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PROTECTING, RESTORING AND VALORISING NATURAL HERITAGE

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ARTEMIS

Accelerating the Restoration of Seagrass Meadows in the
Mediterranean area through Innovative ecosystem-service based
Solutions

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PILOTS ECOSYSTEM SERVICES BASELINE

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Deliverable 1.2.1

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1 Introduction

Seagrasses are among the most valuable coastal ecosystems worldwide, providing a wide range of ecological and societal benefits (do Amaral Camara Lima et al., 2023). Their global extent, however, remains uncertain, with estimates ranging from 277,000 km² based on known distribution (McKenzie et al., 2020) to 917,169 km² based on modelled potential habitat (Gouvêa et al., 2024). As ecosystem engineers, seagrasses play a fundamental role in shaping coastal environments by forming structurally complex, three-dimensional habitats that sustain high levels of biodiversity. They support marine communities of ecological, recreational, and commercial importance by providing food, shelter, and nursery (Bertelli & Unsworth, 2014; Jr et al., 2003; Unsworth et al., 2022). Beyond biodiversity support, seagrasses are considered highly efficient long-term carbon sinks. Despite occupying less than 0.2 % of the seafloor, seagrass meadows may account for up to 18 % of the total carbon burial in the ocean, significantly contributing to climate change mitigation and adaptation storage (Duarte et al., 2013; Fourqurean et al., 2012; Macreadie et al., 2021). In addition, seagrasses provide a number of other ecosystem services, including sediment stabilization, nutrient cycling, water quality regulation and coastal protection (Mtwana Nordlund et al., 2016). The global economic value of these services has been estimated at approximately US\$6.4 trillion annually (Costanza et al., 2014).

Despite their ecological and societal importance, seagrass meadows are declining worldwide due to multiple anthropogenic pressures, including pollution, coastal development, mechanical damage (e.g. anchoring, dredging, and trawling), and climate change (Grech et al., 2011; Orth et al., 2006). Although targeted management actions have resulted in some localized recoveries, overall losses continue to outweigh gains, resulting in a net decline of 19.1% in surveyed global seagrass area since 1880 (Dunic et al., 2021), alongside an annual loss rate of approximately 1.5% in recent decades (since 1990) (Waycott et al., 2009). The degradation and eventual loss of seagrass meadows compromises both their ecological functions and associated ecosystem services (Unsworth et al., 2022). Habitat fragmentation and increased patchiness reduce structural complexity, exposing organisms to higher predation risk and lowering overall survival rates (Van Der Heide et al., 2012). High fragmentation in seagrasses has also been linked to increased evenness and shift in species composition (Riera et al., 2025). Reduced shoot density and cover have been significantly associated with lower Shannon Wiener Diversity (H') and species richness (McCloskey & Unsworth, 2015). While complete cover loss has also led to a shift in fish assemblage, including reduced number of species, density and biomass (Pihl et al., 2006). Likewise, seagrass degradation can reduce their ability to capture and sequester carbon (Duarte et al., 2013) and trigger the release of previously stored carbon (Lovelock et al., 2017). Once destabilized, the carbon in the sediment may be eroded, resuspended, and remineralized, potentially transforming these ecosystems from long-term carbon sinks to sources of greenhouse gases emissions (Dahl et al., 2023; Marbà et al., 2015; Serrano et al., 2016; Stankovic et al., 2021). The economic consequences of the loss or degradation of seagrass carbon stocks alone are projected to have an estimated societal cost of approximately \$213 billion (2020 US dollars) (Krause et al., 2025).

Long overlooked due to their low visual appeal and the limited public awareness compared to other habitats (Duarte et al., 2008), seagrass ecosystems are now increasingly recognized as nature-based solutions for their potential to simultaneously address the intertwined biodiversity and climate crises (Duarte et al., 2025; Fu et al., 2024). While mitigating pressures and protecting seagrass meadows is a priority to avoid further loss and set the conditions for seagrass recovery (Duarte et al., 2020), active restoration is now widely considered necessary to help accelerate natural recovery and to maintain or enhance the ecosystem services provided by these habitats (Gräfnings et al., 2024; Orth et al., 2020; Thorhaug et al., 2017; Unsworth et al., 2025). Restoration initiatives have increased globally, as reflected by the United Nations General Assembly which proclaimed 2020 - 2030 the United Nations Decade on Ecosystem Restoration. This initiative seeks to facilitate global cooperation to prevent, halt and reverse the degradation of ecosystems all around the world. By building political momentum for restoration, they positioned restoration as a major nature-based solution towards meeting a wide range of global goals and priorities as those set by the Paris Agreement (2015) under the United Nations Framework Convention on Climate Change (UNFCCC) and the more recent Kunming–Montreal Global Biodiversity Framework (KM-GBF). In Europe, this momentum is reflected in the recently approved EU Nature Restoration Regulation (NRR), which, together with the EU Biodiversity Strategy for 2030 and the EU Climate Law, further strengthens Europe’s commitment to protect, restore and monitor degraded ecosystems across Member States, including marine habitats such as seagrass meadows.

Among European seagrass, the iconic *Posidonia oceanica*, endemic to the Mediterranean, stands out as the species with the highest capacity for carbon storage among seagrass species worldwide (Kennedy et al., 2022) and provide more substantial and a wider variety of ecosystem services compared to other species due to its large size (Mtwana Nordlund et al., 2016). Despite being listed as a priority habitat under Annex I of the Habitat Directive (92/43/EEC) and in Annex II of the NRR, *P. oceanica* meadows are under threat (Marbà et al., 2014) rendering their conservation and restoration necessary to avoid emissions from stored carbon and to enhance future carbon sequestration potential (Dahl et al., 2023).

The overall aim of restoration is not only to restore the habitat but to restore the ecosystem functions and services it provides. Achieving this requires robust baseline assessments to quantify initial ecological conditions against which restoration outcomes can be measured. For BC, this requires *in situ* measurements, since global or national estimates of BC, while valuable for policy development and GHG inventories, can introduce substantial uncertainties when applied to local-scale assessments (Dahl et al., 2025) as BC stocks and accumulation rates vary spatially and temporally, and across species and hydrodynamic energy (Mazarrasa et al., 2021; Novak et al., 2020). Quantifying these parameters allows for the estimation of additionality under different management scenarios by comparing restored conditions with their own pre-restoration baseline (Figure 1.1).

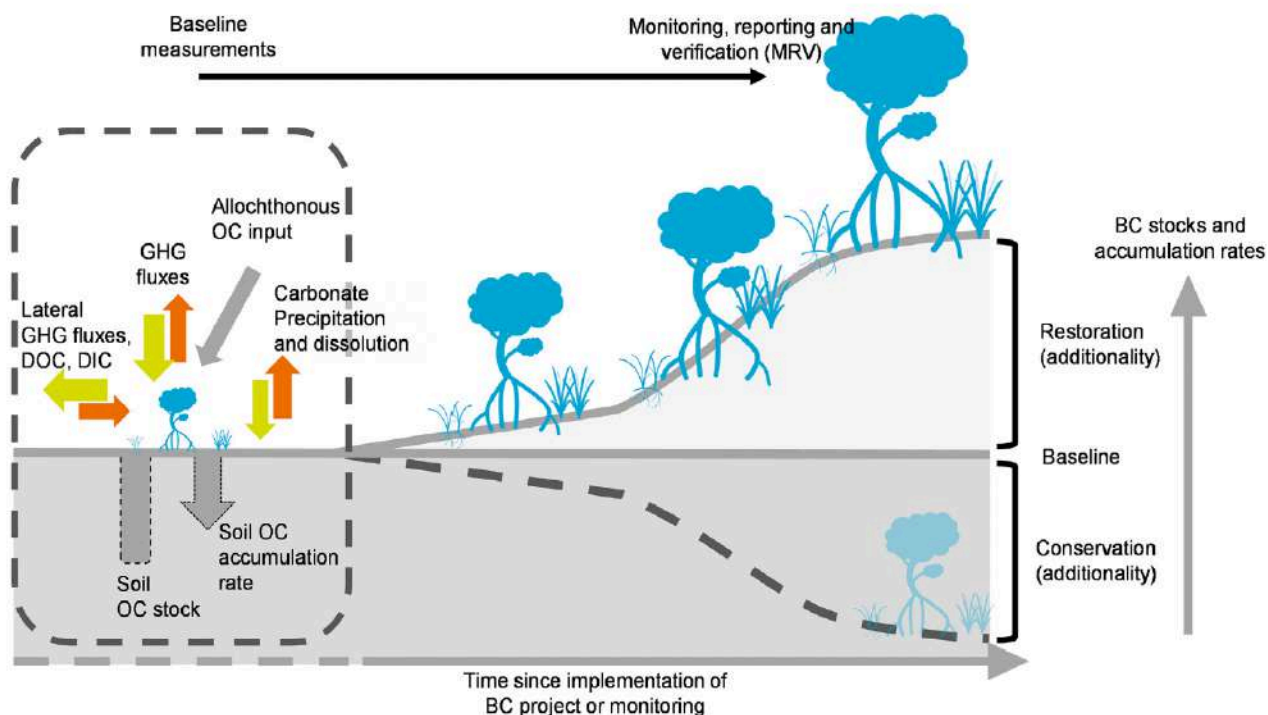


Figure 1.1 Conceptual figure of blue carbon (BC) baseline measurements and pathways for assessing climate benefits from conservations and restoration activities. BC baseline measurements including the quantification of BC stocks and accumulation rates, OC provenance (allochthonous and autochthonous carbon sources), and carbonate precipitation and dissolution processes as well as additional measurement of GHG and lateral fluxes of dissolved organic and inorganic carbon (DOC and DIC) for a comprehensive assessment of the net CO₂ sink capacity of BC habitats. The baseline BC assessments (dashed box) can be quantified within project boundaries for estimating additionality scenarios following different management activities or to estimate BC stocks and/or accumulation rates (either through avoided emissions or enhanced sequestration). From Dahl et al., 2025.

Seagrass-associated biodiversity—especially diverse grazer, macroalgal, and sessile-invertebrate assemblages—enhances seagrass functioning by improving epiphyte control, boosting secondary production, and increasing habitat complexity (Fig. 1.2). Diverse grazers also resist predation more effectively, stabilizing seagrass dominance. Cross-habitat species linkages further supply nutrients and consumers, reinforcing productivity and resilience in seagrass ecosystems (Duffy, 2006). For biodiversity, baselines should assess ichthyofauna, leaf epibionts, epibenthic macrofauna and macroalgae, as well as sediment endofauna, using classical indices of richness and diversity.

