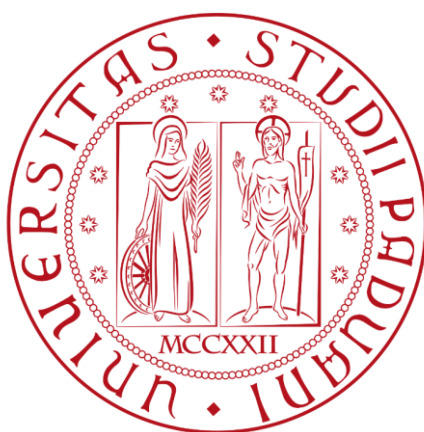


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TESI DI LAUREA

Assessment of Benthic macrofaunal communities: setting a baseline for flat oyster (*Ostrea edulis*) reef restoration in the Northern Adriatic Sea

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List of contents

Abstract.....	
1. Introduction	1
1.1 Introduction.....	1
1.2 The Northern Adriatic Sea and its benthic environment.....	2
1.3 Flat oyster reefs as habitat-forming ecosystems.....	4
1.4 Historical decline of <i>Ostrea edulis</i> and loss of oyster reef habitat	5
1.5 Habitat restoration and the recovery of oyster reefs	6
1.6 The PNRR MER initiative and <i>Ostrea edulis</i> reef restoration in the Northern Adriatic ..	7
1.7 Benthic macrofaunal communities as an ecological baseline	9
2. Aim and objectives of the thesis.....	10
3. Materials and methods.....	11
3.1 Study area	11
3.2 Field sampling.....	12
3.3 Laboratory analysis and identification.....	12
3.4 Statistical analysis	14
4. Results	15
5. Discussion of results and conclusions.....	32
5.1 Discussion of results.....	32
5.2 Limitations	36
5.3 Conclusions.....	37
Bibliography.....	
APPENDIX	
APPENDIX 1.....	
APPENDIX 2.....	
APPENDIX 3.....	

Abstract

In the past, oyster reefs of European flat oyster (*Ostrea edulis*) were common type of habitat in the coastal areas of the north Adriatic Sea. They served as biodiversity hotspots, shelter and hunting grounds for other animals, but also as coastal protection and climate change indicators. Now, after years of overfishing that decreased oyster population and destroyed their natural habitat as well as pathogens that caused mass mortality, the ability of natural restoration is unfairly limited. In this study, which is a part of PNRR MER project, is a large-scale assessment aiming to set a pre-intervention baseline for planned active restoration of native oyster beds in the north Adriatic Sea. The purpose of the baseline is to assess the habitat characteristics during 2024 and 2025, so as to quantify any subsequent changes in the system as a consequence of the restoration actions. In each of three sites, 3 stations for sediment collection were set. For each station, 3 replicate sediment samples were collected using a Van Veen grab. Laboratory analysis included: infauna species identification and abundance assessment, and sediment properties analysis (grain-size composition, loss on ignition, total organic carbon, and total nitrogen). Community diversity and composition were examined using diversity metrics and multivariate ordination. Across the two sampling campaigns, 159 taxa and 7,393 individuals were recorded. The three sites differed markedly in their assemblage composition and sediment characteristics. Tegnùe was associated primarily with sandy sediments, whereas Lignano and the LNG Terminal contained larger proportions of fine sediment. Principal Coordinates Analysis showed consistent spatial separation of the sampled communities in both years. Total organic carbon was strongly associated with community composition in 2024 and 2025, while total nitrogen showed a weaker and less consistent relationship. Although sampling effort differed slightly between years, the results reveal substantial spatial variability among the restoration sites, as well as some temporal variation within them. Future monitoring should therefore evaluate each site relative to its initial conditions and consider background environmental variability when assessing changes potentially associated with oyster-reef restoration.

Keywords: European flat oyster, fauna assessment, biodiversity, restoration, Adriatic Sea

1. Introduction

1.1 Introduction

Marine ecosystems are increasingly affected by human activities and climate-driven environmental changes, resulting in widespread habitat degradation and changes in biological communities (Cardinale et al., 2012; Worm & Lotze, 2021). Although biodiversity loss is one of the major environmental concerns of the present century, changes in species diversity are often the consequence of broader processes of habitat degradation and habitat loss (Villéger et al., 2010). Increasing temperatures, ocean acidification, eutrophication, pollution, and physical disturbance can alter the environmental conditions required by marine organisms and progressively simplify biological communities (Bagheri & Gholamhosseini, 2026; Brierley & Kingsford, 2009)

Climate change represents one of the major drivers of these changes. Rising temperatures are already affecting species distributions, with warm-adapted organisms expanding their ranges while less thermally tolerant species may lose suitable habitat (Somero, 2010). Such shifts have been documented in several marine groups, including fish, phytoplankton, seaweeds, and zooplankton (Worm & Lotze, 2021). At the same time, increasing atmospheric CO₂ concentrations contribute to ocean acidification, which can be particularly detrimental to calcifying organisms and benthic communities, including reef-forming species (Trebilco et al., 2022).

Marine habitats are also directly affected by human activities. Intensive fishing, particularly trawling, can physically disturb the seafloor and damage benthic communities (Jones, 1992), while agricultural runoff and the associated input of nutrients can promote eutrophication and contribute to the degradation of coastal ecosystems (Cardinale et al., 2012). Chemical contaminants may further affect marine organisms through direct toxicity and bioaccumulation. For example, pesticides can reduce organism survival and may also have consequences for subsequent generations through the transfer of residues (Iliff et al., 2019). In addition to these pressures,

pathogens represent an important source of mortality particularly in oyster populations (Okon et al., 2023), with bacterial, viral, and parasitic diseases capable of causing extensive mortality in both natural populations and aquaculture systems (King et al., 2019). The distribution and severity of some diseases may also be influenced by increasing temperatures, as warmer conditions can favour the development and transmission of pathogens (Bougrier et al., 1995; Leung & Bates, 2013).

When habitat-forming organisms like oyster decline extensively, the physical structure and ecological functions associated with their habitats can also be lost (McAfee & Connell, 2021). This is particularly important for benthic ecosystems, where the presence of three-dimensional structures can determine the availability of shelter, attachment surfaces, food resources, and ecological niches for other organisms (Pygas et al., 2020). Consequently, the degradation or disappearance of habitat-forming species may lead to changes in benthic community composition and, ultimately, to a reduction in local biodiversity (Teagle & Smale, 2018).

1.2 The Northern Adriatic Sea and its benthic environment

The Adriatic Sea is a semi-enclosed basin connected to the Ionian Sea through the Strait of Otranto and represents one of the shallowest regions of the Mediterranean Sea (Benjamin et al., 2017). Its bathymetry is characterised by a strong north-south gradient, with the shallowest areas occurring in the northern Adriatic and progressively greater depths towards the south. The average depth of the Adriatic is approximately 253 m, while more than half of the seabed is shallower than 100 m. The deepest areas reach approximately 1200-1270 m, depending on the source (Vrdoljak et al., 2021).

The northern Adriatic is characterised by strong environmental variability associated with its shallow waters and substantial freshwater input from major rivers. During summer, sea-surface temperatures are relatively stable and generally range between approximately 26 and 28 °C, depending on latitude, while temperatures at 100 m depth remain considerably lower (Janeković et al., 2010). Riverine inputs contribute to pronounced spatial variation in salinity, particularly in coastal areas influenced by estuaries, whereas open-sea surface salinity is approximately 38 due to the inflow of

warmer and more saline Mediterranean waters (Mihanović et al., 2021). The Adriatic Sea is a basin that extends for 138,595 km² (including islands), representing about the 4.6% of the total area of the Mediterranean (Buljan & Zore-Armanda, 1971). It is a semi-enclosed basin, whose major axis extends in the northwest direction, and has a length of 800 km and a width of 150-200 km.

The seabed of the Adriatic is largely dominated by soft sediments, particularly sands ranging from approximately 50-2000 µm and silts between 2 and 50 µm, with finer clay fractions also occurring locally (Wang & Pinardi, 2002). These soft-bottom environments support diverse benthic communities and play an important role in biogeochemical processes (Haselmair et al., 2021).

The granulometry and sediment mineralogy are the main factors that define the types of populations and their distribution on the Adriatic seabed (Di Camillo et al., 2022). On the west side, the slight slope of the seabed determines the distribution of the biocoenosis along parallel bands to the coast and are generally dominated by endobenthonic organisms; like bivalves and Polychaetes. On the east side, the bottom biocoenosis are characterized by a mosaic structure and the epifauna prevails (sponges and hydrozoa) (Bastari, 2016).

The benthic communities of soft bottoms can play important roles in carbon cycling (Rohlfert et al., 2026). Organic matter can be incorporated into the sediment through biological processes, including the deposition of faeces and pseudofaeces, contributing to carbon storage within the seabed (Evans et al., 2026). Benthic organisms also contribute to the cycling of nutrients and carbon through processes such as bioturbation and bioirrigation (Bustamante et al., 2019; Norkko & Shumway, 2011).

However, these habitats are increasingly exposed to anthropogenic pressures. Fishing activities represent an important source of physical disturbance. In the northern Adriatic, rapido trawls can penetrate and disturb the sediment, directly affecting benthic organisms and reducing the availability of prey for higher trophic levels (Armelloni et al., 2021). Historical overexploitation has also reduced populations of species that provide important ecosystem functions, including habitat provision and water filtration (Lotze et al., 2011). In addition, riverine inputs transport nutrients and sediments into the Adriatic, with the Po River representing a particularly important

source (Wang & Pinardi, 2002). Although management initiatives such as the Water Framework Directive and cooperation between Italy and Croatia have contributed to reducing these pressures, anthropogenic impacts remain an important component of the environmental context of the northern Adriatic (Çevirgen et al., 2020; Volf et al., 2018).

1.3 Flat oyster reefs as habitat-forming ecosystems

The European flat oyster, *Ostrea edulis*, is a native bivalve of the Mediterranean Sea and is an important example of a marine ecosystem engineer (Pogoda et al., 2019). Adult oysters are sessile organisms that attach permanently to suitable substrates, although the species can occur on both hard and relatively soft seabeds. They are generally found at depths of up to approximately 80 m and tend to favour stable surfaces that provide suitable attachment conditions (Lapègue et al., 2006; Perry & Jackson, 2017).

The ecological importance of *O. edulis* extends beyond its role as a filter-feeding bivalve. By filtering suspended particles from the water column, oysters contribute to water purification and influence the transfer of organic matter and carbon to the seabed (Grabowski et al., 2012). More importantly for the present study, aggregations of oysters form structurally complex three-dimensional habitats (Ysebaert et al., 2019). Oyster beds provide surfaces for settlement and shelter for a variety of associated organisms, creating ecological niches that are otherwise limited or absent in unconsolidated soft sediments (Smyth & Roberts, 2010; Ysebaert et al., 2019; Zhou et al., 2024).

Consequently, they can support diverse assemblages of associated fauna, including polychaetes, amphipods, isopods, decapods, gastropods, and other bivalves (Pogoda et al., 2019). The structural complexity of oyster reefs provides refuge and suitable settlement surfaces for prey species while also increasing the availability of food for predators (Lown et al., 2021).

Oyster reefs can therefore be understood not only as populations of oysters, but as habitats supporting complex biological communities. Their loss may consequently result in the disappearance of physical structure and ecological niches, followed by

changes in species composition and abundance (Cardinale et al., 2012; Worm & Lotze, 2021). The decline of oyster populations should thus be considered in terms of both species' loss and habitat loss, with biodiversity changes representing one of the ecological consequences of this process.

1.4 Historical decline of *Ostrea edulis* and loss of oyster reef habitat

The decline of European flat oyster populations is closely linked to centuries of human exploitation (Airoldi & Beck, 2007; Beck et al., 2011). Molluscs have been an important component of the human diet for thousands of years, and historical evidence of their consumption in Europe dates back to at least the Uruk period (4000-3100 BC) (Bonsall et al., 2009). Their abundance and nutritional value subsequently contributed to their continued exploitation, eventually resulting in oysters becoming an important delicacy (Voultsiadou et al., 2010). Aristotle also referred to oysters as “limnostreon”, reflecting their occurrence in estuarine environments (Perry & Jackson, 2017).

The most substantial decline of flat oyster populations occurred during the twentieth century, when increasing fishing effort and the development of more efficient fishing gears resulted in intense exploitation and limited opportunities for population recovery (Thurstan et al., 2024a). Historically, healthy oyster reefs could extend across areas of several square kilometres. Nowadays, oyster habitats have globally decreased by 85% of their historical habitat (Beck et al., 2011; Koutsoubas et al., 2007). This represents not simply a reduction in oyster abundance, but a profound loss of habitat-forming structures and the ecological communities associated with them.

Bottom-disturbing fishing gears further contributed to this process by removing the three-dimensional structures required for oyster recruitment. The loss of suitable settlement surfaces can prevent oyster larvae from attaching successfully and therefore limit the natural rebuilding of reefs (Thurstan et al., 2024). In this way, habitat degradation can create a feedback mechanism in which the loss of adult oysters reduces habitat complexity, which in turn reduces the availability of suitable habitat for new generations of oysters.

The decline of *O. edulis* has also been associated with disease. During the 1970s, major mortality events affected both wild and farmed populations, with the parasites *Marteilia refringens* and *Bonamia ostreae* identified as important causes of mortality (King et al., 2019; Leung & Bates, 2013). The consequences of these declines are particularly evident in aquaculture production, including the substantial reduction in oyster production recorded in France between the 1970s and recent decades (Pouvreau et al., 2023). Attempts to compensate for the decline of native oysters have also included the introduction of the non-native Pacific oyster, *Magallana gigas*. Although this species was selected for its rapid growth and resilience, its establishment and escape from aquaculture have raised concerns regarding interactions with native species and further changes to native oyster communities (Stagličić et al., 2020).

The historical decline of *O. edulis* therefore represents a broader process of habitat degradation and loss. As oyster populations disappear, so do the three-dimensional structures they create, together with the ecological niches and benthic communities that depend on them. Restoring oyster populations can consequently contribute not only to the recovery of the target species, but also to the restoration of habitat complexity and associated biodiversity (Coen et al., 2007).

1.5 Habitat restoration and the recovery of oyster reefs

The degradation and loss of marine habitats have increased the importance of ecological restoration as a conservation strategy (Higgs et al., 2018). Habitat restoration encompasses a range of interventions aimed at recovering ecological structure and function in degraded environments. In marine systems, one approach is the creation or restoration of artificial reef structures that provide suitable surfaces for the settlement of algae and sessile organisms and subsequently create food and shelter for other species (Ambrose & Anderson, 1990; Hale et al., 2019).

