, while that of polychaetes got increased.

Schlacher et al. [53] also documented the trampling influence on upper shore macrofauna through experimental trampling in Venus Bay, Australia. Surface-active community structure was found to be changed while the number of species declined from 28 to 22 as trampling intensity increased with rare species facing a significant decline. The same kind of negative impact of trampling was found by Machado et al. [21] by assessing the trampling effect on two beaches with urbanized and non-urbanized sectors at Rio de Janeiro, Brazil. However, the community structure between the two beaches was different, which could possibly be due to the different level of tourism pressure, mainly trampling. The study gives very useful information about the sensitivity of certain species, such as the amphipod *Atlantorchestoidea brasiliensis*, the polychaetes *Hemipodia* *californiensis* and *Scolelepis sp.*, which could be good indicators for analyzing recreational activities. Reis and Rizzo [13] investigated the influence of trampling by comparing two beaches, one with intense use (Urca beach) and the other (Fora beach) with restricted use. The overall species density in sediments was found to be higher at Urca beach, probably because disturbances other than trampling (such as beach cleaning) could have an impact on macrofauna communities in Fora beach.

The sand hopper (*Talitrus spp.*) has been used as an important indicator to evaluate trampling effects [35,36,54]. Veloso et al. [36] compared urbanized sites with higher number of visitors to protected ones, both in in Brazil and Spain, and found significantly higher density of talitrid amphipods at protected sites. Minor seasonal fluctuations in density were found in Brazilian sites, while higher seasonal variation was found in Spanish sites with no increase in Talitrus amphipods, even when the beach was not visited anymore in winter. The same negative relation was found in another study, since the number of amphipods was highest at lowest tourist pressure, while lowest at highest tourist pressure [54]. This is the only study which has an assessment based on three years for five out of eight considered sites. Although the author did not exclude the possibility of other influences that could play a role in declining the abundance of sand hoppers, they demonstrated with a trampling simulation experiment that only 60% of sand hoppers in the sample survived soon after trampling, but could not survive more than 24 h after the experiment.

Decapods are indicators used to assess trampling impacts [55,56]. Burrow counting has been used in most of the cases when crabs are used as indicators to analyze the impacts of beach activities. A couple of studies demonstrated lower burrow density at trampled plots with more significant impacts on large sized burrows by simulating pedestrian trampling [57–59]. The burrow density and size were reported to decrease suddenly after trampling but burrow counting may not correspond to the size of the sampled population of crabs. According to Silva and Calado [60], the burrow counts may be challenged by the existence of multiple openings, multiple burrows by the same crab or the “secret chambers”, i.e., special chambers made by crabs inside the main burrow to avoid detection. For burrow counting, it must be important to consider the burrowing behavior of the crab, otherwise the results could potentially become biased. The results could also become biased if there are obscured burrow openings [55]. Kim et al. [56] analyzed the effect of trampling on the behavior of intertidal crabs in South Korea by applying three-leveled trampling; no trampling, moderate trampling (i.e., 20 steps), heavy trampling (60 steps). The same trend was reported i.e., with increasing trampling intensity the number of crabs declined, while no crabs returned to the trampled plots even 30 min after the trampling. Along with beach cleaning, trampling has been found to be an important driver in disappearance of crabs [61]. As mentioned earlier, the understanding of behavior especially of fast-moving organisms is really important here, as mentioned in the paper: the crabs started to hide as the people approached even before trampling. Additionally, since the study was conducted in a conservation area with remarkable crab population and permission was allowed only for specific purpose, still the disappearance of crabs for half an hour after trampling reveals the magnitude of influence on behavior. García-García et al. [62] used the gastropod *Cyclope neritea* as an indicator of the trampling impacts on macrofauna living in sandy beaches of southern Spain, which displayed very peculiar results. Unlike other species, the density of *C. neritea* was found to be significantly high at the trampled spots, except during flooding periods. *C. neritea* gastropods stay buried in the sand during low tide, but they are stimulated by the resuspension due to trampling, since it helps them to find food faster [62]. The increase in number of other gastropods with trampling has been reported by Rossi et al. [51] focusing only on juveniles. The study also related the number of *C. neritea* to the ebbing and flooding phenomenon, showing its sensitivity to tidal cycle. The use of *C. neritea* can be a good indicator at beaches with limited use but at urbanized beaches, intensive trampling might be lethal to them, resulting in biased data.

4.2. Studies on Breakwater Barriers

The rising sea level and storm frequency are globally increasing the demand for placement of hard coastal defenses [63,64]. But, at the same time, hard structures, such as breakwater barriers, are thought to be weak surrogates of natural habitats [65] and do not fully reach the expected results. These structures influence the sediment transportation by reflection, refraction, diffraction and dissipation of waves [66] and could affect the community structure by physical alterations [67]. However, little has been understood about their impacts on macrofaunal communities in soft-bottom environments.

Among the examined papers on breakwaters, Bertasi et al. [68] has used the term "artificial defence structures"; Martin et al. [69] has referred them as "coastal defence structures", while the most recent two articles [70,71] included the term 'breakwater', showing some uniformity of the term with time.

Three of the four studies about breakwater barriers (Table S1) were conducted entirely in Italy [68,70,71], while one was done collectively in Italy, Spain and England [69]. Out of the four studies in Italy, three were conducted at or nearby the beach 'Lido di Dante', on the northern Adriatic coast, while one was done on Tyrrhenian coast. In all the studies found, the overall macrofaunal community was considered rather than some particular indicator species. The first study focusing on breakwaters' impacts on soft-bottom macrofaunal communities was conducted by Martin et al. [69]. They suggested a difference in the community structure with an increase in macrofaunal diversity at breakwater barriers compared to the control sites and attributed it to the presence of species belonging to the rocky substrate and altered dominance relationship among species. The comparison in the first study was made among landward side, seaward side and a controlled site. At Lido di Dante, the species' number at the landward side was always significantly higher than the seaward and the control sites. Moreover, differences among infaunal abundance at controlled, landward and seaward side were also reported at other locations covered in Spain and UK.

Another study at the same location in Italy, i.e., Lido di Dante, was done on a breakwater connected to land by two groynes making a sheltered location between the breakwater and the shore [68]. This study also considered a comparative analysis of the seaward side, landward side (sheltered) and an adjacent fully exposed reference site. The number of taxa found on landward sheltered site was significantly higher than the other two sites with a dominance of bivalves (50.8%) and polychaetes (43.3%). Breakwater barriers actually make pools where the environmental conditions are different than the surrounding [70] and could provide favorable ground for new species to thrive. Furthermore, the community composition at landward side was found to be significantly different (50.8% bivalves, 43.3% polychaetes) than the seaward (98.3% bivalves) and reference sites (91.6% bivalves). Just like the shallow northern Adriatic Sea, communities at Lido di Dante are poor in species and the quantitative dominance could be found only in few species [72].

These results were further supported by Munari et al. [70], who conducted a study at a small distance from the site studied by Martin et al. [68] at Lido di Dante. They reported seasonal differences in species on seaward side and not on landward side. Significant differences between landward and seaward side were reported for summer (June) only. For October, some species could be found exclusively either on landward (six species) or seaward (four species) side. The seasonal changes at seaward side and stability at landward side could be possibly related to the fact that breakwaters in the intertidal zone are expected to provide a more homogenous habitat at micro-spatial scale than the natural habitat [73].

Some further advancement was made by Becchi et al. [71], who investigated the ecological impacts of breakwater barriers at two locations by adapting a new strategy. They conducted sampling while considering sample points at different distance from the breakwater, i.e., at 5, 15, 50 and 100 m. The abundant species found were crustaceans, polychaetes and molluscs, while other taxa were found in small percentages. The distance from breakwater did not influence the species' abundance significantly, but the species' composition was affected significantly. A higher level of dissimilarities was reported in macrobenthic assemblages among the study sites, showing the dependence of soft-bottom communities on specific environments. The response of benthic communities to breakwaters or coastal defense structures could potentially be influenced by local conditions and infrastructure.

4.3. Studies on Mechanical Beach Cleaning

Mechanical beach cleaning has been one of the basic concerns for beach managers to satisfy beach visitors. At the same time, the impact of mechanical cleaning practices is one of the most under-investigated topics in beach management [14]. Out of the total eight studies found, two have reported insignificant values for impact resulting from mechanical beach cleaning on macrofaunal population [74,75], while five have favored the argument that mechanical beach cleaning does have a negative impact on macrofauna [59,76–78]. Among the studies conducted, two are using single indicator species, while the rest are comparing multiple species to investigate the problems associated with mechanical beach cleaning. The indicator species used in two studies are the ghost crab (*Ocypode cordimanus*) [59] and sand hopper (*Talitrus saltator*) [79].

In studies using the ghost crab, burrow counting has been considered in several studies to analyze human impacts on sandy beaches [61,80]. All the studies found had considered multiple beaches for conducting a comparison of macrofaunal communities or species, based on the frequency or presence and absence of mechanical cleaning activities. Some of the beach organisms like Donax spp. are reported in the literature to be affected mainly through physical injuries from beach vehicular traffic [81,82], while others like talitrid amphipods from the removal of the beach wrack [76]. Most of the authors have associated the wrack or organic matter removal resulting from beach cleaning as a reason for decrease in abundance. Several studies [61,76–79] have reported 25% more abundant macrofauna, such as amphipods and isopods, on ungroomed beaches compared to the groomed ones, and related it to the macrophyte wrack on the beach. Dugan et al. [76] reported significant differences in species' richness, abundance and biomass due to bottom-up effects of the macrophyte wrack subsidies. Gilburn [77] associated the grooming-driven decline in macrofaunal diversity to beach award status and stated that award beaches are groomed more often (69% groomed) than non-award beaches (6% groomed). Out of 8 taxa considered as indicators, on average 4.86 could be found on ungroomed beaches, while 1.13 on groomed beaches. The study concluded the beach award status as the major factor in the loss of macrofaunal diversity. Another study [78] also correlated the macrofaunal taxa richness to the depth of wrack and revealed a positive relationship between wrack depth and macrofaunal diversity. In this year-long study at 104 sites, it is also reported that taxa richness is high on ungroomed beaches even in winter, when the wrack depth becomes similar on both groomed and ungroomed beaches. The impact could also be long lasting as a result of other activities, since groomed beaches are the ones which are intensely used by visitors. As an indicator, the ghost crab is another species found to be negatively affected by mechanical beach cleaning and other related beach activities [59,62,80]. The relationship between ghost crab density and mechanical beach cleaning has further been explored by Stelling-Wood et al. [59], who found a decline in burrow density of ghost crabs with increase in frequency of mechanical beach cleaning by comparing three beaches with high, medium and low cleaning frequency. The burrow density was found to be higher in beaches cleaned three or less than three times a week. In contrast, a couple of studies did not report significant effects of mechanical beach cleaning on macrofaunal communities [74,75]. No significant impact of cleaning was reported on macrofaunal assemblages in Sweden by Malm et al. [74]; however, the organic matter and total biomass was found to be significantly lower on the intensively cleaned beaches. Among the macrofauna, the polychaetes, mysids and mussels were the most commonly found groups, with abundances significantly higher at uncleaned sites. The study also related the presence of these groups to the decomposing beach casts on uncleaned beaches.

Morton et al. [75] also did not report any significant difference in the community structure (at partially cleaned beaches), but related it to the frequency of cleaning and supplemented the conclusions of Stelling-Wood et al. [59], i.e., the density of organisms declines with an increasing frequency of cleaning and vice versa. Beaches that are cleaned moderately have similar organic matter and total animal biomass as uncleaned ones [74], however, more frequent cleaning (more than three days) has been reported to have more prominent impacts [59].

5. Conclusion

Considering our findings, we conclude that the work done focusing the impacts of individual three human disturbances, i.e., trampling, installation of breakwater barriers and mechanical beach cleaning, on sandy beach macrofauna is scarce and very region-specific. In our observation, the reason for this limited number of detected studies is that, in most of the cases, the impacts are not evaluated independently and are collectively grouped as human disturbances or human activities, probably due to difficulties in separating them. In the case of trampling, almost all the articles had reported negative impact on benthic macrofauna of sandy beaches. Exceptionally, one article did not report significant impact and justified it with limited trampling pressure on the beach. Almost all of the authors have reported a significant influence of breakwater barriers on benthic macrofauna, whose species composition and abundance were deeply altered. Mechanical beach cleaning was also found to be significantly influencing benthic macrofaunal communities, where 6 out of 8 studies reported a negative impact of cleaning activities. In beach cleaning studies, the species are mostly reported to be affected by direct physical injuries or loss of biomass which they inhabit and exploit.

Among all the three impacts, "breakwater barriers" is the most under-investigated issue. Additionally, there is deficiency of data on the subject in general. Although all the three studies about breakwaters are involving the Mediterranean Sea, the studies on trampling and mechanical beach cleaning have been scarcely conducted in this basin. Similarly, the impacts of breakwaters still need to be investigated in other parts of the world. The studies on trampling impacts are rather uniform with time and had followed comparatively more simplified methods with well-explained scales of impact, such as number of visitors in beaches. However, the studies on mechanical beach cleaning impacts lack information about the magnitude and type of beach cleaning machinery.

We highlighted that the considered impacts affect macrofaunal assemblages—which could be shaped by meiofauna [83], and in its turn, shape the communities feeding on macrofauna, for example, the Kentish plover, Charadrius alexandrinus Linnaeus, 1758 (Chordata, Aves, Charadriiformes), a species included in the Annex I of the Bird Directive 2009/147/EC [84] (Nature 2000 code: A138) and in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species as LC (Least Concern) [85].

The current and generalized engineering approach to protect coasts with breakwaters and the use of beaches for touristic purposes without an ecosystem-based management asks urgently for further studies on their impact on biodiversity of beach ecosystems, especially regarding to breakwater barriers. While dune systems and sandbanks are considered by Habitat Directive [86], the intertidal zone (except for mud- and sandflats) is excluded by environmental protection regulations, notwithstanding its priority ecological role in land-sea connectivity [87]. With this paper, we would stress the importance of the intertidal zone, and underline that anthropocentric [88] beach usage is not suitable to maintain the biodiversity and ecosystem functions of this habitat. Further researches on impacts on beach ecosystems should be promoted all around the world to find appropriate macrofauna indicator species and to address a more sustainable use of beaches.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-1312/8/6/405/s1.

Table S1: Main Anthropogenic Impacts on Benthic Macrofauna of Sandy Beaches.

Author Contributions: Conceptualization, C.G.D.C. and C.C.; Methodology, A.A., G.L. and C.G.D.C.; Software, A.A., C.G.D.C., T.P.M, C.R.; Validation, C.C., B.C. and S.P.; Writing—Original draft preparation, A.A. and C.G.D.C.; Writing—Review and editing, all authors; project administration, C.D.C and C.C.; funding acquisition, C.G.D.C and C.C. All authors have read and agreed to the published version of the manuscript.

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Chapter 2: Temporal variations in macrofauna of the intertidal zone in two sites along the Adriatic coast

Abstract

Benthic macrofauna is comprised of complex and diverse taxa playing an important role in shaping, modifying, and maintaining sandbanks in marine coastal ecosystems. In particular, macrofauna living in the intertidal zone is subjected to temporary but intense disturbances such as beach tourism as well as permanent alteration in the habitat by engineering structures like breakwater barriers. However, information about diversity and abundance of macrozoobenthos of the intertidal zone in the Mediterranean sandy coasts is still scant.

Main objective of this study is to contribute to fill this knowledge gap by defining structure and dynamics of macrozoobenthic communities of two sandy Adriatic beaches: Palombina, a highly frequented urban beach characterized by breakwater barriers and Torre del Cerrano, a Marine Protected Area. The hypothesis that macrofauna composition and abundance in each site changes over time, likely in relation to seasonal variations in biotic and abiotic factors, was tested.

Results showed that bivalves were the dominant group at both the sites, even if *Lentidium* spp. were mainly found in Palombina while *Donax* spp. and *Chamelea gallina* were more common in Torre del Cerrano. Difference in the community was found mainly for crustaceans and polychaetes —which declined at Palombina in the summer months— and for gastropods, that increased in summer only at the urbanized site. A possible combined effect of breakwater barriers and beach activities has been theorized to explain main discrepancy between the two sites at summertime.

Notwithstanding the two beaches are subjected to different kinds and intensities of human impacts, both beaches were labelled as "high or good quality" by the Multivariate Marine Biotic Index (M-AMBI), suggesting that i) sampling tools more efficient than hand-corers should be tested to collect macrofauna in the intertidal zone, or ii) at local scale, specific functional groups should be considered to assess the ecological status of beaches using benthic communities.

Keywords: Sandy beaches, macrozoobenthos, biodiversity, artificial barriers, marine protected areas

1. Introduction

Benthic macrofauna living in soft substrates is a diverse and complex community of organisms, playing a pivotal role in different contexts. Mainly they are involved in the mixing, ventilation, oxygenation and irrigation of sediments (phenomena commonly known as a whole as *bioturbation* (Aller, 1988; Meysman *et al.,* 2005; Huhta, 2007; Kristensen *et al.* 2012). The bioturbation activity improves 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 (e.g., redox) are positively influenced (Aller, 1988) and the depth of the oxic layer is extended over the anoxic one (Koike & Mukai, 1983; Aller, 1988).

All these benefits can contribute to the increase or the maintenance of biodiversity, thanks even to the moderate disturbance that the bioturbation creates (Widdicombe *et al.*, 2000), but in particular to the complex environment these organisms shape (Aller, 1988). Macrofauna can indeed act as "ecosystem engineer" (Jones *et al.,* 1994; Meysman *et al.,* 2006; Kristensen *et al.* 2012; Passarelli *et al.,* 2014), that modifies, maintains and creates the habitat and its complexity, being able to model the sediment structure (e. g. constructing tubes, digging channels and burrows) (De Smet *et al.,* 2015).

The macrofauna is involved in complex trophic relationships with microbiome, meiofauna and megafauna. In particular, macrofauna in the intertidal zone represents a food source for shorebirds (Aarif et al. 2021), such as the Kentish plover, *Charadrius alexandrinus* Linnaeus, 1758, a species included in the IUCN Red List of Threatened Species as LC (Least Concern). Kentish plover's diet includes small crustaceans, molluscs and polychaetes (Castro et al. 2008); therefore, macrofauna from the intertidal zone may be particularly relevant in moderately urbanized beach areas where supratidal habitats —such as lagoons, salinas or dune systems— suitable for foraging waders (Masero et al. 2000) are reduced or absent.

Community structure of macro-, meio- and microfauna hosted in beach environments is influenced by several natural 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). Thus, the alteration of natural beach dynamics due to human impact, such as trampling, bait collection, mechanical beach cleaning, (Rossi et al., 2007; Schlacher *et al.,* 2016, Zielinski et al, 2019), could potentially affect ecological traits of these organisms and overall functioning of beach ecosystem (Thrush *et al.,* 2017).

Ecological status of submerged sandy beaches can be assessed by analyzing the composition and abundance of benthic macrofauna and through the elaboration of biotic indexes (Borja *et al.*, 2000; Muxika *et al.,* 2007). Macrofauna living in the intertidal zone can be particularly vulnerable to beach activities (Vieira *et al.,* 2012; Bessa *et al.,* 2013; Bessa *et al.,* 2014; Reis & Rizzo, 2019). Even if the data on specific impacts influencing macrofauna is limited, the number of studies regarding the response of macrofaunal communities and populations towards physical disturbances has increased in recent decades (see for e.g., 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; Afghan *et al*., 2020). Some of the studies are based on single indicator species, while others are focused on the overall macrofaunal community. Especially the organisms living buried in the sand, such as polychaetes, molluscs and crustaceans are directly damaged by beach activities (Reis & Rizzo, 2019). However, different taxa have different levels of sensitivity to disturbance, since certain species have been reported to be more vulnerable than others (Veloso *et al.,* 2006).

Main objective of this study is to contribute to fill the knowledge gap about macrofauna of the intertidal zone of the Italian Adriatic sandy coast by defining structure and dynamics of macrozoobenthic communities of two sandy beaches. The chosen sites present different levels of anthropogenic impact: one, Torre Cerrano which is a marine protected area; and the other one, Palombina is a touristic beach characterized by the presence of breakwater barriers. Ecological beach status of each site has been defined by applying the M-AMBI index (Borja *et al.,* 2000; Muxika *et al.,* 2007).

The hypothesis that macrofauna composition and abundance in each site changes over time, likely in relation to seasonal variations in biotic and abiotic factors, was tested. The obtained results help in characterizing the macrofaunal community of the mentioned sites that could be used to set future research to highlight differences in macrofaunal communities in response to anthropogenic impacts.

2. Materials and methods

2.1 Study areas

The two selected sites are Palombina (43°36'59" N, 13°25'46" E) and Torre del Cerrano (thereinafter: Torre Cerrano, 42°35'5" N, 14°5'26" E), along the North-Central Adriatic Sea. Both the beaches are dissipative beaches i.e., high energy beaches with medium to wide surf zone and consisting of sandy sediments.

Palombina (Ancona, Italy) is characterized by many touristic facilities, while its backshore is neighbored by a railway. Here, breakwater barriers, distant about 150 m from the shoreline, modifying the water exchange (Bertasi et al., 2007), favor sand accumulation by preventing erosion (Munari et al., 2011) and, likely due to reduced depth, dense algal blooms are observed in warmer periods of the year.

Sometimes, sand is accumulated by bulldozers on the backshore during non-swimming season to prevent beach erosion. The sand is then re-distributed on the beach at the beginning of the swimming season. After sea storms, vegetal detritus and huge quantity of plastic materials above all from nearby mussel culture plants are found stranded commonly along this beach.

Torre Cerrano is a small Marine Protected Area located southern to Palombina (Silvi-Pineto, Italy). Established in 2010, it extends up to 3 NM out into the sea, 7 km along the coast. The area is divided into three different zones with increasing degree of protection (D, C, B respectively, no reserve zone A is present). The sampling area is located into the B zone, whose backshore is characterized by the presence of sand dunes, a bike trail adjacent to railway track, and pine trees. No beach facilities are present inside the B Zone; however, the site is frequented by bathers from May to September. Litter of marine and land origin (mainly brought by nearby rivers) is periodically removed through manual collection activities.