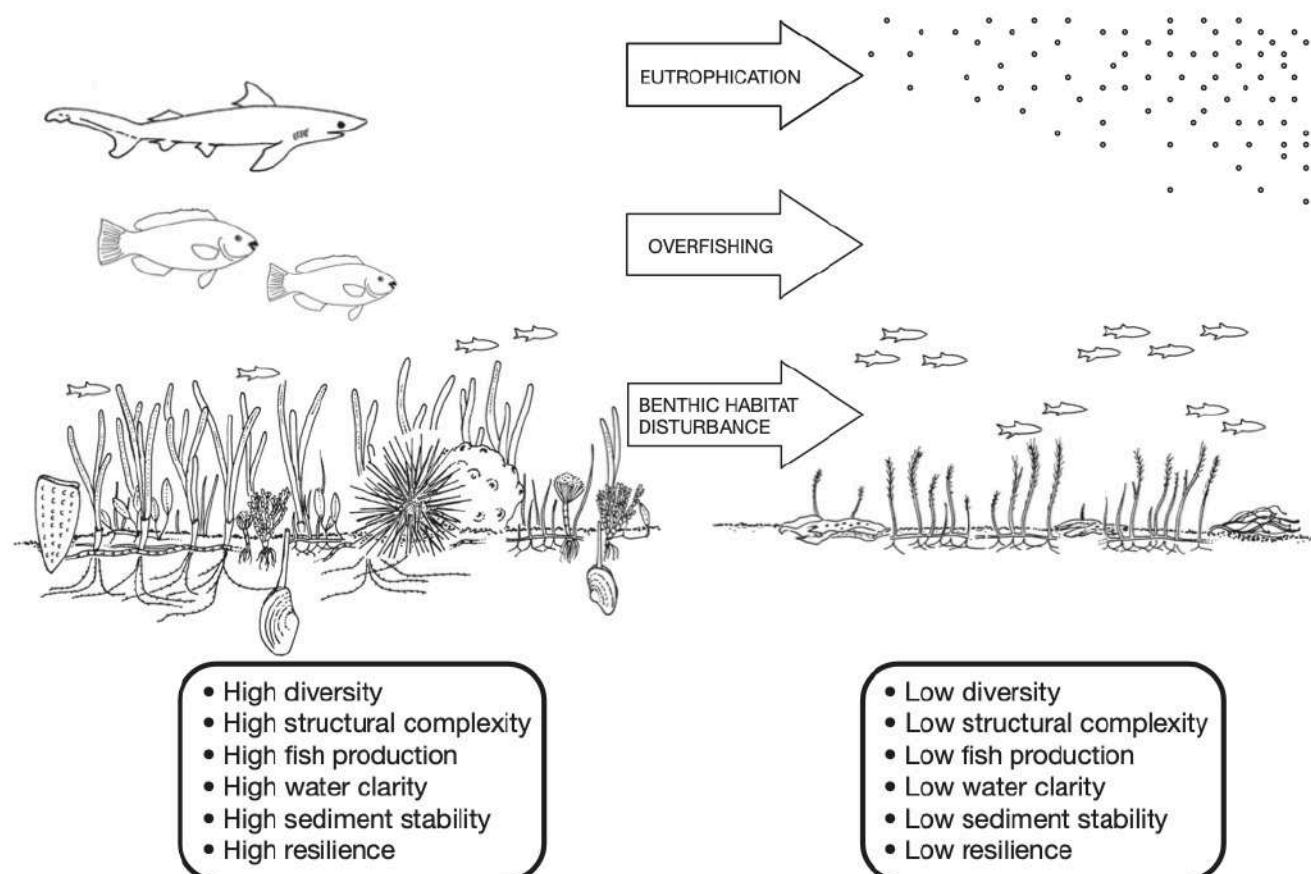


Figure 1.2. Schematic illustration of shifts in seagrass habitat structure and ecosystem functioning resulting from major human-induced pressures—including eutrophication, overfishing, and seabed disturbance—and how these stresses collectively reduce biodiversity, simplify habitat architecture, and weaken key ecological processes (from Duffy, 2026).

To this end, the **ARTEMIS** project “Accelerating the restoration of seagrass meadows in the Mediterranean area through innovative ecosystem service-based solutions”, conducts research in **four transnational pilot sites**, covering different restoration stages and policy contexts. Within Work Package 1 (WP1): “Seagrass restoration pilots for ecosystem service (ES) enhancement”, Action 1.2: “Establish an ecosystem service baseline per pilot”, Deliverable 1.2.1 aims to provide a baseline assessment of the extent, conditions and key ecosystem services at each pilot site. From the broad range of ecosystem services, the ARTEMIS consortium focused on two in particular: **blue carbon** and **biodiversity**.

The four pilot sites are:

- Atzikiary bay, Crete (Greece)
- Ciutadella, Menorca (Spain),
- Capo Testa-Punta Falcone, Sardinia (Italy)
- Panzano Bay, Gulf of Trieste (Italy)

These sites are located within distinct environmental regimes. The Western Mediterranean Sea (WMS) is generally colder than both the Eastern Mediterranean Sea (EMS) and the Adriatic Sea (AS) (Pisano et al., 2020). The AS is further characterized by significant freshwater inflow, resulting in

lower salinity compared to the rest of the basin (Vodopivec et al., 2022). The EMS is more oligotrophic than the WMS (Powley et al., 2018).

The sites mainly host *P. oceanica* meadows, with the exception of Monfalcone (Panzano Bay), where three other smaller seagrass species dominate. In terms of ecological traits, *P. oceanica* meadows in the WMS generally exhibit higher primary production compared to those in the EMS (Litsi-Mizan 2023, PhD thesis). Mean shoot length tends to be higher in the EMS than in the WMS (Gerakaris et al., 2021).

The type of anthropogenic impact affecting the ecosystem also differs across sites. In Atzikiary bay, a fish farm caused extensive *P. oceanica* loss. In Ciutadella, construction of a water collector for the desalination plant, together with an incidental bentonite spill during construction, caused direct losses of *P. oceanica*. In Capo Testa-Punta Falcone, unregulated boat anchoring has caused significant fragmentation of *P. oceanica* meadows. In Panzano Bay pressures affecting seagrasses include anchoring and fishing activities. Finally, the level of protection also varies across sites. Atzikiary bay, although lies in a Natura 2000 site, it has not received management measures yet, Menorca Island was declared a UNESCO Biosphere Reserve in 1993, Capo Testa-Punta Falcone is located inside a Marine Protected Area (MPA), and Panzano Bay contains two Natura 2000 sites.



2 General Methodology

At the pilot sites of Crete, Menorca and Sardinia, field assessments were carried out, while at Panzano Bay, where no active restoration is planned during the project, a bibliographic review was conducted to gather existing ecological and functional information on the seagrass species present in the bay, *Cymodocea nodosa*, *Zostera noltei* and *Ruppia spp*, providing a foundation for future restoration initiatives.

The overall assessment involved separating each pilot site into two areas: the impacted one and the control. The impacted area included stations with different seagrass cover, ranging from complete loss of seagrass to sparse patches. Standard methodologies were applied across all sites to assess the seagrass extent and conditions and the key ecosystem services targeted by ARTEMIS. Ecological status of the meadow was assessed with the national, intercalibrated indices applied in the corresponding country for each partner, i.e. the “Posidonia oceanica Rapid Easy Index” (PREI) for Italy (Gobert et al., 2009) and “Weighted POSidonia index” (WePOSI) for Greece (Gerakaris et al., 2021). The status of the ecosystem was evaluated using an ecosystem-oriented status assessment based on the Ecosystem-Based Quality Index (EBQI). Biodiversity was assessed by scientific divers following standard methodologies for biodiversity data collection in seagrass systems (Buia et al., 2004), utilising transects, quadrats and core sampling. This included the assessment of associated faunal and algal groups (fish, macroinvertebrates and macroalgae) - and the production of a comprehensive species list with their relevant abundances. Carbon storage was estimated following the standard Blue Carbon methodologies outlined in *Coastal Blue Carbon: methods for assessing carbon stocks and emission factors in mangroves, tidal salt marshes, and seagrass meadows* (Howard et al., 2014). This included the assessment of vegetative carbon stocks, and sedimentary carbon stocks and accumulation rates.

3 Atzikiari Bay, Crete

3.1 Study Area

The study was conducted in Atzikiari Bay, located in Eastern Crete, Greece. The bay is within a NATURA 2000 site (GR4320006). The area previously hosted a fish farm that operated for 21 years (1996 to 2017) (Figure 3.1), producing over 1,000 tons of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) (Pitta et al., 2009). Over the years, multiple studies and environmental assessments have been carried out in the area, including 2007 and 2008 while the fish farm was still operational, and in 2019 and 2021, approximately two and six years after its cessation, respectively (Dounas & Psochiou, 2008; Dounas et al., 2019; Koulouri et al., 2023).



Figure 3.1 Top view of the aquaculture facility in Atzikiari bay in 2003.

The first environmental assessment, carried out in 2008 by HCMR (Dounas & Psochious, 2008), concluded that the bay was heavily polluted with organic matter, primarily originating from the aquaculture facility, leading to extensive anoxic zones on the seabed. The area beneath and around the cages was severely degraded and covered by chemotrophic bacterial mats. Twelve years after farm installation, the previously dense and widespread *P. oceanica* meadows, were highly degraded or absent, particularly near the cages.

The second assessment, carried out in 2019 by HCMR (Dounas et al., 2019), two years after the removal of the ~10 ha of aquaculture facilities, indicated that a large portion of the seabed remained severely degraded. Total organic carbon concentration in surface sediments was 11.9 mg/g, 3 times lower than in October 2008 (32.2 mg g⁻¹), yet 7 times higher than a reference site

selected by the study (Elounda, North Crete). Extensive areas of the seabed were covered by bacterial mats, devoid of macrofauna. In some areas these mats coexisted with dense meadows of the non-indigenous seagrass, *Halophila stipulacea*. Across nearly the entire bay, *P. oceanica* was almost completely lost, leaving dead *P. oceanica* matte. Solid wastes were still present on the seabed contributing to the persistence of anoxic and azoic conditions.

The third and most recent assessment was conducted in 2023 by HCMR (Koulouri et al., 2023), 3.5 years after the last study in 2019 and two years after the cessation of the fish farm activities. Results show that concentration of organic matter, nutrients, chlorophyll pigments, dissolved oxygen, and associated hydrological parameters in the water column have returned to normal seasonal levels, comparable to those observed in other coastal areas of northern Crete. Sediment organic carbon concentrations decreased 2-fold since 2019, indicating recovery of superficial sediments, though levels remained 3 times higher than those measured at the reference site (Elounda, North Crete), reflecting an intermediate state of organic pollution (Hyland et al., 2005). Bacterial mats that had covered large parts of the seabed in 2019 had disappeared, while *H. stipulacea* continued to expand across the bay. *P. oceanica*, although showing signs of recovery in the more exposed parts of the bay, has not recolonized the areas near the former fish farm cages, where only dead *P. oceanica* matte remained.

3.2 Methods

3.2.1 Seagrass extent and condition

In order to determine the current extent of the meadow, and the magnitude of seagrass damage and loss due to the fish farming activities at Atzikiari bay, habitat mapping was carried out in April 2024 using both aerial (Fig. 3.2A) and underwater drones (Fig. 3.2B) and applying modern image analysis techniques (photogrammetry) and spatial classification. Drone flights were conducted at low altitude under optimal flight conditions. During the aerial surveys, high-resolution vertical images (aerial photographs) were captured (Fig. 3.3A), which, after appropriate processing and composition, were used to generate a precise orthophotomosaic of the study area. Seabed identification and classification (habitat categories) were carried out within a Geographic Information System (GIS) environment. The accuracy of identification and classification of seabed characteristics for their correct representation in the final map was ensured through an appropriately designed network of underwater data collection (ground truthing) stations (Fig. 3.3B). At these locations, data collection was carried out using underwater drones and, where necessary, through SCUBA diving.