Restoration is particularly challenging when historical reference conditions have been lost. The Shifting Baseline Syndrome describes the process through which successive generations perceive increasingly degraded environmental conditions as normal, resulting in progressively lower expectations of what constitutes a healthy

ecosystem (Soga & Gaston, 2018). As human perception changes over time, the historical reference point for ecosystem condition may be lost, making it increasingly difficult to recognise the magnitude of ecological degradation (Papworth et al., 2009). Establishing quantitative ecological baselines is therefore essential for determining whether restoration interventions result in measurable improvements over time.

Large-scale restoration initiatives, including the UN Decade on Restoration (United Nations Environment Programme, 2020), European restoration programmes such as Rest-Coast (Marino et al., 2026), and initiatives supported by the National Oceanic and Atmospheric Administration in the United States (US Department of Commerce, 2025), reflect the increasing importance of ecosystem restoration. Within Italy, the PNRR MER (Marine Ecosystem Restoration) initiative represents a major national effort aimed at restoring and monitoring marine ecosystems (ISPRA, 2023).

1.6 The PNRR MER initiative and *Ostrea edulis* reef restoration in the Northern Adriatic

The PNRR MER initiative is part of the Italian National Recovery and Resilience Plan and is implemented through cooperation between the Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) and the Ministero dell'Ambiente e della Sicurezza Energetica (MASE). The project, covering the period 2022-2026, includes several interventions aimed at improving knowledge, monitoring, and restoration of Italian marine ecosystems. Among its objectives is the restoration of habitats associated with the European flat oyster, *Ostrea edulis*, in the northern Adriatic Sea.

The restoration approach is based on the creation of intervention units (UDIs) designed to provide suitable conditions for oyster settlement and the development of reef-associated communities. Five intervention sites, designated A1-A5, were established, with four UDIs at each site. Each UDI covers an area of 50 x 50 m and contains a central 16 x 16 m metal frame supporting adult and sub-adult oysters. The surrounding area includes different substrate types, namely stones, bioclasts, bricks produced from crushed oyster shells, and the original substrate of each site. The centres of neighbouring units are approximately 100 m apart. Each intervention site is

additionally equipped with a GPS beacon and anti-trawling devices. The simplified overlook of an UDI is presented on the following figure (fig. 1).

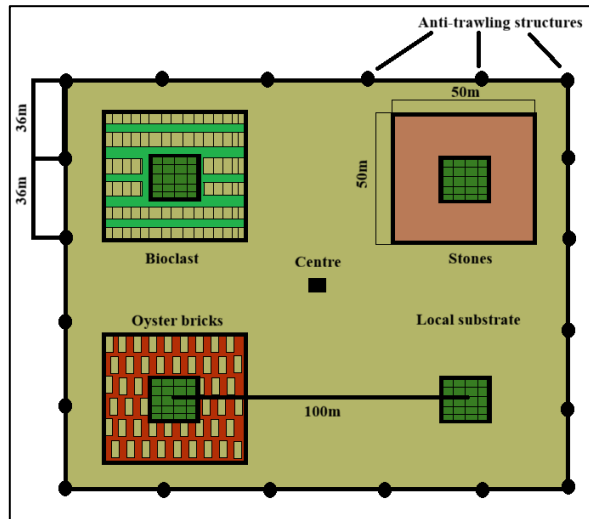


Figure 1 Intervention Unit (UDI) after installation on the seabed

The present thesis focuses on three of these intervention sites, A1-A3, located in the coastal areas of Friuli Venezia Giulia and Veneto (fig. 2). Site A1 is located near Lignano, site A2 in the Tegnue di Chioggia area, and site A3 in the vicinity of the LNG Terminal (Terminale GNL Adriatico). These sites represent the spatial context in which the baseline benthic communities investigated in this study are characterised.

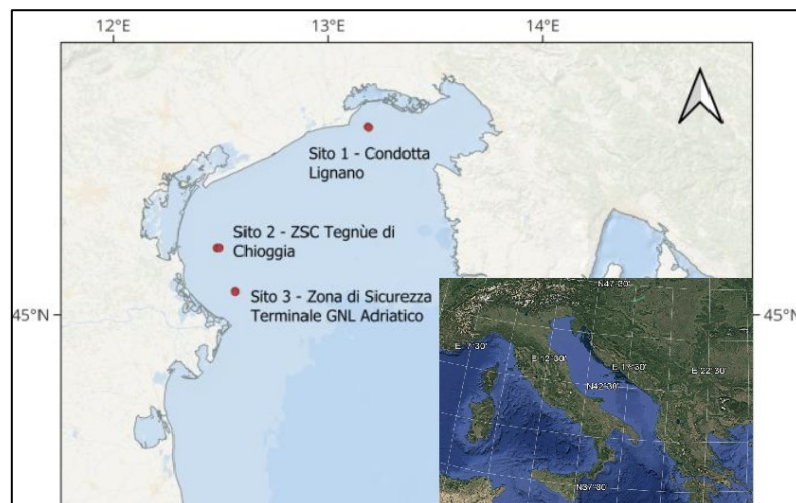


Figure 2 Map of North Adriatic region with marked intervention sites: 1 - Lignano, 2 - Tegnue, 3- LNG Terminal

1.7 Benthic macrofaunal communities as an ecological baseline

The restoration of oyster reefs is expected to produce changes not only in oyster abundance, but also in the structure of the wider benthic community. The introduction of hard and structurally complex substrates into predominantly soft-bottom environments may provide new settlement surfaces and refuges, potentially facilitating the establishment of epifaunal species that are less abundant or absent from the surrounding sediment.

At the same time, the existing soft-bottom communities provide an important reference against which future changes can be evaluated. Processes such as bioturbation and bioirrigation demonstrate the importance of benthic organisms in maintaining sediment functioning. Bioturbation involves the physical reworking of sediment by organisms, while bioirrigation facilitates the exchange of oxygenated water and dissolved substances between the sediment and the overlying water (Bustamante et al., 2019; Norkko & Shumway, 2011). Together, these processes contribute to benthic-pelagic coupling and the cycling of nutrients such as nitrogen, phosphorus, and silicates (Griffiths et al., 2017).

The composition of benthic communities reflects the ecological characteristics of their habitat. Soft-sediment environments can support organisms occupying different ecological niches, including suspension-feeding bivalves, polychaetes, amphipods, oligochaetes, decapods, and other organisms occupying different trophic positions (Moller et al., 1985). Consequently, changes in species richness, abundance, and community composition can provide valuable information on the ecological response of the environment to habitat modification.

Establishing a baseline before the development of the restored reef communities is therefore essential. Without a description of the initial benthic assemblages, future changes cannot be reliably distinguished from the natural spatial variability already present among the restoration sites. The assessment of macrofaunal communities can consequently provide a reference against which the ecological development of the restored habitats can be evaluated during subsequent monitoring.

2. Aim and objectives of the thesis

The main objective of this thesis is to characterise the benthic macrofaunal communities associated with selected *Ostrea edulis* restoration sites in the Northern Adriatic Sea and establish an ecological baseline for future reef-restoration monitoring. The study focuses on sites A1-A3 and evaluates the benthic communities using biological descriptors including species richness, abundance, and biodiversity indices. In addition, sediment characteristics are investigated through grain-size composition, total organic carbon (TOC), and nitrogen content in order to characterise the environmental conditions of the study areas and explore their potential relationship with benthic community structure.

The baseline established by this study will provide a reference for future annual surveys and will allow subsequent changes in species composition, abundance, and community structure to be evaluated in relation to the development of the restored oyster reefs. In particular, the restoration structures are expected to increase habitat complexity and promote the gradual establishment of hard-substrate-associated epifaunal communities alongside the existing soft-bottom assemblages.

The central ecological expectation is therefore not simply an increase in biodiversity, but a change in habitat structure and benthic community composition associated with the recovery of an oyster reef habitat. As *O. edulis* populations develop and form increasingly complex three-dimensional structures, these habitats may provide additional settlement surfaces, refuges, and trophic resources, potentially supporting a more structurally complex and functionally diverse benthic assemblage.

By documenting the initial state of benthic macrofaunal communities and the environmental characteristics of the restoration sites, this study provides a foundation against which the long-term ecological effectiveness of *O. edulis* reef restoration can be assessed. The results will therefore contribute to understanding whether the restoration intervention is accompanied by measurable changes in benthic community

structure and will provide an ecological reference for future monitoring of oyster reef development in the Northern Adriatic Sea.

The following chapters describe the study area, sampling design, laboratory procedures, and statistical analyses, followed by the presentation and discussion of the results in the context of existing literature. The thesis concludes by summarising the main findings and their implications for the future restoration and long-term monitoring of *O. edulis* reef habitats.

3. Materials and methods

3.1 Study area

The study was conducted at three sites located in the central-northern Adriatic Sea, along the coasts of Friuli Venezia Giulia and Veneto (Italy). The sites differed in water depth, sediment characteristics, benthic assemblages, degree of protection, and presence of natural or artificial hard substrates.

The Lignano Pipeline site (45°37.696' N, 13°11.387' E) is located offshore of Lignano Sabbiadoro at a depth of approximately 16 m. The seabed is flat and consists mainly of muddy sand with a relatively high organic-matter content. Near-bottom visibility is generally low. The area contains several artificial structures, including anti-trawling blocks and artificial reefs, some of which have partially collapsed or become entangled in fishing nets. The benthic fauna observed in the area includes holothurians, ophiuroids, decapod crustaceans and ascidians, while the fish assemblage is relatively sparse and primarily represented by gobies. The site lies within an area where bottom trawling is prohibited.

The Tegnùe di Chioggia site (45°13.475' N, 12°29.808' E) is situated within the Natura 2000 Special Area of Conservation IT3250047 and the associated Fisheries Restricted Area, at a depth of approximately 24 m. The intervention area is characterised by a flat, medium-grained sandy seabed containing small proportions of silt and organic matter. Dense aggregations of ophiuroids and numerous holothurians occur on the soft bottom, whereas mobile fauna is comparatively scarce. Less than 1

km northwest of the site, the seabed includes natural rocky outcrops locally known as *tegnùe*. These biogenic formations support structurally complex and highly diverse epibenthic communities. The study area was positioned away from these ecologically valuable rocky habitats.

The Adriatic LNG Terminal site (45°04.716' N, 12°34.336' E) is located approximately 15 km offshore of Porto Levante, within the safety zone surrounding the Adriatic LNG terminal, at a depth of approximately 27 m. The seabed consists predominantly of sandy mud and is largely protected from bottom trawling because navigation, fishing, anchoring and unauthorised diving are prohibited within the safety zone. ROV surveys documented a comparatively rich soft-bottom benthic community, including extensive ophiuroid aggregations, bivalves and holothurians. Fish were also more abundant than at the other study sites, with gobies and scorpionfish among the taxa observed. Artificial reef structures intended to promote fish repopulation are present near the study area.

3.2 Field sampling

Each intervention site was divided into three sampling stations internal (inside the future UDI), near (area close the future UDI) and distant (area far from the future UDI). In 2024, four replicate sediment samples were collected from each station using a Van Veen grab sampler. The sediment collected from each grab was used for both infaunal analysis and sediment characterisation, including grain-size composition, organic matter, total organic carbon (TOC), and nitrogen (N) content. In 2025, three replicates per station were collected using the same approach. Additionally, sediment bulk density was assessed exclusively in 2025 by collecting sediment with scuba diving by using a syringe of known volume (60 mL). It was only possible to obtain samples from Lignano and *Tegnùe* since diving in the area of LNG Terminal is not allowed.

3.3 Laboratory analysis and identification

After collection, sediment samples intended for sediment analyses were frozen until further processing. Sediment samples intended for infaunal analysis were sieved

to retain the macrofaunal organisms and subsequently preserved in ethanol until laboratory identification. Each sample was placed on 500 µm sieves, spread evenly and divided into two or four parts depending on the volume of the sample. From each sample, one of divided parts was selected and taken for the additional sieving on a sieving collum consisting of 3 sieves: 2 mm on top, 1 mm in the middle and 500 µm at the bottom. Separated fractions were then taken for stereomicroscopic analysis. Using a microscope, every organism with remaining head was taken out and later identified to the lowest possible taxonomic level. After the identification the number of organisms was multiplied by the number of parts (x2 or x4). Identified organisms were preserved in 70% EToH solution.

Sediment organic matter content was determined by loss-on-ignition (LOI). Approximately 20 g of wet sediment was dried at 85 °C for 24 h to remove moisture, after which the dry sample was weighed. The samples were then combusted in a muffle furnace at 450 °C for 8 h. Following combustion, the samples were allowed to cool to room temperature in a desiccator and reweighed. Organic matter content was determined from the mass loss following combustion and expressed as a percentage of the initial dry sediment mass. For elemental analysis, sediment samples were stored at –20 °C prior to laboratory analysis. Carbon and nitrogen concentrations were determined using a CHNS/O elemental analyser. Total organic carbon (TOC) and total nitrogen (TN) contents were quantified and expressed as percentages of dry sediment weight (% dry weight).

Sediment bulk density was determined by measuring the dry mass of a known volume of sediment. An initial volume of 60 mL of wet sediment was collected, dried to constant mass, and weighed. Bulk density was calculated as the ratio between the dry sediment mass and the initial wet sediment volume:

$$\text{Bulk density} = \frac{\text{dry sediment mass (g)}}{\text{wet sediment volume (cm}^3\text{)}}$$

To quantify sediment grain-size composition, Sediment samples were treated with a sodium hexametaphosphate (NaHMP) solution to promote particle dispersion, diluted with distilled water, and analysed using a laser particle-size analyser. Three replicate

measurements were performed for each sample. Grain-size distributions were used to calculate the relative proportions of clay (<0.004 mm), silt ($0.004\text{--}0.062$ mm), and sand ($0.062\text{--}2$ mm).

3.4 Statistical analysis

All statistical analyses were performed using R version 4.3.2 (R Core team, 2024). Prior to statistical analysis, the dataset was organized and formatted in Microsoft Excel for subsequent import into R. Standard errors were calculated in Excel, and plots were generated to illustrate the dominance of higher taxonomic groups across sites. Biological diversity indices were calculated in RStudio using the vegan package (put the citation that I gave you). The package was used to calculate commonly applied diversity metrics, including the Hill numbers N_1 and N_{10} , and richness S . The indices were calculated for each sampling station based on the observed species abundances.