2.2 Sampling design and fieldwork

The samplings have been carried out monthly at Palombina (Site 1) and Torre Cerrano (Site 2) from February 2019 to January 2020. The distance of sampling points from the shore was kept constant throughout by using a fixed reference point on the emerged beach. In each site, 3 transects parallel to the coast have been set: A, B, C respectively, where one transect was designated at a distance of 5 meters from the next transect. Five replicates were collected for each transect using a PVC corer (⌀: 9.5 cm, h: 70 cm) as given in **Figure 1**. Once the samples of sediments were collected from the intertidal zone and gently sieved using a double net, composed 500 µm mesh. The collected sediments samples containing macrofaunal component were preserved in 95% alcohol and transported carefully to the laboratory for sorting. The data for macrofauna for twelve months was presented into four quarters i.e., Q1 (January, February, March) Q2 (April, May, June), Q3 (July, August, September) and Q4 (October, November, December). In order to detect eventual variations in sand granulometry, three further samples of sediment have also been collected in February, March, July, November and December in every transect and dried for successive analysis. Sampling sessions were conducted in low tide conditions where wave heights and tide coefficients were taken from different sources (www.meteopesca.com; www.ilmeteo.it/portale/meteo-mare).

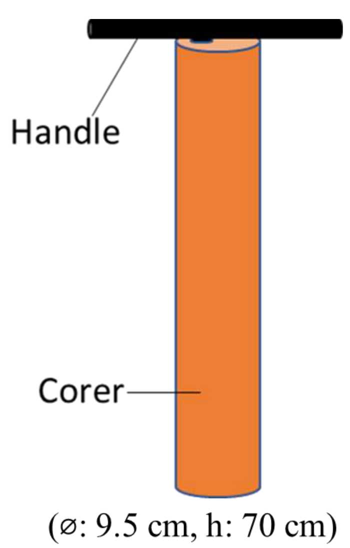

Figure 1: Sampling corer for macrofauna collection

2.3 Laboratory work

In order to determine temporal variations in abundance of macrofauna (expressed as number of items/m^3), the collected organisms were identified to the lowest possible taxonomic level and counted using a stereomicroscope. Living (i. e. organisms still alive during the samplings) and non-living (**Table 1)** components were considered separately.

Table 1: Categories of non-living components.

Items	**Description**
Tubes (soft + hard)	Empty tubes belonging to polychaetes (Serpulidae, Sabellidae, Spionidae,...), mollusks (Vermetidae, Scaphopoda,...)
Sea urchin fragments	Fragments of tests $\geq$ 5 mm
Gastropod shells	Entire, empty shells
Sea urchin spines	With the joint part not broken
Anthropogenic litter	Every kind of rubbish
Natural detritus	Wood, seeds, vegetal and algal fragments $\geq$ 5 mm
Ophiuroidea vertebrae	Complete vertebrae

The sediment samples were dried in an oven and granulometric analysis were performed using a sieve shaker. The limits of the different granulometric classes have been fixed following the Udden-Wentworth scale.

2.4 Definition of the beach status using benthic communities

To assess the ecological status using benthic communities, some methodologies have been recently developed in Europe according to the European Water Framework Directive (Borja *et al.,* 2008).

Many of them are based on the AMBI index (AZTI Marine Biotic Index; Borja *et al.,* 2000), such as M-AMBI (Multivariate-AMBI; Muxika *et al.,* 2007): it is derived from the proportions of organisms' abundance into five ecological groups, which are related to the degree of sensitivity/tolerance to an environmental stress gradient (Borja *et al.,* 2000; Borja *et al.,* 2008). The M-AMBI relies upon a statistical multivariate tool, the Factor Analysis (FA), which includes richness, Shannon's diversity and AMBI (Borja & Muxika, 2005; Borja *et al.,* 2008).

In this research, the M-AMBI has been applied to monthly data of macrobenthos collected in the two sites using the AZTI Marine Biotic Index software. In **Table 2** the details about functional groups are shown.

Table 2: Functional groups of AMBI and M-AMBI (elaboration from Borja et al., 2000)

Functional groups	**Definition and examples**	**Examples**
Group I (blue)	Species very <u>sensitive</u> to organic enrichment and present under unpolluted conditions	*Bathyporeia guilliamsoniana, Echinogammarus stocki, Pontocrates altamarinus, Donax trunculus, Tritia neritea, Amphiura chiajei, Magelona* sp., *Eurydice* sp., *Chamelea gallina*
Group II (green)	Species <u>indifferent</u> to enrichment, always present in low densities with non-significant variations with time	*Lentidium mediterraneum, Eteone* sp., *Diogenes pugilator, Sigalion mathildae, Cumopsis* sp., *Pseudocuma* sp., *Glycera* sp., Mysida
Group III (yellow)	Species <u>tolerant</u> to excess organic matter enrichment; they may occur under normal conditions, but their populations are stimulated by organic enrichment	*Scolelepis sp., Gilvossius candidus, Apseudopsis latreilliid*
Group IV (orange)	<u>Second-order opportunistic</u> species	Cirratulidae
Group V (red)	<u>First-order opportunistic</u> species	*Capitella capitata*

The distribution of these ecological groups, according to their sensitivity to pollution stress, provides a Biotic Index with eight levels, from 0 (best status) to 7 (worst status), with decreasing environmental quality (Borja *et al.,* 2000). These values are compared with Shannon diversity index (0 = minimum diversity, 1 = maximum diversity) and species' richness (number of taxa) to give a cumulative score.

2.5 Statistical analysis

Nonparametric multivariate techniques contained in the PRIMER (Plymouth Routines In Multivariate Ecological Research) package (Clarke & Warwick, 1994) was used to analyse the abundance data of the living component. Ranked lower triangular similarity matrices were constructed using the Bray-Curtis similarity measure on square root transformed, standardized (%) abundance data. Analysis of similarity (ANOSIM) permutation test was used to check the significance of differences between treatments (Clarke & Green, 1988; Clarke, 1993). Bootstrap averages were performed as well for factor 'Quarters' (Q1-Q4) where Q1 averages are abundance data of, Q1= January, February, March; Q2=April; May, June; Q3= July August, September; Q4= October, November, December.

3. Results

3.1 Qualitative analysis of benthic macrofauna

A total of 32 taxa have been identified among living benthic macrofauna of the two sites (**Table 3**). Most abundant taxon was that of bivalves smaller than 5 mm, mainly belonging to *Lentidium mediterraneum* (with some *Donax* sp. and *Chamelea gallina*). Larger bivalves ranging from 5 to 10 mm were more common in Palombina and they are mainly represented by *Lentidium mediterraneum*, with minor contributes from the Veneridae, Donacidae, Tellinidae and Mactridae family. Veneridae and Donacidae were represented particularly by the species *Chamelea gallina* and *Donax* sp.

The gastropods found belonged to only two species: *Tritia neritea*, observed exclusively in Palombina and *Neverita josephinia* which was more abundant in Torre Cerrano. The eggs envelopes of the latter have also been observed in the intertidal zone and on the beach together with detritus accumulated on the shorelines (wrack).

Crustaceans found were characterized by ostracods, amphipods, cumaceans, tanaids, mysids and isopods. All the tanaids belong to the same species (*Apseudopsis latreillii*); the same goes for the mysids (subfam. Mysinae), while the cumaceans have been identified as *Cumopsis* sp. and *Pseudocuma* sp. in both the sites. The amphipods found are *Echinogammarus stocki, Bathyporeia guilliamsoniana* and *Pontocrates altamarinus*, present in both the beaches together with the isopod *Eurydice* sp. and *Idotea* sp.; the later species is not typical of soft bottoms, but it is often associated to beach-cast macroalgae or vegetal detritus, usually found on sediment surface or on the beach (see ahead, paragraph about Non-living component).

The polychaetes were represented particularly by the species *Scolelepis* sp., followed by *Sigalion mathildae* and *Glycera* sp., with minor contributions from *Magelona* sp., *Eteone* sp. and *Capitella capitata*. Other frequently found components were the Spionidae family, with some sporadic records of Cirratulidae and Terebellidae. *Glycera* sp. (fam. Glyceridae) was also found at both sites.

Tab 3: List of total taxa. Exclusive taxa are in red.

Species (macrobenthos)	**Taxon**	**Palombina**	**Torre Cerrano**
Amphiura chiajei Forbes, 1843	brittle star	X	
Apseudopsis latreillii (Milne Edwards, 1828)	tanaid	X	X
Bathyporeia guilliamsoniana (Spence Bate, 1857)	amphipod	X	X
Bivalvia ≤5 mm	bivalve	X	X
Capitella capitata (Fabricius, 1780)	polychaete	X	
Caprellidae	amphipod	X	
Chamelea gallina (Linnaeus, 1758)	bivalve	X	X
Cirratulidae	polychaete	X	
Cumopsis sp.	cumacean	X	X
Diogenes pugilator (P. Roux, 1829)	hermit crab	X	
Donacidae/Tellinidae/Veneridae/Mactridae >5 mm ≤10 mm	bivalve	X	X
Donax trunculus Linnaeus, 1758	bivalve	X	X

Echinogammarus stocki G. Karaman, 1970	amphipod	X	X
Eteone sp.	polychaete	X	
Eurydice sp.	isopod	X	X
Gilvossius candidus (Olivi, 1792)	thalassinid	X	
Glycera sp.	polychaete	X	X
Idotea sp.	isopod	X	X
Lentidium mediterraneum >5 mm ≤10 mm	bivalve	X	X
Magelona sp.	polychaete		X
Mysinae (tribe Mysini/Dyamisini/Paramysini)	mysid	X	X
Neverita josephinia Risso, 1826	gastropod		X
Ophiuroidea	brittle star	X	
Ostracoda	ostracod	X	X
Pontocrates altamarinus (Spence Bate & Westwood, 1862)	amphipod	X	X
Priapulida	priapulid	X	
Pseudocuma sp.	cumacean	X	X
Scolelepis sp.	polychaete	X	X
Sigalion mathildae Audouin & Milne Edwards, 1832	polychaete	X	X
Spionidae	polychaete	X	X
Terebellidae	polychaete		X
Tritia (Cyclope) neritea (Linnaeus, 1758)	gastropod	X	
Unknown fragmented polychaete	polychaete	X	X
Species (non-living component)	**Taxon**	**Palombina**	**Torre Cerrano**
Antalis vulgaris (da Costa, 1778)	scaphopod		X
Ditrupa cornea (Linnaeus, 1767)	serpulid	X	X
Echinocardium cordatum (Pennant, 1777)	sea urchin	X	X
Fustiaria rubescens (Deshayes, 1826)	scaphopod		X
Paracentrotus lividus (Lamarck, 1816)	sea urchin	X	
Sabellaria sp.	polychaete		X

Some species have been found exclusively at one site, i.e., *Capitella capitata* (polychaete), *Diogenes pugilator* (hermit crab, a host of empty shells belonging to *Tritia neritea* or *Ocenebra edwardsii*) and *Gilvossius candidus* callianassid in Palombina. In particular, the callianassid shrimp was found in June parasitized by the isopod *Ione thoracica*. On the other hand, *Neverita josephinia* gastropod and *Magelona* sp. polychaete were found only in Torre Cerrano.

Non-living component included fragments of both hard and soft tubes. The soft tubes found in Palombina were qualitatively comparable with those of Torre Cerrano, while the hard tubes found were dissimilar. In Torre Cerrano, the hard tubes mainly comprised of sand serpulids (i.e., *Ditrupa cornea*), scaphopod fragments (i.e., *Antalis vulgaris* and *Fustiaria rubescens*) and possibly fragments belonging to *Sabellaria* which normally break because of sand accumulation. In Palombina, the tubes of organisms attached to breakwaters were predominant (e.g., rock serpulids, vermetids).

The sea urchin fragments were likely composed of the skeleton of *Echinocardium cordatum*, a sand sea urchin, which were found in both sites, and of *Paracentrotus lividus*, a species typical of hard substrates, found only in Palombina.

3.2 Temporal variations in abundance

Torre Cerrano living component

Variation in abundance of living organisms is shown in **Figure 2** and in Figure 1 of supplementary material. The abundance at Torre Cerrano is mainly dominated by smaller bivalves while bigger bivalves are not as prevalent as in Palombina (**Figure 2)**. Furthermore, the changes in smaller bivalves' abundance are also different than that of Palombina since in Torre Cerrano, the peak abundance is observed in Q4, which is the end of summer touristic period. Another important feature at this site is the comparatively higher abundance of crustaceans in spring and summer periods (Q2 and Q3). Amphipods were found rather abundant in Q2 and Q3 while the abundance of gastropods was found to be very low throughout at Torre Cerrano.

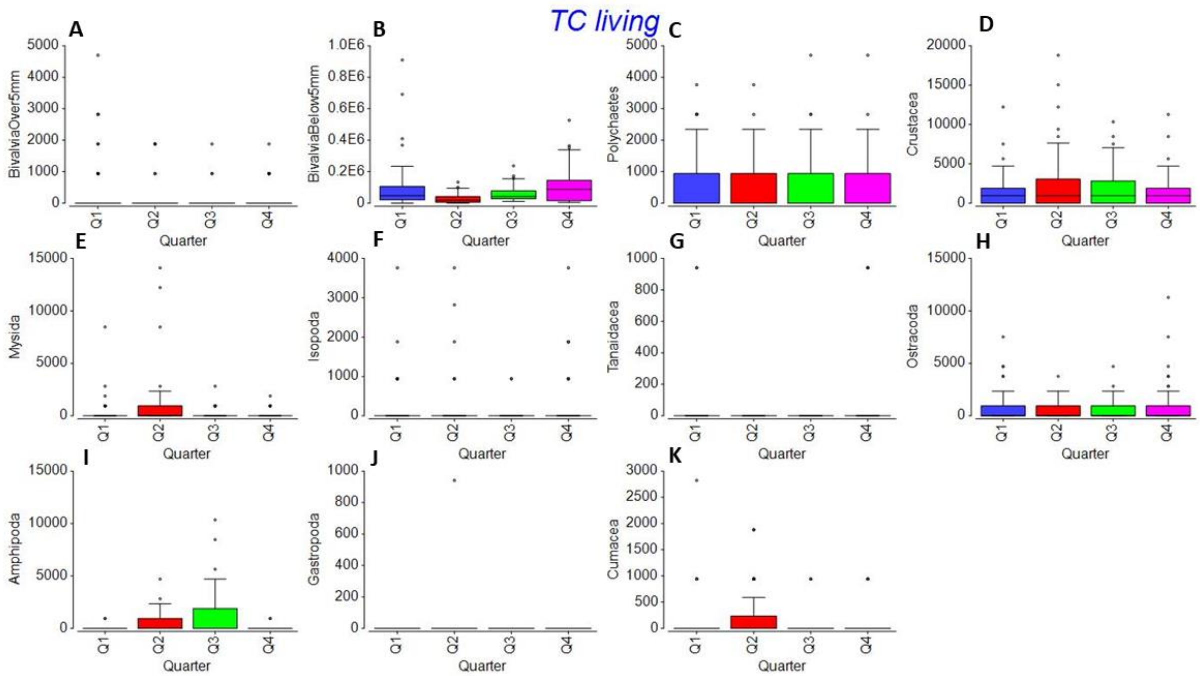

Figure 2: Living component Torre Cerrano (ind. m^{-3}) A) Bivalves over 5mm B) Bivalves below 5mm C) Polychaetes D) Crustacea E) Mysidacea F) Isopoda G) Tanaidacea H) Ostracoda I) Amphipoda J) Gastropoda K) Cumacea

Two-ways crossed ANOSIM tests for differences between unordered Transect groups and between unordered Quarter groups both give a low R value (0.057 and 0.073 respectively), indicating that there are not significant differences with the null hypothesis value of R = 0, indicating no significant differences among transects or quarters.

Palombina living component

Considering the intertidal macrofaunal community at Palombina, it is quite obvious that bivalves are the most dominant group compared to others, especially smaller size bivalves (<5 mm). The peak abundance of bigger bivalves (>5 mm) could be observed Q3, followed by a sharp decline in Q4 **(Figure 3)**. More or less the same pattern is shown by smaller bivalves (<5 mm) however the abundance decline in Q4 is not as much as bigger bivalves. Unlike bivalves, the crustaceans and polychaetes were found to have lower abundance in Q3. overall, apart from ostracods and cumaceans, the other crustaceans were found rather in very small numbers. However, gastropods were found mainly confined to Q3. Ostracods were found more in number compared to Torre Cerrano; however, a decline was observed for them as well in Q3. Amphipods, which are one of the most important crustaceans of sandy beaches were found very less in abundance compared to Torre Cerrano.

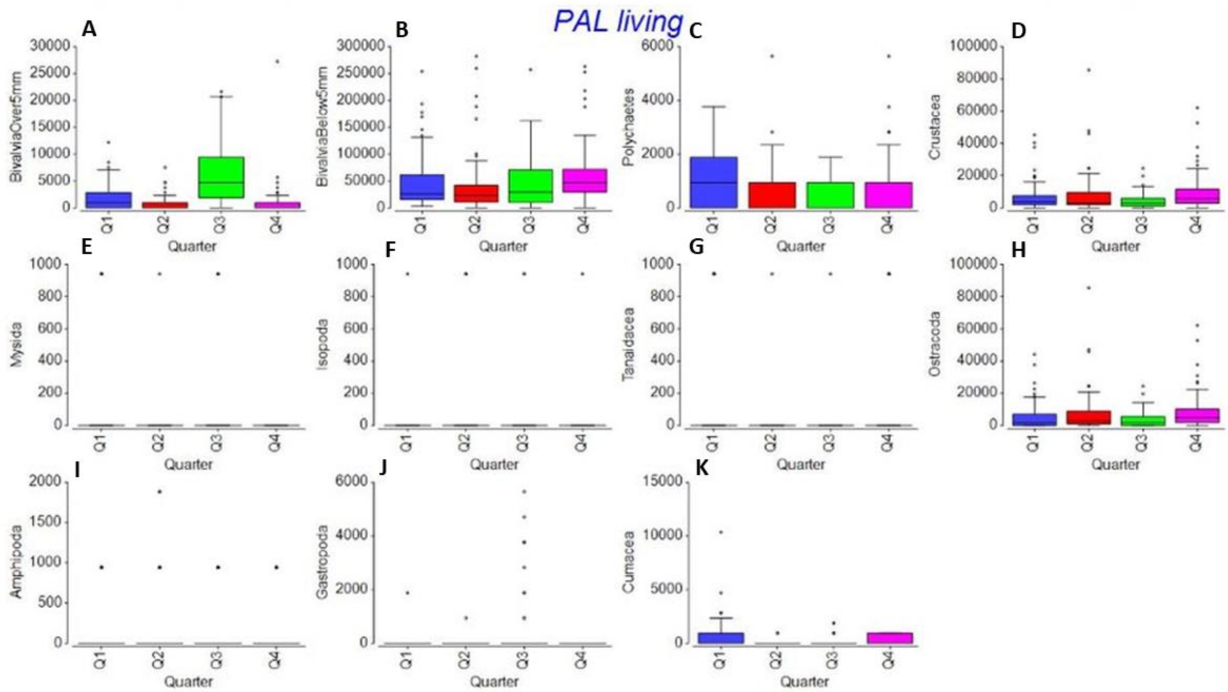

Figure 3: Living component at Palombina (ind. m^{-3}) A) Bivalves over 5mm B) Bivalves below 5mm C) Polychaetes D) Crustacea E) Mysidacea F) Isopoda G) Tanaidacea H) Ostracoda I) Amphipoda J) Gastropoda K) Cumacea

Two-ways crossed ANOSIM tests for differences between unordered Transect groups and between unordered Quarter groups both give a low R value (0.046 and 0.099 respectively), indicating that there are not significant differences with the null hypothesis value of R = 0, and therefore no significant differences among transects or quarters are evident.

3.3. Statistical comparison between Palombina and Torre Cerrano

Two-ways crossed ANOSIM test for differences between unordered Quarter groups of the two sites gives a low R value (0.067), indicating that there are not significant differences among quarters. A certain significance is found between sites (R=0.428) with a 0.1% significance level of sample statistics.

Bootstrap analyses

The bootstraps analyse further visualises the difference among the quarters where Q2 and Q3 appear to be the most distant ones. In contrast, Q1 and Q3 show more similarity. However, the variation among quarters is not significant statistically. The sites in bootstrap presentation appear to be different from each other, expressing the same significant difference between the two sites.

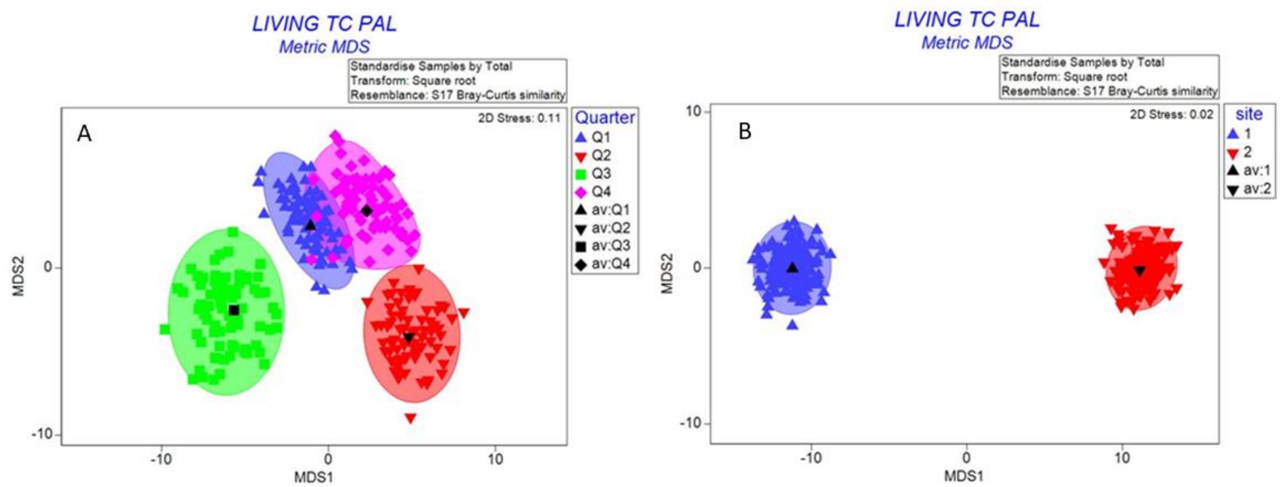

Figure 4: Bootstrap analyses for macrofaunal abundance among A) Quarters B) Sites

3.4 Non-living component at both the sites

The non-living component present in the collected sediments was showing changes with time at both sites. In Torre Cerrano, sea urchin fragments were found more often especially from June onwards and was the main contributor to the overall non-living component (**Figure 5**). On the other hand, in Palombina, natural detritus was the major contributor especially from June till September. At both the sites anthropogenic litter was found with lower contribution. Polychaete and scaphopod tubes were found almost throughout at Torre Cerrano while at Palombina, these tubes declined remarkably between May and September. Dead gastropod shells were found frequently at Palombina as well as at Torre Cerrano where living gastropods were not found that commonly.