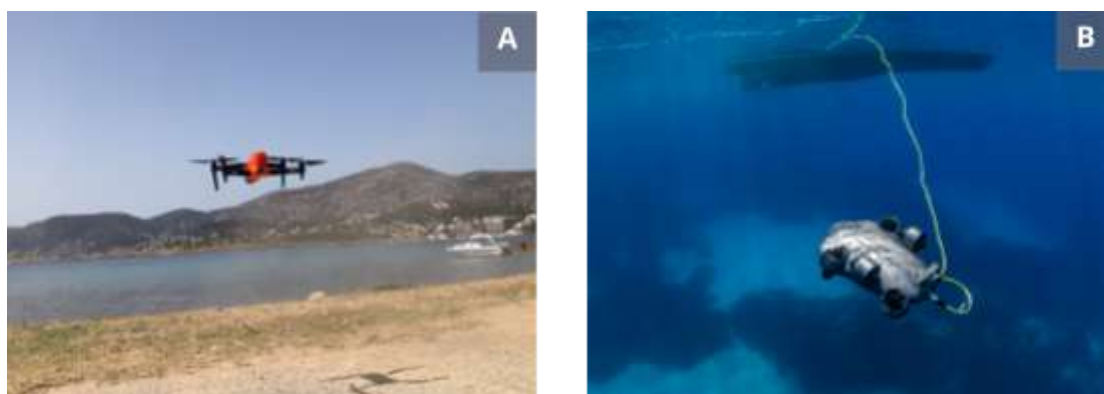


Figure 3.2 (A) The aerial drone used during the mapping. (B) The underwater drone used during the mapping.

As a final product, a high-resolution marine habitat map was generated (Fig. 3.4). This map served as the basis for designing the sampling strategy required to assess the ecological status of the *P. oceanica* meadow, as well as to select the stations for determining the key ecosystem services targeted by the project, namely biodiversity and blue carbon.

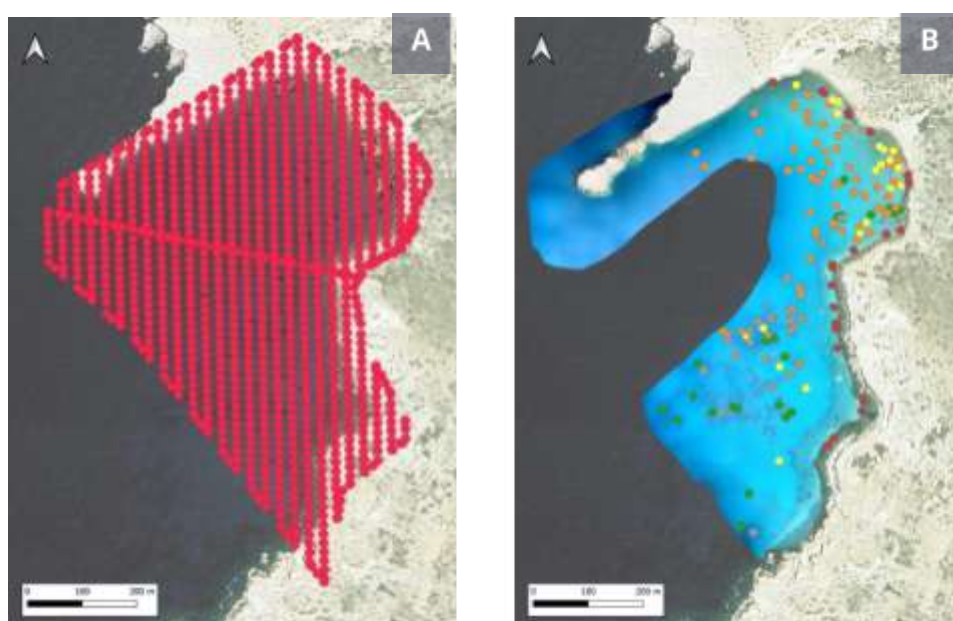


Figure 3.3 (A) Aerial photographs capture positions during drone flights (April 2024). (B) Network of ground-truthing validation stations using SCUBA diving and underwater drone, along with all reference points utilized for the production of the final map.

3.2.2 Sampling Strategy

To ensure a comprehensive representation of the bay, data were collected from multiple stations both within the impacted area and within the control, capturing different levels of seagrass cover. In the impacted area, stations included sites where *P. oceanica* had been completely lost and dead matte was colonized primarily by *H. stipulacea* but also *C. nodosa* and *Caulerpa* spp. (hereafter ST1), as well as sites with dead matte interspersed with small remnant patches of *P. oceanica* (hereafter ST2 and ST3). Within the control area, stations were selected to reflect varying levels of seagrass

density and canopy cover, ranging from areas where *P. oceanica* was intermixed with dead matte (hereafter ST4) to a continuous *P. oceanica* meadow (hereafter ST5) (Fig. 3.4). Sampling was conducted at depths of 6 to 15 m during the field campaigns in July and September 2024.

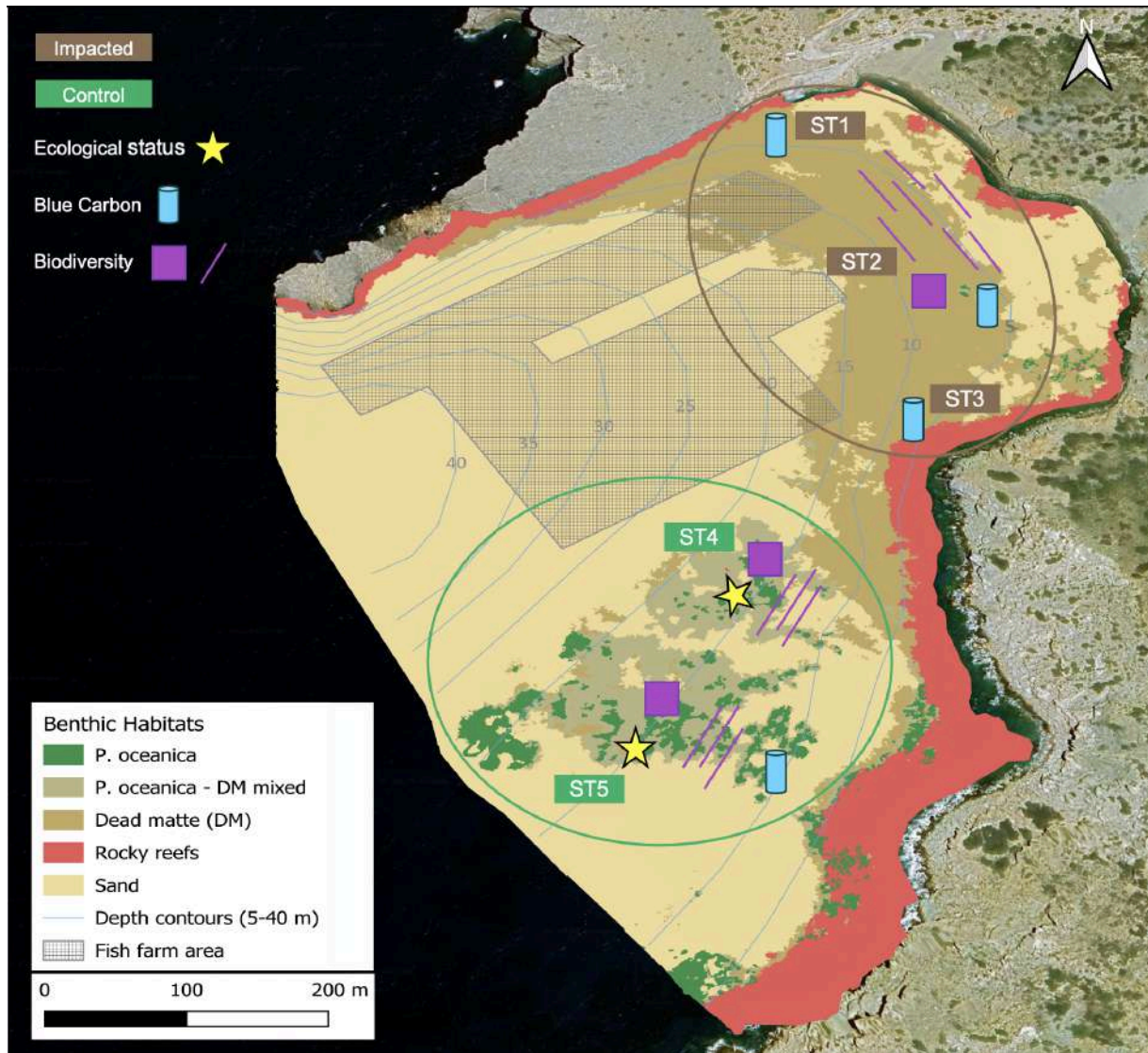


Figure 3.4 Sampling map including the impacted area and the control and the station numbers with the corresponding analysis.

3.2.3 Ecological Status

The ecological status assessment employed a multi-index approach, ensuring a robust and holistic evaluation of *P. oceanica* meadows by capturing both their structural integrity and functional dynamics, in line with European monitoring frameworks and advanced ecosystem-based assessment methodologies (Gerakaris et al., 2021; Montefalcone et al., 2007; Moreno et al., 2001; Personnic et al., 2014). Data collection was conducted through SCUBA diving and included collection of shoots, in situ measurements and observations along underwater transects.

3.2.3.1 WePOSI (Weighted POSidonia Index)

The primary index applied was the Weighted Posidonia Index (WePOSI) (Gerakaris et al., 2021), a calibrated biotic index officially used in Greece for the implementation of the of the WFD (2000/60/EC), the MSFD (2008/56/EC) and the HD (92/43/EU) Directives. The WePOSI index integrates several metrics by assigning different weights according to their biological level: population (meadow), individual (plant), and community (Table 3.1). Measurements were carried out at the 15m depth and at the deeper edge of the meadows (lower limit).

Table 3.1 Metrics used in the biotic index WePOSI.

Organization level	Metrics
Population (Meadow)	Lower Limit Depth (m)
	Lower Limit Type
	Meadow Cover (%)
	Dead matte Cover (%)
	Shoot Density (shoots m ⁻²)
	Plagiotropic rhizomes (%)
Individual (Plant)	Shoot Length (cm shoot ⁻¹)
Community	Epiphytic Biomass (g shoot ⁻¹)

For the application of the WePOSI index, reference values (RVs) for Greek coastal waters as proposed by Gerakaris et al. (2021) were employed. These RVs, tailored to the spatial distribution of the studied *P. oceanica* meadows, are presented in Table 3.2. In Fig. 3.5A and 3.5B, views of the underwater transects are presented.

Table 3.2. Reference values for the examined metrics according to the distribution of the *P. oceanica* meadows studied.

Metrics	Insular areas & continental areas with limited river outflows	
	"Best" values	"Worst" values
Lower Limit Depth (m)	41	24
Meadow Cover (%)	100	65
Dead Matte Cover (%)	5	100
Shoot Density (shoots m ⁻²)	750	150
Plagiotropic Rhizomes (%)	0	45
Shoot Length (cm shoot ⁻¹)	105	30
Epiphytic Biomass (g shoot ⁻¹)	0	0.5
Lower Limit Type	Progressive	Regressive

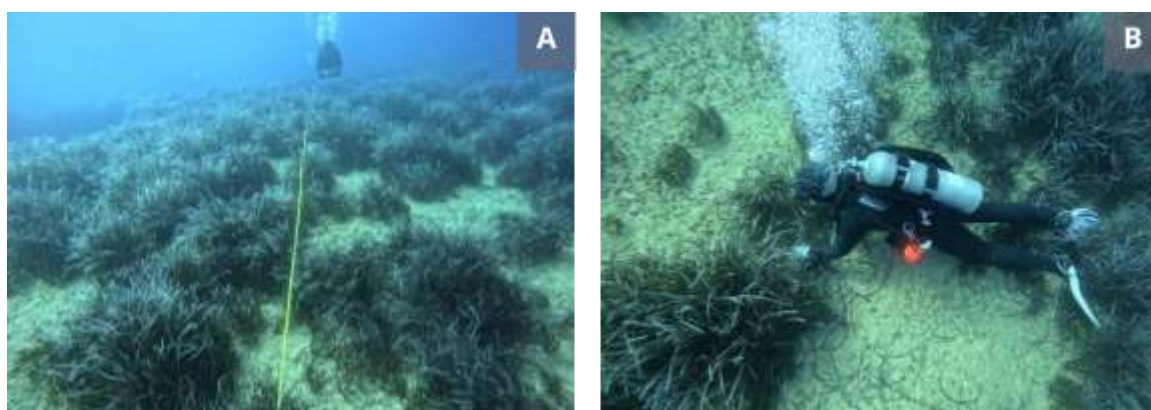


Figure 3.5 (A) Underwater transect for data collection using SCUBA diving. (B) Sampling station for measuring ecological status of the *P. oceanica* meadow.