Principal Coordinates Analysis (PCoA) (Gower, 1966) based on Bray–Curtis dissimilarities (Bray & Curtis, 1957) were performed in R version 4.3.2 (R Core team, 2024) to explore and visualize patterns of variation in the taxonomic composition of benthic assemblages among the three intervention sites. PCoAs were conducted separately for 2024 and 2025, as well as on the combined dataset including both years. Lingoes correction was applied to account for negative eigenvalues using the ape package (Paradis & Schliep, 2019). Principal Component Analysis (PCA) of sediment grain-size composition was performed to investigate differences in sediment characteristics among the three study sites

To assess the relationship between benthic assemblage composition and sediment nutrient content, nitrogen (N) and total organic carbon (TOC) concentrations were fitted as environmental vectors onto the PCoA ordinations using the envfit function implemented in the vegan package (Oksanen et al., 2026). The significance of the fitted vectors was assessed using 999 permutations.

4. Results

Across all collected samples it was possible to identify 159 different taxa. Organisms were identified to the lowest possible taxonomical level. The number of taxa per year was slightly larger in 2025 as it was equal to 98 in comparison to 94 taxa in the previous year. A total of 7393 individuals was collected, 5021 in 2024 and 2372 in 2025, also reflecting the lower the number of collected replicates per site in 2025. Further comparisons were done using the number of individuals per square meter in each sample.

Table 1 depicts number of taxa for each station of site 1 – Lignano in 2024 and 2025. More detailed table with specific taxa, abundance and standard error is included in appendix 1 (Appendix 1). There is a great decrease in individuals per square meter from 2024 to 2025. During the time of first collection there were 498 individuals per square meter, during the second one, this value was only 272. However, the number of observed taxa was higher in 2025, as it was 38 which is 3 more than in 2024 (35 taxa). In both years, the two most dominant classes were Polychaeta and Malacostraca. They presented the highest number of species, as well as the highest number per m². Worth noticing fact is that in 2025 there were present taxa that were not observed in 2024, including: Asteroidea, Cnidaria, Ostracoda and Sipunculidae. The comparison of higher taxa abundance is presented in the graph below (fig. 3). Withing “other” category such taxa are included: Asteroidea, Cnidaria, Gastropoda, Holothuroidea, Nematoda, Ophiuroidea, Ostracoda, Sipunculidae, as they presented very little or no abundance.

Table 1 Number of taxa present in stations 1,2 and 3 in Lignano in both 2024 (4 replicates per station) and 2025 (3 replicates per station)

Lignano	2024			2025		
Station	1	2	3	1	2	3
No. Taxa	13	25	18	19	22	19

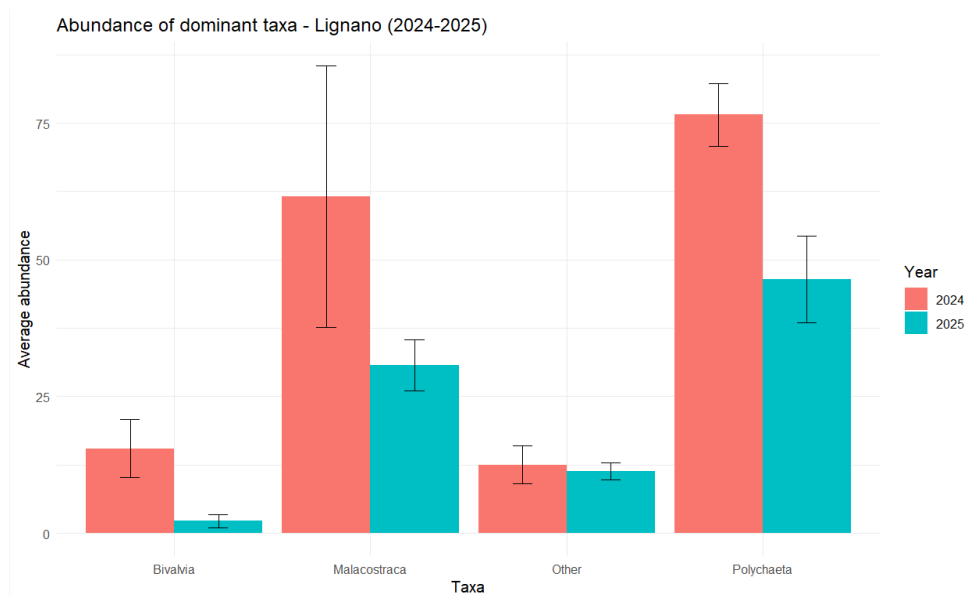


Figure 3 Comparison of total number of individuals per square meter (abundance) of dominant in years 2024 - 2025 at site 1 - Lignano

The number of taxa at site 2 - Tegnùe is represented by the next Table 2. Detailed table with individual taxa, and their abundance with SE is attached as Appendix 2. In this site both abundance and number of species is higher in the samples from 2024. There is a visible shift in dominance of higher taxa. Previously, this area was dominated by polychaetes (595,8 individuals/m²), however this changed in the next year when their abundance dropped to 120 individuals/m², being replaced Bivalvia and Malacostraca. This great loss of polychaetes is strongly influenced by the genus *Aricidea*, whose abundance dropped from 420 individuals per m² to barely 18,7. Similarly to site 1, in 2025 we detected taxa not observed in 2024. Those are: Asteroidea, Echinoidea, Gastropoda, Holothuroidea, Ostracoda and Priapulida. On the other hand, copepods, that were present in 2024, were absent in 2025. The abundance of main taxa is presented in Figure 4. Less abundant taxa were grouped into “other” category.

Table 2 Number of taxa present in stations 1,2 and 3 in Tegnùe in both 2024 (4 replicates per station) and 2025 (3 replicates per station)

Tegnùe	2024			2025		
Station	1	2	3	1	2	3
No. Taxa	30	22	32	28	29	33

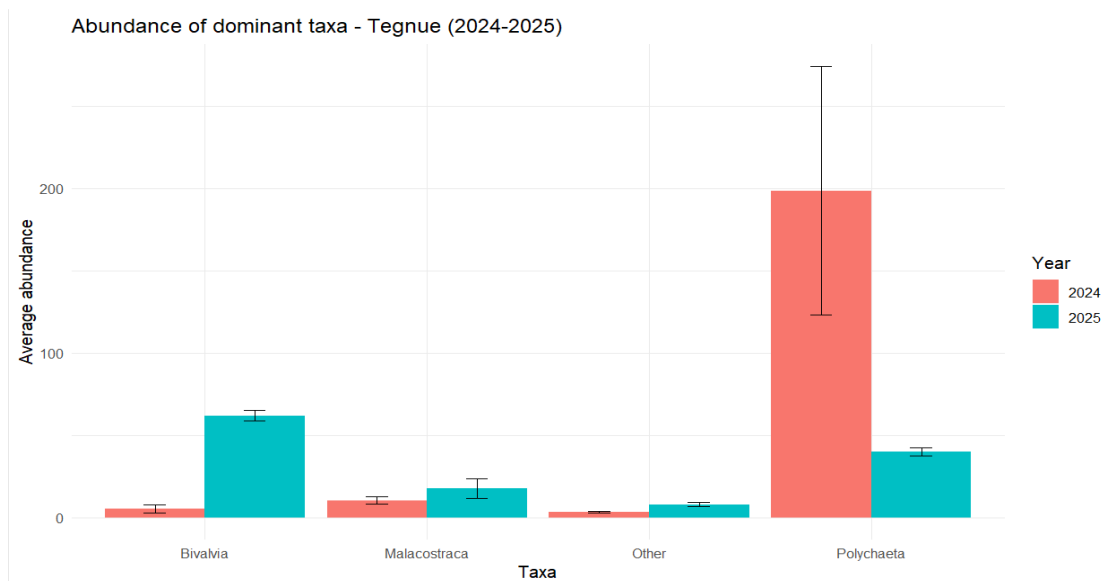


Figure 4 Comparison of total number of individuals per square meter (abundance) of dominant in years 2024 - 2025 at site 2 - Tegnùe

Lastly, site 3 – LNG Terminal, presented in Table 3. The full list of present taxa with abundance and SE is included in Appendix 3. This site is the one showing the least abundance out of the three, but the greatest richness in taxa. In both years the most represented taxa included Polychaeta and Ophiuroidea, while Malacostraca were less represented than at other sites. The ones that were absent in 2024 and present in 2025 are: Cnidaria, Copepoda, Echinoidea, Gastropoda, Holothuroidea and Nematoda. The abundance of the dominant taxa is illustrated in Figure 5.

Table 3 Number of taxa present in stations 1,2 and 3 in LNG Terminal in both 2024 (4 replicates per station) and 2025 (3 replicates per station)

LNG	2024			2025		
Station	1	2	3	1	2	3
No. Taxa	24	19	24	29	22	16

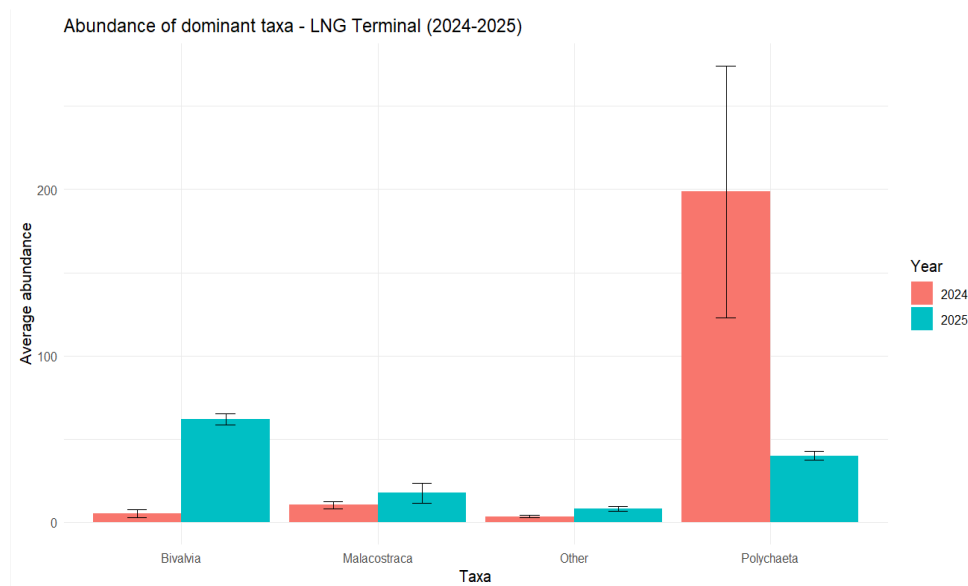


Figure 5 Comparison of total number of individuals per square meter (abundance) of dominant in years 2024 - 2025 at site 3 – LNG Terminal

The graphs below show three biodiversity metrics calculated for each sampling station across the three study sites. Hill number N1, which is an exponential of the Shannon Entropy, it weights all species proportionally to their frequency of appearance. Hill number N10 is more sensitive to the most abundant (dominant) species and strongly discounts rare species. If the community has only few highly abundant species and larger number of rare species, the index will decrease to match the number of the dominant species. Finally, richness S index, represents the number of species present in specific sample.

For Lignano in 2024 (fig. 6) we can observe that the indexes vary among the stations. The highest values for N1 and S are visible in station 2. Site 3 presents moderate value for N1 and slightly lower S than station 2. The lowest value for both N1 and S are presented in site 1. N10 was on the similar level across all stations, which indicates similar richness. The difference in N1 and S shows that the dissimilarity in diversity was caused by low evenness of species. Looking at the standard error bars, we can observe that station 2 presents the greatest variability among replicates. Both 1 and 3 represent fewer variable measurements with station being the most stable.

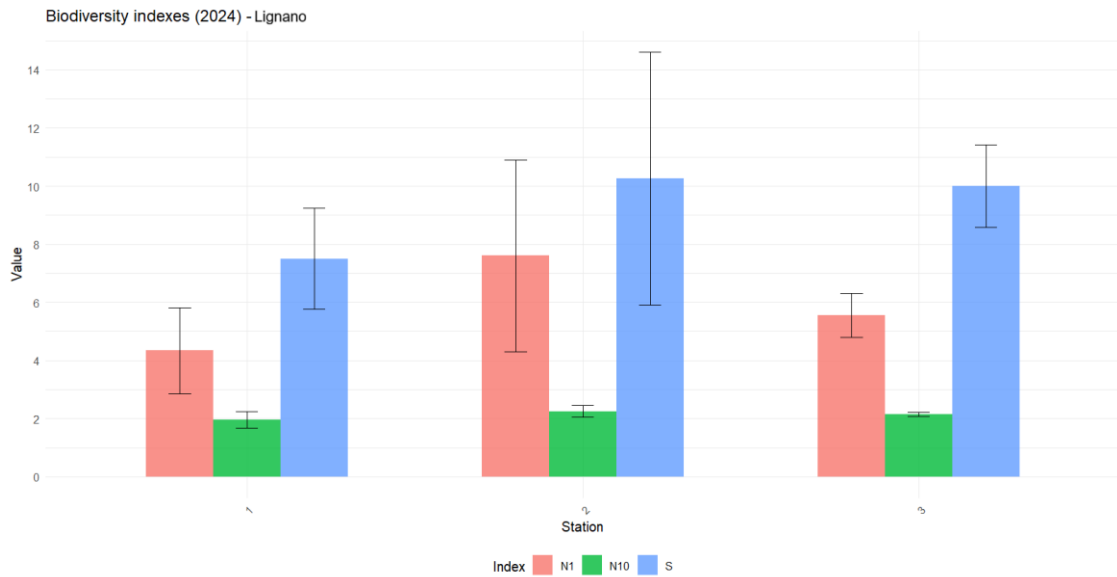


Figure 6 Mean (+ standard error) of the indices of A) Species diversity expressed as the effective number of species (Hill number N1) (pink), B) the corresponding evenness component (Hill number N10) (green), and C) species richness (S) (blue) at the three sampling stations of intervention Site 1 – Lignano in 2024, $n = 4$ for each sampling station

In 2025 differences in biodiversity metrics were observed among the three stations at the same study site (Figure 7). Station 1 showed the highest values of N1 and S, while Station 2 displayed slightly lower but broadly comparable values. The N10 values were similar at these two stations. In contrast, Station 3 showed the lowest values for all three metrics, suggesting a community characterised by lower taxonomic richness and diversity. What is similar between stations 1 and 3 is that they both present high variability and this time station 2 is the most stable within replicates. In comparison to the previous year, we can observe improvements in station 1, relatively stable conditions in station 2 with only minor decrease, and a decline in station 3.