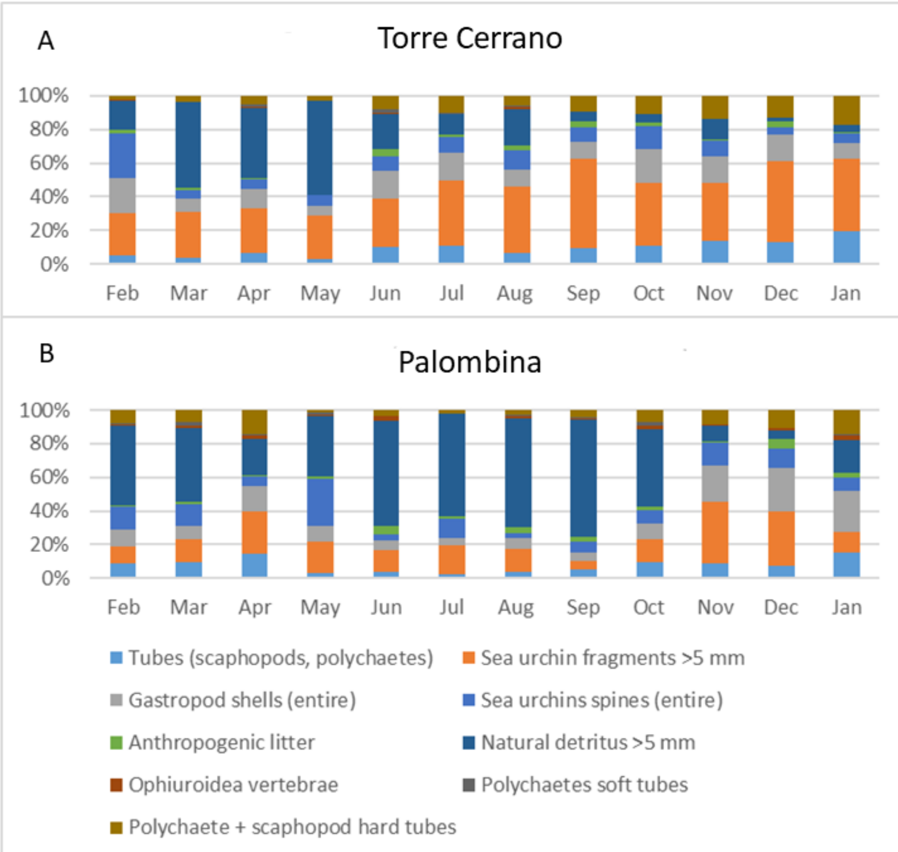

Figure 5: Percent composition of non-living component found at A) Torre Cerrano B) Palombina

3.3 Granulometry

The granulometry of February, March, July, November and December is presented in **Figure 6**.

In Palombina the sediment can be defined as "very fine sand" according to the Udden-Wentworth scale, having the 63-150 µm as the most represented fraction in every season in each transect, while in Torre Cerrano the "fine sand" fraction was the most abundant one (151-250 µm). in Palombina the contribution of very fine sand (63-150 µm) was found to be slightly decreasing from February towards December. Especially in March and December there was more contribution of sediment with larger size. In Torre Cerrano, the changes were not as much like Palombina however the contribution of larger size sediments was found to be increasing from Transact A towards Transact C. Specifically, the month of July was found to have fine sand compared to other months in all the three transacts.

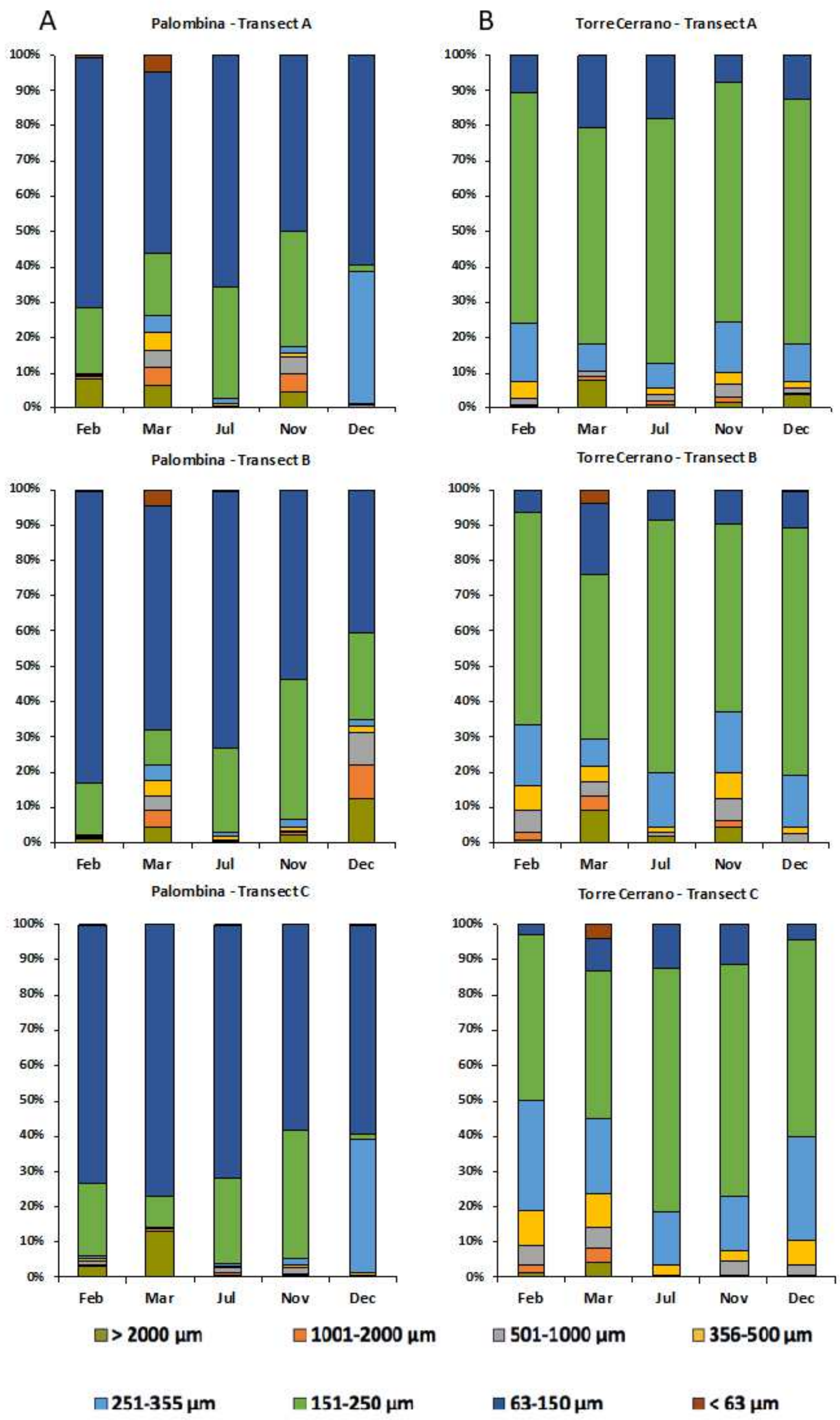

Figure 6: Granulometry of the two sites through five months A) Palombina B) Torre Cerrano

The ratio between the different granulometric fractions in each site was not constant and a variation was visible in each season for every transect. The month of March could be seen to have more share of different fractions in the granulometry.

3.4 Definition of the beach status using benthic communities

The complete results of the application of M-AMBI to monthly data are reported in the Supplementary material. The graphs of M-AMBI are shown in **Figure 2** to **Figure 6**.

The months are referred to as “stations”.

Tab 4: Legend for AMBI graphs.

4. Discussion

Living component

This research aimed to characterize benthic macrofaunal communities from two sandy beaches along the Italian side of the Adriatic Sea. In each site, the macrofauna abundance and composition varied over time. However, the total abundance of macrofauna among in different quarters was also not significant. The most abundant category in both the sites throughout the year was represented by bivalves smaller than 5 mm. Most of the bivalves at both sites but mainly at Palombina belonged to *Lentidium mediterraneum* which is one of the most commonly found specie in the Northern-Central Adriatic beaches and can comprise up to 95% of the community (Bertasi et al., 2007). These bivalves prevailed specifically in Q3 i. e., during the summer months, suggesting that *Lentidium mediterraneum* is tolerant to disturbance linked to the touristic season (trampling, beach cleaning); probably due to its ability to go under the surface rapidly (Targusi et al., 2018). In the MPA Torre Cerrano, most of the collected bivalves belonged to the *Donax* genus. The abundance of polychaetes at Torre Cerrano didn’t change remarkably through quarters except in May, when a sharp decrease of this taxon occurred. Since no sea storms occurred in May, and touristic pressure is still low at that period, the polychaete decrease was related to other factors. Kentish plover’s diet includes polychaetes (Castro et al. 2008) and it is likely that *Charadrius alexandrinus* and its chicks, common in the protected area, feed on this protein food source above all during reproductive and hatching season (Imperio et al. 2020) as highlighted by several amateur photographers (**Figure 8**).

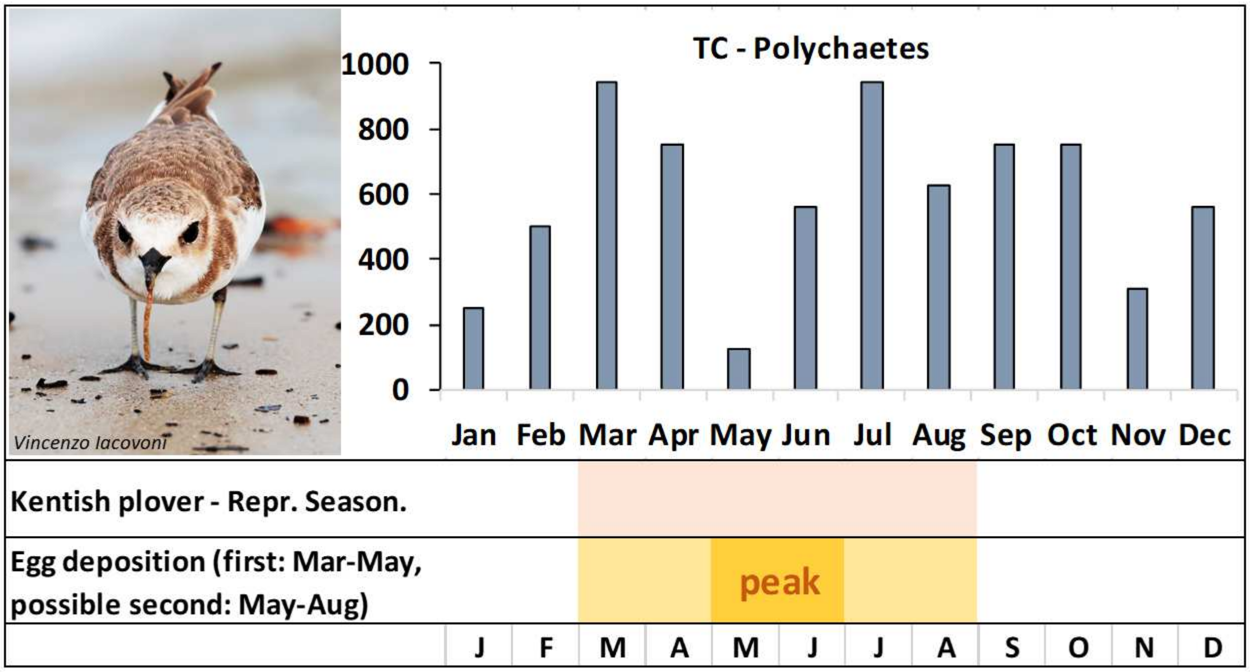

Figure 8: Polychaete abundance and reproductive period of Charadrius alexandrines. Note that in the picture in the inset the Kentish plover is feeding on Owenia fusiformis. (Photo: Vincenzo Iacovoni).

A decline was observed for crustaceans in Q3 at Palombina while at Torre Cerrano, the crustaceans instead increased in that period. This change could be linked to the increase in disturbances and sensitivity of crustaceans since several studies have reported a negative relation between physical anthropogenic disturbances and the abundance of these groups (Veloso et al., 2006; Ugolini et al., 2008; Schlacher et al., 2016). The complete absence of polychaetes in Palombina was observed in December, when trampling intensity was very low, but beach accumulation with heavy machinery occurs in this site between October and December usually, involving the use of bulldozers to collect the sand in the backshore before winter and to re-distribute the sand at the beginning of the touristic season (**Figure 9)**.

Figure 9: Sand accumulation in in Palombina (in November) to be redistributed before summer season

The trend in abundance of *Tritia neritea* is likely due to a combination of factors such as its seasonality (since they were observed at juvenile stages in early summer and mature towards the end of summer), tolerance to disturbances due to its thicker shell and opportunistic behaviour of feeding on other dead organism (Southward et al., 1997). García-García *et al*. (2015) has documented in a field experiment that this gastropod increases in abundance with increase in the presence of shellfishers activities. The preliminary results suggest differences between the sites, but it is important to further assess the community with more efficient sampling equipment and methods as well as advanced statistical techniques to test all the comparisons in detail. A potential limitation in this work could be the use of sampling corer which in our observation, may not be the right equipment to collect an appropriate macrofauna sample as the taxa found here were distributed rather close to the surface rather than the depth.

Definition of the beach status using benthic communities

Overall, the results of M-AMBI labelled both the beaches as “high or good quality”, except for transect C in September at Torre Cerrano beach and transect A in November and December at Palombina beach, which were labelled as “moderate quality”. In both the cases, the moderate quality status depends on a low species' richness and diversity value. Although, there were similarities between the two sites likely due to the presence of the small bivalve *Lentidium mediterraneum*, which was assigned to group II (“species tolerant to organic enrichment”, Borja *et al.,* 2000). However, the observations conducted in this study leads to think that high abundance levels of *L. mediterraneum* is due to the ability of the species to withstand physical disturbances such as sediment resuspension due to sand movements or high frequentation levels of the beach visitors.

The results of M-AMBI are conducted considering the species' richness, diversity and AMBI index. M-AMBI groups the species into functional categories, considering the tolerance/sensitivity to organic enrichment and pollutants (i.e., heavy metals). However, the M-AMBI is not an appropriate tool for local scale assessments and rather other functional groups could be found for assessing the health of coastal ecosystems locally. Moreover, the sensitivity to direct physical disturbances coming from beach visitors and swimmers are not taken into account.

Non-living component

Comparing the non-living component between the two sites, the most evident difference was the presence of high quantities of vegetal detritus in Palombina, that was often the most abundant component over the year especially between June and October hinting about the relation between detritus transportation and beach exploitation. The origin of this material can be the fragmentation of tree branches and plants from the backshore or transportation by the sea from other areas. In contrast, sea urchin spines and echinoderm spines were rather less compared to Torre Cerrano which hints about more suitable conditions for the presence of echinoderms at Torre Cerrano which has a certain level of protection from disturbances. Just like living polychaetes, the non-living polychaete tubes and scaphopods were frequently found at Torre Cerrano but were on the decline from May till September. Even there were fewer living gastropods in Torre Cerrano, dead gastropod shells were found there quite frequently. However, the it is worth mentioning that in Palombina the gastropod shells mainly belonged to *Tritia neritea* while in Torre Cerrano the gastropod shells belonged to other families.

Granulometry

Overall, the granulometry of Palombina was found finer than Torre Cerrano which supports the idea of less hydrodynamic activity toward the landward side of the breakwaters. In Torre Cerrano, there is usually stronger wave action leading to exchange of sediments as well as formation and displacement of sand bars which makes a more dynamic environment. While the presence of breakwaters is more than capable of influencing the granulometry by neutralizing the wave action and holding sediments (Vona et al., 2020; Karapurkar et al., 2022). In Palombina, the lack of proper exchange of sediments due to breakwaters is leading to a more stable conditions characterized by finer sand particles.

5. Conclusion

Lentidium mediterraneum has been found to be the most abundant macrofaunal group in the study sites especially at early life stages. Some organisms have been seen to be more sensitive to anthropic disturbance for example crustaceans especially amphipods which experienced a decline in their abundance in summer months at Palombina. At the same time, some were also found to be positively influenced by physical disturbance such as *Tritia neritea* which are adapted to withstand physical disturbances because of their ability to hide under surface and thicker shells. Additionally, they are scavengers feeding on dead organisms. The influence of breakwaters is potentially more on the sand composition and indirectly on macrofauna composition. The skeletal fragments of organisms belonging to hard substrate indicated the ability of breakwaters to provide habitat to those hard-bottom macrofauna. Furthermore, the finer granulometry at Palombina hints about the stable environment and limited hydrodynamic action on the landside of the breakwaters. It is hereby worth mentioning that one of the important factors in getting unbiased data is choosing the sampling equipment with maximum efficiency. We have used corers with specifications mentioned earlier, however we suggest that the corer which works more with depth rather than surface area may not portray the most appropriate biological picture of an area. An equipment covering more surface area could potentially provide more rigorous data. In future, new aspects should be considered, including the consideration of additional human disturbance over the year, such as trampling, assessment of frequency and magnitude of mechanical beach cleaning, beach nourishment, in particular at more urbanized beaches like Palombina.

About the Kentish plover, long-term studies conducted along the west coast of India demonstrated that invertebrate prey depletion is one of main factors causing declines in abundance and delays in migration in shorebirds (Aarif et al. 2021). According to these Authors, the birds need more time to assimilate enough high-quality food to build up food reserves prior to departure. These considerations put in evidence the importance of preserving the macrofauna of the intertidal zone in beaches characterised by dune systems and associated avifauna.

Also, the use of indexes such as AMBI may not be the best approach for assessing impacts on organisms, as it takes into account only the tolerance/sensitivity of a species towards organic enrichment and pollutants, but not the human physical activities. To explore these impacts further, more study sites should be included but importantly, the methods of sample collection should be more efficient.

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Supplementary material

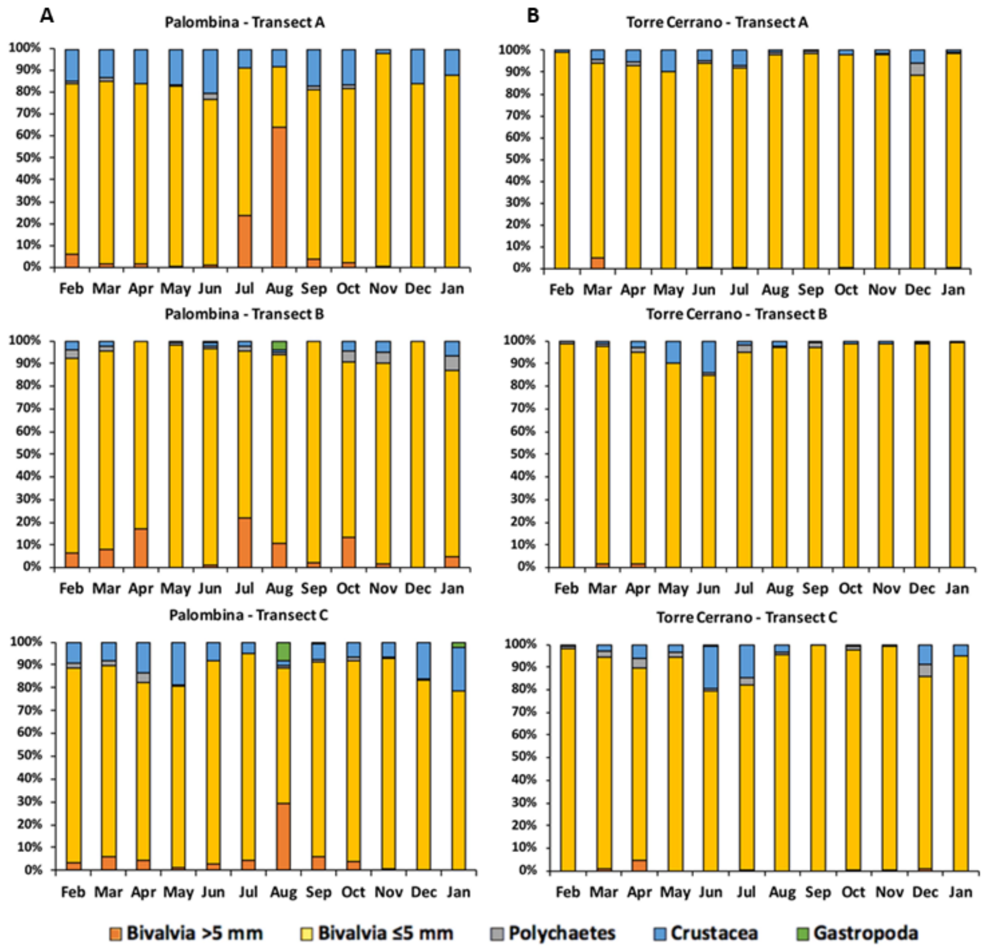

Figure 1: Abundacne of taxa in the three transacts A) Palombina B) Torre Cerrano

M-AMBI

The months are referred to as “stations”, the replicates as numbers (1, 2, 3, 4, 5):

Months	Stations
FEB	1
MAR	2
APR	3
MAY	4
JUN	5
JUL	6
AUG	7
SEP	8
OCT	9
NOV	91
DEC	92

Torre Cerrano Tr A

Stations	AMBI	Diversity	Richness	X	Y	Z	M-AMBI	Status
Bad	6	0	0	3.9838	2.4582	-3.2614	1.98E-16	Bad
High	1.42	0.79	11	-1.1879	-1.7359	1.9808	1	High
1	1.4912	0.10634	5	-0.02152	1.0587	-0.43371	0.57656	Good
2	1.5692	0.69815	6	-0.41037	-0.97435	0.29689	0.77662	High
3	1.5036	0.52652	7	-0.4886	-0.5035	0.50321	0.76982	Good
4	2.5	0.63797	5	0.43333	-0.56193	-0.3048	0.64786	Good
5	1.4577	0.5214	11	-1.0134	-0.84298	1.7735	0.92016	High
6	1.4249	0.78936	6	-0.56677	-1.2954	0.4015	0.81427	High
7	1.5073	0.18068	7	-0.29706	0.64008	0.24783	0.6706	Good
8	1.5025	0.12305	4	0.10114	1.0925	-0.73988	0.5434	Good