3.2.3.2 Additional Ecological Indices (CI, SI, PSI)

In addition to WePOSI, three further ecological indices were applied: the Conservation Index (CI), the Substitute Index (SI), and the Phase Shift Index (PSI). They provide complementary insights, particularly regarding meadow conservation status, potential degradation processes, and community shifts (Montefalcone et al., 2007; Moreno et al., 2001). Details on the methodology, calculation procedures, and ecological status classification scales for all indices (WePOSI, CI, SI, PSI) are provided in Annex 1 and 2.

3.2.3.3 Ecosystem-Based Quality Index (EBQI)

To capture functional ecosystem-level dynamics, the Posidonia Ecosystem-Based Quality Index (EBQI) was applied following Personnic et al. (2014). This framework integrates multiple structural and functional components of the *P. oceanica* ecosystem, each assigned a relative weighting to reflect its ecological significance. The functional compartments and associated parameters used in the assessment are detailed in Table 3.3. Except for the seabirds, data for which were obtained

from (Stavros Xyrouchakis - NHMC, personal communication), all other parameters were studied *in situ*, in September 2024.

Table 3.3 Functional compartments of the *P. oceanica* ecosystem included in the EBQI framework, with their corresponding weightings and ecological parameters (adapted from Personnic et al., 2014).

Box n°	Functional compartment	Weighting	Parameter
1	Roots and rhizomes ('rhizomes')	3	Growth of orthotropic rhizomes (mm yr ⁻¹)
2	<i>P. oceanica</i> leaves ('leaves')	5	Density (shoots m ⁻²) / Cover (%)
3 - 4	MPOs, filter- and suspension-feeders leaf epibiota (leaf epibiota)	4	Biomass (g DM shoot ⁻¹)
5	Benthic filter-feeder: <i>Pinna nobilis</i> (bivalve) ('Pinna')	2	Density (individuals 100 m ⁻²)
6	Other benthic filter- and suspension-feeders (HOM LOM')	2	HOM / LOM
7	Litter detritus: dead leaves and broken rhizomes (litter')	2	Biomass (g DM m ⁻²)
8	Detritus-feeders 2 and 3 (<i>Holothuria</i> spp.) (' <i>Holothuria</i> ')	2	Density (individuals 10 m ⁻²)
9	Herbivores 1 (herbivores')	5	Density (<i>Paracentrotus lividus</i> m ⁻²) / Grazing index %
10	Predatory teleosts, cephalopods and seastars ('predators')	5	Biomass (kg 100 m ⁻²)
11	Piscivorous teleosts ('piscivores')	5	Biomass (kg 100 m ⁻²)
12	Planktivorous teleosts ('planktivores')	3	Biomass (kg 100 m ⁻²)
9 - 12	All teleosts ('SRDI')	3	Specific Relative Diversity Index (SRDI)
13	Sea birds *	1	Distance to the nearest nesting site (km)

3.2.4 Biodiversity

3.2.4.1 Collection of samples

The biodiversity assessment was conducted in multiple stations along the impacted area at ST2 and the control at ST4 and ST5. Data was collected using a combination of visual census, quadrat sampling, and core sampling to collect the biota associated with the seagrass. This involved the assessment of ichthyofauna densities with the use of 7 x 5 m wide strip transects with a duration of 5 min each covering an area of 100 m² inside the impacted area, 3 transects in low seagrass cover meadow and 3 transects in high seagrass cover meadow. Epibenthic macrofauna and macroalgae densities were assessed with the use of 7 x 5 m wide strip transects with a duration of 5 min each inside the impacted area, 7 x 0.16 m² (40 cm x 40 cm) quadrats in low seagrass cover and 7 x 1 m² (1m x 1 m) quadrats in high seagrass cover. Endofauna was assessed with the collection of 3 x 13 cm diameter cores in high seagrass cover and another 3 similar cores in the impacted area. Leaf epifauna in seagrass was assessed with the collection of shoots from the high

seagrass cover area. *P. oceanica* samples were fixed in 96 % alcohol and core samples were cleaned from *P. oceanica* rhizomes, sieved (500 µm mesh size) and fixed in 96 % alcohol.

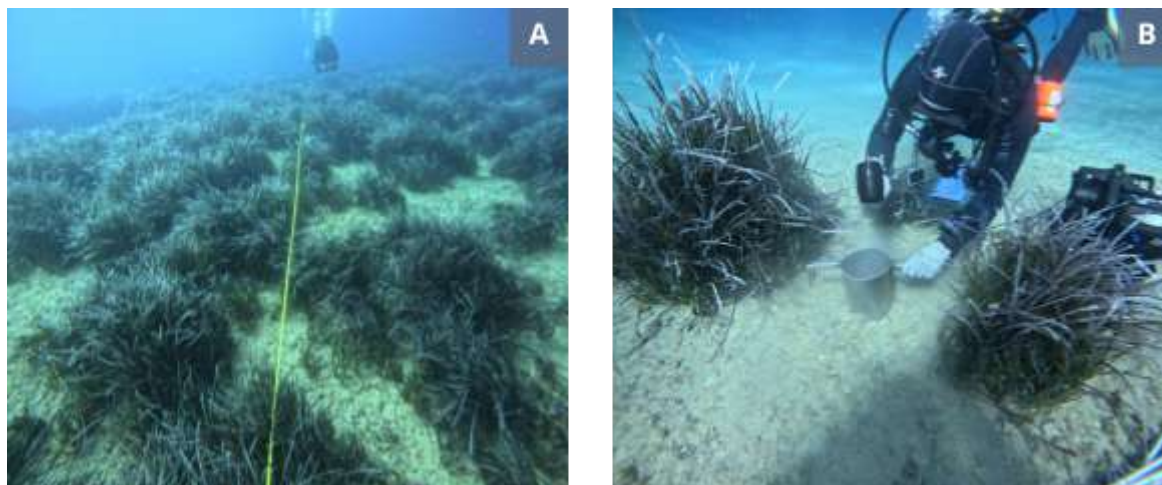


Figure 3.6 (A) Visual census over a transect. (B) Core sampling

3.2.4.2 Laboratory Procedures

Ichthyofauna data from strip transects were processed in order to produce species lists with relative abundances and fish biomass. Fish biomass was calculated with the use of the Bayesian length – weight equation where $\text{weight} = a \cdot (\text{length})^b$ (weight in gr, length in cm). The values of a and b were obtained from Fishbase and are unique for each species of fish. Invertebrate data from quadrats and strip transects (epibenthic macrofauna) were processed in order to produce species lists with relative abundances. Leaf epibionts, as well as sediment endofaunal samples were sorted and identified to the nearest taxonomic level possible. Densities of ichthyofauna were standardized to count 10m^{-2} and benthic macrofauna were standardized to count m^{-2} .

3.2.4.3 Numerical procedures

Abundances were statistically analysed with ANOSIM and MDS, in order to assess differences in biodiversity between the habitat conditions of Atzikiari bay. Ecological indexes (Shannon & Simpson), concerning ichthyofauna and epibenthic macrofauna, were statistically analyzed by ANOVA.

3.2.5 Blue Carbon

3.2.5.1 Collection of samples

Collection of samples was conducted at ST1, ST2 and ST3 along the impacted area and ST5 within the continuous *P. oceanica* meadow.

3.2.5.1.1 Water sample collection

Bottom water (5 liters) was collected in triplicates at each station using a Niskin bottle. The water was filtered using pre-combusted 25 mm glass microfiber filters (GF/C™) and stored at -20 °C until further analysis.

3.2.5.1.2 Seagrass and macroalgae collection

Seagrass density (shoot m⁻²) was measured in triplicate at ST2, ST3, and ST5 by counting the number of *P. oceanica* shoots within a 40 x 40 cm quadrant randomly tossed over the meadow. *P. oceanica* shoots and other macrophytes present at each station (i.e., *C. nodosa*, *H. stipulacea*, *Caulerpa* sp.) were collected by hand and kept frozen until further analysis. Seagrass litter was collected in triplicates at ST1 and ST2 using a 15 cm diameter corer and at ST5 using a frame of 20 x 20 cm.

3.2.5.1.3 Sediment core collection

At each station, sediment cores were collected in triplicate by SCUBA divers (Fig. 3.7) using manual percussion and rotation, with each replicate taken a few meters apart to assess spatial variability. Sampling was performed using PVC pipes (2 m in length, 7.5 cm in diameter). Sediment cores were inserted down to 1 m or deeper where possible. To account for sediment compaction during coring, the outer, inner, and total core length were measured *in situ* prior to extraction. Compaction ranged from 11 % to 35 %.



Figure 3.7 (A) Two divers retrieving sediment cores using manual percussion and rotation. (B) Retrieved sediment cores prepared to be transported to the surface.

3.2.5.2 Laboratory procedures

3.2.5.2.1 Suspended particulate organic matter (SPOM)

The glass microfiber filters (GF/C™) were oven dried at 60 °C until constant weight. The filters were weighed and acidified with 4 % HCl in silver capsules to expunge all carbonates. Organic carbon (C_{org}) and stable isotope of carbon (δ¹³C) determination were done using an isotope ratio mass spectrometer (Thermo IRMS Delta Plus XP) coupled with an elemental analyser (Thermo EA-1112).



Analytical uncertainty based on replicate measurements of internal standards (Acetanilide for C, International Atomic Energy Agency IAEA- CH-6 for $\delta^{13}\text{C}$) was 0.02 % and 0.1 ‰ for C and $\delta^{13}\text{C}$, respectively.

3.2.5.2.2 Seagrass and macroalgae

P. oceanica seagrass leaves were scraped with a blade to remove epiphytes. The seagrass shoots were then separated into leaves, rhizomes and roots. The *P. oceanica* compartments, seagrass litter, epiphytes, and other macrophytes were oven-dried at 60 °C until constant weight. An aliquot of each dried tissue was milled to a fine powder and used for the determination of carbon content and $\delta^{13}\text{C}$ as explained above.

3.2.5.2.3 Sediment

Sediment cores were either opened by cutting the PVC pipe longitudinally or extruded vertically (Fig. 3.8). The cores were sliced at 1-, 5- or 10 cm intervals. After slicing, a compression correction factor was applied to adjust the thickness of the sediment slices, accounting for compaction during coring. All subsequent analyses were conducted using the decompressed thickness values. All sediment samples were oven-dried at 60 °C until constant weight was reached. Selected sediment slices from each replicate were quartered to obtain subsamples for subsequent analyses.

The dried sediment was passed through a 1 mm mesh sieve, and the weight of both, > and < 1 mm fractions were recorded. The < 1 mm fraction was subsequently analyzed for grain size distribution using a laser-diffraction particle analyzer (Mastersizer 2000; Malvern Instruments Ltd., UK). Grain size fractions were classified as follows: very coarse sand and gravel (> 1 mm), coarse sand (> 0.5 – < 1 mm), medium sand (> 0.25 – < 0.5 mm), fine sand (> 0.063 – < 25 mm), and silt/clay (< 0.063 mm).