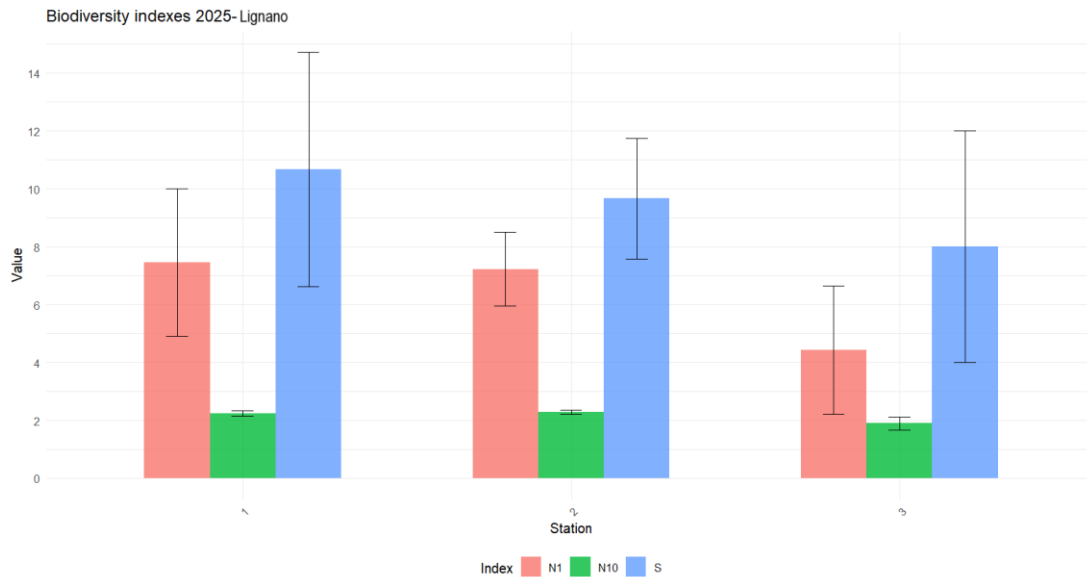


Figure 7 Mean (+ standard error) of the indices of A) Species diversity expressed as the effective number of species (Hill number N1) (pink), B) the corresponding evenness component (Hill number N10) (green), and C) species richness (S) (blue) at the three sampling stations of intervention Site 1 – Lignano in 2025, $n = 3$ for each sampling station

Tegnùe site in 2024 (Figure 8) presents distinguishable differences between sites. In comparison to the previous stations, there is no station that would dominate both N1 and S. The highest N1 and N10 values are visible in station 3, that would mean the greatest diversity and richness among stations, the S value is the lowest but still on the high level. Station 2 presents the lowest overall values for N1, and S. Station 1 presents the highest and overall moderate values for N1 and N10, which could mean the lower species dominance and high overall diversity. In all cases the variability for N1 and S is very high with the most variable measurements in station 1, particularly for N10 that was notably more variable than in other stations. For stations 2 and 3 values of N10 are very stable. Stations 2 low measurements were influenced by both evenness and richness. Station 1 presents really balanced community, and station 3 is the richest according to both N1 and N10.

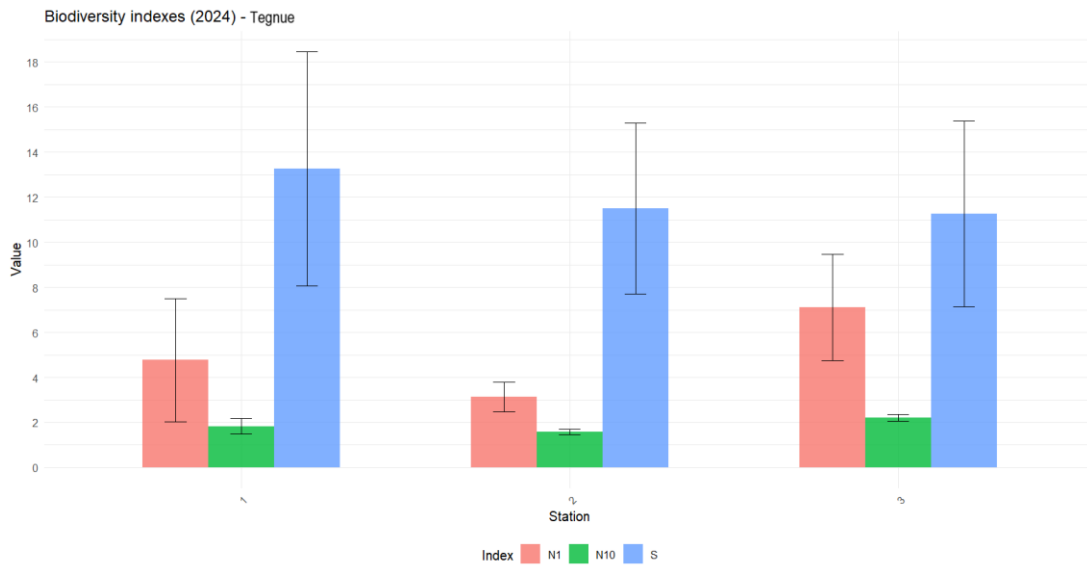


Figure 8 Mean (+ standard error) of the indices of A) Species diversity expressed as the effective number of species (Hill number N1) (pink), B) the corresponding evenness component (Hill number N10) (green), and C) species richness (S) (blue) at the three sampling stations of intervention Site 2 – Tegnue in 2024, $n = 4$ for each sampling station

For the same site in 2025, all indexes were the highest in station 3 Figure 9. The lowest values are in station 2, however station 1 presents only slightly higher values. In comparison to the previous year, station 1 shows slight decline in N, and stable position of N10 and S. There is an improvement in every index within stations 2 and 3. The variability is on a stable level for most of the indexes with only S in station 2 being more variable.

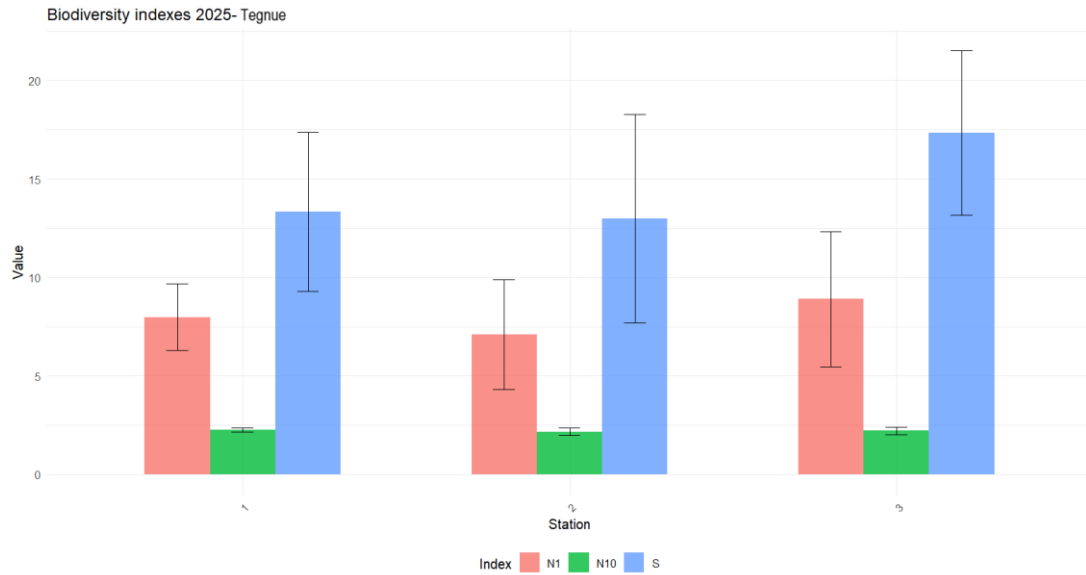


Figure 9 Mean (+ standard error) of the indices of A) Species diversity expressed as the effective number of species (Hill number N1) (pink), B) the corresponding evenness component (Hill number N10) (green), and C) species richness (S) (blue) at the three sampling stations of intervention Site 2 – Tegnue in 2025, $n = 3$ for each sampling station

LNG Terminal in the year 2024 is presented in figure 10 (fig. 10). The highest value of N1 is present in station 1. N1 is of similar value in both station 2 and 3. N10 is very similar among the stations. Stations 1 and 3 are represented by equally high value of S. The lowest value of S is in station 2. It appears that station 1 is the most balanced and diverse of the stations. Station 2 is lacking in the evenness of the replicates. Error bars indicate that in most indexes, there are large variabilities in the measurements.

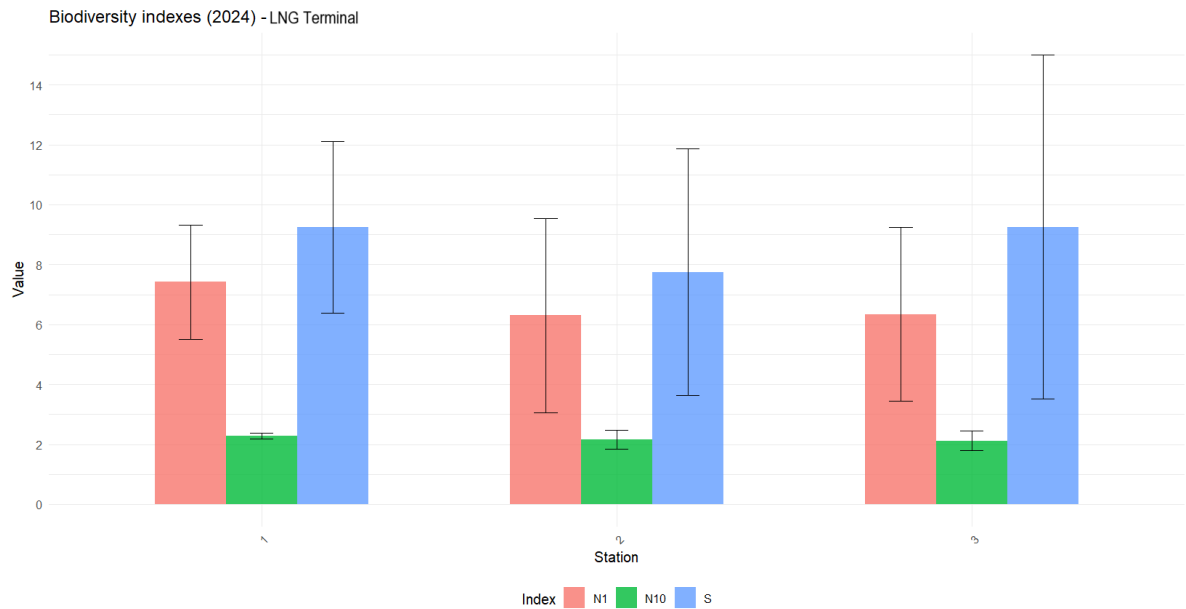


Figure 10 Mean (+ standard error) of the indices of A) Species diversity expressed as the effective number of species (Hill number N1) (pink), B) the corresponding evenness component (Hill number N10) (green), and C) species richness (S) (blue) at the three sampling stations of intervention Site 3 – LNG Terminal in 2024, n = 4 for each sampling station

Lastly, site 3 in the year 2025 (fig. 11). As observed in 2024, N10 values are similar across stations. Both N1 and S are the highest in station 1 and the lowest in station 3. Those indexes in station 2 are on the moderate level, however they are closer to the lower end of the spectrum. There is large variability visible in N1 in station 3 and in S in stations 2 and 3. The rest of measurements seem to be on a stable level. Since the N10 values are similar across stations, stations 2 and 3 seem to struggle with low evenness and possible with high dominance of few species. In comparison to the previous year, station 1 prospers and improved in N1 and S, station 2 looks stable, without any major changes in the index values, and station 3 presents a decline in diversity and evenness, visible with decrease in both N1 and S.

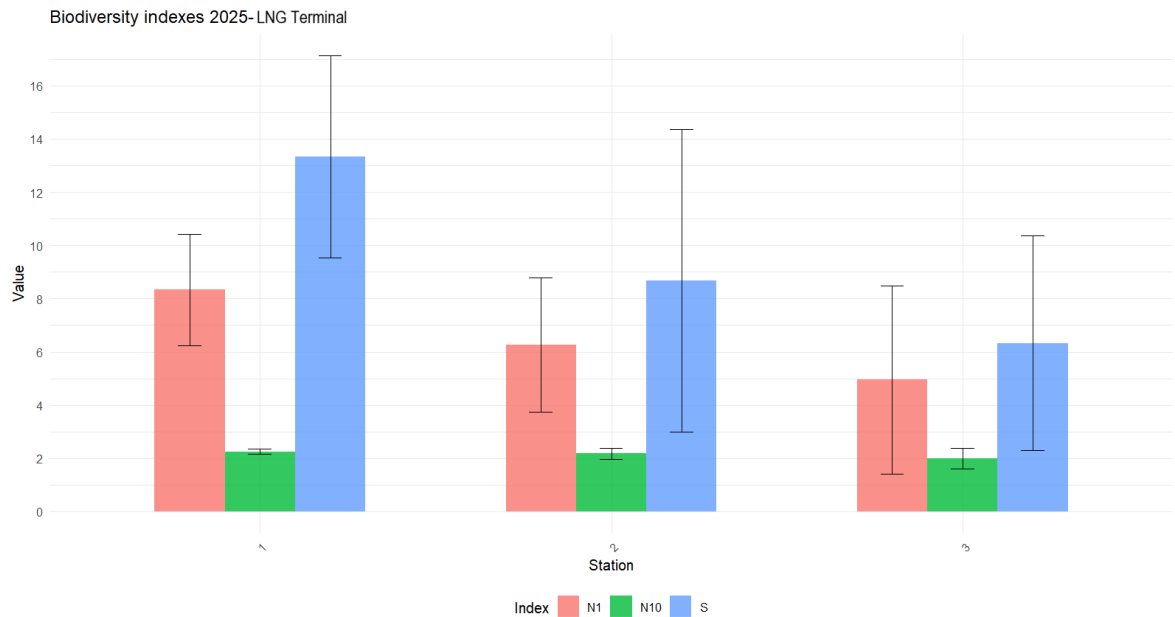


Figure 11 Mean (+ standard error) of the indices of A) Species diversity expressed as the effective number of species (Hill number N1) (pink), B) the corresponding evenness component (Hill number N10) (green), and C) species richness (S) (blue) at the three sampling stations of intervention Site 3 – LNG Terminal in 2025, $n = 3$ for each sampling station

The first principal component (PC1) explained 96.9% of the total variance, whereas the second principal component (PC2) accounted for only 3.1%, indicating that most of the variation among samples was driven by a single environmental gradient. Overall, the PCA revealed a clear sedimentological differentiation among the study sites, with Tegnùe characterised by sand-dominated substrates, whereas Lignano and LNG were associated with relatively finer sediments. This pattern provides an important environmental context for interpreting differences in benthic community structure and the performance of subtidal oyster reef restoration across the three sites (fig. 12).