9	1.4951	0.20041	6	-0.19346	0.66074	-0.04999	0.64019	Good
91	1.4964	0.17092	6	-0.1764	0.75839	-0.07203	0.63165	Good
92	1.56	0.68427	4	-0.16276	-0.75464	-0.34196	0.69933	Good

Torre Cerrano Tr B

Stations	AMBI	Diversity	Richness	X	Y	Z	M-AMBI	Status
Bad	6	0	0	4.1429	2.3402	3.2454	9.66E-17	Bad
High	1.43	0.92	9	-1.0846	-2.304	-1.7205	1	High
1	1.5272	0.12949	6	-0.17588	0.6007	0.009231	0.63524	Good
2	1.5132	0.22922	7	-0.3847	0.15192	-0.41795	0.70725	Good
3	1.5119	0.51061	9	-0.82244	-1.0012	-1.3381	0.87329	High
4	1.4667	0.58177	5	-0.29363	-0.70299	-0.07024	0.73129	Good
5	1.4291	0.92106	8	-0.93639	-2.1754	-1.3861	0.95877	High
6	1.5161	0.35685	4	0.003161	0.14545	0.47643	0.61972	Good
7	1.5	0.20391	4	0.066059	0.62658	0.6077	0.57601	Good
8	1.5263	0.20951	4	0.083064	0.61205	0.60927	0.57561	Good
9	1.5032	0.14128	8	-0.4985	0.29638	-0.67816	0.72379	Good
91	1.492	0.14277	6	-0.20879	0.55453	-0.01123	0.64187	Good
92	1.5111	0.13175	4	0.10976	0.85581	0.67427	0.55393	Good

Torre Cerrano Tr C

Stations	AMBI	Diversity	Richness	X	Y	Z	M-AMBI	Status
Bad	6	0	0	2.4979	-4.0465	-3.1006	0	Bad
High	1.3	1.13	11	-1.8305	1.1447	1.7894	1	High
1	1.5237	0.1758	7	0.71966	0.15219	-0.01293	0.64073	Good
2	1.6644	0.56438	9	-0.32719	0.42268	0.77057	0.78106	High
3	1.5486	0.93222	6	-0.81546	0.33195	0.21343	0.76551	Good
4	1.4857	0.52861	5	0.16519	0.10598	-0.32095	0.65012	Good
5	1.5147	1.0421	5	-0.94989	0.29302	0.018099	0.75725	Good

6	1.3034	1.1258	11	-1.8209	1.1405	1.7858	0.99884	High
7	1.4845	0.38348	8	0.15172	0.37524	0.41304	0.72262	Good
8	1.5	0.045244	2	1.5501	-0.42862	-1.481	0.4426	Moderate
9	1.5178	0.22391	7	0.61399	0.1762	0.020813	0.65146	Good
91	1.5172	0.13129	5	1.0355	-0.07925	-0.59567	0.56288	Good
92	1.6054	0.96559	7	-0.99008	0.41196	0.49997	0.80248	High

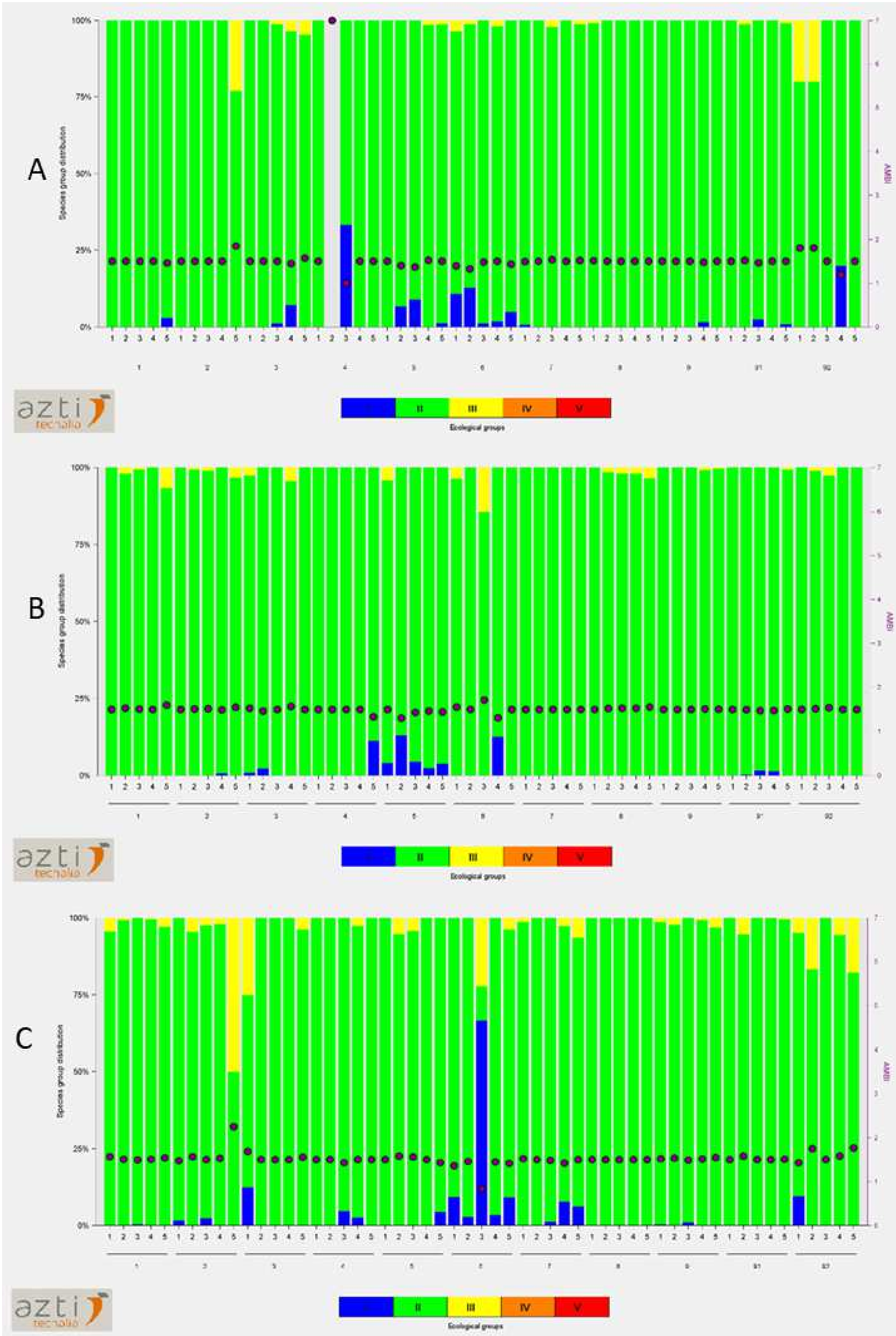

Figura 2: AMBI Torre Cerrano (Transact A, B and C)

Palombina Tr A

Stations	AMBI	Diversity	Richness	X	Y	Z	M-AMBI	Status
Bad	6	0	0	3.055	-4.0826	3.0693	0	Bad
High	1.46	1.46	11	-1.9518	1.0505	-1.7737	1	High
1	1.5095	0.87167	11	-1.5185	0.67818	-0.63184	0.87163	High
2	1.5273	0.81572	7	-0.35593	0.31042	-0.1061	0.73467	Good
3	1.5043	0.75274	6	-0.03532	0.21119	0.11422	0.69218	Good
4	1.5071	0.79323	5	0.21576	0.15153	0.14049	0.6696	Good
5	1.4625	1.2985	8	-0.99586	0.71477	-1.1515	0.87281	High
6	1.5	1.2076	4	0.19565	0.31232	-0.55464	0.72693	Good
7	1.487	1.4582	5	-0.26708	0.54554	-1.1432	0.81193	High
8	1.5278	1.0733	6	-0.26168	0.37619	-0.49813	0.75824	Good
9	1.5172	0.93232	8	-0.72156	0.46505	-0.43611	0.79107	High
91	1.5	0.1979	3	1.2025	-0.34346	1.492	0.48225	Moderate
92	1.5	0.25835	2	1.4387	-0.38964	1.4793	0.46412	Moderate

Palombina Tr B

Stations	AMBI	Diversity	Richness	X	Y	Z	M-AMBI	Status
Bad	6	0	0	4.0945	4.5868	3.2251	0	Bad
High	1.36	1.41	10	-1.7846	-1.1725	-1.3904	1	High
1	1.5766	1.0192	6	-0.05865	-0.2531	-0.19328	0.76451	Good
2	1.564	1.2379	9	-1.2089	-0.75876	-0.92427	0.91106	High
3	1.5	0.60998	2	1.6289	0.46719	0.97858	0.54574	Good
4	1.5044	0.65024	5	0.74744	0.14876	0.57839	0.64528	Good
5	1.4746	0.92702	10	-1.0414	-0.61481	-0.4809	0.86771	High
6	1.4946	1.4135	9	-1.4854	-0.98213	-1.2606	0.9612	High

7	1.3632	1.0215	8	-0.65842	-0.60167	-0.4587	0.84042	High
8	1.5	0.6595	5	0.7327	0.13642	0.56051	0.64798	Good
9	1.6217	1.2373	9	-1.1951	-0.71545	-0.91159	0.90669	High
91	1.5734	0.91224	9	-0.72306	-0.43299	-0.32475	0.82683	High
92	1.4965	0.69597	4	0.95196	0.19225	0.60194	0.62775	Good

Palombina Tr C

Stations	AMBI	Diversity	Richness	X	Y	Z	M-AMBI	Status
Bad	6	0	0	3.5442	4.294	-3.5529	0	Bad
High	1.35	1.47	13	-2.0414	-1.3141	1.9627	1	High
1	1.5172	1.0616	9	-0.72799	-0.53342	0.60872	0.7939	High
2	1.5847	0.9272	7	-0.23187	-0.209	-0.00764	0.70805	Good
3	1.6248	1.0133	5	-0.23744	-0.0761	-0.43817	0.67486	Good
4	1.4553	0.89696	7	-0.19539	-0.27876	-0.0007	0.71047	Good
5	1.5148	0.53807	5	0.80281	0.21476	-0.79752	0.57361	Good
6	1.4944	0.58309	7	0.51878	-0.00613	-0.26523	0.63551	Good
7	1.3512	1.4704	9	-1.6861	-0.97145	0.98126	0.89987	High
8	1.478	0.82298	13	-0.55769	-0.71789	1.4058	0.84203	High
9	1.5281	0.76871	12	-0.33472	-0.55405	1.1042	0.8009	High
91	1.5136	0.46409	6	0.87962	0.18598	-0.61197	0.58173	Good
92	1.5021	0.73563	6	0.26717	-0.03383	-0.38854	0.64497	Good

AMBI Palombina (TrA, TrB, TrC)

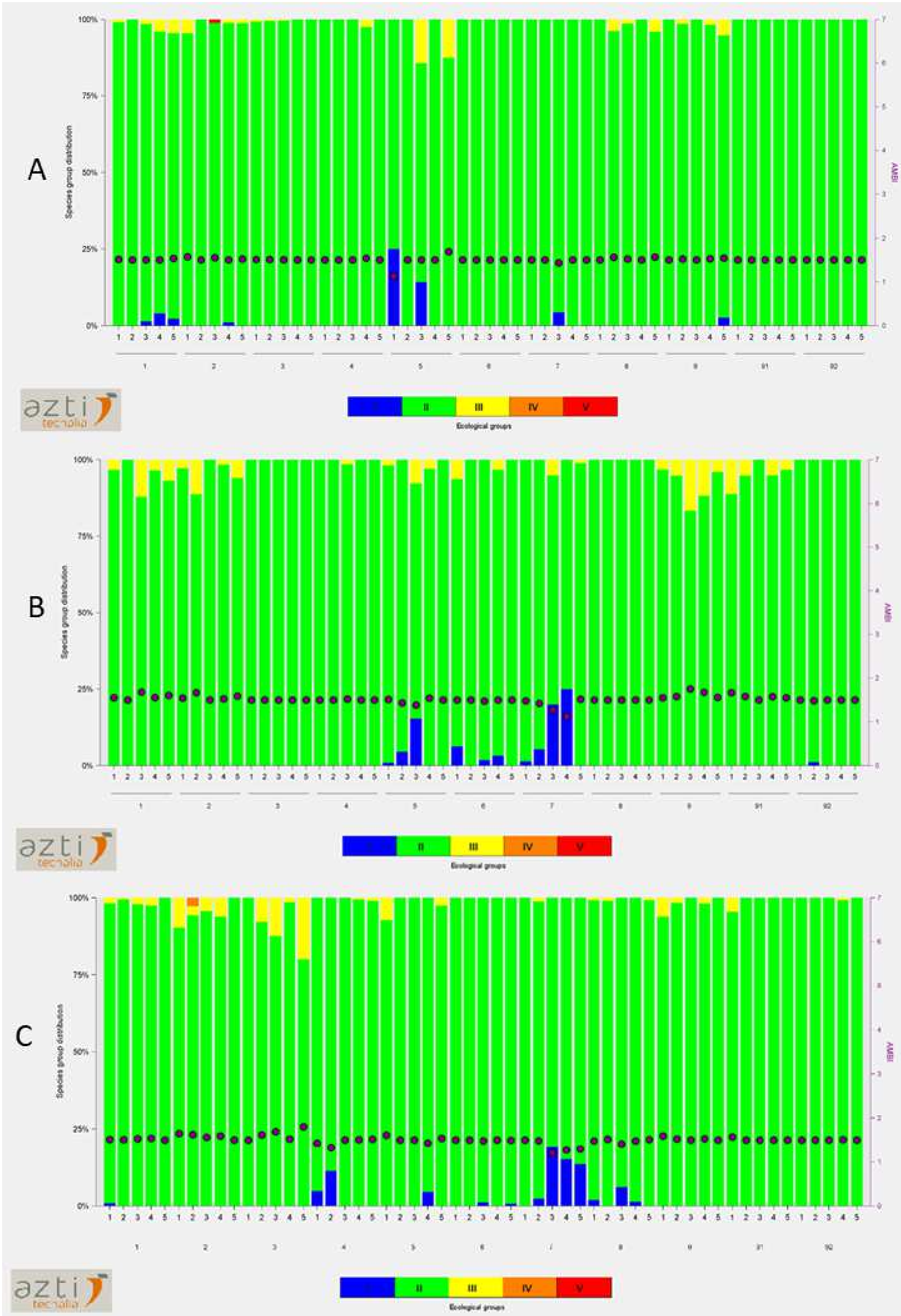

Figura 3: AMBI Torre Cerrano (Transact A, B and C)

Chapter 3: Evaluating the seasonal fluctuations in macrofaunal community from four Adriatic beaches with different levels of anthropogenic disturbances

Abstract

Adriatic sandy beaches are faced by certain disturbances related to touristic activities and infrastructure. These disturbances influence the macrofaunal abundances of sandy beach ecosystem which needs to be explored. This work was conducted through three seasons, on four Adriatic beaches with different levels of disturbances; 1) Torre del Cerrano, a marine protected area 2)Palombina and 3) Senigallia, two commercial beaches with proper touristic setup and 4) Montemarciano, a small beach in urban locality but without touristic infrastructure. Macrofaunal abundance and touristic facilities and trampling pressure were evaluated. The bivalves were the most dominant taxa in all the sites except Torre del Cerrano in June. *Lentidium mediterraneum* was the most dominant group at Palombina while *Donax sp.* was occupying Torre del Cerrano mainly. The amphipods were found to be possibly the most sensitive group towards disturbances since they were found mostly in periods with restricted beach use and in protected area. Gastropod *Tritia neritea* was found in lower abundance and restricted mainly to Senigallia and Palombina. Statistically, Torre del Cerrano was found to be the most different site compared to the other three, while Palombina and Montemarciano were having weakest differences in pairwise tests. Senigallia and Palombina were having the highest level of trampling pressure and touristic facilities while in Montemarciano and Torre Cerrano, no touristic facilities existed, and were faced by less trampling pressure.

However, statistical differences among sites are based on the abundance of macrofaunal community and could appear peculiar since the abundance of these macrofaunal taxa was likely influenced by COVID-19 lockdown and restrictions on beach use in spring (May 2020).

Keywords: Adriatic beaches, sandy beaches, macrofauna, intertidal, beach tourism

1. Introduction

Sandy beaches represent very dynamic environments occupied by a wide variety of life forms (Escrivà et al., 2020; McLachlan et al., 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). Furthermore, they 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). Coastal engineering (Dafforn et al., 2015; Pioch et al., 2018; Morris et al., 2019) and several other anthropogenic activities like trampling, mechanical beach cleaning, playing areas on the beaches are other factors that damage the intertidal 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 further affect the ecological traits of these organisms living and overall functioning of beach ecosystem (Thrush et al., 2017). The main form of coastal engineering considered in this study is the presence of breakwater barriers which are responsible for altering the natural habitat by minimizing the wave action and dynamics of the sediments (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).

In recent decades, there are more studies coming globally based on understanding the response of macrofaunal communities and populations towards certain physical disturbances (see for e.g., Defeo & de Alava, 1995; Veloso et al., 2006, 2008, 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 fewer and the comparative assessment of these communities in line with the anthropogenic factors is limited (Afghan et al., 2020). Furthermore, the COVID-19 pandemic has caused restrictions in the last year that could alter the situation and influence unexpected and unpredictable changes in the community structure. The aim of this study is to characterize and compare the macrozoobenthic communities of four 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 COVID-19 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 analyze possible temporal variations in the macrofaunal assemblage from the chosen sites.

2. Materials and methods

2.1 Study sites

To test our hypotheses, four sites along the North-Central Adriatic Sea have been chosen: two touristic beaches with breakwater barriers and many touristic facilities (Palombina and Senigallia), a beach with breakwater barriers but no touristic infrastructure (Montemarciano), although surround by industrial and urban area. The fourth one, is inside the B Zone of the Torre del Cerrano (thereinafter: Torre Cerrano) which is a marine protected area (MPA); it is a low-impacted beach with a backshore and without breakwater barriers or touristic facilities. The selected sites are distributed along the North-Central Adriatic Sea where 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 exposed to different levels of anthropogenic pressure. Besides other impacts, Torre Cerrano is the only site without breakwater barriers (**Figure 1**).

Figure 2: Study sites A) Torre Cerrano B) Palombina C) Montemarciano D) Senigallia

2.2 Sampling design and collection

The sampling activities have been carried out in Spring (May 2020), Autumn (late September 2020) and winter (January 2021). In the study further, spring refers to May 2020, Autumn refers to late September and Winter refers to January 2021. Field work was conducted in low tide conditions where the samples were taken from the intertidal zone. Three replicates were taken each time from each sampling site parallel to the shore at an average distance of 35 meters from a fixed point on the beach. Each sample was taken with a sampler having a width of 35 cm, pushed into the intertidal surface for 8 cm and dragged for five meters. The sample design and equipment is given in **Figure 2**.

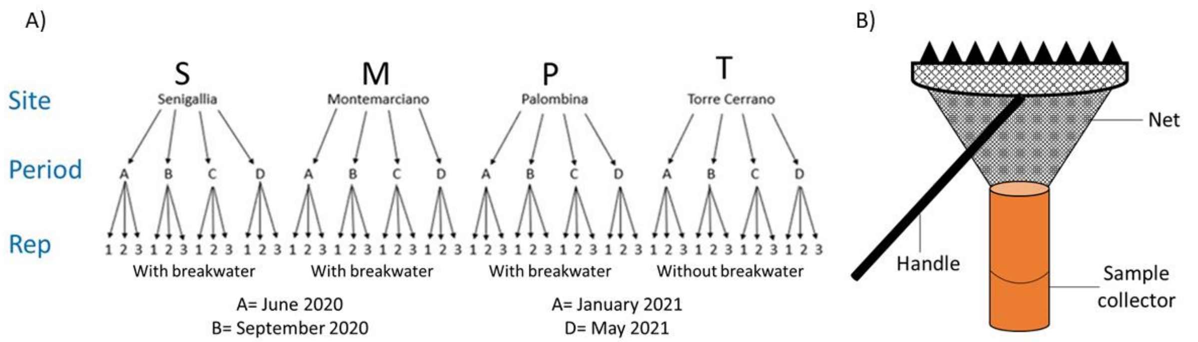

Figure 3: A) Sample design B) Sampling gear

The sampling gear (**Figure 2B**) was connected to a a 500 µm mesh to collect macrofauna and 10 mm mesh to collect shell fragments. The distance between two sampling points was kept as 5 meters.

The shells were placed in closed sachets while the organisms in plastic jars with seawater and alcohol 95% for preservation. People were counted on hourly basis on each of the sampling day at all the sites to assess the trampling pressure. Counting was considered for both the people walking/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.

It is important to mention here that the sampling equipment in this study was changed since this equipment has certain advantages over the previously used corer for sampling (mentioned in Chapter 2). It was observed during the field activities that the macrofauna studied were rather found horizontally near the surface rather than depth. Since this tool works more with surface rather than depth (as corer does), it is more appropriate for conducting sampling for macrofauna. Additionally, there is no loss of samples during collection which makes it more reliable, while in case of corer, there is often a possibility of losing fractions of samples. Moreover, it requires less physical effort and is easy to use compared to the corer.

2.3 Laboratory work

The samples were observed with a stereomicroscope, where the organisms were counted and their densities were calculated (number of items/m^3). 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/m^3).

2.4 Statistical Analyses.

PRIMER-E, version 7 was used to process the 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

3.1 Living component

The macrofauna assemblage is dominated mainly by bivalves in all the considered periods. In particular, the samples collected in spring (May 2020) at all sites had higher abundance of bivalves by many folds especially at Palombina (10781 ind. m^{-3} 5520± SD), compared to other taxa except Torre Cerrano. Most of the bivalves (usually having a size of 5-10 mm) usually belonged to *Lentidium mediterraneum* at all the sites with smaller contribution from *Donax sp.* and *Chamelea gallina*. In autumn (late September) however, the abundance of bivalves had declined at other sites but increased at Torre Cerrano (reaching 2493 ind. m^{-3} 173 ± SD) which mainly belonged to *Donax sp.* The bivalves didn't experience drastic changes in community from spring and autumn but their percent contribution mainly changed because of changes in abundance of other taxa **(Figure 3)**. The highest abundance for gastropods was recorded at Palombina in spring (98 ind. m^{-3} 61 ± SD).