For the analysis of organic carbon (C_{org}) content and stable carbon isotopic composition ($\delta^{13}\text{C}$), sediment subsamples were milled into a fine powder. For the determination of organic carbon (C_{org}), the sample aliquot was acidified in silver capsules and analyzed using an isotope ratio mass spectrometer (Thermo IRMS Delta Plus XP), coupled to an elemental analyzer (Thermo EA-1112) according to Nieuwenhuize et al., (1994) to remove carbonates. For the determination of carbon stable isotope ($\delta^{13}\text{C}$), the sample aliquot was acidified with HCl 2N until the bubbling stopped to remove carbonates and centrifuged. The supernatant was removed by pipetting, and the sample was then washed with Milli-Q water, centrifuged, oven dried after supernatant removal and weighed in tin capsules.

To calculate short-term sediment (100 years) accumulation rates (SARs), the activity of ^{210}Pb was determined in selected subsamples from one replicate core per station for stations ST2, ST3 and ST5. The activity was measured via alpha spectrometry by quantifying its granddaughter isotope, polonium (^{210}Po). A known quantity of ^{209}Po was added to each previously milled sample as a tracer. Samples were then acid-digested, and Po isotopes were plated onto polished silver discs.

Emissions were measured over three days using a Canberra Alpha Spectrometry Quad System. The supported ^{210}Pb activity was considered to equal the values obtained at the bottom of the core (> 60 cm sediment depth). To estimate the long-term SARs (> 500 years) radiocarbon (^{14}C) dating was used. Seagrass sheaths from the lower sections of one replicate core per station were analyzed for ^{14}C -AMS radiocarbon at the Mini Carbon Dating System (MICADAS) at Yale University, following standard protocols (Stuiver & Polach, 1977). Sheaths were rinsed in ultrapure MQ water to remove fine sediment particles, inspected under a stereomicroscope for attached reworked materials, and dried at 60°C to a constant weight before radiocarbon dating. Resulting ages of ST2 were inconsistent, most likely due to sediment mixing, and are therefore considered unreliable.

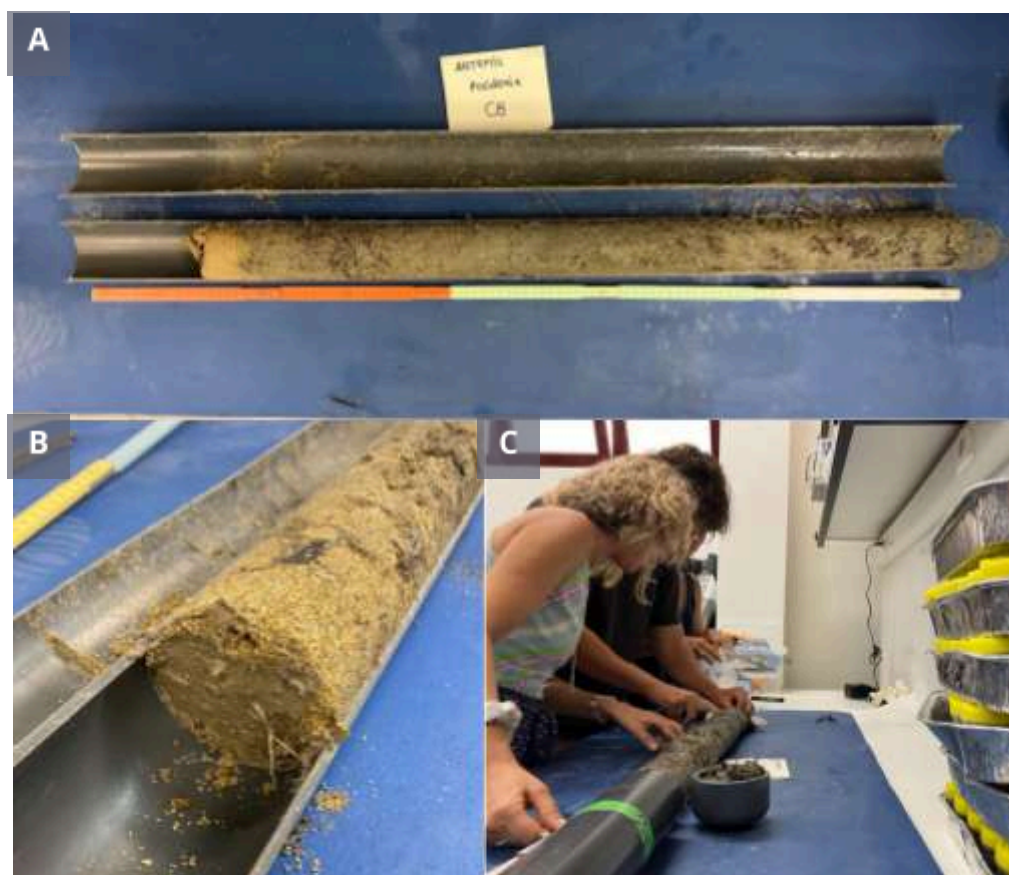


Figure 3.8 A) Longitudinally opened sediment core, B) Top view of a sediment core from ST1 and C) Eugenia T. Apostolaki, Pavlos Efthymidis and Catalina A. Garcia-Escudero slicing a sediment core in the laboratory.

3.2.5.3 Numerical Procedures

Dry bulk density (DBD, g cm^{-3}) for each sediment slice was calculated by dividing the dry weight by the volume of the corresponding wet sample. Living carbon biomass was calculated by multiplying the average dry weight of above- and below-ground biomass by shoot density (shoots m^{-2}) and by the measured carbon content of living tissues (% dw). Similarly, litter carbon biomass was estimated by multiplying the average dry weight biomass of seagrass litter and its corresponding carbon content. The vegetative carbon stocks (kg C m^{-2}) at ST2, ST3, and ST5 were then obtained by adding the living and litter carbon biomass.



The C_{org} content (% dw), for slices that were not directly analyzed was estimated as the average of the values from the adjacent upper and lower slices. The mass of each slice was estimated by multiplying the DBD with the corresponding elemental content and the decompressed sediment slice thickness (in cm). Sedimentary C_{org} stocks ($kg\ m^{-2}$) were calculated as the cumulative carbon mass and standardised to 1 m of sediment thickness. For cores C2 (94 cm) and C7 (78 cm), which were <1 m long, stocks were standardized to one meter by fitting a linear regression of cumulative stock per slice against sediment depth. This approach was validated by comparing the extrapolated 1-m stocks to the measured stocks in 1 m from the cores with at least 1 m of sediment depth. The extrapolated stocks at 1 m depth showed a strong correlation with measured values.

Short-term sediment accretion rates (SAR, $cm\ yr^{-1}$) and mass accumulation rates (MAR, $g\ m^{-2}\ yr^{-1}$) were derived from ^{210}Pb profiles, using the Constant Flux: Constant Sedimentation CF: CS model (Robbins, 1978). Age-depth models were produced from radiocarbon ages (Cal yr BP) with the R package “Bacon” (Blaauw & Christeny, 2011), calibrated using the Marine13 radiocarbon age calibration curve and corrected with a local reservoir correction of $\Delta R = -113 \pm 95$ years (McCormac et al., 2002). Radiocarbon ages are reported as calibrated years before present (Cal yr BP), where “present” corresponds to the year of sampling (2024). The ^{14}C age-depth models were used to estimate the long-term SARs ($cm\ yr^{-1}$) per core. For replicate cores at each station not subjected to ^{210}Pb or ^{14}C dating, SARs were assumed to be equivalent to those of the corresponding dated core, or the average of the two dated cores for stations in which more than one core was dated.

Organic carbon accumulation rates (CAR, $g\ m^{-2}\ yr^{-1}$) were calculated over three timescales: the last 100, 500 or 1,000 years. Short-term CARs were estimated by multiplying the cumulative mass of C_{org} over 100 years by the corresponding SAR derived from ^{210}Pb dating. Long-term CARs were estimated by multiplying the cumulative mass of C_{org} over 500 and 1,000 years by the corresponding SAR derived from ^{14}C dating.

3.2.5.4 Statistical analysis

To assess differences among stations, one-way analysis of variance (ANOVA) was conducted for C_{org} stock, and both short-term (100-year) and long-term (500-year) C_{org} accumulation rates. Prior to each ANOVA, the Shapiro–Wilk test was used to verify the assumption of normality. When significant differences were detected ($p < 0.05$), post hoc pairwise comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to identify which stations differed significantly.

Stable isotope mixing models based on $\delta^{13}C$ values were used to estimate the relative contributions of different organic matter sources to sedimentary organic carbon at each station. Models were implemented using the R package “*simmr*” (Stable Isotope Mixing Models in R; Parnell et al., 2013). To minimize the influence of post-depositional diagenesis, mixing models were restricted to the top 5 cm of sediment. The end-members included were the $\delta^{13}C$ values from *P*.

oceanica leaves, other macrophytes present at the bay (*H. stipulacea*, *C. nodosa*, and *Caulerpa sp.*), SPOM and terrestrial material). Mean $\delta^{13}\text{C}$ signature for the terrestrial material derived from the literature (Kennedy et al., 2010). To assess potential isotopic overlap among sources, t-tests were performed using the “*vegan*” R package (Oksanen et al., 2001). Significant differences were found between *P. oceanica* leaves and epiphytes at ST5 and therefore they were grouped to reduce uncertainty in the mixing model outcome. Data visualization of all analysis was performed using the R package “*ggplot2*” (Wickham, 2016) or Microsoft Excel.

To estimate bay-scale C_{org} stocks, regression-based upscaling techniques were employed (Ladd et al., 2022). The modelling depicted the relationship between C_{org} stock and water depth, integrating the spatial distribution of *P. oceanica* meadows and dead matte habitats across various depths from the high-resolution habitat map generated through drone surveys (April 2024).

A Generalized Additive Model (GAM) was employed for upscaling C_{org} stock values, selected for its ability to capture non-linear relationships between depth and carbon storage through flexible smoothing functions (Wood, 2017). The GAM was fitted using a Gaussian family distribution with a smooth term for depth ($s(\text{Depth}, k)$), where the smoothing parameter (k) was determined based on the number of unique depth values in the dataset to ensure appropriate model complexity while avoiding overfitting.

The spatial covariate used for predictive analysis was a 1 m resolution bathymetric raster derived from the photogrammetric processing of aerial drone imagery collected in April 2024. Given the strong predictive power of continuous spatial data (spatially continuous surfaces), such as bathymetry raster, when correlated with the response variables (Hengl and MacMillan, 2019), the bathymetric raster was resampled to a uniform 5 m cell-size to ensure uniformity across the bay while maintaining sufficient spatial resolution for the small-scale study area. Site locations (ST1, ST2, ST3, and ST5) were overlaid onto these covariate rasters, allowing for the extraction of cell values, which served as predictor variables in the regression model.

The model coefficient was then used to predict C_{org} stock values onto the spatial covariate surface (Ladd et al. 2022). Mapping of C_{org} stock values across Atzikiari Bay was achieved by converting geospatial vector data into raster format using Geographic Information Systems (GIS), maintaining a 5 m resolution to ensure data consistency. The map was then refined by clipping with polygons delineating the spatial extent of *P. oceanica* meadows and dead matte habitats as identified through the habitat classification process. The total C_{org} stock at the top meter of sediment of the bay was calculated by aggregating the values of raster cells multiplied by their respective areas.

Spatial and predictive analyses were performed in R packages ‘mgcv’, ‘raster’, ‘sf’, ‘sp’, and ‘caret’.

3.3 Results

3.3.1 Seagrass extent and condition

In Fig. 3.9, the resulting high-resolution orthophotomosaic is shown, which was produced from a total of 1,018 aerial photographs taken at an altitude of 90 m. The geospatial resolution of the orthophotomosaic reached 2.5 cm per pixel, based on the mosaicking of 759 photographs, ensuring a detailed mapping of the marine habitats.