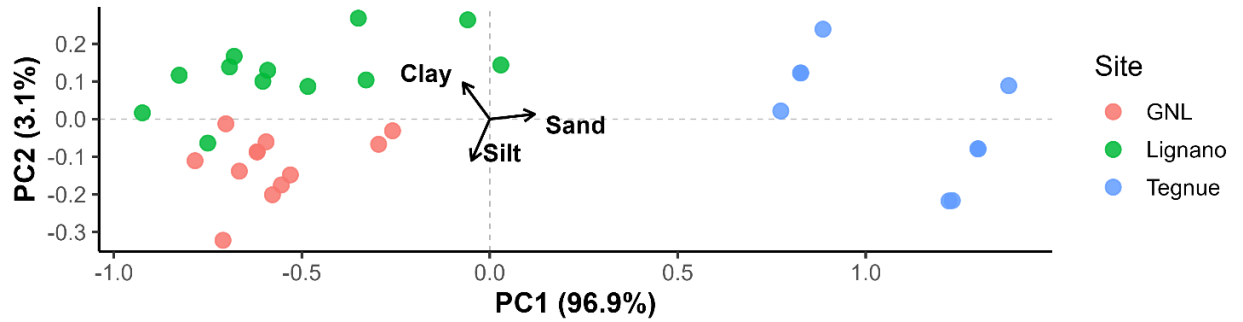


Figure 12 Principal Component Analysis (PCA) biplot of sediment grain-size composition based on centred log-ratio (CLR)-transformed proportions of sand, silt, and clay for 2024. Samples are coloured according to study site (LNG Terminal, Lignano, and Tegnùe). Arrows represent the loading vectors of the sediment fractions, indicating the direction and relative contribution of each variable to the principal components. PC1 explained 96.9% of the total variance and primarily represented a gradient from fine-grained sediments (clay and silt) to sand-dominated sediments, while PC2 explained 3.1% of the variance and mainly reflected the relative contribution of clay versus silt.

Samples from Tegnùe were clearly separated along the positive side of PC1, in the direction of the sand vector, confirming that this site was characterised by predominantly sandy sediments. In contrast, Lignano and LNG clustered on the negative side of PC1, indicating relatively higher proportions of fine sediment fractions. The second principal component explained only a small proportion of the total variance and mainly described the relative contribution of silt and clay (fig.13).

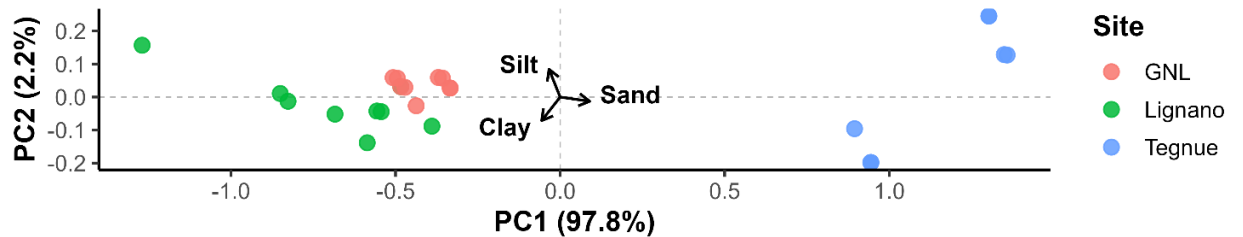


Figure 13 Principal Component Analysis (PCA) biplot of sediment grain-size composition in 2025 based on centred log-ratio (CLR)-transformed proportions of sand, silt, and clay for 2025. Each point represents one sediment sample coloured according to study site (LNG Terminal, Lignano, and Tegnùe). Arrows indicate the loading vectors of sediment fractions. PC1 explained 97.8% of the total variance and primarily represented a gradient from fine-grained sediments to sand-dominated sediments, whereas PC2 explained 2.2% of the total variance.

The highest values of density are visible in Tegnùe , where all replicates surpass 1,5 g/cm³ with greatest density at station 3 (around 1.75 g/cm³). Bulk density in Lignano was much lower, slightly exceeding 1,0 g/cm³ and the lowest density at station 2 with approximately 0,75 g/cm³. The values are presented in the following figure (fig. 14).

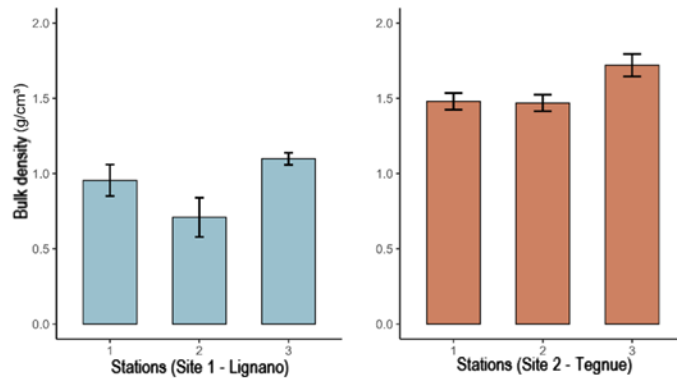


Figure 14 Mean bulk density at sampling stations 1–3 of the Lignano and Tegnùe sites in 2025. Values are expressed in g cm⁻³. Error bars represent standard error (SE); n = 4 replicates per sampling station

For the year 2024 (fig. 15) the largest loss of organic matter (around 4%) is visible at station 2 in Tegnùe , however this station has also the largest variability between replicates. The remaining stations do not exceed 2% loss, with the samples from LNG Terminal keeping steady approximate 1% loss throughout all the stations.

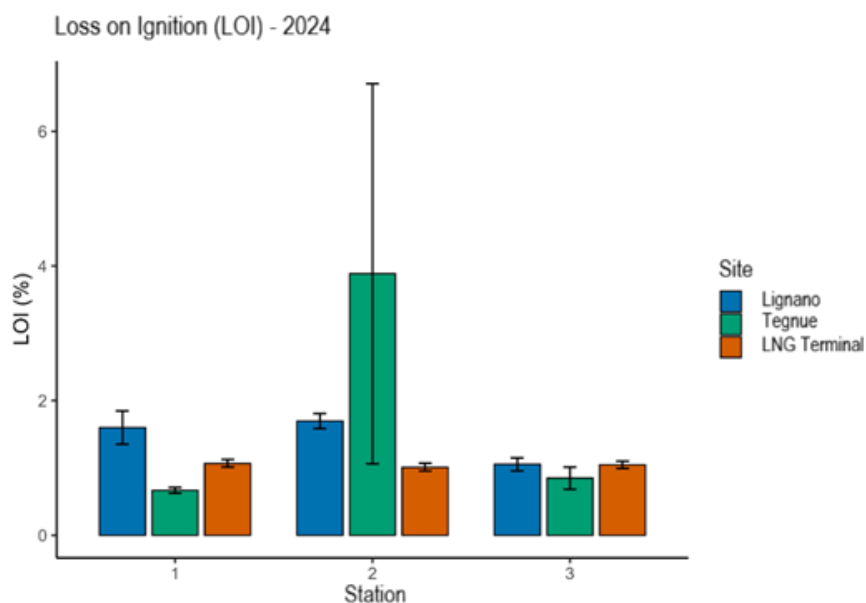


Figure 15 Mean ($\pm$ SE) concentrations of Loss on Ignition of organic matter content measured at all sites (Lignano, Tegnùe, and LNG Terminal) in 2024. Values are expressed as percentages (%); error bars represent the standard error (SE), ($n = 4$ replicates for each sampling station).

The results from 2025 are presented in the next figure (fig. 16). The highest LOI is present at station 2 of LNG Terminal site, although it expresses the highest variability among replicates. This site in general shows higher levels of weight loss, with one exception of station 3 of Lignano. The most uniform LOI is visible at site in Tegnùe, that is around 0,3% loss.

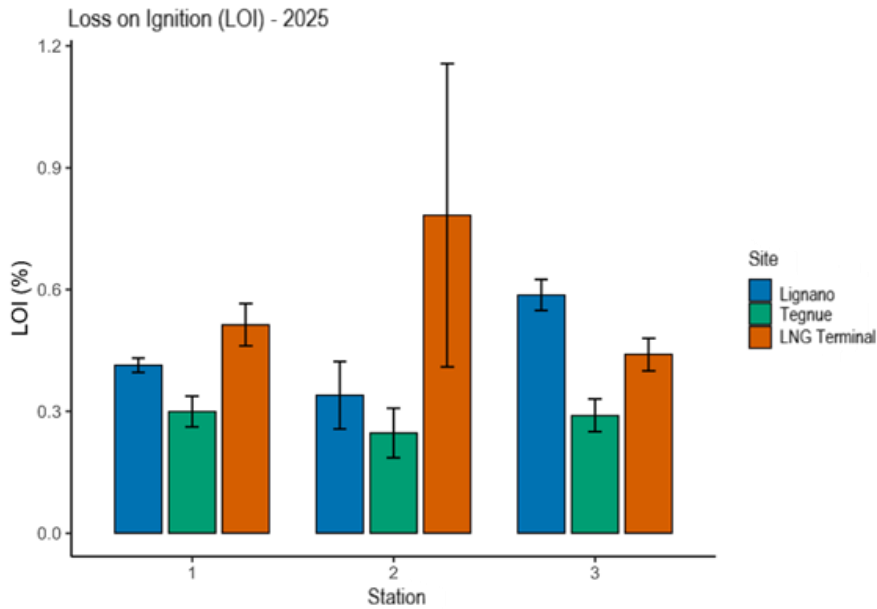


Figure 16 Mean ($\pm$ SE) concentrations of Loss on Ignition measured at all sites (Lignano, Tegnùe, and LNG Terminal) in 2025. Values are expressed as percentages (%); error bars represent the standard error (SE), ($n = 3$ replicates for each sampling station).

The levels of Total Organic Carbon (TOC) and nitrogen (N) for 2024 are presented below (fig. 17). The highest concentrations of TOC are present in Lignano, especially at station 3, where it reaches approximately 2%. In the remaining sites concentrations remain lower than 0.5%, with the lowest values in LNG Terminal where at all stations the concentration is lower than 0.25%. Nitrogen contents remained on very low levels in all sites. The highest concentration are visible in Lignano, however at all stations values are close to 0% of concentration.

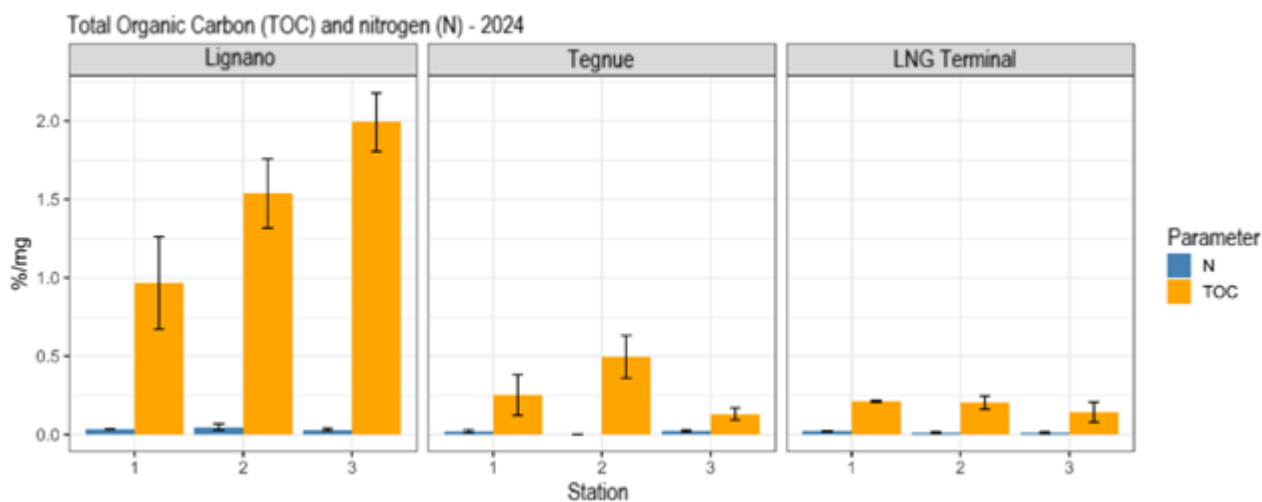


Figure 17 Mean ($\pm$ SE) concentrations of Total Organic Carbon (TOC) and nitrogen (N) measured at all sites (Lignano, Tegnùe, and LNG Terminal) in 2024. Values are expressed as percentages (%); error bars represent the standard error (SE), ($n = 4$ replicates for each sampling station).

The concentration levels for TOC and N in 2025 are displayed in the following graph (fig. 18). Similarly to the previous year, the highest concentrations of TOC are present in Lignano, but also values are similar to each other, all surpassing 2% of concentration. In comparison to the first site, the remaining sites, Tegnùe and LNG Terminal, present similarly low values, not exceeding 0,5% of TOC concentration. The N levels in all cases are low and oscillate around 0% of content.