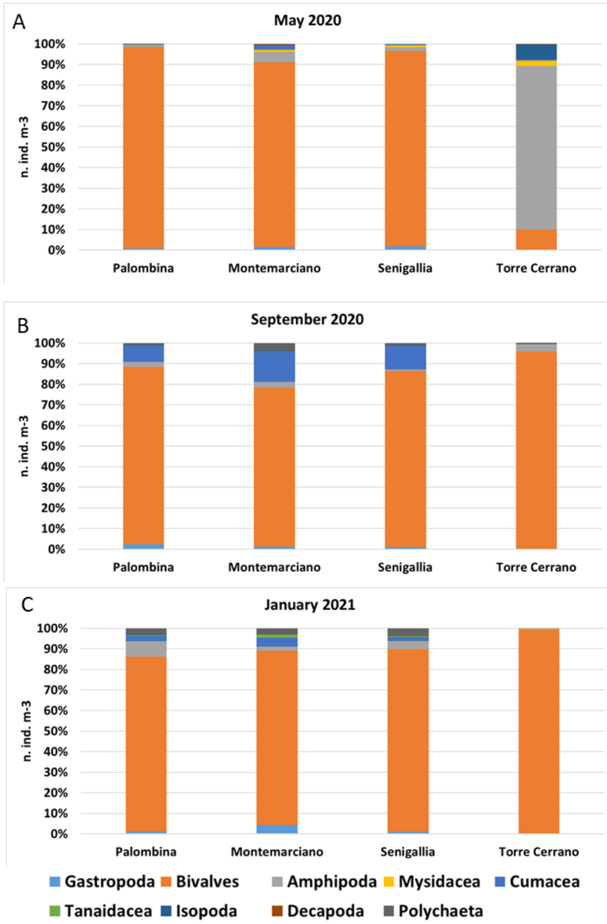

Figure 4: Variations in percent composition of macrofaunal abundance in different sites through A)Spring, B)Autumn, C)Winter

Excluding the taxon 'Bivalves' from the graphs (**Figure 4**), it was possible to highlight a trend of less abundant taxa, i. e., Gastropods, Amphipoda, Tanaidacea, Isopoda, Decapoda, Mysidiacea, Cumacea and Polychaeta. Interestingly in spring, there is presence of most of the macrofaunal groups especially at Montemarciano and Torre Cerrano, which declines in upcoming period (autumn), except for cumacea which increased at Montemarciano (152 ind. m^{-3} 109 ± SD) as well as in Palombina and Senigallia. Amphipods had the highest abundance in spring at Torre Cerrano (612 ind. m^{-3} 384 ± SD), mainly represented by *Bathyporeia guilliamsoniana,* while there was contribution from *Pontocrates altamarinus* and *Echinogammarus stocki*.

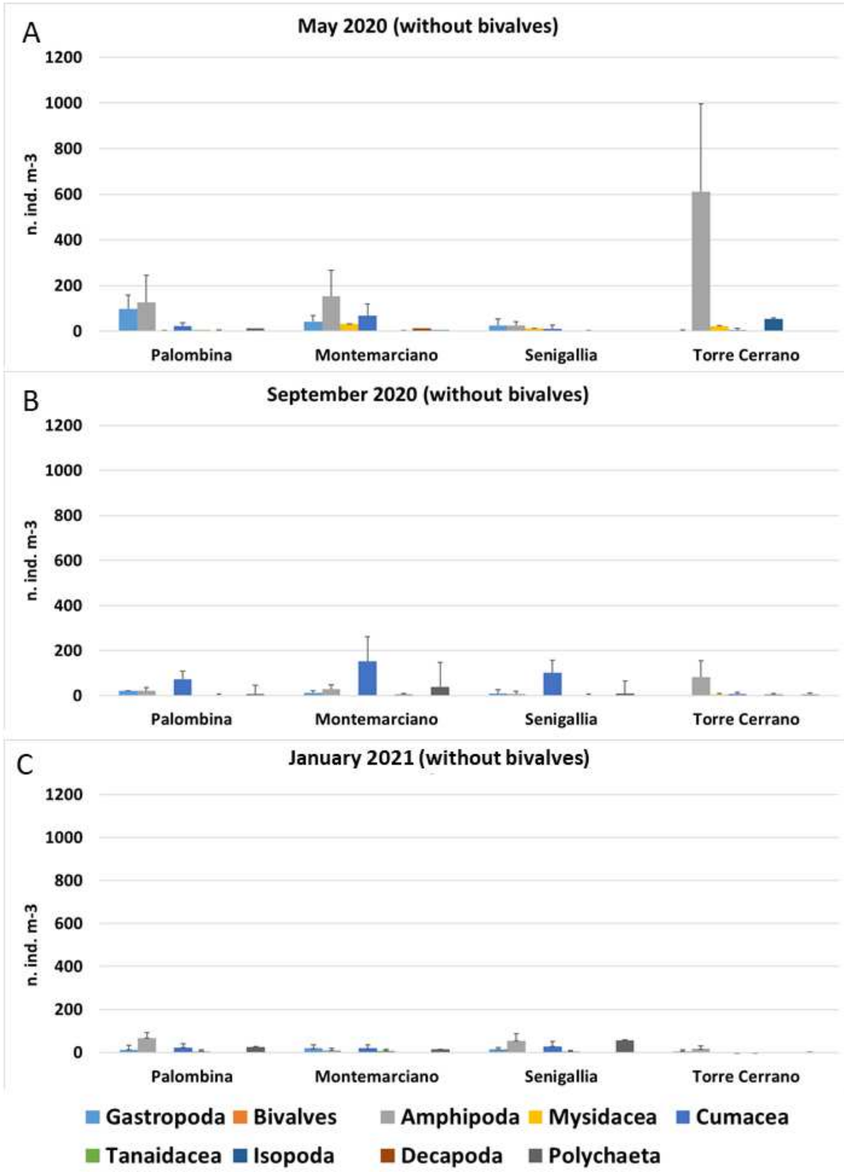

Figure 5: Abundance of taxa (ind. m^{-3}) without bivalves through A)Spring, B)Autumn, C)Winter

Overall, polychaetes are rarely found at Torre Cerrano and in lower abundances at other sites. Apart from Torre Cerrano, Montemarciano had comparatively higher abundance of crustaceans (amphipods and cumacea). Mysids and Tanaidacea were found scarcely, though both in the spring season which can be observed to have more diversity especially at Montemarciano and Torre Cerrano. The percent composition of taxa without bivalves (**Figure 5**) gives a clearer picture of the fluctuations in abundance of different taxa.

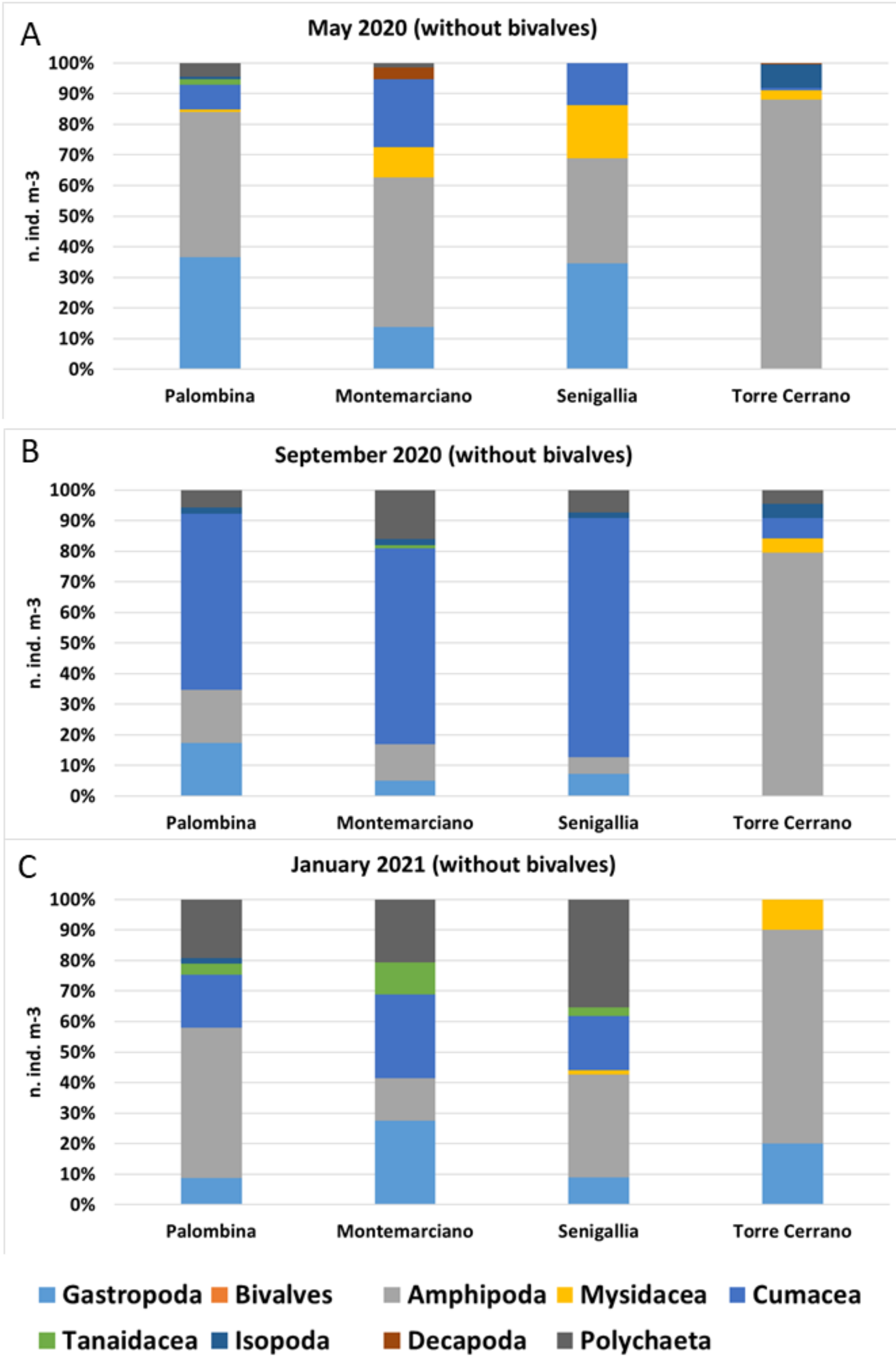

Figure 6: Percent composition of macrofaunal community without bivalves through A)Spring, B)Autumn, C)Winter

The list of the taxa found is presented below in **table 1**.

Table 1: List of taxa found at all the four sites; Senigallia (Sen), Montemarciano (MM), Palombina (Pal), Torre Cerrano (TC)

Species/group	**Taxon**	**Senigallia**	**Montemarciano**	**Palombina**	**Torre Cerrano**
Apseudopsis sp.	Tanaid	x	x	x	
Apseudopsis latreilliid (Milne Edwards, 1828)	Tanaid	x	x		
Bathyporeia guilliamsoniana (Spence Bate, 1857)	Amphipod	x	x	x	x
Bittium sp.	Gastropod	x	x	x	
Chamelea gallina (Linnaeus, 1758)	Bivalve	x	x	x	x
Comopsis sp.	Cumacean	x	x	x	x
Diogenes pugilator (P. Roux, 1829)	hermit crab		x	x	
Donax trunculus Linnaeus, 1758	bivalve	x	x	x	x
Echinogammarus stocki G. Karaman, 1970	amphipod		x		x
Eriphia verrucose (Forskål, 1775)	crab		x		
Eurydice sp.	isopod			x	x
Gammaroidea	amphipod	x	x	x	x
Glycera sp.	Polychaete	x	x	x	
ldotea sp.	Isopod		x	x	
Lentidium mediterraneum (O. G. Costa, 1830)	Bivalve	x	x	x	x
Mactra stultorum (Linnaeus, 1758)	Bivalve			x	
Metapenaeus monoceros (Fabricius, 1798)	Shrimp	x			
Mysinae	Mysid	x	x	x	x
Nassariidae	Gastropod	x	x	x	x
Ophiuroidea	brittle star	x			
Peringia ulvae (Pennant, 1777)	Gastropod	x	x	x	
Pontocrates altamarinus (Spence Bate & Westwood, 1862)	Amphipod	x	x	x	x
Pseudocuma sp.	Cumaceans	x	x	x	x
Sabellidae	Polychaete	x			
Scoleiepis sp.	Polychaete		x		
Sphaeroma serratum (J. C. Fabricius, 1787)	Isopod				x
Spionidae	Polychaete		x	x	
Tritia neritea (Linnaeus, 1758)	Gastropod	x	x	x	
Unknown fragmented polychaete	Polychaete	x	x	x	x
Veneridae	Bivalve	x	x	x	x

3.2 Statistical analyses for macrofauna

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.

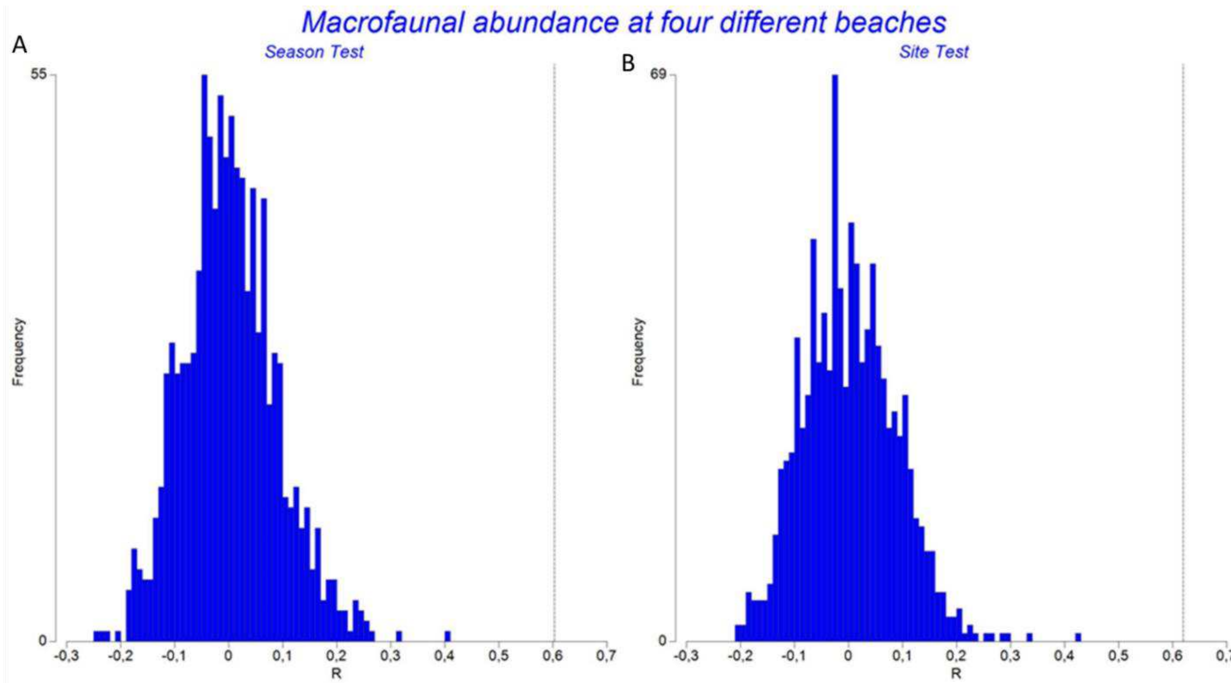

Figure 7: Two-way ANOSIM for A) seasons B) sites

The test for the studied three seasons as shown on the left side of the plot, shows a significant but moderate difference of macrofaunal abundance among the seasons by the R value (0.603). On the right side, the graph shows the macrofaunal differences based on the sites which are slightly stronger (0.619) than the differences based on seasons.

Table 2: Pairwise test among the studied months (seasons)

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
May2020, Sep2020	0,87	0,1	10000	999	0
May2020, Jan2021	0,704	0,1	10000	999	0
Sep2020, Jan2021	0,407	0,2	10000	999	1

Table 2 above shows the pairwise comparison among different periods. The strongest differences could be found between the May and the January. The moderate differences could be found between September and January but are still significant which were not significant statistically.

Table 3: Pairwise test for the sites (P: Palombina M: Montemarciano S: Senigallia T: Torre Cerrano).

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
P, M	0,259	5,7	1000	999	56
P, S	0,346	5,2	1000	999	51
P, T	1	0,3	1000	999	2
M, S	0,222	11,3	1000	999	112
M, T	0,815	0,4	1000	999	3
S, T	0,938	0,3	1000	999	2

Table 3 above shows the pairwise comparison of the sites. The differences among sites could be seen significantly high from the histogram given earlier but here more interesting comparisons could be observed as the differences between Torre Cerrano which is an (marine protected area) MPA, and other sites in pairwise test are significant and stronger displaying higher R value; while the differences among between other sites display low R value and non-significant P value, highlighting similarity between them. The highest differences could be found between Palombina and Torre Cerran. The overall picture portrays that considering the macrofaunal abundance, the protected area Torre Cerrano tends to be the most different one.

3.3 Other data

Shell fragments

The amount of shell fragments at different sites in the three seasons was assessed which contained whole shells or fragments larger than 1 cm. The amount of shells was observed to be very low in May 2020 at all the sites (**Figure 7**).

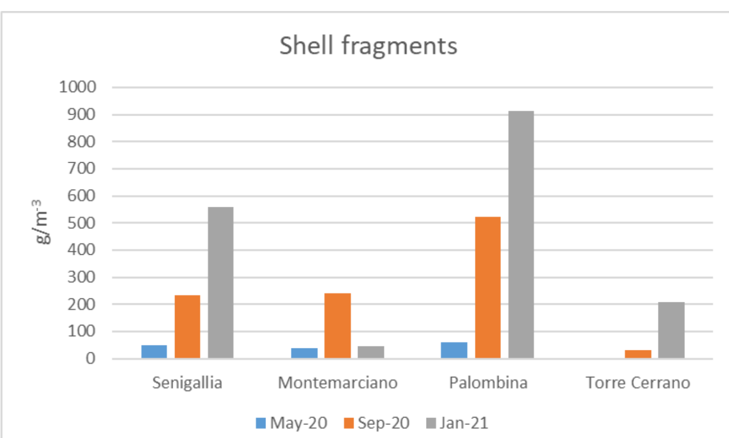

Figure 8: Shell fragments found at all sites through the three seasons; Spring (May-20), Autumn (Sep-20), Winter (Jan 21).

In the later months, the amount of shells could be seen to increase constantly till autumn and subsequently winter at Senigallia and Palombina. In winter, all the sites had increased quantity of shell fragments except Montemarciano where the quantity declined. Overall Montemarciano and Torre Cerrano were found to have comparatively less abundance of shell fragments.

Trampling activity and touristic infrastructure

Trampling activity of beach visitors along the shore as well as in the intertidal beach was evaluated on hourly basis. It can be observed that the trampling is very high in Senigallia (380/hour) during the spring, while it is totally absent in Montemarciano in all the year. Furthermore, the minimum trampling for all four sites is in winter period. Torre Cerrano (54/hour) and Palombina (108/hour) had rather moderate trampling pressure. The trampling activity at all the sites through the studied period was observed to change without any specific trend or pattern except Montemarciano where no trampling activity was detected (**Figure 8**).

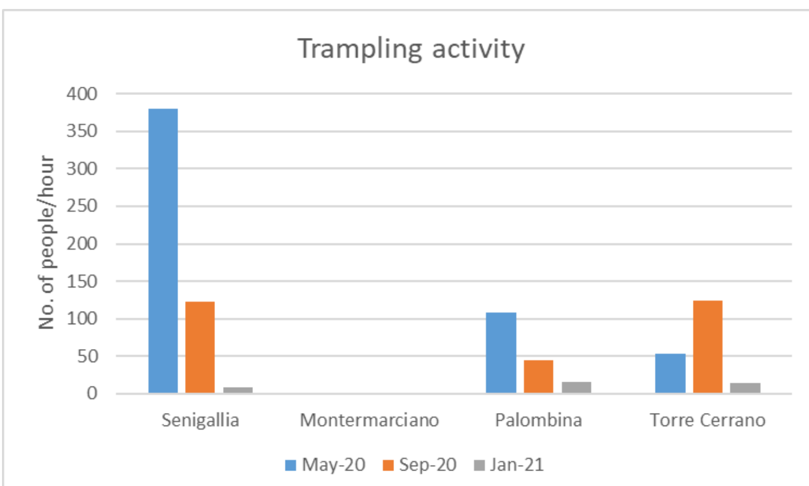

Figure 9: Trampling activity at all the sites

Touristic infrastructure such as restaurants, bars and playing areas were counted on the beach. Palombina and Senigallia were found to be hosting more touristic infrastructure (**Table 4).**

Table 4: Touristic facilities at the studied sites

Site	bars/restaurants	Playing areas/ courts
Senigallia	49	25
Montemarciano	0	0
Palombina	51	18
Torre Cerrano	0	0

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 or 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.