Figure 3.9 High-resolution orthophotomosaic used for seabed habitat mapping.

Based on the complete dataset from the network of ground-truthing stations and the reference points used to generate the final map (Fig. 3.10), five seabed habitat categories were identified: 1) *P. oceanica* meadows, 2) Mixed *P. oceanica* meadows with dead matte, 3) Dead matte, 4) Soft sediments (unvegetated or dominated by the seagrasses *Cymodocea nodosa* and *Halophila stipulacea*), and 5) Rocky reefs.

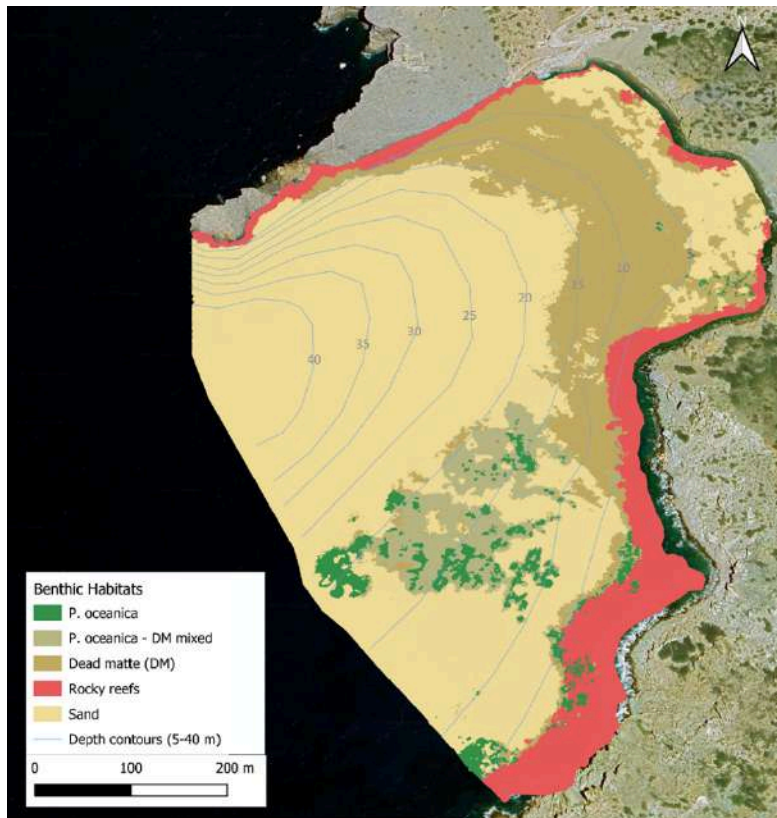


Figure 3.10 Habitat distribution map within Atzikiari bay.

The total mapped seabed area within the bay was 36.2 hectares. Table 3.4 presents the extent and percentage coverage of each habitat type within the study area. The dominant seabed type was soft sediments (sandy substrate), extensively distributed throughout the study area and occupying the deepest part of the western zone (20 – 40). In the inner bay, at depths 5 and 15 m, continuous dead matte formations prevailed. Along the outer margins, at depths of 10 – 22 m, mixed habitats of *P. oceanica* and dead matte were observed, whereas the southern-central part of the bay, between 15 and 22 m depth, supported well-developed *P. oceanica* meadows.

Table 3.4 Extent and percentage coverage of the identified seabed habitat types within the study area, based on the high-resolution orthophotomosaic and ground-truthing surveys.

Habitat	Extent (ha)	%
<i>P. oceanica</i> meadow	1.1	3
<i>P. oceanica</i> meadow & Dead matte	2.2	6.1
Dead matte	6.5	18
Soft sediments	20.3	56.2
Rocky reefs	6.1	16.7

3.3.2 Ecological status

3.3.2.1 WePOSI (Weighted POSidonia Index)

According to the results of the WePOSI, the *P. oceanica* meadows within Atzikiari bay are characterized as having a “Moderate” ecological status in ST4 (EQR = 0.479) and “Good” ecological status in ST5 (EQR = 0.739). Table 3.5 summarizes the mean ($\pm$ SD) values of *P. oceanica* metrics examined for the calculation of WePOSI. Fig 3.11 presents the photomosaics of the fixed transects used for the calculation of the ecological indices at stations ST4 and ST5.

Table 3.5 Mean values ($\pm$ SD) *P. oceanica* metrics examined for the calculation of WePOSI at ST4 and ST5.

Metrics	ST4	ST5
Lower Limit Depth (m)	22	24
Lower Limit Type	Regressive	Progressive
Meadow cover (%)	45 $\pm$ 8.8	45 $\pm$ 8.8
Dead matte cover (%)	55 $\pm$ 8.8	14.3 $\pm$ 7.2
Shoot density (shoots m ⁻²)	632.8 $\pm$ 167.0	695.3 $\pm$ 274.8
Plagiotropic rhizomes (%)	13.4 $\pm$ 21.5	6.4 $\pm$ 6.9
Shoot Length (cm shoot ⁻¹)	43.8 $\pm$ 7.4	59.7 $\pm$ 12.2
Epiphytic biomass (g shoot ⁻¹)	0.012 $\pm$ 0.006	0.006 $\pm$ 0.003

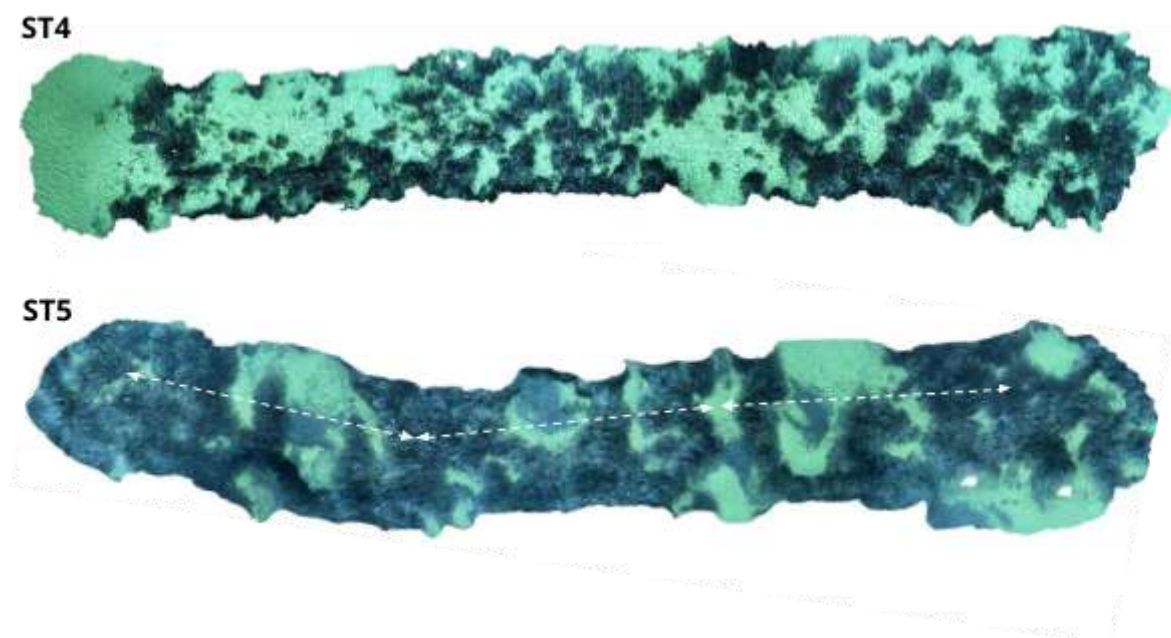


Figure 3.11 Photomosaics of the fixed transects used for the purposes of the ecological indices (WePOSI, CI, SI, PI).

3.3.2.2 Ecological Indices (CI, SI, PSI)

According to the results of the ecological indices CI, SI, and PSI, the *P. oceanica* meadows within Atzikiari bay are characterized as having a “Poor” conservation status in ST4 and “Good”

conservation status in ST5 (Table 3.6).

Table 3.6 Mean values ($\pm$ SD) and classification of the conservation status of *P. oceanica* meadows based on the CI, SI, and PSI indices in ST4 and ST5.

Station	Conservation Index (CI)	Conservation status (CS)
ST4	0.843 ± 0.08	Good
	Substitution Index (SI)	Substitution level
	0	no
	Phase Shift Index (PSI)	Phase Shift level
ST5	0.024 ± 0.01	no
	0.45 ± 0.09	Poor
	Substitution Index (SI)	Substitution level
	0	no
	Phase Shift Index (PSI)	Phase Shift level
	0.092 ± 0.02	low

3.3.2.3 Ecosystem-Based Quality Index (EBQI)

Based on the EBQI, the ecosystem status of *P. oceanica* meadows was classified as "Bad" (EBQI = 3.1; CI = 4) (Fig. 3.12). The results of EBQI calculation for the different functional compartments are presented in Annex 3, Table 8.2. Out of the total of 13 compartments of *P. oceanica* ecosystem considered, three were classified as in Good or High status (i.e., 'Rhizomes', 'Leaves' and 'HOM / LOM'), while the remaining nine compartments were classified in "Poor" or "Bad" status. The greatest contribution to the resulting "Bad" status comes from the functional absence of all key benthic macrofauna, as well as the remarkably low biomass and density of all fish trophic groups.

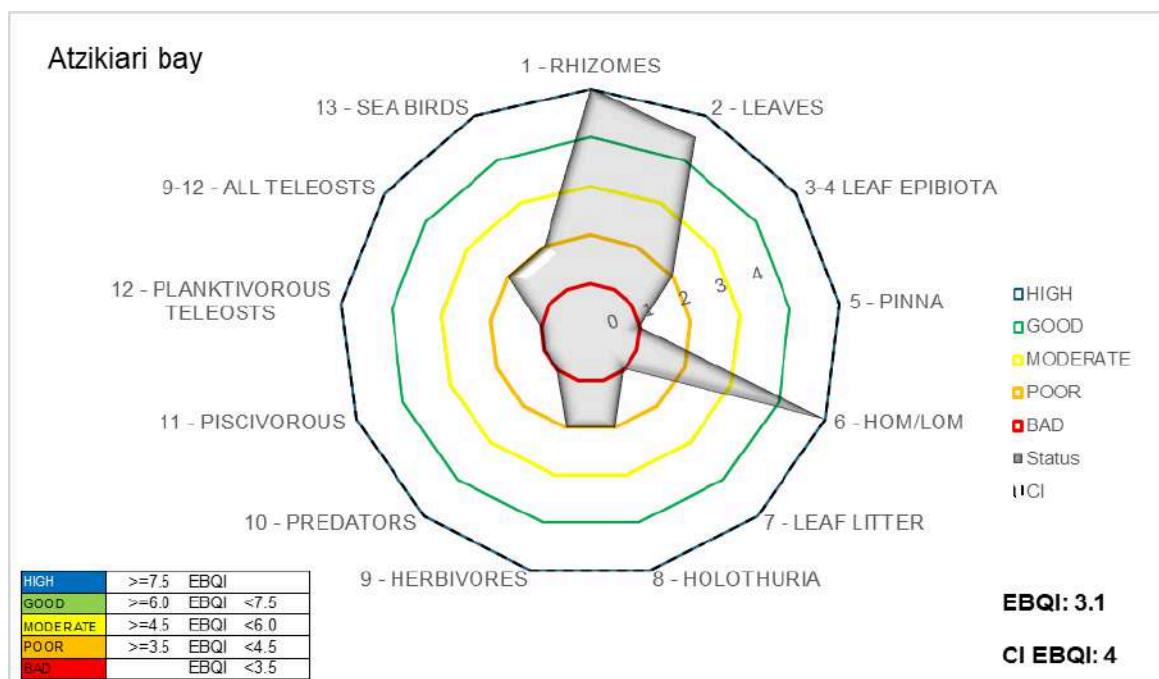


Figure 3.12 Ecosystem-based ecological assessment of *P. oceanica* meadow. The radar chart represents the ecological status

(semi-quantitative scale from 0: Bad to 4: High) of each ecosystem's compartment and their corresponding confidence index (semi-quantitative scale from 0: lowest to 4: highest).