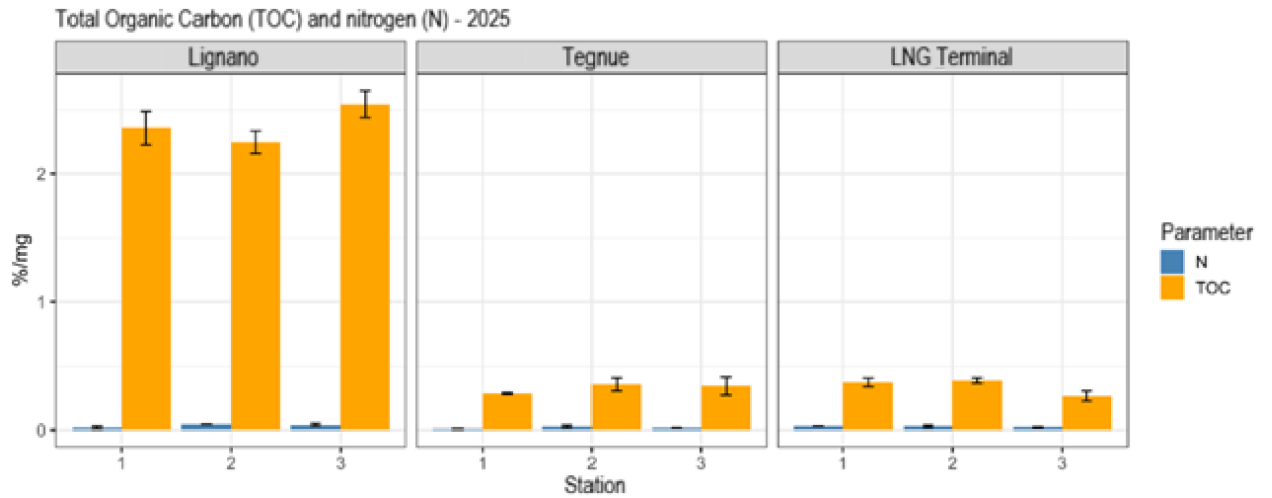


Figure 18 Mean ($\pm$ SE) concentrations of Total Organic Carbon (TOC) and nitrogen (N) measured at all sites (Lignano, Tegnùe, and LNG Terminal) in 2025. Values are expressed as percentages (%); error bars represent the standard error (SE), ($n = 3$ replicates for each sampling station).

In the 2024, the Principal Coordinates Analysis (PCoA) revealed a clear separation of benthic community composition among the three study sites (fig. 19). Samples from Lignano clustered on the negative side of PCoA1, whereas GNL and Tegnùe were separated along the positive side of PCoA1 and further differentiated along PCoA2, indicating distinct community assemblages among sites. Environmental fitting (envfit) identified total organic carbon (TOC) and total nitrogen as significant correlates of community composition. TOC exhibited the strongest relationship with the ordination ($R^2 = 0.79$, $p < 0.001$), with its vector pointing towards the Lignano samples, indicating that this site was associated with relatively higher organic carbon content. Total nitrogen was also significantly correlated with community composition ($R^2 = 0.23$, $p = 0.01$), although its explanatory power was substantially lower than that of TOC. The similar orientation of the TOC and nitrogen vectors suggests that these variables covary and define an environmental gradient associated with differences in benthic assemblage structure across the study sites.

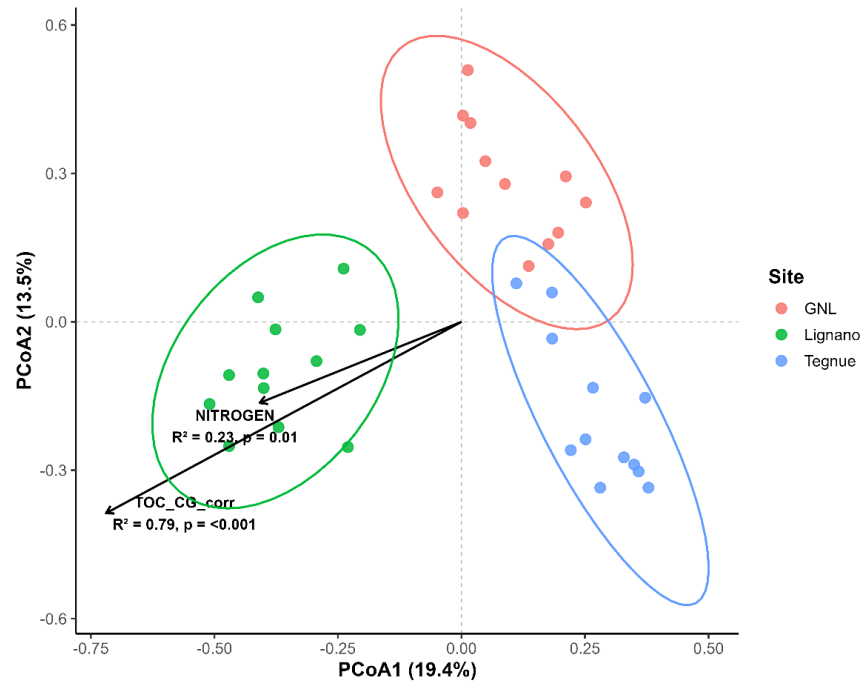


Figure 19 Principal Coordinates Analysis (PCoA) of benthic community composition at the three study sites (LNG Termianl, Lignano, and Tegnùe) for 2024. Points represent individual samples and ellipses indicate the dispersion of samples within each site. Arrows show significant environmental variables fitted to the ordination using *envfit*. Vector length is proportional to the strength of the correlation with community composition.

Also, in the 2025 the Principal Coordinates Analysis (PCoA) showed a clear separation in benthic community composition among the three study sites (fig. 20). Samples from Lignano clustered on the negative side of PCoA1, whereas Tegnùe was separated along the positive side of PCoA1. Samples from GNL occupied an intermediate position along the first axis but were clearly differentiated from the other two sites along PCoA2, indicating distinct benthic assemblages across all study sites. Environmental fitting (*envfit*) identified total organic carbon (TOC) as the only environmental variable significantly associated with community composition ($R^2 = 0.83$, $p < 0.001$). The TOC vector pointed towards the Lignano samples, indicating that this site was associated with relatively higher sediment organic carbon content. In contrast, total nitrogen showed a weaker and non-significant relationship with the ordination ($R^2 = 0.15$, $p = 0.15$), suggesting that nitrogen contributed little to the observed variation in benthic community structure. The similar orientation of the TOC and nitrogen vectors indicates that both variables followed the same general

environmental gradient, although only TOC significantly explained differences in community composition.

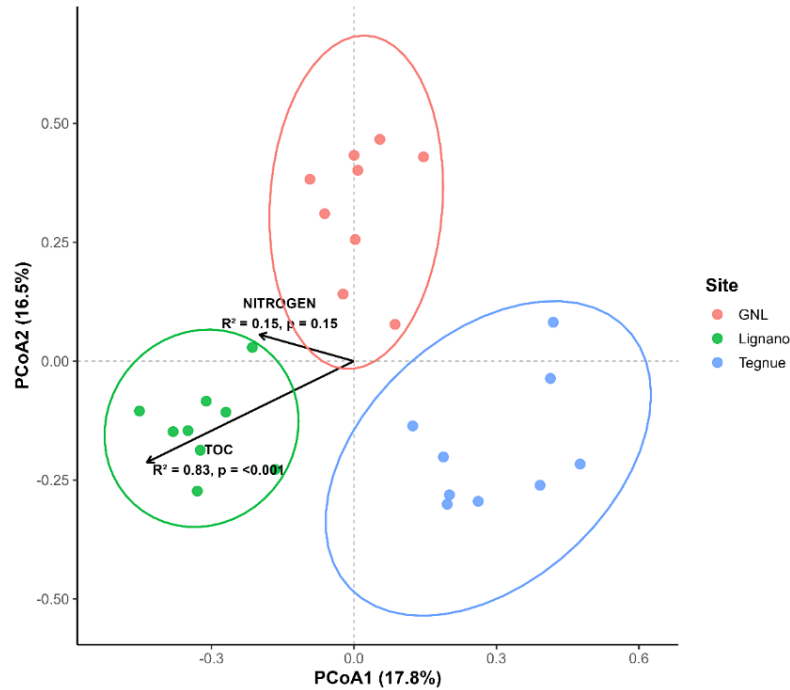


Figure 20 Principal Coordinates Analysis (PCoA) of benthic community composition in 2025. Points represent individual samples and ellipses indicate the 95% confidence interval for each study site (LNG Terminal, Lignano, and Tegnùe). Arrows show environmental variables fit ted to the ordination using envfit. Vector length is proportional to the strength of the correlation between environmental variables and community composition. Total organic carbon (TOC) was strongly and significantly associated with community composition ($R^2 = 0.83$, $p < 0.001$), whereas total nitrogen showed a weaker and non-significant relationship ($R^2 = 0.15$, $p = 0.15$). PCoA1 and PCoA2 explained 17.8% and 16.5% of the variation in community composition, respectively.

5. Discussion of results and conclusions

5.1 Discussion of results

The main and foremost crucial finding of the thesis is that sites in Lignano, Tegnùe and LNG Terminal strongly differ among each other. This is an important piece of information that needs to be kept in mind, that neither the sites, nor stations cannot be directly compared with each other, as the stations do not present the exact same assemblages as their counterparts at other sites. Additionally, the sampling efforts

performed in 2024 were higher than in 2025, since the number of collected replicates per station decreased from 4 to 3 replicates. However, we can distinguish differences observed within the sites that occurred between sample collections in 2024 and 2025, but also the differences between the sites in order to strengthen the argument that each site should be considered separately with its own starting point. Present differences in infaunal assemblages at those sites are being seen in such parameters as: identified taxa, their abundance, and the most dominant genera.

All identified taxa were already observed in the Adriatic Sea, with most of them being common and characteristic for the Adriatic Sea region and the Mediterranean Sea (Faulwetter et al., 2017; Mikac, 2015; Petović et al., 2017; Scipione & Zupo, 2010; Thurstan et al., 2024). Present in the samples were species that are endemic to the Mediterranean Sea, e.g. *Apseudopsis mediterraneus*, and those which first sighting was in the Venetian Lagoon – *Prionospio caspersi* (Britayev et al., 1994). There are species like *Onuphis eremita*, *Owenia fusiformis*, *Ophiura ophiura*, and *Varicorbula gibba* that are present across multiple sites. It could imply that these organisms are a part of core community in this region, making them a backbone for assemblage forming. Several of the identified species strongly support the oyster reef restoration efforts. Bioturbators like *Aricidea* sp, *Magelona* sp, and *Sipunculus nudus* promote remineralization of nutrients and supply oxygen into sediment.

As previously stated, the number of collected replicates could have potentially impacted the number of identified taxa and their abundance. However, we can still notice the differences between and within sites in 2024 and 2025. In both years, highest number of identified taxa, as well as the highest abundance, were recorded in Tegnùe. Also, this site stood out because of the most noticeable change in the most dominant genera. In 2024, undeniable dominance in abundance presented Polychaeta, however in 2025 a large decrease in observed individuals from *Aricidea* genus was recorded. At the same time, representatives of Bivalvia and Malacostraca started to be observed more frequently. Lignano was characterised by the lowest number of identified taxa, but fairly high abundance dominated in both years by Polychaeta and Malacostraca. Site in LNG Terminal, throughout the study period, presented relatively high number

of identified taxa (43 in 2024 and 45 in 2025), however it also has shown the lowest abundance. What sets this site apart from the remaining two is high abundance of Ophiuroidea, both in 2024 and 2025. Worth noticing fact is that during the analysis of 2025 samples we observed taxa that were not present in 2024. They were mainly representatives of Echinodermata (e.g. Asteroidea, Echinoidea and Holothuroidea). Although, it is not a sign of those taxa not being present in 2024, because with relatively few replicate grab samples, rare taxa can easily be present but not sampled.

Considering all those parameters, we cannot set a uniform baseline for the entire project, nor can we compare the future changes to total diversity and abundance of the sites. Each site should be considered as separate entity with unique characteristics and properties, including environmental conditions. Noticing that is crucial for the future restoration efforts and the PNRR MER project. The best support for this claim is the results of Principal Coordinates Analysis. In both years, the clusters representing the interventions sites are separated and occupy opposite sides of the ordination space. In 2024, the highest variability was presented in Tegnùe. It may suggest higher structural complexity of the site, in comparison to more homogenous Lignano. In 2025, the clusters were fully separated, proving that the special composition differences are constant over time. In this year the separation was even more pronounced. Large variability within Tegnùe show high heterogeneity of the present community. In contrast, and similarly to the previous year, Lignano presented the highest homogeneity within habitat. However, the separation does not prove that the differences are constant over time. It shows a pattern of separation in the sampled communities.

As for the indexes of biological diversity, obtained values suggest relatively high overall biodiversity across the sites. The common characteristic of all sites in both years is they maintain similar levels of species evenness. As for Shannon and Simpson Indexes, considerable small-scale spatial, and interannual variability was present during the baseline period. In comparison to similar studies of benthic macrofauna diversity in the region of North Adriatic, conducted by Occhipinti-Ambrogi et al. in 2005, we can notice an upward trend that started in the 2000's after a decline in the late 90's. The long-term studies conducted in the waters of Ravenna have shown the

Shannon-Wiener going from 1,5 in July 2000 to approximately 3,75 in July 2002. In comparison, the lowest value in this study was about 3 in site 2 in Tegnùe in 2024 that later climbed to around 7,5. What sets apart the compared study from mine is the relatively high evenness of species throughout the climb period (excluding samples from July 2001), what appeared to be a more prominent struggle for assemblages assessed by me (Occhipinti-Ambrogi et al., 2005). Overall, we can observe a great leap in diversity of the region that may suggest that the communities are being rebuilt and the habitats repopulated, which is a great news for the future conservation efforts in the region.

A good anchor point helping us to understand the spatial structure of assemblages are the environmental differences observed between the sites. Data obtained from sediment analysis provides useful context for each site. The simplest and one of the first noticeable differences is the most dominant sediment fraction at intervention areas. Tegnùe was strongly associated with sandy sediment. On the other hand, both Lignano and LNG Terminal have shown higher association with finer sediment fractions like clays and silts. Going deeper into factors shaping the assemblage formations, we analysed how impactful and significant are Total Organic Carbon and Total Nitrogen in the sediment. As it turns out, in 2024, both TOC and TN were significant factor, with TOC having $R^2 = 0.79$ and $p < 0.001$ and TN having $R^2 = 0.23$ and $p = 0.01$. However, while being significant statistically, TN presented much weaker association than TOC. In 2025, TOC remained strongly associated with community composition ($R^2 = 0.83$, $p < 0.001$), whereas TN did not appear to be significant ($R^2 = 0.15$, $p = 0.15$). These findings provide a very important piece of evidence that the differences in these particular benthic communities are driven by a sedimentary gradient, that is Total Organic Carbon. In means of future assessments TOC gradient should be considered as an important and reliable factor used in characterisation of the intervention sites. On the other hand, TN levels were expected to appear rather low as most sediment mass is mineral material, though due to its statistical insignificance it explains very little of the multivariate patten observed in the

area. It still plays very important role in the environment but does not significantly impact the assemblage formations.