4. Discussion

Temporal variation at four beaches with different levels of anthropogenic disturbances and touristic facilities was assessed to investigate the response of the macrofaunal assemblages in different scenarios. For all the fluctuations in abundance of macrofauna in the studied months and sites, it is important to mention that the whole study period was withing the COVID-19 pandemic and the periodic restrictions on human activity as well as the use of the beach could possibly produce results that are different from what could be observed in other years without any pandemic related restrictions. Several studies (Ormaza-Gonzalez 2021; Soto et al., 2021) have reported the changes in abundance of coastal communities influenced by COVID-19 lockdowns. In this current study, the abundance of macrofaunal taxa changes at sites over time, where bivalves are found to be the most dominant group in all the sites through all the periods except Torre Cerrano in spring (May 2020). Bivalve specifically *Lentidium mediterraneum* are in huge numbers compared to other taxa and masks the whole community in Northern-Central Adriatic beaches (Bertasi et al., 2009), hence they were excluded from the data to highlight the changes in the rest of the community. In this study, they were found to be more than 90% of the community at all the sites and periods except for Torre Cerrano in Spring. The bivalves in Palombina were mainly *Lentidium mediterraneum* while at Torre Cerrano they were mainly *Donax sp*. In Montemarciano and

Senigallia, the bivalves found belonged to *Lentidium mediterraneum*, *Donax sp.* and *Chamelea gallina*. Apart from bivalves, most of the taxa were present in the spring season especially in Montemarciano and Torre Cerrano. These both are the sites without commercial touristic facilities which potentially result in minimum disturbances providing comparatively better conditions for organisms to thrive. Reis and Rizzo, (2019) have reported that the macrobenthic organisms prevail in absence of disturbances. Gastropods which belonged to *Tritia Neritea* were found in summer spring season mainly at Palombina and Senigallia which are more disturbed sites compared to the other two; however, this specie of gastropods can escape physical disturbance since it has thicker shell and is capable to rapidly hiding in surface from threats. Additionally, it's a scavenger specie feeding on dead organism which could be more in sites with physical disturbances due to food availability. The mysids and isopods were rarely observed in autumn and winter period while the amphipods and cumacea were found to experience more fluctuations in their abundance compared to the other taxa. After being abundant in spring, the amphipods declined in abundance while the cumacea increase at Palombina, Montemarciano and Senigallia in the following period, autumn (late September). The decline in abundance could be related to the sensitivity of amphipods towards disturbances as reported in literature (Ugolini et al., 2008). Since beach visitors were allowed after May, it could have played a role in population decline of amphipods. However, the higher abundance of amphipods in spring could also be related to the biological cycle of certain species. In case of Cumacea, there highest abundance was found in autumn. The increase in cumaceans abundance could be possibly due to their resilience or rapid recovery in population. (Veloso et al., 2008; Machado et al., 2017). Beach cleaning activities are also one of the major cause responsible for the loss of crustaceans from beaches (Morton et al., 2015; Zielinski et al., 2019) which could have played a role in the overall decline in population of crustaceans (except cumacea) in autumn since with the end of summer season, heavy machinery is used on the beaches especially Palombina for maintenance.

The abundance of shells was found to on the rise at Palombina and Senigallia from spring till winter. However, at Montemarciano and Torre Cerrano the abundance of shell fragments was much less which could be attributed to the fewer disturbances at these sites. The abundance of shells could be also related to the presence of dredging and fishing activities since dredging has been very damaging to bivalve shells (Kar Soon and Ransangan, 2018). Palombina was found not only to have highest abundance of shell but also a constant increase in the shell's abundance in the proceeding periods. In the three Marche sites the quantity of shells could be related to fishing events involving vessels with hydraulic dredges while in Torre Cerrano MPA this type of fishing practice is banned among 3 NM which could be the reason for less weight of shells.

Trampling activity was assessed along the beach in the form of number of people walking or running per hour along the shore as well as the intertidal zone. The highest trampling was observed in the spring in Senigallia and Palombina which are certainly the most renowned urban beaches having touristic facilities and bars. Torre Cerrano had considerable level of trampling in autumn however in Montemarciano there were zero trampling activity. Probably during the summer and spring in Torre Cerrano, people prefer to go to beaches that have more facilities and comforts, such as children's play areas and bars. Torre Cerrano is frequented by people who go for walks, often accompanied by their dogs. The trampling activity is somehow related to touristic infrastructure and facilities as sites with more facilities and bars/restaurants are receive more visitors and people jogging/walking on the shoreline. Senigallia and Palombina has almost the same number of playing areas and touristic facilities that are attraction factors for more visitors. Hence, more facilities and visitors are likely to reflect the abundance of intertidal macrofaunal organisms; however, since all the data for this study was taken during pandemic with on and off restrictions, there could be possibly some peculiarity regarding the abundance of certain taxa.

5. Conclusion

Overall, it could be observed based on the statistical analyses that Torre Cerrano, the MPA is significantly different from the other sites located in urban setup as far as macrofaunal abundance is concerned. The bivalves are the most dominant group especially *Lentidium mediterraneum* in this part of the Adriatic Sea. In Torre Cerrano, the bivalves mainly belonged to *Donax sp*. Usually, Palombina and Senigallia are among the beaches with maximum visitors and commercial touristic infrastructure, but the higher number of bivalves and the presence of different crustaceans in spring 2020 could be due to COVID-19 related restrictions since that period was after lockdown. Montemarciano beach is next to industrial infrastructure but is faced by very limited tourism pressure since its lacking touristic facilities and was found to have more crustaceans than other impacted beaches i.e., to Palombina and Senigallia. Torre Cerrano was dominated by crustaceans, mostly amphipods throughout the study period (excluding bivalves). Amphipods appear to one of the sensitive groups towards disturbances since they exist mainly in spring when beach visitors were restricted. The gastropods were mostly found in Palombina which belonged to *Tritia Neritea,* especially in summer.

Shell fragments could be found frequently increasing from spring till winter at Palombina and Senigallia. While at Torre Cerrano and Montemarciano the shell fragments were rather less abundant. 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. 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 Torre Cerrano was is the beach with more different characteristics as compared to the other three. The data found to compare the sites is portraying an interesting picture, however it can't be said as the last word since data could potentially change in normal years as the study period was hit by lock downs time to time which had kept a limit on beach visitors. Further work could be done to explore the research problem further and possibly, the future data could be compared to ours or other work conducted during lockdown to portray the macrofaunal abundance of these sites in different scenarios.

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Chapter 4: Evaluating the role of summer tourism and COVID-19 lockdowns in shaping macrofaunal communities: a study of Northern-Central Adriatic sandy beaches

Abstract

The effects of human impacts in natural beach ecosystems need to be investigated thoroughly, with particular regards to benthic macrofaunal communities in the intertidal zone. This study is intended to compare five sandy beaches in the North-Cental Adriatic —three highly and two scarcely frequented, among which the B zone of a Marine Protected Area (MPA)— by evaluating variations in macrofaunal communities in relation to different levels of human disturbance. Considering that presence of tourists peaked in mid-summer in the study area, the hypothesis that the macrofaunal assemblage varied in relation to touristic pressure and in particular to human trampling was tested by monitoring the communities in three different periods: before-, mid-summer and after-summer. The COVID-19 related restrictions, entered into force in the 'before-summer' period of 2021, were also taken into account.

Significant changes in the macrofaunal communities were found among the three studied periods, likely mainly related to people trampling in the intertidal zone. Amphipods, cumaceans and *Donax* sp. were found to be the most sensitive taxa to disturbances as they disappeared with the start of beach activities at the end of COVID-19 lockdown. On the contrary, *Lentidium mediterraneum* and the gastropod *Tritia neritea* were the most resistant taxa to trampling.

Considering the macrozoobenthic assemblages in relation to sites, significant differences between the MPA and the other localites were observed only in the 'before-summer' period likely due to the pandemic restrictions, reduced leisure and economic activities, with a consequent improvement of the environmental quality. After the lockdown and hence when the economy restarted, no differences among sites were detected, suggesting that the reserve effectiveness failed when human pressures increased again. This result underlines the importance of increasing the protection level of the few semi-natural Adriatic sandy beaches and of developing complementary measures to limit cumulative detrimental effects of economic activities.

1. Introduction

Despite certain threatening anthropogenic impacts on coastal ecosystems, beaches remain one of the main attraction points for human due the sun, sea and sand (i. e., 3S tourism, Zielinski et al., 2018). Sandy beaches are the ecosystems that are more frequently accessed by humans for leisure or to create economic opportunities globally (Amaral et al. 2016). Because of these possible benefits, disturbances related to human activities on sandy beaches are constantly increasing and making the ecosystem more susceptible and prone to anthropogenic threats (He and Silliman 2019; Park and Kim, 2021) and rising sea temperature (Scapini et al., 2019). These cumulative impacts are in fact resulting from increasing demand for ocean and its resources as the human population and migration towards coastal areas increase (Halpern et al., 2015).

Characterized by their sand, wave and tidal regimes, sandy beaches also provide habitat to the diversity of different faunal species, especially burrowing invertebrates, molluscs, crustaceans and polychaetes, encompassing scavengers, predators and filter feeders (Defeo et al., 2009). Even if beaches are the major source of nutrient recycling, provide water filtration and mineralize organic matter in high quantities, these ecosystems are often neglected compared to other coastal habitats like coral reefs (Limongi and Carranza 2020).

Biodiversity of sandy beaches and their ecosystem services are mined by impacts such as dredging activities (Zilinskas et al., 2020), artificial barriers (Martin et al., 2005; Bertasi et al., 2007; Munari et al., 2011; Becchi et al., 2014), trampling (Schlacher and Thompson 2012; Schlacher et al., 2016, Reyes-Martínez et al., 2018), mechanical beach cleaning (Gilburn et al., 2012; Morton et al., 2015; Griffin et al., 2018), playing beach games (Moffet et al., 1998), release of chemicals into the sea, offroad vehicles on the beach (Celliers et al., 2004; Schlacher et al., 2008; Petch et al., 2018) etc. Over the last two decades, several studies have been conducted on evaluating these impacts. Some studies have evaluated the impacts on a specific taxon (Fanini et al., 2005; Ugolini et al., 2008; Stelling-Wood et al., 2016) while some have assessed these impacts by considering the whole macrozoobenthic assemblage (Veloso et al., 2006; Rossi et al., 2007). At the same time, some studies are focused on overall threats to sandy beach macrofauna (Barros 2001; Bessa et al., 2014) while some are focused on certain particular impacts (Malm et l., 2004; Fanini et al., 2014; Schlacher et al., 2016). The resident macrofaunal species responds to these disturbances in the form of alterations in their behaviour, interactions (predation, competition and facilitation) and abundances and hence can indicate the health of local ecosystem (Heaven and Scrosati, 2008;

Costa et al., 2020). Because of their ability to adjust their structure and composition, the macrofaunal community qualify as one of the most important groups in assessment of human activities (Vesal et al., 2021).

Considering the Adriatic beaches, there is limited work done on the benthic macrofaunal communities of sandy beaches and very few indicator species have been detected to determine the anthropogenic impacts on benthic macrofauna of intertidal sandy beaches (Afghan et al. 2019). Using indicator species as a surrogate to track changes in the biodiversity and environment is one the most cost-effective way since studying every species and its environment could be financially and logistically impossible (Lindenmayer and Likens, 2010). Additionally, as one indicator species may not be a suitable indicator in another region, it increases the need for exploring the topic further.

In the last two years, i.e., 2020 and 2021, people were not allowed to Adriatic beaches during lockdown periods because of COVID-19 pandemic. The controlling and reduction of anthropogenic activities has a potential capability to improve the environmental health (Patterson Edward et al., 2021). Recently, numerous urban beaches all over the world have been assessed before and after COVID-19 lockdown regarding stressors such as pollution, noise, human physical activities and user density which have revealed improvements in the biological components of the coasts as well as other environmental factors (Soto et al., 2021). Because of absence of such disturbances, some beaches had even appeared to have —at least transitorily— characteristics like protected areas (Ormaza-Gonzlez et al., 2021).

In this study, five beaches from the North-Central Adriatic Sea —three highly and two scarcely frequented, among which the B zone of a Marine Protected Area (MPA)— were considered to test the following hypotheses: macrofaunal communities vary 1) in relation to different levels of human disturbance, 2) with increase in disturbances as the touristic period proceeds. To test these hypotheses, field work was conducted in three different periods —before-, mid- and after-summer— i. e., just before, during and just after the touristic period. The 'before summer' period coincided with the restrictions due to Covid-19 pandemic, and a possible lockdown effect has been explored.

These beaches are subjected to cumulative impacts such as trampling, beach nourishment, mechanical beach cleaning, presence of breakwater barriers and touristic infrastructure such as restaurants and playing areas. Even if it was not possible to clearly separate the effects of each pressure, the study is providing evidence of consequences resulting from human trampling on benthic macrofauna and supplying a baseline to the future research for assessing the effects of breakwater barriers. Moreover, indicator species for the intertidal beaches of northern Adriatic coast were provided.

2. Materials and methods

2.1 Study sites

The study was conducted in 5 different beaches along the northern Adriatic coast in 2021. All the beaches exhibit different characteristics (Table 1), one way or the other and faced by different levels of human pressure. The beaches we studied are namely, Torre Cerrano (thereinafter: Torre Cerrano), Palombina, Montemarciano, Senigallia and Senigallia 2. Torre Cerrano is a natural beach and a marine protected area situated between the towns of Silvi and Pineto of Abruzzo region. The protected area was recognized in 2010 having an extension of 7 kilometers parallel to the shore and 3 NM towards the sea. The zonation of the area can be divided into B, C and D zones according to the protection level, no A zone is present. The samples were taken from B zone. The backshore of the B zone is characterized by sand dunes, a botanical garden, a bike trail, and a railway track.

Table 1: Main impacts present in each of the studied localities

Beach	Breakwaters	Trampling activity	Restaurants/Playing areas	Adjacent to railway line
Torre Cerrano		x		x
Palombina	x	x	x	x
Montemarciano	x			x
Senigallia	x	x	x	
Senigallia 2		x	x	

Palombina beach in the northern-most beach situated in Ancona, detached from residential area by a road and a railway line. The beach is characterized by several bars, restaurants, volleyball courts and lots of visitor facilities. The sand is accumulated on the banks in winter season to save it from erosion and it’s re-distributed on the beach in swimming season using heavy machinery and trawlers.

Montemarciano is a beach without any restrictions next to quite urbanized locality but no tourist infrastructure and commercial activities (bars, restaurants, playing areas etc.) is available here. Montemarciano beach is also having a railway track with a small station that separates it from the residential area. Compared to other beaches mentioned above, there is more plastic and non-plastic waste, branches, and pieces of wood likely transported by water.

The fourth beach we considered for our study is Senigallia, that too, is a commercial beach with proper touristic infrastructure and urbanized neighbourhood like Palombina. There are more beach bars, restaurants and playing areas than the other mentioned beaches, that makes it the beach facing maximum touristic pressure. The fifth and last beach studied is also in the same locality termed as "Senigallia 2" which displays almost the same infrastructural setup.

Except Torre Cerrano and Senigallia 2, all the three beaches are characterized by the presence of breakwater barriers for the shoreline protection against erosion. Except Torre Cerrano where the backshore is characterized by agricultural lands, the rest of four beaches have their backshore characterized of urbanized setup. Except both the beaches at Senigallia, the rest of the beaches are located next to a railway track. An aerial view of the sites is given below in Figure 1.

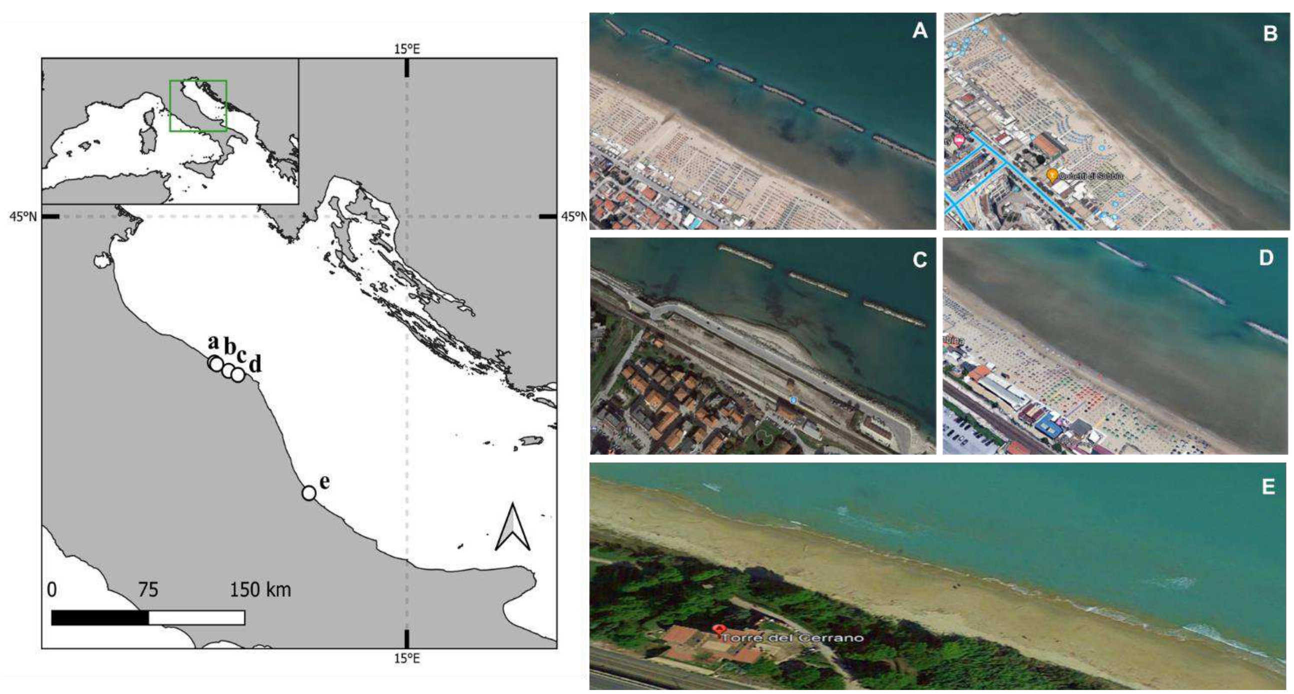

Figure 10: Map and aerial view of the studied sites displaying the surrounding scenario: a) Torre Cerrano b) Palombina c) Montemarciano d) Senigallia e) Senigallia 2

2.2 Sampling method

In order to test hypotheses 1) and 2), , sediments containing macrofauna were collected in three sessions, one just before-summer (May), the second mid-summer (August, peak touristic period) and the third one just after-summer (second half of September). Three replicates for each period were taken from the intertidal zone at each site, on average about 35 meters from a fixed point on the emerged beach in the low tide conditions. In order to check the tide levels, and other weather-related data before sampling sessions, the website https://meteopesca.com/ was used. A major reason of choosing this sampling equipment was that in our previous field activities, we had observed that the macrofaunal communities stay more near the surface rather than the depth. Additionally, this method completely rules out the loss of sediment containing macrofauna, hence the human error in data collection is avoided. The sampling equipment (**Figure 2**) used was having a width of 35 cm, which was pushed into the intertidal surface for 8 cm and dragged along for 5 meters, parallel to the shore, collecting the sediment sample containing macrofaunal component. A distance of 5 meters was left between each two sampling points. The sample collecting part of the equipment was attached with a 500 µm mesh which would sieve the sample for collection of benthic macrofauna. additionally, sediment samples were collected from each site in plastic tanks for granulometry purpose. The sample design is presented in **Figure 3**.

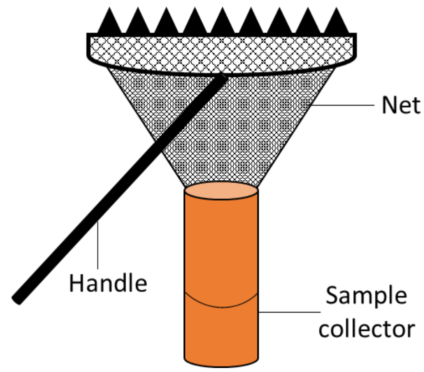

Figure 11: The sampling gear used

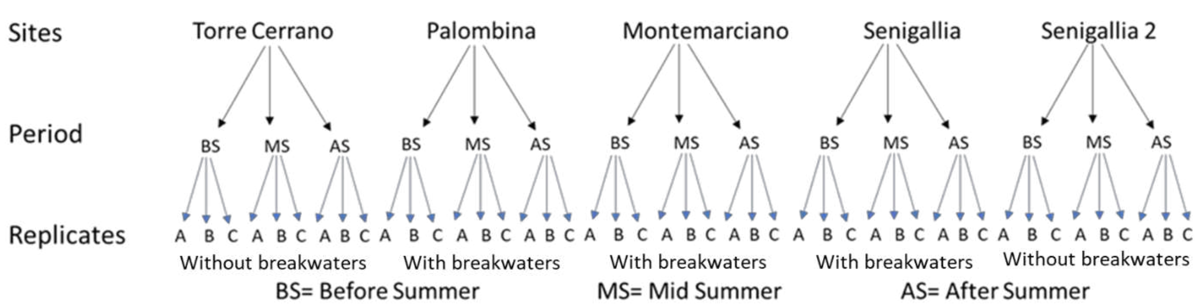

Figure 3. Sampling design for taking sediment containing macrofauna

The samples were preserved on the spot with 95% alcohol. A wash bottle with seawater was used in the sieving process. The samples were taken to the lab and were analyzed under a stereomicroscope to extract main taxa (i.e., bivalves, gastropods, polychaetes and crustaceans belonging to different families). Density for each taxon was expressed as number of individuals m^{-3}.

2.3 Shell fragments, trampling and granulometry

Apart from main data about macrofaunal community, other data was also taken into account to supplement the evidence of fluctuations in these communities. In order to assess the pressure caused by the beach users, trampling activity, that included running and walking along the shore and in the intertidal zone, was quantified and compared among all the sites in different periods since its one of the most important disturbances of the coastal ecological system (Veloso et al., 2006). People were counted (Reis and Rizzo, 2019) on hourly basis on each of the sampling day at all the sites. It was made sure that the counting was done on weekdays since the visitor's data on weekends could be possibly higher.

Shell fragments and dead bivalves were commonly observed along sandy beaches of the Adriatic coast; however, factors driving presence and abundance of shells on shore were not fully understood. The shell fragments were collected simultaneously to sediment collection through the same sampling gear used for macrofauna collection which presented two nets, one with a larger mesh (10 mm) for collecting items over 10 mm, such as shell fragments, and one net with a size of 500 µm for collecting macrofauna. The fragments were dried and weighed for all the sites and periods.

To assess granulometry for all the sites, sediment samples were taken once using simple falcon tubes (45 ml) in August from each of the sites. Three replicates were then made from each sample and stored for drying in Eppendorf tubes. Mastersizer 3000 laser diffraction analyser having a lens range of 0.01-20000 µm was used, following the ISO 13320:2020(E) guidelines (Bainbridge et al., 2021). All the samples were ultrasonically dispersed for 30 seconds using a refractive index of 1.52. The three replicates were analysed for quality assurance and the average was calculated. The results were obtained as grain size distribution in percentage.

Statistical analyses for macrofaunal component

To process our data and understand the differences and similarities among sites as well as the mentioned periods, PRIMER-E (Plymouth Routines In Multivariate Ecological Research) version 7 package (Clarke and Gorley 2015) was used. The abundance results were transformed by square root in order to minimize the effect of dominant species and lower triangular resemblance matrices were obtained using the Bray-Curtis similarity measure on square root transformed data. A two-way Analyses of Similarities (ANOSIM) was run to see the pairwise differences between each of the sites and between each of the period. In order to display the compositional pattern of the data and visually evaluate the similarities among the sites, ordination by Non-metric Multi-Dimensional Scaling (nMDS) (Kruskal & Wish, 1978; Clarke & Green, 1988) was also performed.