3.3.3 Biodiversity

3.3.3.1 Ichthyofauna

Overall, 19 species of indigenous and 5 species of non-indigenous fish were recorded in total over Atzikiari bay (Figure 3.13, Annex 3, Table 8.3 and Annex 4, Table 8.5, 8.6, 8.7). In ST4 and ST5 which were placed in the control area over the seagrass meadow, planktotrophic fish were the most abundant in terms of numbers (Fig. 3.14) and biomass (Fig. 3.15) while the number of non-indigenous fish was found to be relatively small. However, in ST2 within the impacted area, herbivorous fish were found to be more abundant (Fig. 3.14). The abundance of non-indigenous fish was found to be similar ($n = 62$) compared to the number of native ones ($n = 60$). However, in terms of biomass the non-indigenous species dominate the dead matte area (Fig. 3.15) with a strong presence of the thermophilic goatfish *Parupeneus forsskali*. It is also worth noting that the total number of fish counts was the smallest in the impacted area.

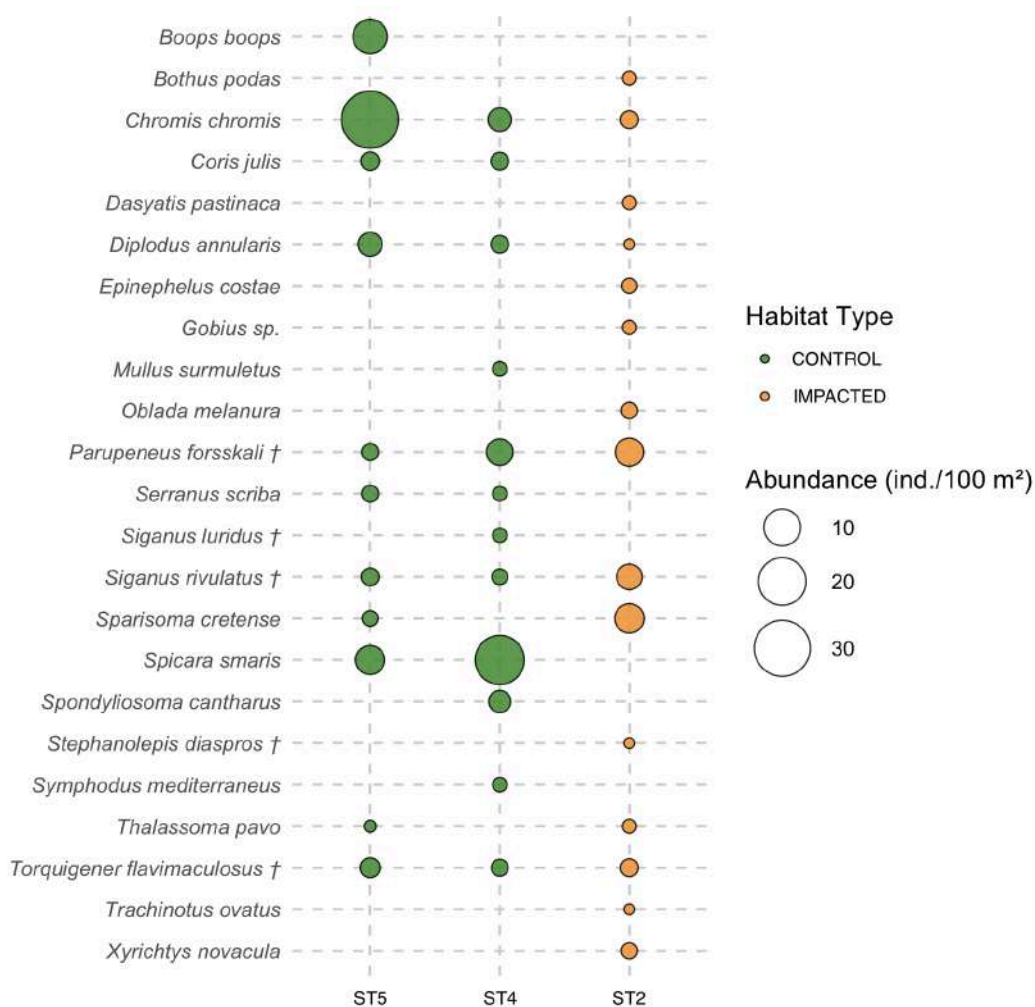


Figure 3.13 Pooled abundances of ichthyofauna across control and impacted sites in Atzikiari bay. The symbol † indicates

invasive species.

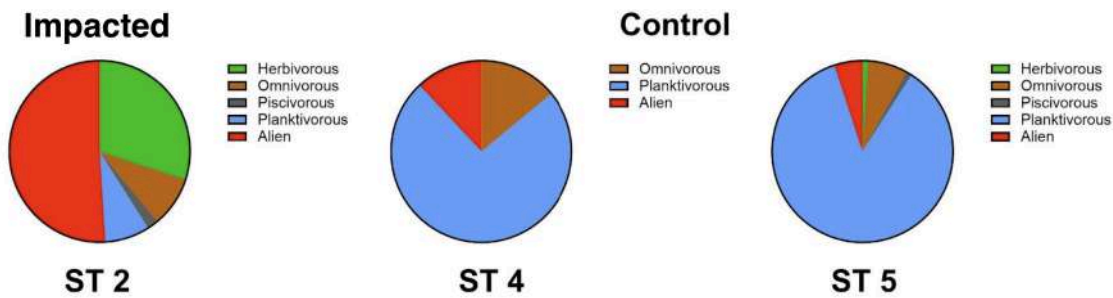


Figure 3.14 Relative abundances of ichthyofauna in ST2, ST4, ST5, respectively.

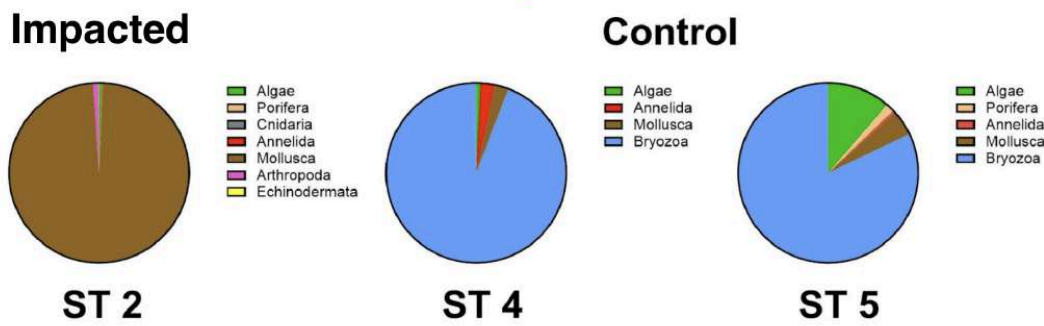


Figure 3.15 Relative biomass of ichthyofauna in ST2, ST4, ST5, respectively.

ANOSIM & MDS analysis

The analysis of similarities (ANOSIM) performed on the ichthyofauna data produced a sample statistic (Global R) = 0.352 with a significance level of 0.1 %. The results of the pairwise tests between the 3 sampling sites are shown in Table 3.7. The multi-dimensional scaling analysis (MDS) visualizes the differences recorded in the ichthyofauna dataset (Fig. 3.16). Both these analyses highlight the significant differences found in the aggregations of ichthyofauna over the *P. oceanica* meadow compared to the dead matte which dominates the parts of the impacted area of former *P. oceanica* meadow.

Table 3.7 Pairwise similarity tests between the 3 sampling sites.

Groups	R statistic	Significance level %
ST2 vs ST4	0.22	4.9
ST2 vs ST5	0.46	0.2
ST4 vs ST5	0.42	0.9

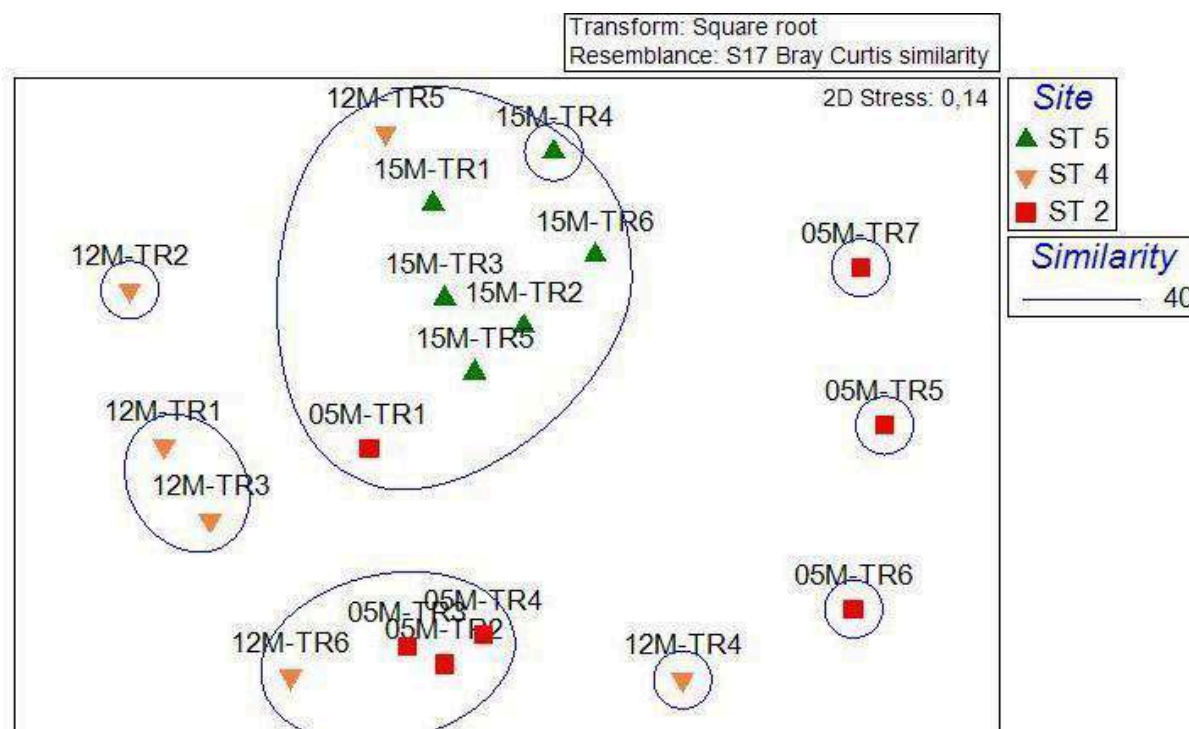


Figure 3.16 Non-metric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarity illustrates differences in fish community composition across stations. Points represent individual transects, grouped by station. Ellipses show 40 % confidence limits around group centroids. The stress value of 0.14 indicates a good ordination fit in two dimensions.

Shannon & Simpson Indexes

Two classic ecological indexes were calculated for the ichthyofauna recorded in the 3 sampling sites of Atzikiari bay based on the number of species of fish (Table 3.8). The number of fish species and the Shannon index were not significantly different between the sampling sites; however, the Simpson Index was significantly different between the impacted area (ST2) and low cover meadow (ST4).