The LOI results show a very large difference between years. Most 2024 values are above 1%, with one approaching 4%, whereas the 2025 values are reportedly all below 1%. The most noticeable and concerning are the results from Lignano, which put together with TOC results present unusual state of matter. Because LOI estimates total combustible organic matter whereas TOC measures organic carbon, one should expect the results to pair the increase of TOC with increased LOI. However, the results at this site suggest that that organic carbon accumulation was not proportional to the bulk organic matter. One of the possible causations of this phenomenon can be differences in sedimentary organic matter sources and composition, as well as its association with mineral particles.

The baseline shows that the three sites differ substantially in community composition and environmental characteristics and that considerable variability also occurs within sites and between sampling years. This means that future monitoring must account for this pre-existing variability before attributing changes in biodiversity, abundance or community composition to oyster-reef restoration. This is an important result in itself. A baseline does not need to show that the ecosystem is improving, but rather its purpose is to tell what was there before the intervention and how variable it already was, so that later we can distinguish a restoration response from background variation. It all comes down to the foundation of the restoration project that says: future changes should be evaluated against the initial benthic assemblages and environmental conditions.

5.2 Limitations

Several limitations should be considered when interpreting the results of this study. Firstly, differences in taxonomic identification between the 2024 and 2025 datasets may have affected the comparison between years. The higher taxonomic

resolution achieved for some 2025 samples may therefore have resulted in a higher number of recorded taxa, potentially leading to an underestimation of species richness in 2024. Secondly, the reduction in the number of replicates from four to three may have reduced sampling effort and affected estimates of infaunal abundance and variability among samples. Finally, diving restrictions within the LNG Terminal area prevented sediment sampling for bulk density measurements at this site. Consequently, bulk density could not be compared with the other intervention sites. Moreover, as this analysis was introduced only in 2025, no corresponding data were available for 2024.

5.3 Conclusions

This study achieved its main goal of establishing a baseline for future assessments of the oyster-reef restoration sites. The identified taxa, their abundance, the biodiversity metrics, and the sediment characteristics provide a reference that can be used to follow changes during future monitoring. The results also show that the three sites have different benthic communities and environmental conditions. For this reason, each site should be considered separately and compared with its own initial state.

Some differences were observed between the 2024 and 2025 sampling campaigns, including changes in the recorded taxa, abundance, and diversity metrics. However, these patterns should be interpreted with caution because sampling effort differed slightly between years and natural variability was observed among stations. The results do not yet show the effects of restoration because the data describe the conditions before the oyster-reef structures were established.

The formation of the benthic assemblages was also associated with sediment characteristics. Total organic carbon showed the strongest relationship with community composition, particularly at Lignano, while Tegnùe was characterised by sandier sediment. These environmental differences will be important when interpreting the results of future surveys.

The presence of different taxa and ecological groups shows that the study areas already support diverse benthic communities. After the restoration structures are installed, hard surfaces and the developing oyster reefs may provide additional habitat for oysters and other organisms. Continued monitoring will be necessary to determine whether these interventions are followed by changes in biodiversity, abundance, and community composition. The baseline established in this study will make it possible to compare these future changes with the conditions that were present before restoration.

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APPENDIX

APPENDIX 1

Appendix 1 List of taxa present in the sediment samples collected during sampling campaigns conducted in years 2024 and 2025 at the stations 1, 2, and 3 of Site 1 - Lignano. The values are expressed as the average of the number of individuals per m² ± standard error

Taxa	2024			2025		
	St. 1-1	St. 1-2	St. 1-3	St. 1-1	St. 1-2	St. 1-3
CNIDARIA						
Hexacorallia						
Haeckel, 1896						
<i>Peachia hastata</i>	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
<i>Gosse, 1855</i>						
ANNELIDA						
Polychaeta Grube, 1850						
<i>Aricidea Webster, 1879</i>	0 ± 0	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Glycera sp. Lamarck, 1818</i>	4 ± 4.0	2 ± 2.0	0 ± 0	1.3 ± 1.3	2 ± 1.2	2.7 ± 2.7
<i>Hilbigneris gracilis (Ehlers, 1868)</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	2.7 ± 2.7
<i>Lumbrineridae Schmarda, 1861</i>	40.5 ± 19.6	38 ± 14.4	39 ± 12.9	0 ± 0	0 ± 0	0 ± 0
<i>Lumbrineris sp. Blainville, 1828</i>	0 ± 0	0 ± 0	0 ± 0	14.7 ± 5.3	6.7 ± 6.7	0 ± 0
<i>Mysta sp. Malmgren, 1865</i>	2.25 ± 1.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Nereididae Blainville, 1818</i>	0 ± 0	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Nereiphylla rubiginosa (Saint-Joseph, 1888)</i>	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0
<i>Onuphidae Kinberg, 1865</i>	29 ± 13.0	24 ± 14.2	24 ± 1.4	0 ± 0	0 ± 0	0 ± 0
<i>Onuphis eremita Audouin & Milne Edwards, 1833</i>	0 ± 0	0 ± 0	0 ± 0	37.3 ± 11.4	18.7 ± 2.9	46.7 ± 25.5
<i>Orbinia sp. Quatrefages, 1866</i>	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
<i>Owenia fusiformis Delle Chiaje, 1844</i>	0 ± 0	4 ± 4.0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
<i>Paraonidae Cerruti, 1909</i>	4.3 ± 3.9	2 ± 2.0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Phyllodocidae Örsted, 1843</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0
<i>Polynoidae Kinberg, 1856</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0

Sigalionidae Kinberg, 1856	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7
<i>Sigambra</i> sp. Müller, 1858	0 ± 0	4 ± 4.0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Sphaerosyllis</i> sp. Claparède, 1863	0 ± 0	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Spionidae Grube, 1850	0 ± 0	4 ± 4.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sipunculidae Rafinesque, 1814						
<i>Sipunculus</i> (<i>Sipunculus</i>) <i>nudus</i> Linnaeus, 1766	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0
ARTHROPODA						
Malacostraca						
Latreille, 1802						
<i>Ampelisca</i> sp. Krøyer, 1842	5 ± 3.7	6 ± 3.8	5 ± 3.0	0 ± 0	0 ± 0	0 ± 0
<i>Ampelisca brevicornis</i> (A. Costa, 1853)	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	4.7 ± 3.7	5.3 ± 3.5
<i>Apseudopsis</i> <i>mediterraneus</i> (Bacescu, 1961)	0 ± 0	20 ± 17.4	1.5 ± 1.5	2.7 ± 2.7	2.7 ± 2.7	0 ± 0
<i>Apseudopsis</i> sp. Norman, 1899	6 ± 6.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Bathynellacea Chappuis, 1915	0 ± 0	0 ± 0	2 ± 1.4	0 ± 0	0 ± 0	0 ± 0
<i>Bodotria scorpioides</i> (Montagu, 1804)	0 ± 0	2 ± 2.0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Caprella</i> sp. Lamarck, 1801	2.5 ± 2.5	32 ± 17.0	62.5 ± 13.0	12 ± 6.1	11.3 ± 2.4	8 ± 2.3
Cumacea Krøyer, 1846	1 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Diastylis cornuta</i> (Boeck, 1864)	0 ± 0	0 ± 0	0 ± 0	2.7 ± 2.7	1.3 ± 1.3	4 ± 4.0
<i>Diastylis</i> sp. Say, 1818	0 ± 0	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gammaridae Latreille, 1802	0 ± 0	0 ± 0	0 ± 0	0 ± 0	13.3 ± 13.3	2.7 ± 2.7
<i>Ilia nucleus</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	1.3 ± 1.3
<i>Iphinoe serrata</i> Norman, 1867	0 ± 0	8 ± 3.3	1 ± 1.0	0 ± 0	0 ± 0	1.3 ± 1.3
<i>Iphinoe trispinosa</i> (Goodsir, 1843)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0
Isaeidae Dana, 1852	0 ± 0	12 ± 7.7	12 ± 3.6	0 ± 0	0 ± 0	0 ± 0
<i>Leucothoe oboa</i> Karaman, 1971	0 ± 0	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Leucothoe</i> sp. Leach, 1814	0 ± 0	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

<i>Leucothoe spinicarpa</i> (Abildgaard, 1789)	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Medicorophium runcicorne</i> (Della Valle, 1893)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0.7 ± 0.7
<i>Microprotopus maculatus</i> Norman, 1867	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0
<i>Photis longicaudata</i> (Spence Bate & Westwood, 1862)	0 ± 0	0 ± 0	0 ± 0	8 ± 4.0	2.7 ± 1.3	0 ± 0
<i>Polybius depurator</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
Ostracoda Latreille, 1802						
Ostracoda Latreille, 1802	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
ECHINODERMATA						
Asteroidea de Blainville, 1830						
<i>Astropecten spinulosus</i> (Philippi, 1837)	0 ± 0	0 ± 0	0 ± 0	4 ± 4.0	0 ± 0	0.3 ± 0.3
Holothuroidea de Blainville, 1834						
<i>Leptopentacta grisea</i> Clark, 1938	0 ± 0	0 ± 0	1.8 ± 0.9	0 ± 0	3 ± 1.5	4 ± 2.3
<i>Thyone fusus</i> (O.F. Müller, 1776)	0 ± 0	2 ± 2.0	1.3 ± 0.3	0 ± 0	3 ± 3.0	0 ± 0
Ophiuroidea Gray, 1840						
<i>Amphiura chiajei</i> Forbes, 1843	0 ± 0	4 ± 2.3	2,8 ± 1.4	0 ± 0	0 ± 0	0 ± 0
<i>Ophiothrix fragilis</i> (Abildgaard in O.F. Müller, 1789)	0 ± 0	2.3 ± 1.9	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
<i>Ophiura ophiura</i> (Linnaeus, 1758)	5 ± 3.8	0 ± 0	0 ± 0	5.3 ± 1.3	1.3 ± 1.3	1.3 ± 1.3
MOLLUSCA						
Bivalvia Linnaeus, 1758						
<i>Abra</i> sp. Lamarck, 1818	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Chamelea gallina</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
<i>Donax trunculus</i> Linnaeus, 1758	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
<i>Lucinella divaricata</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0

<i>Nucula nitidosa</i> Winckworth, 1930	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
<i>Tellina</i> sp. Linnaeus, 1758	0 ± 0	4 ± 2.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Varicorbula gibba</i> (Olivi, 1792)	24 ± 15.3	10 ± 10.0	8.5 ± 5.3	0 ± 0	0 ± 0	1.3 ± 1.3
Gastropoda Cuvier, 1795						
<i>Melanella polita</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0
<i>Naticarius hebraeus</i> (Martyn, 1786)	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
<i>Neverita josephina</i> Risso, 1826	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Retusa umbilicata</i> (Montagu, 1803)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
NEMATODA DIESING, 1861						
Nematoda Diesing, 1861	8 ± 8.0	10 ± 7.6	0 ± 0	0 ± 0	2.7 ± 2.7	0 ± 0

APPENDIX 2

Appendix 2 List of taxa present in the sediment samples collected during sampling campaigns conducted in years 2024 and 2025 at the stations 1, 2, and 3 of Site 2 - Tegnùe . The values are expressed as the average of the number of individuals per m2 ± standard error

Taxa	2024			2025		
	St. 2-1	St. 2-2	St. 2-3	St. 2-1	St. 2-2	St. 2-3
ANNELIDA						
Polychaeta Grube, 1850						
<i>Ampharetidae</i> Malmgren, 1866	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Ampharete acutifrons</i> (Grube, 1860)	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0.7 ± 0.7	0 ± 0
<i>Aonides oxycephala</i> (Sars, 1862)	0 ± 0	0 ± 0	0 ± 0	6.7 ± 6.7	16 ± 16.0	0 ± 0
<i>Aricidea (Acmira)</i> <i>meridionalis</i> Laubier & Ramos, 1974	0 ± 0	0 ± 0	0 ± 0	0 ± 0	9.3 ± 6.6	9.3 ± 6.6
<i>Aricidea</i> Webster, 1879	127 ± 43.9	259 ± 44.6	34 ± 9.3	0 ± 0	0 ± 0	0 ± 0
<i>Boccardia</i> <i>polybranchia</i> (Haswell, 1885)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 0,7
<i>Capitella capitata</i> (Fabricius, 1780)	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	1.3 ± 1.3	0 ± 0
<i>Capitellidae</i> Grube, 1862	0 ± 0	4 ± 2.8	2.5 ± 2.5	0 ± 0	0 ± 0	0 ± 0

Cossuridae Day, 1963	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Eulalia</i> sp. Savigny, 1822	3.5 ± 2.1	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Glycera</i> sp. Lamarck, 1818	3.5 ± 2.6	8.8 ± 3.7	4.75 ± 1.8	3.3 ± 1.8	2 ± 1.2	0.7 ± 0.7
Hesionidae Grube, 1850	0 ± 0	2.3 ± 2.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Hilbigneris gracilis</i> (Ehlers, 1868)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	4.7 ± 3.7
Lumbrineridae Schmarda, 1861	4.5 ± 2.1	5 ± 2.9	2.5 ± 1.5	0 ± 0	0 ± 0	0 ± 0
<i>Lumbrineris</i> sp. Blainville, 1828	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
<i>Magelona filiformis</i> Wilson, 1959	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0.7 ± 0.7
<i>Magelona minuta</i> Eliason, 1962	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0
<i>Magelona</i> sp. F. Müller, 1858	0 ± 0	6 ± 4.8	5 ± 3.0	0 ± 0	0 ± 0	0 ± 0
Maldanidae Malmgren, 1867	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0
<i>Mediomastus fragilis</i> Rasmussen, 1973	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
<i>Nephtys</i> sp. Cuvier, 1817	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	2.7 ± 2.7
Nereididae Blainville, 1818	0 ± 0	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
Onuphidae Kinberg, 1865	0 ± 0	3 ± 1.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Onuphis eremita</i> Audouin & Milne Edwards, 1833	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	2 ± 2.0	6.7 ± 1.8
<i>Onuphis</i> sp. Audouin & Milne Edwards, 1833	1.5 ± 1.0	4 ± 2.8	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Orbinia sertulata</i> (Savigny, 1822)	4 ± 4.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Orbinia</i> sp. Quatrefages, 1866	0 ± 0	0 ± 0	0 ± 0	11.3 ± 11.3	0 ± 0	3.3 ± 3.3
<i>Owenia fusiformis</i> Delle Chiaje, 1844	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0.7 ± 0.7
<i>Oxydromus pallidus</i> Claparède, 1864	0 ± 0	0 ± 0	0 ± 0	4 ± 4.0	0 ± 0	0.7 ± 0.7
Paraonidae Cerruti, 1909	0 ± 0	0 ± 0	2 ± 1.2	0 ± 0	0 ± 0	0 ± 0
<i>Paucibranchia belli</i> (Audouin & Milne Edwards, 1833)	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	2.7 ± 2.7	0 ± 0