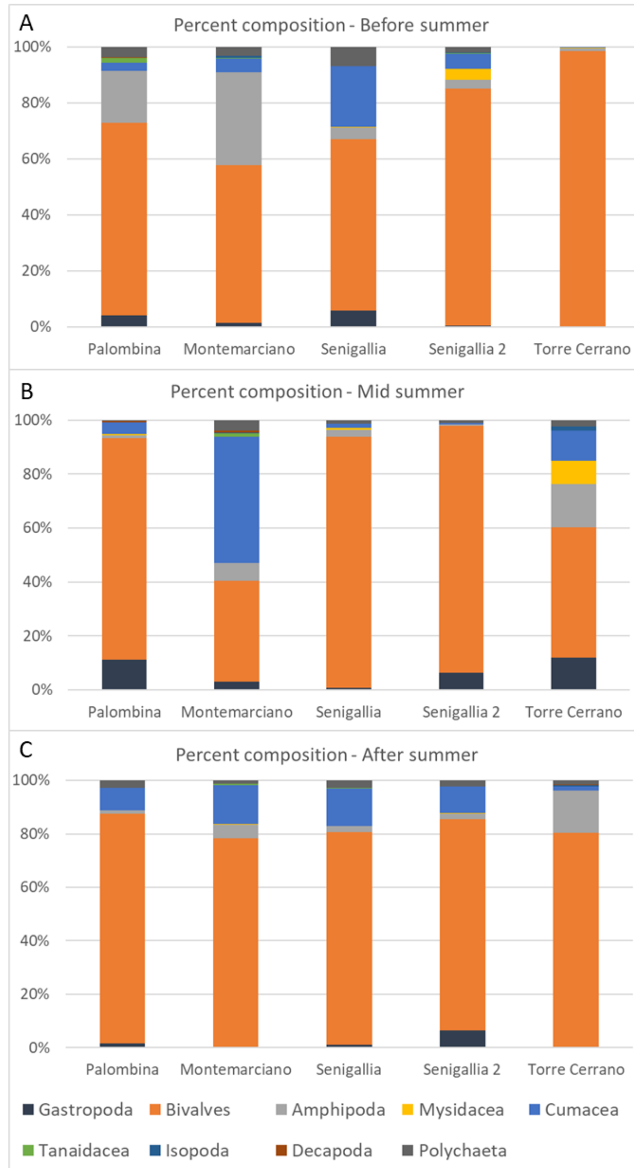

Figure 4: Percent composition of macrofaunal taxa in A) Before-summer B) Mid-summer C) After-summer periods

3. Results

3.1 Macrofaunal abundance

The results obtained were showing quite significant differences in the composition of community at the studied sites (Figure 4). Overall, bivalves were found to be the dominant taxa at all sites and all periods except Montemarciano in mid-summer period where cumacea were found as the most dominant group (502 ind. m^{-3} ± 562 SD) (Figure 4B). Montemarciano and Torre Cerrano beaches were the sites displaying more variations in community composition through the three periods. In Montemarciano, amphipods (257 ind. m^{-3} 237 ± SD) and bivalves (436 ind. m^{-3} 143 ± SD) were the dominant taxa before summer, cumaceans were dominant in mid-summer period while bivalves were taking over again as dominant taxa in after summer period (3024 ind. m^{-3} 1341± SD). Torre Cerrano can be observed to have changes in community composition from before-summer to mid-summer period since the dominance of bivalves has been decreased by amphipods, cumaceans, polychaetes and gastropods as shown in the percent contribution graphs in **Figure 4.**

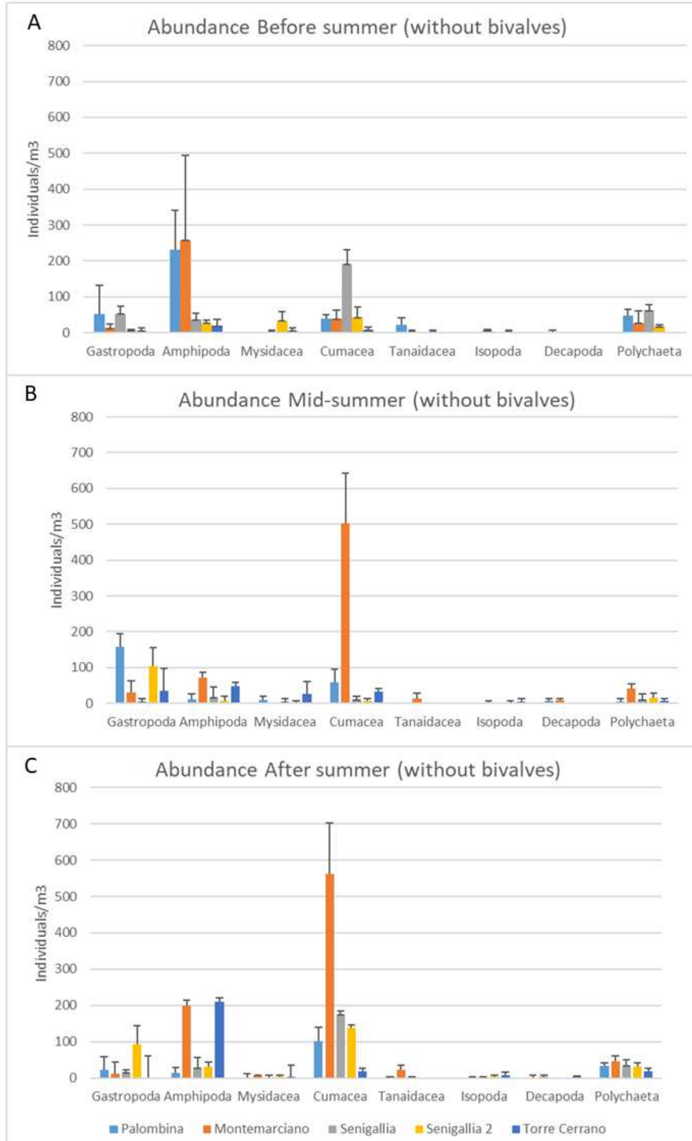

Figure 5: Abundance of macrofauna (ind. m^{-3})without bivalves A) Before-summer B) Mid-summer C) After-summer

Having a look at the **Figure 5** without bivalves could clear the picture even more displaying the actual number of individuals through all the periods. Bivalves are considered separately from the other taxa since they were masking the other taxa because of their dominance in most of the periods and sites. Among most of the sites, cumaceans and amphipods were the dominant groups in the periods before-summer and after-summer with changes in community happening periodically. In the mid-summer the gastropods could be observed prevailing mainly at Palombina and Senigallia which are the more impacted sites. In the after-summer period, the dominance of gastropods could be seen to have a decline as the cumaceans increases in abundance. Amphipods are the most dominant taxa at Palombina, Montemarciano and Torre Cerrano in the before-summer period while this composition change in the after-summer period, as the cumaceans dominate all the four sites except Torre Cerrano where the amphipod presence increase even more. At sites Senigallia 2 and Torre Cerrano (both sites without breakwaters) tanaidaceans could be found throughout the three sampling periods, even if their composition changes. Polychaetes are present in all sites and all periods except in the period before-summer in Torre Cerrano. Isopods were found to be one of the least abundant taxa throughout the study and were found in Senigallia 2, throughout all the periods but in very small number. In Torre Cerrano they were found only in mid-summer and after-summer, while in Senigallia they were found only after summer. Decapods were found in Palombina and Montemarciano before-summer and mid-summer while in after-summer, they were found only in Torre Cerrano.

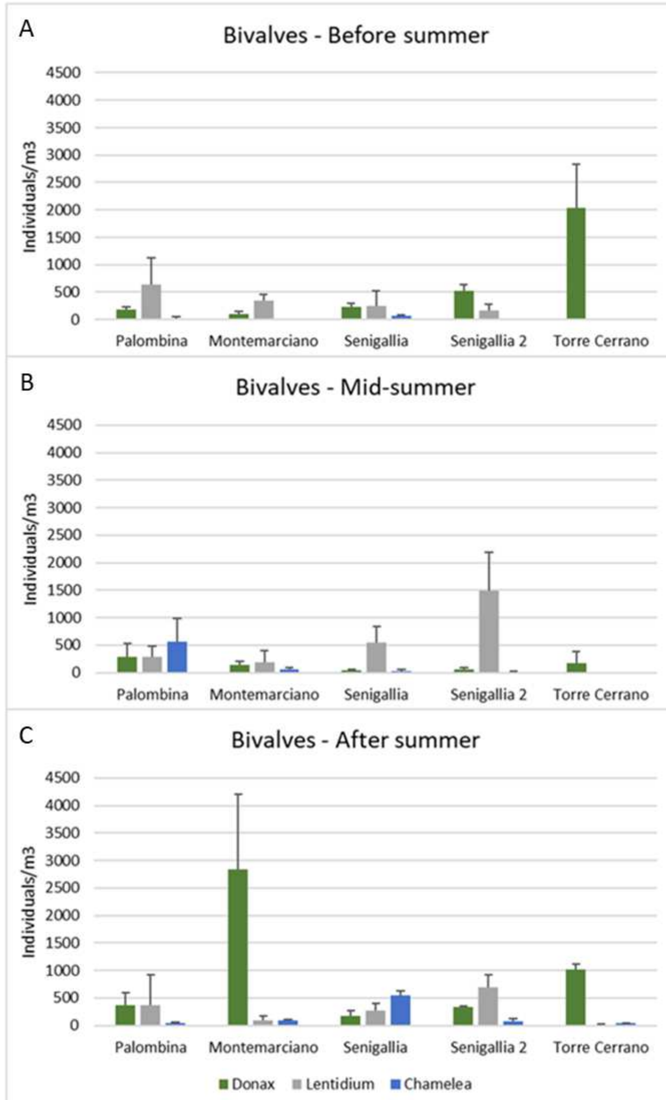

Figure 6: Abundance of Bivalves (ind m^{-3}) A) Before-summer B) Mid-summer C) After-summer

Bivalves, which constitute a major part of the benthic macrofaunal community, were found to have very interesting fluctuation. Three types of bivalves were found in our study; *Lentidium mediterraneum*, *Chamelia gallina* and *Donax* sp. which varied among sites and periods. In the period before-summer, *Lentidium mediterraneum* was found to be the dominant taxa in all the sites except Senigallia 2 and Torre Cerrano which are both the sites without breakwaters and dominated by *Donax* sp. (519 ind. m^{-3} 112 ± SD and 519 ind. m^{-3} 2029 805± SD respectively) while *Chamelea gallina* was found rarely. In mid-summer period, abundance of *Donax sp.* decline drastically (to (181 ind. m^{-3} 207± SD) at Torre Cerrao. *Lentidium mediterraneum* was rather found more frequently at all sites except Torre Cerrano where they were found in fewer number only in after-summer period. They were mainly found in mid-summer and after-summer periods and in sites with touristic infrastructure i.e., Palombina and both the Senigallia sites (with highest abundance of 1490 ind. m^{-3} 697± SD at Senigallia 2). *Donax* sp. were displaying the opposite picture as they were more frequent in sites without touristic facilities i.e., Montemarciano and Torre Cerrano. While the abundance of Donax sp. got increased in after summer period at Torre Cerrano, it reached the highest value at Montemarciano (2833 ind. m^{-3} 1371± SD). *Chamelea gallina* was rather found considerably only in Palombina before-summer (562 ind. m^{-3} 417± SD) and Senigallia after-summer (555 ind. m^{-3} 74± SD).

3.2 Statistical analyses

The two-way ANOSIM (Suppl. mat. **Figure 12**) shows that both the period-based and site-based comparison exhibit significant differences for the macrofaunal communities (R= 0.595 and R=0.614 for period and site tests, respectively)

Overall, the period-based differences are significantly high, as all the three exhibit a significance level of 0.1% (p-value = 0.001). The R value suggest the strongest differences between before-summer and mid-summer periods. Among sites, the most significant differences i.e., lowest p-value and highest R value can be seen between Senigallia 2 and Torre Cerrano which are interestingly the sites without breakwater barriers. Another interesting observation is the higher and significant difference (significance level 0.4%) between Senigallia and Senigallia 2, which are the sites literally adjacent to each other in similar urban setup; however, the only and important difference between them is the presence and absence of the breakwater barriers. The weakest difference can be found between Palombina and Senigallia 2, with a significance level of 2.9% and an R value of 0.383. Overall, there are significant differences among all the sites across the three periods; however, the strength of differences can be found from relatively weak (only between Palombina and Senigallia 2) to moderate or high.

The same two-way ANOSIM was run on bivalves' data by considering sites and periods as factors. Higher values of R were found in both cases displaying stronger differences among the site-based and period-based data as given in Suppl. Mat. **Figure 13** . In period-based comparison, the R value was found to be 0.715, while in site-based comparison, this value reached 0.76, revealing stronger differences.

The strongest differences for bivalves in period-based analyses were found in before summer and after summer periods with a significance level 0.1%. In case of site-based differences, Palombina and Montemarciano were found to have moderate differences with a significance level of 1.4% (p-value = 0.14). Overall, all the sites were found to have significant and moderate to stronger differences in all the pairs indicated by R values given in the pairwise test. Torre Cerrano was found to have the maximum differences with other sites in the pairwise test.

Comparison between 2020 and 2021: a possible lockdown effect

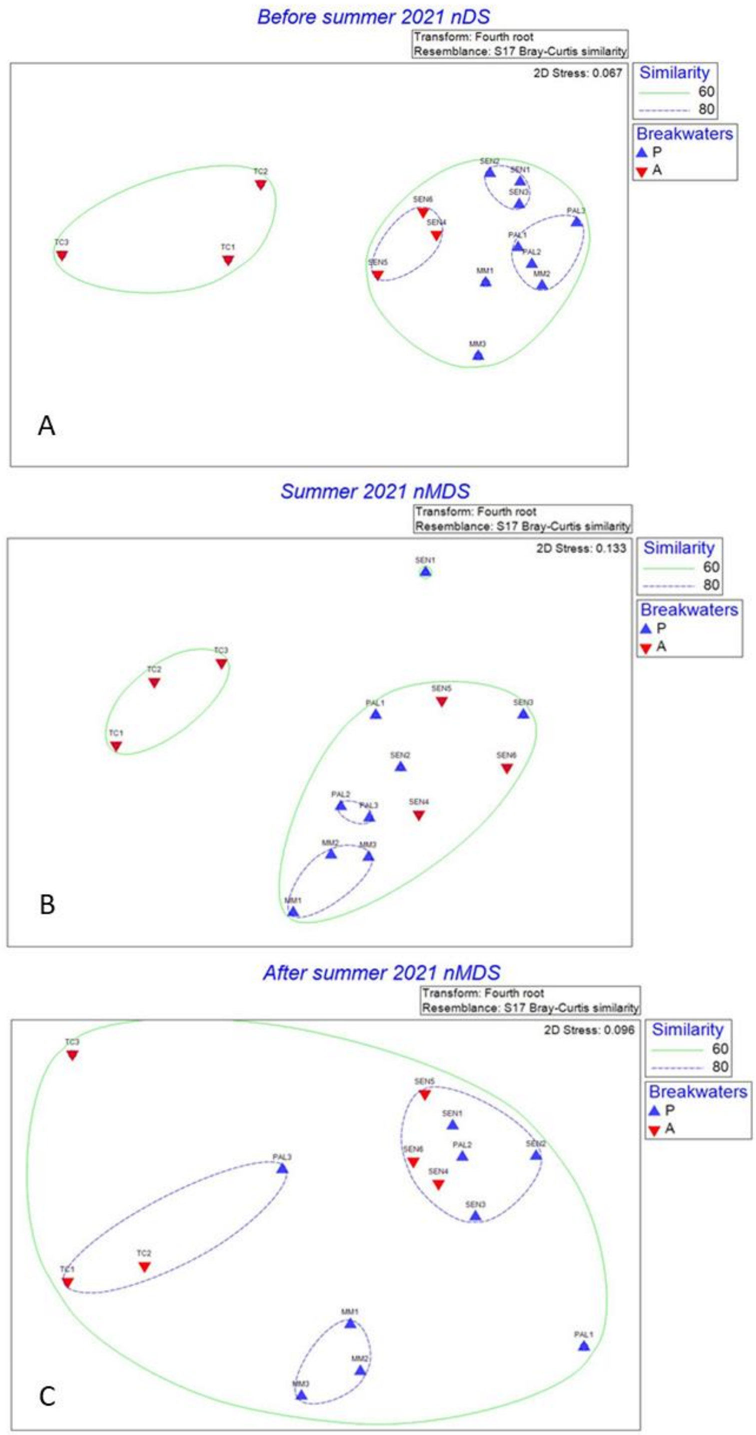

Figure 7: nMDS for the three summer periods; A)Before-summer, B)mid-summer and C: After-summer

Non-metric Multi-dimensional Scaling (NMDS) was utilized which displays the changes in data through the three periods. Before summer, Senigallia 2 tends to appear comparatively closer to Torre Cerrano which is also an area without breakwaters like Torre Cerrano. Palombina and Senigallia tends to appear closer as well; however, Montemarciano can be found different among all the sites. In mid-summer, the MPA Torre Cerrano appears to be easily distinguishable from other sites which are high impacted as the touristic period ascends as shown in **Figure 7 A, B and C**.

The same data was taken for the same sites except Senigallia 2, before summer and after summer in 2020 (Afghan et al., in prep) which was a period with more strict restrictions due COVID-19. Before as well as after the summer period, the protected area i.e., Torre Cerrano was found to be distinct than the other sites (**Figure 8**). Palombina and Montemarciano appear closer to each other which are geographically the closest sites to each other.

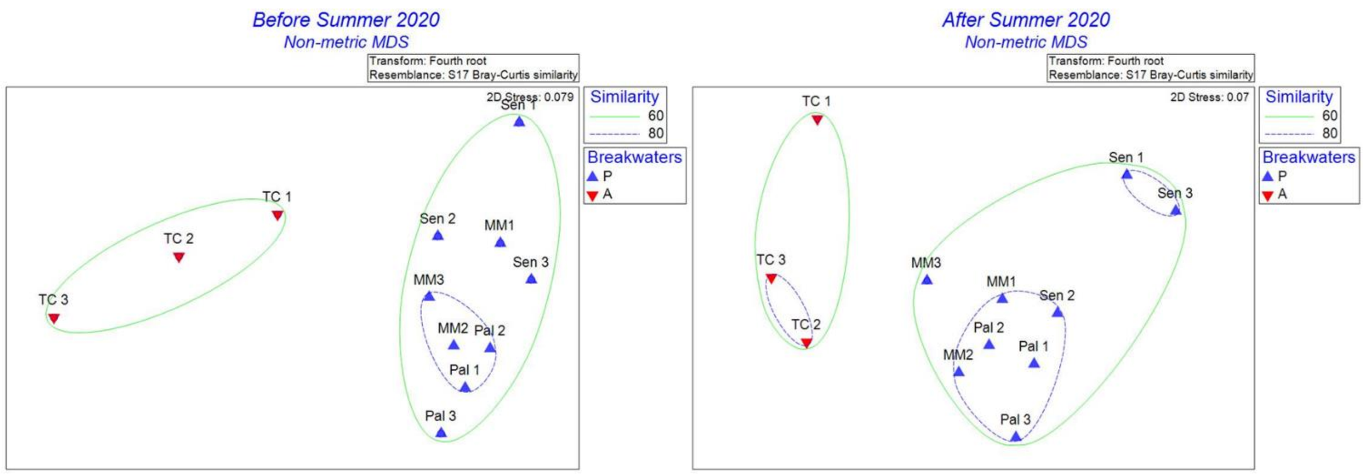

Figure 8: nMDS Before summer 2020 and after summer 2020 (without Senigallia 2)

3.3 Shell fragments, trampling and granulometry

The amount of non-living shells and shell fragments found in samples were compared through the considered three periods and five sites. Differences in shell quantity through the three periods were more evident at Palombina where, an increase in shell fragments was observed in middle and after summer (**Figure 9**).

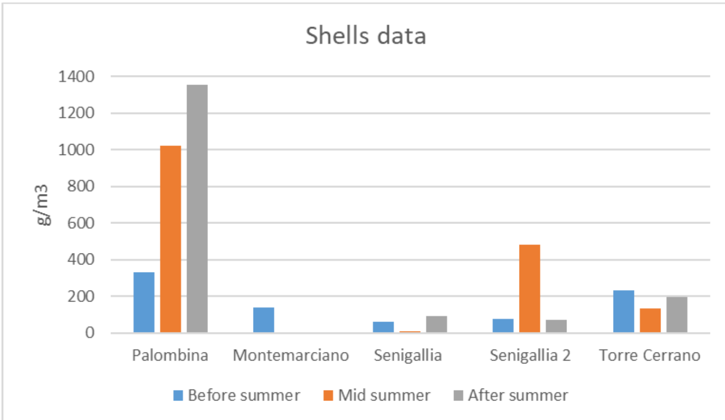

Figure 9: Abundance of shells (g/m^3)

Trampling activity was found more intense in the mid-summer period at all the sites, particularly at both the sample beaches in Senigallia (up to 450 hr^{-1}) and in Palombina (max 350 hr^{-1}), while Montemarciano was found to have the minimum trampling pressure (24 hr^{-1} only in mid-summer).

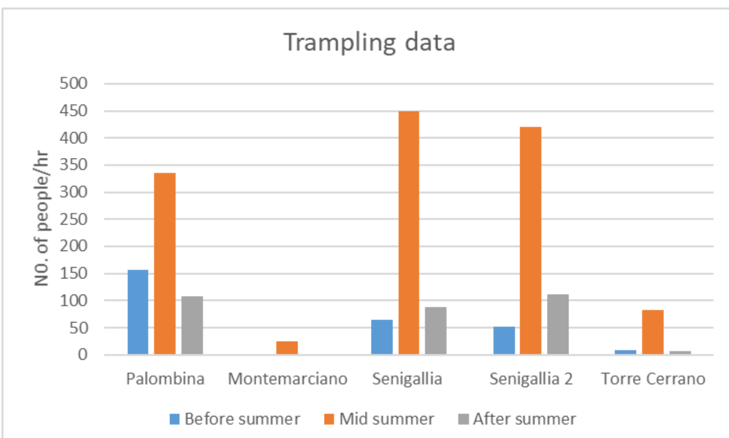

Figure 10: Trampling pressure on hourly basis (No. people/hour)

Torre Cerrano also had very limited trampling pressure (82 hr^{-1} max in mid-summer) throughout in comparison to Palombina and both the sites at Senigallia **(Figure 10)**. Trampling activity was found to be almost zero at Montemarciano in before-summer and after-summer period.