<i>Phyllodoce</i> sp. <i>Lamarck, 1818</i>	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Phyllodocidae Örsted, 1843	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7
<i>Podarkeopsis</i> sp. <i>Laubier, 1961</i>	2 ± 2.0	0 ± 0	1.5 ± 1.5	0 ± 0	0 ± 0	0 ± 0
Polynoidae Kinberg, 1856	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Sabellidae Latreille, 1825	0.3 ± 0.3	5.3 ± 2.8	1.5 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Salvatoria</i> sp. <i>McIntosh, 1885</i>	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0.7 ± 0.7
Serpulidae Rafinesque, 1815	2 ± 1.8	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
Sigalionidae Kinberg, 1856	2.5 ± 0.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7
<i>Sigambra</i> sp. Müller, 1858	27.3 ± 13.7	121 ± 15.3	12 ± 12.0	0 ± 0	0 ± 0	0 ± 0
<i>Sphaerosyllis</i> sp. <i>Claparède, 1863</i>	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	1.3 ± 1.3	0 ± 0
Spionidae Grube, 1850	2.5 ± 1.9	0 ± 0	2 ± 2.0	0 ± 0	0.7 ± 0.7	2 ± 1.2
<i>Spiophanes bombyx</i> (<i>Claparède, 1870</i>)	0 ± 0	0 ± 0	4.5 ± 2.6	1.3 ± 1.3	2 ± 2.0	5.3 ± 3.5
Sipunculidae Rafinesque, 1814						
<i>Sipunculus</i> (<i>Sipunculus</i>) <i>nudus</i> <i>Linnaeus, 1766</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7
Sipunculidae Rafinesque, 1814	0.3 ± 0.3	0 ± 0	1.5 ± 0.1	0 ± 0	0 ± 0	0 ± 0
ARTHROPODA						
Copepoda Milne						
Edwards, 1840						
Calanoida Sars G.O., 1903	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Malacostraca						
Latreille, 1802						
<i>Ampelisca</i> sp. Krøyer, 1842	0.5 ± 0.5	5 ± 1.9	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Ampelisca brevicornis</i> (<i>A. Costa, 1853</i>)	0 ± 0	0 ± 0	0 ± 0	9.3 ± 3.5	5.3 ± 3.3	4.7 ± 3.7
<i>Ampelisca diadema</i> (<i>A. Costa, 1853</i>)	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Apseudopsis</i> <i>mediterraneus</i> (<i>Bacescu, 1961</i>)	1 ± 1.0	3 ± 1.0	2.5 ± 1.5	2 ± 2.0	1.3 ± 1.3	2 ± 1.2
<i>Caprella</i> sp. Lamarck, 1801	0.5 ± 0.5	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0

<i>Corystes cassivelaunus</i> (Pennant, 1777)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7
<i>Diastylis cornuta</i> (Boeck, 1864)	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	1.3 ± 0.7
<i>Diastylis</i> sp. Say, 1818	0.5 ± 0.5	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Ebalia cranchii</i> Leach, 1817	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gammaridae Latreille, 1802	3 ± 2.4	1 ± 1.0	0.5 ± 0.5	0 ± 0	1.3 ± 1.3	0 ± 0
<i>Iphinoe serrata</i> Norman, 1867	0 ± 0	0 ± 0	3 ± 3.0	0 ± 0	0 ± 0	0 ± 0
Isopoda Latreille, 1816	0 ± 0	2 ± 1.2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Leucothoe incisa</i> Robertson, 1892	0 ± 0	0 ± 0	0 ± 0	2 ± 1.2	0 ± 0	0.7 ± 0.7
<i>Leucothoe</i> sp. Leach, 1814	0 ± 0	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Microprotopus maculatus</i> Norman, 1867	0 ± 0	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Natatolana borealis</i> (Liljeborg, 1851)	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0.7 ± 0.7
<i>Paradexamine</i> sp. Stebbing, 1899	0 ± 0	0 ± 0	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0
<i>Photis longicaudata</i> (Spence Bate & Westwood, 1862)	0 ± 0	0 ± 0	0 ± 0	10.7 ± 7.1	0.7 ± 0.7	1.3 ± 1.3
Phoxocephalidae G.O. Sars, 1891	0 ± 0	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Platysquilla eusebi</i> (Risso, 1816)	0.5 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7
<i>Pleonexes</i> sp. Spence Bate, 1857	0 ± 0	0 ± 0	0 ± 0	2.7 ± 2.7	2.7 ± 2.7	0 ± 0
Ostracoda Latreille, 1802						
Ostracoda Latreille, 1802	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0.7 ± 0.7
ECHINODERMATA						
Asteroidea de Blainville, 1830						
<i>Astropecten spinulosus</i> (Philippi, 1837)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3
Echinoidea Schumacher, 1817						
<i>Ova canalifera</i> (Lamarck, 1816)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.7 ± 0.9	1 ± 0.6

<i>Naticarius hebraeus</i> (Martyn, 1786)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
PRIAPULIDA THÉEL, 1906 Priapulida Théel, 1906	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0

APPENDIX 3

Appendix 3 List of taxa present in the sediment samples collected during sampling campaigns conducted in years 2024 and 2025 at the stations 1, 2, and 3 of Site 3 – LNG Terminal. The values are expressed as the average of the number of individuals per m2 ± standard error

Taxa	2024			2025		
	St. 3-1	St. 3-2	St. 3-3	St. 3-1	St. 3-2	St. 3-3
CNIDARIA Hexacorallia Haeckel, 1896 <i>Peachia hastata</i> Gosse, 1855	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0
ANNELIDA Polychaeta Grube, 1850 <i>Aonides oxycephala</i> (Sars, 1862)	0 ± 0	0 ± 0	0 ± 0	17.3 ± 9.8	11.3 ± 10.3	2.7 ± 2.7
<i>Aricidea (Strelzovia) suecica</i> Eliason, 1920	0.8 ± 0.5	3.5 ± 2.1	6 ± 2.4	3.3 ± 3.3	0.7 ± 0.7	0 ± 0
<i>Capitellidae</i> Grube, 1862	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Ceratocephale</i> sp. Malmgren, 1867	0 ± 0	0 ± 0	2 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Cirratulidae</i> Ryckholt, 1851	0 ± 0	0.5 ± 0.5	0 ± 0	2 ± 0.0	0.7 ± 0.7	0 ± 0
<i>Cossuridae</i> Day, 1963	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Dodecaceria</i> Örsted, 1843	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Dysponetus</i> sp. Levinsen, 1879	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0
<i>Glycera</i> sp. Lamarck, 1818	0.3 ± 0.3	2.3 ± 1.0	4.3 ± 1.3	2 ± 0.0	0.7 ± 0.7	2.7 ± 2.7
<i>Hesionidae</i> Grube, 1850	0.5 ± 0.5	1.5 ± 1.0	2.5 ± 2.5	0 ± 0	0 ± 0	0 ± 0
<i>Inermonephtys inermis</i> (Ehlers, 1887)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
<i>Lumbrineridae</i> Schmarda, 1861	1 ± 0.6	2.5 ± 1.9	2.5 ± 1.3	0 ± 0	0 ± 0	0 ± 0
<i>Lumbrineris</i> sp. Blainville, 1828	0 ± 0	0 ± 0	1 ± 1.0	2.7 ± 0.7	1.3 ± 0.7	0.3 ± 0.3
<i>Magelona filiformis</i> Wilson, 1959	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0

<i>Magelona minuta</i> <i>Eliason, 1962</i>	0 ± 0	0 ± 0	0 ± 0	2.7 ± 1.3	2.7 ± 1.8	2.7 ± 2.7
<i>Magelona sp. F.</i> <i>Müller, 1858</i>	1.3 ± 1.3	2.5 ± 2.5	1 ± 1.0	0 ± 0	0.7 ± 0.7	0 ± 0
Maldanidae Malmgren, 1867	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
<i>Mysta sp. Malmgren,</i> <i>1865</i>	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Nephtys sp. Cuvier,</i> <i>1817</i>	0.5 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Onuphis eremita</i> <i>Audouin & Milne</i> <i>Edwards, 1833</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0
<i>Onuphis sp. Audouin</i> <i>& Milne Edwards,</i> <i>1833</i>	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Orbinia sertulata</i> <i>(Savigny, 1822)</i>	0.8 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Orbinia sp.</i> <i>Quatrefages, 1866</i>	0 ± 0	0 ± 0	0 ± 0	2.7 ± 2.7	0.7 ± 0.7	0 ± 0
Orbiniidae Hartman, 1942	0 ± 0	0 ± 0	0 ± 0	3.3 ± 3.3	0 ± 0	0 ± 0
<i>Owenia fusiformis</i> <i>Delle Chiaje, 1844</i>	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	1.3 ± 1.3	0 ± 0
Paraonidae Cerruti, 1909	2.8 ± 1,3	2.5 ± 1.9	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Paraonis sp. Grube,</i> <i>1873</i>	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
Parergodrilidae Reisinger, 1925	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
<i>Paucibranchia belli</i> <i>(Audouin & Milne</i> <i>Edwards, 1833)</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.3
<i>Phyllodoce sp.</i> <i>Lamarck, 1818</i>	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Podarkeopsis sp.</i> <i>Laubier, 1961</i>	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0.7 ± 0.7	0 ± 0
<i>Prionospio caspersi</i> <i>Laubier, 1962</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	3.3 ± 3.3	0 ± 0
<i>Prionospio cirrifer</i> <i>Wirén, 1883</i>	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	1.3 ± 1.3	0 ± 0
<i>Prionospio fallax</i> <i>Söderström, 1920</i>	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3	0 ± 0	0 ± 0
<i>Prionospio sp.</i> <i>Malmgren, 1867</i>	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Questinae Hartman, 1966	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Sigambra sp. Müller,</i> <i>1858</i>	0.5 ± 0.3	1 ± 0.6	2.5 ± 0.5	0.7 ± 0.7	0.7 ± 0.7	0 ± 0

Spionidae Grube, 1850	1 ± 0.4	2 ± 1.2	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Spiophanes bombyx</i> (Claparède, 1870)	0.3 ± 0.3	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
<i>Spiophanes sp.</i> Grube, 1860	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Sipunculidae Rafinesque, 1814						
<i>Sipunculus</i> (Sipunculus) nudus Linnaeus, 1766	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
Sipunculidae Rafinesque, 1814	0.3 ± 0.3	1 ± 1.0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
ARTHROPODA						
Copepoda Milne Edwards, 1840						
Calanoida Sars G.O., 1903	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	1.3 ± 1.3
Malacostraca Latreille, 1802						
<i>Ampelisca sp.</i> Krøyer, 1842	0.8 ± 0.5	0 ± 0	2.5 ± 1.5	0 ± 0	0 ± 0	0 ± 0
<i>Ampelisca brevicornis</i> (A. Costa, 1853)	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0.7 ± 0.7	0 ± 0
Bathynellacea Chappuis, 1915	0 ± 0	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Caprella sp.</i> Lamarck, 1801	0 ± 0	0 ± 0	1 ± 1.0	0 ± 0	0 ± 0	0 ± 0
<i>Diastylis sp.</i> Say, 1818	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Gammaridae Latreille, 1802	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
Isaeidae Dana, 1852	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Leptocheirus pilosus</i> Zaddach, 1844	2.5 ± 1.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Leptocheirus sp.</i> Zaddach, 1844	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Metaphoxus simplex</i> (Spence Bate, 1858)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
<i>Microprotopus maculatus</i> Norman, 1867	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
<i>Paralacydonia paradoxa</i> Fauvel, 1913				0.7 ± 0.7	0 ± 0	0 ± 0
<i>Pisidia bluteli</i> (Risso, 1816)	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0
ECHINODERMATA						
Echinoidea Schumacher, 1817						

<i>Ova canalifera</i> (Lamarck, 1816)	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0.7 ± 0.3
Holothuroidea de Blainville, 1834						
<i>Leptopentacta grisea</i> Clark, 1938	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0
Ophiuroidea Gray, 1840						
<i>Amphiura chiajei</i> Forbes, 1843	2.75 ± 0.9	8.8 ± 2.0	16.5 ± 11.4	0 ± 0	0 ± 0	2.7 ± 2.7
<i>Ophiothrix fragilis</i> (Abildgaard in O.F. Müller, 1789)	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	1 ± 0.6	0 ± 0	0 ± 0
<i>Ophiura ophiura</i> (Linnaeus, 1758)	1 ± 0.3	0.8 ± 0.8	0 ± 0	2.3 ± 1.5	5.3 ± 3.5	9.3 ± 5.3
MOLLUSCA						
Bivalvia Linnaeus, 1758						
<i>Donax trunculus</i> Linnaeus, 1758	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
<i>Flexopecten glaber</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0
<i>Lembulus pella</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
<i>Lentidium mediterraneum</i> (O. G. Costa, 1830)	0.3 ± 0.3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
<i>Macomangulus tenuis</i> (da Costa, 1778)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0
<i>Nucula nitidosa</i> Winckworth, 1930	0 ± 0	0.5 ± 0.5	2 ± 2.0	0 ± 0	0 ± 0	0 ± 0
<i>Peronaea planata</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.3 ± 1.3
<i>Pharus legumen</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	0.7 ± 0.7	0 ± 0	0 ± 0
<i>Talochlamys multistriata</i> (Poli, 1795)	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	0 ± 0
<i>Tellina sp.</i> Linnaeus, 1758	0 ± 0	0 ± 0	0.5 ± 0.5	0 ± 0	0 ± 0	1.3 ± 1.3
<i>Varicorbula gibba</i> (Olivi, 1792)	0 ± 0	0 ± 0	0.5 ± 0.5	0.7 ± 0.7	1.3 ± 1.3	0 ± 0
Gastropoda Cuvier, 1795						
<i>Hexaplex trunculus</i> (Linnaeus, 1758)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.3 ± 0.3
NEMATODA						
DIESING, 1861						
Nematoda Diesing, 1861	0 ± 0	0 ± 0	0 ± 0	6.7 ± 3.5	3.3 ± 2.4	1.3 ± 1.3