The granulometry analyses showed fewer dissimilarities in granulometry of the sites. Only Torre Cerrano was the standing out site with a considerable fraction of medium sand while, at all the sites including Torre Cerrano, fine sand was having more representation by percentage. Interestingly, Palombina and Senigallia 2 (Without breakwaters) had almost similar sand characteristics. Even Senigallia and Senigallia 2, a dissimilarity could be found in the granulometry composition.

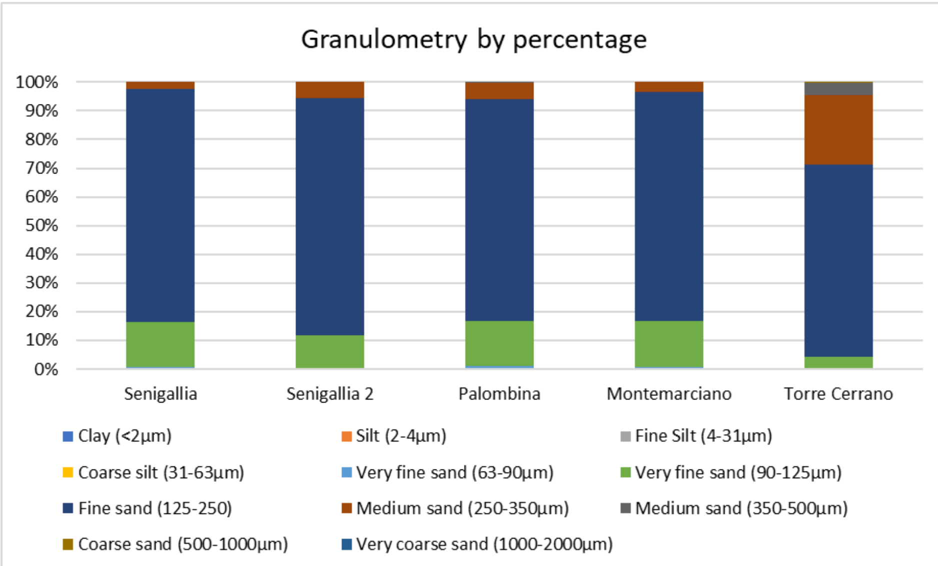

Figure 11: Granulometry composition of the studied sites

4. Discussion

The main purpose of the study was to evaluate abundance and composition of macrofaunal communities of some Adriatic beaches before, during and after touristic peak season 2021. In before-summer period, the communities at all the sites were mainly represented by bivalves; however, at Palombina, Montemarciano and Senigallia, the community also had a major share from other organisms, mainly amphipods, cumaceans and polychaetes. These taxa are usually sensitive groups and an inverse relation between their abundance and the intensity of human pressure on the beaches was reported by several Authors (Ugolini et al., 2008; Veloso et al., 2008, Machado et al., 2017). This is leading us to think that the shift in species composition observed in the mid-summer period is mainly due to the increase in touristic activity intensity rather than a natural effect of seasonality.

In confirmation of what has been hypothesized, in Montemarciano and Torre Cerrano, the cumaceans and amphipods were more prevalent even in mid-summer period which could have resulted from absence of commercial facilities and a minor effect of people trampling.

The bivalve *Lentidium mediterraneum* is one of the most dominant species on the North-Western Adriatic coast that is masking other communities and can be up to 95% of the individuals in total assemblage (Bertasi et al., 2009). In this study, it was found more frequently in mid-summer and after summer period in Senigallia 2, which is a site without breakwaters but with high levels of trampling. However, its prevalence in touristic period, especially in the disturbed sites hints about its ability to escape physical disturbances, as they are capable of going deeper in sediments rapidly and regulating their position in depths with respect to the shore to avoid the disturbances (Targusi et al. 2018).

The bivalve *Donax* sp. was found more dominantly in Torre Cerrano through all periods, while in Senigallia 2 it was observed before-summer and in Montemarciano in after-summer period, suggesting that even in this case the abundance of the species could be influenced by different levels of human pressure.

The gastropod *Tritia neritea*, characterized by a small but thick shell, peaked in the summer touristic period even in sites with the highest impact level (Palombina, Senigallia1). Smaller and hence juvenile stages were noticed in the before-summer period while, bigger and mature individuals were present at the end of summer, highlighting that the intermediate growth stages observed in mid-summer are able to resist the physical disturbances occurring in the touristic season. Besides having thicker shells, an important characteristic is that they can submerge quickly and move horizontally while keeping the inhalant siphon out (Southward et al. (1997).

In this study, Senigallia 2 and Torre Cerrano are possibly the sites resembling each other in terms of community structure in before-summer period, i. e., when the frequentation of people was low. Both sites are without breakwaters. According to Munari et al., (2011), the presence of breakwaters could limit water exchange, and therefore favour deposition of fine sand on landward side, and amplify the consequences of summer temperature increase. The granulometry of the Torre Cerrano's beach resulted larger than the other sites: this could be due to the combined action of waves and winds that are not weakened seaward by artificial barriers and landward by buildings, and, according to their direction, they move fine sediments by and towards the dunes.

This research highlighted that trampling is the most intense impact during mid-summer. The highest trampling intensity at Palombina and both the Senigallia sites are potentially due to the availability of touristic facilities and their easily accessibility. Montemarciano is situated between Palombina and Senigallia however, the absence of commercial tourism and the limited accessibility due to the presence of the railway are a deterrent for tourist. Many Adriatic sandy beaches are subjected to beach cleaning and beach nourishment (Targusi et al., 2018) usually just before the beginning of the touristic season, and these other two anthropic pressure may synergically impact with trampling on macrofaunal communities.

It is conceivable that the abundance of shell fragments present in the considered sites was linked to hydraulic dredging. In Palombina, the amount of shell fragments was observed to increase from mid-summer through after-summer period, i.e., when the COVID-19 associated restrictions were lifted. In Torre Cerrano, the shell fragments throughout the three summer periods didn't experience any drastic change since it's a protected area and fishing activities are forbidden.

The considered beaches have different set of impacts, however, it is likely that the COVID-19 related lockdowns could have played a role in shaping communities even if it was temporary, since a shift in the abundance of different taxa could be observed as the restrictions were lifted and summer touristic period proceeded. A general improvement is coastal ecosystem has been reported recently in several regions following Covid pandemic restrictions. With reduction in pollution, the observation of different organisms near the shore has been found to be more compared to previous years (Ormaza-Gonzales et al., 2021). Soto et al., (2021) has reported similar results regarding the COVID-19 lockdowns covering 29 urban beaches from seven countries revealing an increase in crustaceans and improved biological health with declined number of beach visitors.

According to the nMDS results, both in 2020 and 2021 the protected area Torre Cerrano resulted distinct from the other sites in the before summer period. This augmented protection effect was still evident just after-summer 2020 but not in the after-summer 2021, suggesting that more stringent measures of the first lockdown (March to May 2020) had a stronger and prolongated protection effect on beach macrofaunal communities.

From overall behaviour of different taxa especially the crustaceans and *Donax sp.* with fluctuations in the intensity of beach exploitation, their potential as indicator groups to evaluate impacts could be play an important role in assessment of sandy coastal systems.

5. Conclusion

The response of different taxa was quite evident towards the presence and absence of disturbances e.g., crustaceans (amphipods and cumacea) and bivalves (*Donax sp.*) were found to be declining as the disturbances appear. On contrary, gastropod such as *Tritia neritea* and *Lentidium* *mediterraneum* were found to be more resistant towards disturbances. Specifically, these changes were found as the lockdown period was over and visitors were frequent at the beaches. The trampling activity was found to be at peak in mid-summer period especially at both the Senigallia sites. The protected area (Torre Cerrano) was found different in before-summer period than the other sites; however, this difference was no more significant in after-summer period highlighting that the protection effectiveness of the MPA was compromised in that period. To mitigate the potential anthropogenic impacts on these ecosystems, the protection level of MPAs needs to be more efficient. From the response of different taxa towards disturbances, their potential use as indicator species to assess certain anthropogenic impacts could be considered.

Further studies should be carried out to understand how much breakwater barriers may influence the development of communities of sandy beaches, especially in those sites where the barriers are very close to the shoreline (Becchi et al., 2014). In order to consider the impacts of breakwaters, a good consideration would be the before-summer period because that was the only period when beach activities were non existing or minimum.

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Supplementary Materials

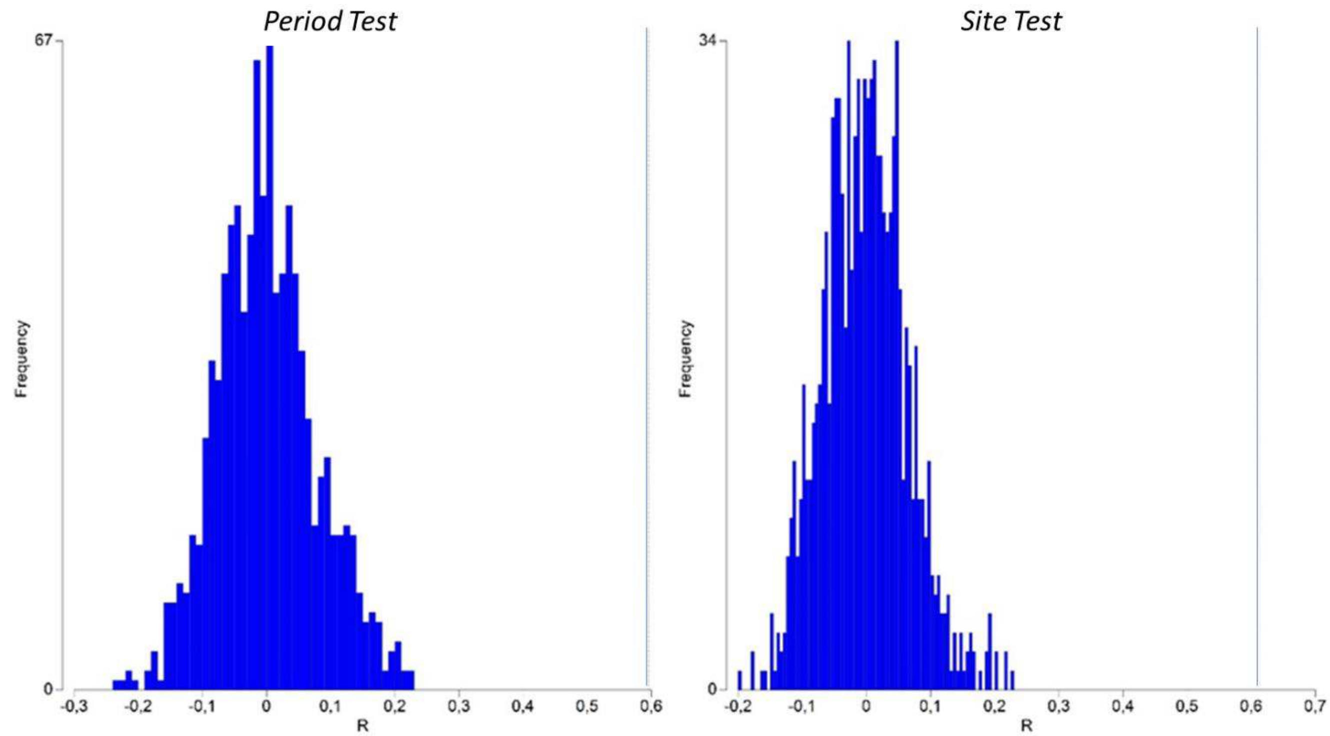

Figure 12: ANOSIM plot for overall community

Significance of the data could be further explained by the results obtained from the test.

ANOSIM for overall community (Two-Way Crossed – AxB)

Factors

Place	Name	Type	Levels
A	Period	Unordered	3
B	Site	Unordered	5

Period levels
BS (Before Summer)
MS (Mid-Summer)
AS (After Summer)

Site levels
P (Palombina)
M (Montemarciano)
S (Senigallia with breakwaters)
S2 (Senigallia without breakwaters)
T (Torre Cerrano)

Tests for differences between unordered Period groups
(across all Site groups)

Global Test
Sample statistic (Average R): 0,595
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Average R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
BS, MS	0,756	0,1	100000	999	0
BS, AS	0,644	0,1	100000	999	0
MS, AS	0,541	0,1	100000	999	0

Tests for differences between unordered Site groups
(across all Period groups)
Global Test
Sample statistic (Average R): 0,614
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Average R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
P, M	0,469	0,7	1000	999	6
P, S	0,494	0,6	1000	999	5
P, S2	0,383	2,9	1000	999	28
P, T	0,63	0,3	1000	999	2
M, S	0,827	0,3	1000	999	2
M, S2	0,852	0,2	1000	999	1
M, T	0,852	0,3	1000	999	2
S, S2	0,605	0,4	1000	999	3
S, T	0,728	0,3	1000	999	2
S2, T	0,889	0,1	1000	999	0

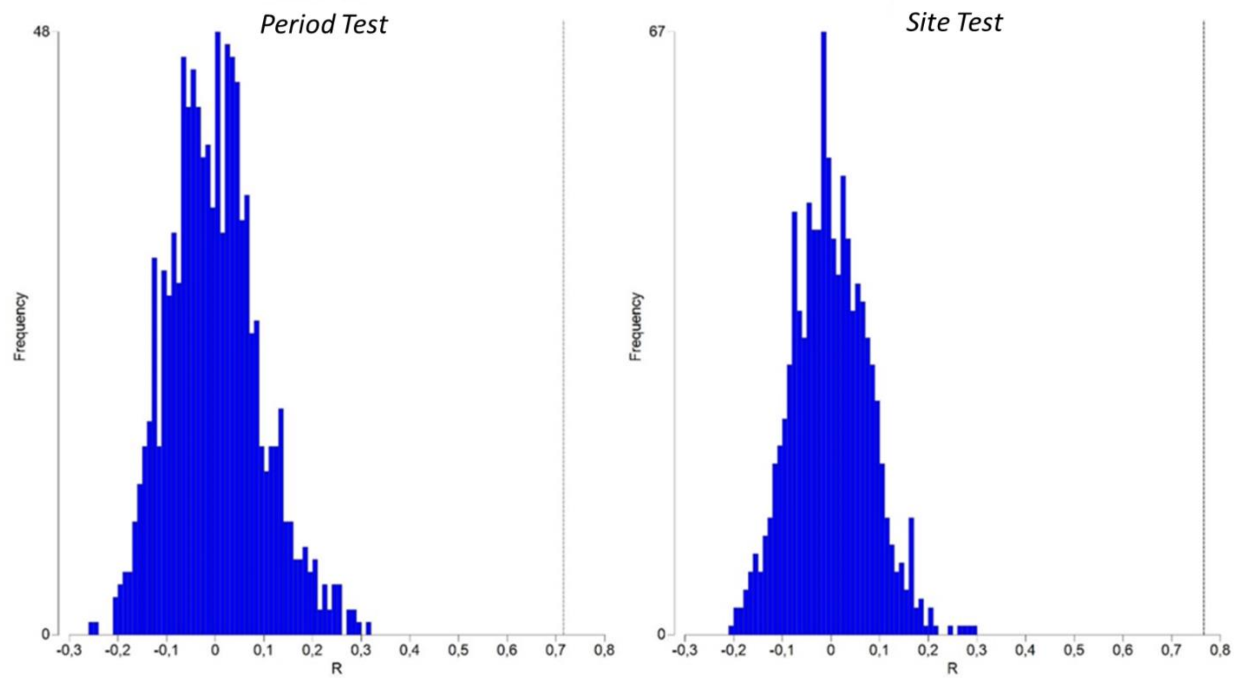

Figure 13: ANOSIM plot for bivalves

ANOSIM for bivalves only (Two-Way Crossed – AxB)

Factors

Place	Name	Type	Levels
A	Period	Unordered	3
B	Site	Unordered	5

Period levels
BS (Before Summer)
MS (Mid-Summer)
AS (After Summer)

Site levels
P (Palombina)
M (Montemarciano)
S (Senigallia with breakwaters)
S2 (Senigallia without breakwaters)
T (Torre Cerrano)

Tests for differences between unordered Period groups
(across all Site groups)
Global Test
Sample statistic (Average R): 0,715
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Average R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
BS, MS	0,711	0,2	100000	999	1
BS, AS	0,793	0,1	100000	999	0
MS, AS	0,696	0,1	100000	999	0

Tests for differences between unordered Site groups
(across all Period groups)
Global Test
Sample statistic (Average R): 0,766
Significance level of sample statistic: 0,1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Average R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
P, M	0,457	1,4	1000	999	13
P, S	0,568	0,7	1000	999	6
P, S2	0,617	0,5	1000	999	4
P, T	0,79	0,1	1000	999	0
M, S	0,802	0,2	1000	999	1
M, S2	0,938	0,1	1000	999	0
M, T	0,877	0,3	1000	999	2
S, S2	0,704	0,3	1000	999	2
S, T	0,988	0,2	1000	999	1
S2, T	1	0,1	1000	999	0

Thesis summary

The first part of the project was to develop a systematic review about the work done before related to the stressors on macrofaunal communities where three stressors were selected as they were the understudied topics especially on the Adriatic Sea. They were trampling, mechanical beach cleaning and presence of breakwater barriers. Overall, Australia, Brazil and Italy were found to have most of the studies however the types of studies differed. For example, in Brazil all the five studies conducted were based on trampling effects. In Italy, four out of six studies were based on impacts of breakwater barriers. Additionally, only two articles were published about trampling effects on sandy beach macrofauna before 2000. On the other hand, the first article about breakwater's effects on macrofaunal community was published in 2005 while the last one in 2014. Polychaetes and bivalves were found in literature to be the most affected ones by breakwaters. after the review part, the experimental work was conducted in the following phases (Chapters). The main findings from all the three phases of the project are demonstrated below.

Phase 1: Monthly study of two sandy beaches for one year

After the literature review, the second chapter consists of the first experimental work of the project to assess the macrofaunal diversity of concerned area. In this part of the study, two beaches, an urban beach with commercial touristic infrastructure (Palombina) and a natural beach (Torre Cerrano), in a marine protected area were studied. The total abundance of the macrofaunal community of both the sites was observed to be dominated by bivalves; however, the types of bivalves were different at both the sites. Besides bivalves, the variation in crustaceans especially amphipods and gastropods were more prominent, yet the differences in four quarters of the study year were not significant. More similarity was found in Q2 and Q3 while Q1 and Q4 were rather distant (the twelve months data was divided into four quarters). The differences between benthic community of two sites were significant; however, the equipment (corer) used for this work appeared to have possible flaws. Corers work with depth while it was observed in our field work that most the considered macrofauna stay more near the surface instead of depth; hence a sampling tool that work with the surface area instead of depth could be more appropriate. The sampling equipment was changed for the further phases of the project.

Phase 2: Season based study covering 4 beaches

After assessing the results from the first experimental work and the efficiency of the methods used, more sites were added in this experimental work with modification of sampling equipment and method. The scope of the study was expanded further and macrofaunal community abundance was evaluated based on seasons covering Spring (May 2020), Autumn (Late September 2020) and Winter (January 2021) at same two sites with the addition of two more sites i.e., Montemarciano (MM) and Senigallia (Sen). Although one was a protected area and three were urban beaches, the results produced were quite interesting since Montemarciano, which is an urban beach, was having less human impact because of absence of tourist infrastructure. In spring, which was the period just before summer, bivalves seem to dominate all the sites except Torre Cerrano (TC) where amphipods were the dominant group while polychaetes and isopods could also be observed. However, in Montemarciano, which is a beach between Palombina and Senigallia, there is more diversity than Palombina and Senigallia as amphipods, cumacea, mysidacea and polychaetes are present. Cumaceans were rather increased mainly in September in all sites except Torre Cerrano. In January, overall abundance of most of the taxa had declined at all the sites. The field activity conducted in spring (May 2020) was conducted just after the COVID-19 lockdown was over. In that period, a higher abundance of crustaceans especially amphipods was observed hinting about its sensitivity towards disturbances as well as its potential to be used as indicator taxa to assess certain impacts. In general, the sites with more touristic facilities i.e., Palombina and Senigallia were found to have more shell fragments and more people using the shoreline for running and jogging. Statistically, Torre Cerrano was found to be the most significantly different other sites regarding macrofaunal abundance highlighting the health status of this beach.

Phase 3: Impact of the summer touristic season

Through the other stages of the study, the data suggested that the summer period plays a crucial role in the alteration of macrofaunal community since the beach are exploited more than the normal months. Hence, different stages of summer season were considered for the study to explore the role of the summer season. In this phase, one more site was added to the existing sites and five sites were studied in total in three stages of summer; before-summer, mid-summer and after-summer to assess the influence of summer touristic season on the macrofaunal community. The additional site was Senigallia 2, a touristic beach in urban setup without breakwater barriers. The dominance of bivalves was the same as usual in all the sites except Montemarciano which amphipods and cumaceans had considerable contribution in before-summer and mid-summer respectively. Montemarciano was the site which experienced more changes throughout the summer compared to other sites. Torre Cerrano specifically exhibited changes regarding amphipods and bivalves *Donax sp*. which were the taxa apparently more sensitive to physical disturbances. This variation in these two sites especially in mid-summer period is likely due to less anthropogenic pressure. Again, a two-way ANOSIM and nMDS were used to check the significance of the difference in data as well as similarity among sites in different period. The sites were found to be significantly different in all seasons. Through nMDS similarity was found between sites without breakwaters (Senigallia 2 and Torre Cerrano) regarding benthic macrofaunal communities for certain periods for example before-summer Senigallia2 was showing similarity to after-summer Torre Cerrano. Even the Senigallia 2 and Torre Cerrano are located in completely different kind of setup, one just in the urban area and the other outside, the similarities of the two sites could possibly be related to the constantly changing environment mainly by the wave action as well as the restriction on beach use in that period due to pandemic. No major difference was found in granulometry of the sites; however, Torre Carrano's sediments were found to be comparatively coarser than the rest. Furthermore, the behaviour of certain taxa such as amphipods, cumaceans and bivalves Donax sp. Hints about their potential utility as indicator species to assess human impacts in Adriatic beaches. Furthermore, the data generated in this work could contribute in filling the knowledge gap regarding the subject as well as open more research questions for future work; and eventually could be utilised as a tool in the management of coastal ecology and beach tourism for better conservation.