Table 3.8 Number of species, Shannon index and Simpson index for the ichthyofauna recorded at the 3 sampling sites of Atzikiari bay.

Site	Total n° of species	Avg. n° ± SD of species/transect	Shannon Index	Simpson Index
ST2	13	4.4 ± 1.1	1.42	0.87
ST4	13	3.8 ± 1.5	1.15	0.75
ST5	11	5 ± 0.9	1.38	0.77

3.3.3.2 Epibenthic macrofauna and macroalgae

In total 26 species of macrofauna and 3 species of macroalgae were recorded in the Atzikiari bay

(Fig. 3.17, 3.18, Annex 3, Table 8.4 and Annex 4, Table 9.8, 9.9, 9.10). The bryozoan *Pentapora ottomulleriana* had by far the most pronounced presence in the rhizomes in ST4 and ST5 of the control area. The gastropod mollusc *Bittium latreillii* was by far the most abundant taxon on ST2 on the dead matte of the former seagrass meadow.

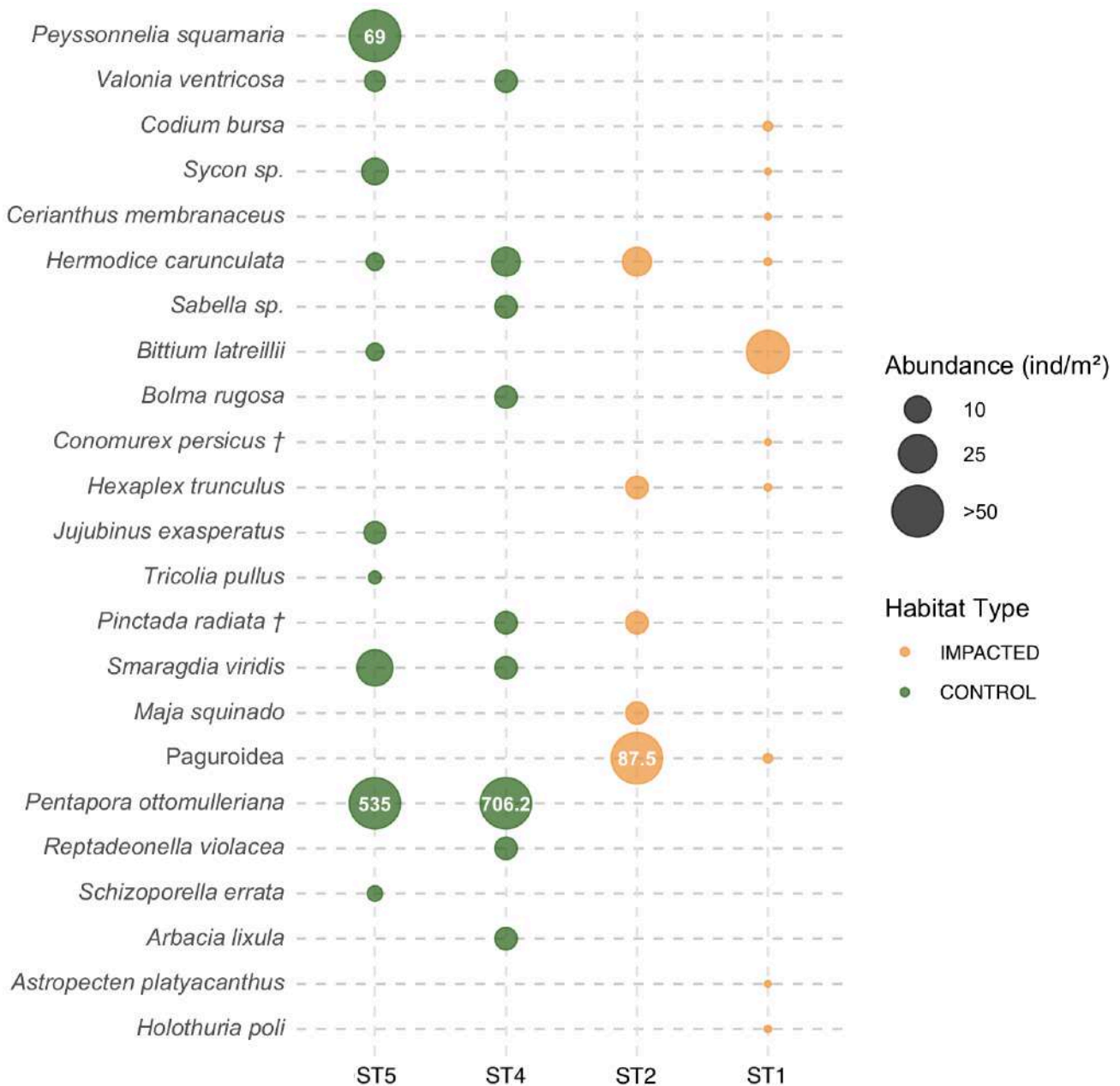
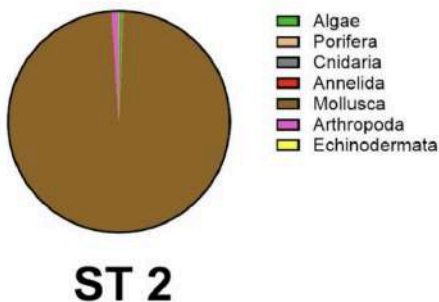


Figure 3.17 Pooled abundances of epibenthic macroalgal and macrofauna taxa across impacted and control sites in Atzikiari bay. The symbol † indicates invasive species.

Impacted



Control

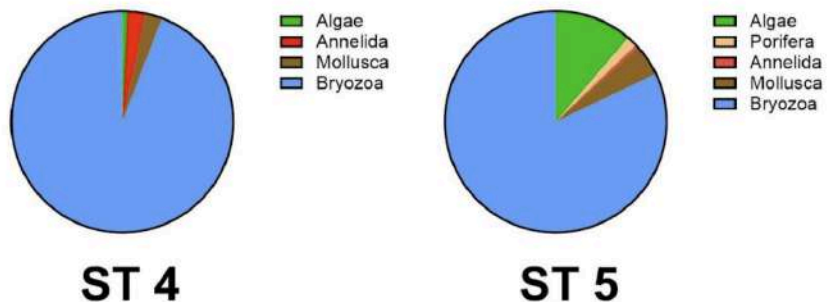


Figure 3.18 Relative abundances of benthic macrofauna taxa recorded in ST2, ST4 and ST5, respectively.

ANOSIM & MDS analysis

The analysis of similarities (ANOSIM) performed on the macroalgae, and macrofauna data produced a sample statistic (Global R) = 0,51 with a significance level of 0,1%. The results of the pairwise tests between the 3 sampling sites are shown in Table 3.9. The multi-dimensional scaling analysis (MDS) visualizes the differences recorded in the macroalgae and invertebrate dataset (Fig. 3.19). Both these analyses highlight the significant differences found in the aggregations of macroalgae and macrofauna over the *P. oceanica* meadow compared to the dead matte which dominates the parts of the destroyed *P. oceanica* meadow.

Table 3.9 Pairwise similarity tests between the 3 sampling sites.

Groups	R statistic	Significance level %
ST2 vs ST4	0.75	0.1
ST2 vs ST5	0.72	0.1
ST4 vs ST5	0.20	0.9

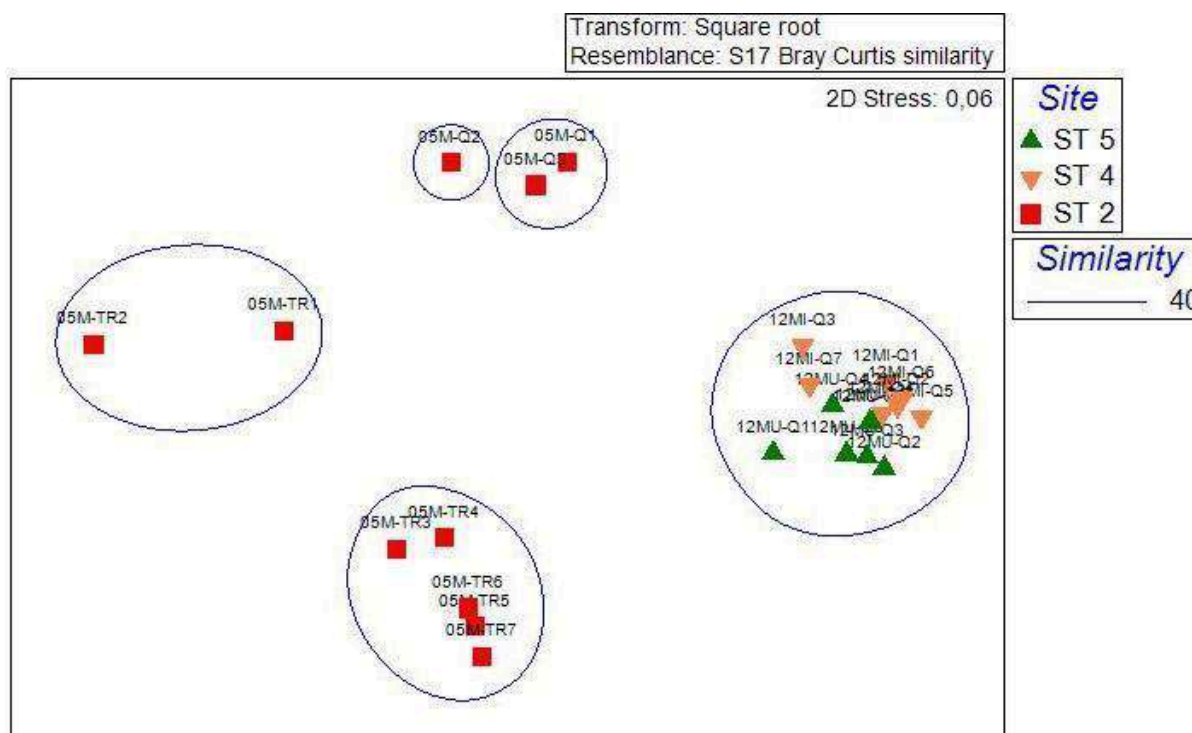


Figure 3.19 Non-metric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarity illustrates differences in benthic macrofauna community composition among three habitat conditions: ST2 (DM), ST4 (LC), and ST5 (HC). Points represent individual transects, grouped by sampling station. Ellipses show 40 % confidence limits around group centroids. The stress value of 0.06 indicates a good ordination fit in two dimensions.

Shannon & Simpson Indexes

Two standard ecological indexes were calculated for the macroalgae and invertebrates recorded in the 3 sampling sites of Atzikiari bay based on the number of species found (Table 3.10). The number of species, the Shannon index and the Simpson Index were not significantly different between the sampling sites.

Table 3.10 Number of species, Shannon index and Simpson index for the macroalgae and invertebrates recorded at the 3 sampling sites of the Cretan pilot.

Station	Total n° of species	Avg. n° ± SD of species/transect	Shannon Index	Simpson Index
Impacted (ST2)	16	4.0 ± 1.2	0.76	0.42
Control (ST4)	8	2.3 ± 1.5	0.54	0.36
Control (ST5)	10	3.9 ± 1.9	0.94	0.53

3.3.3.3 Epifauna

In total 20 families of Annelid worms, and 8 families of Molluscs were found and identified in the leaf canopy of the control meadow (ST5) (Annex 4, Table 9.11 and 9.12). The relative abundances

of each group can be seen in Fig. 3.20 and Fig. 3.21. The most abundant annelids were representatives of the family Syllidae, followed by the families *Fabriciidae*, *Sabellidae* and *Serpulidae*. Additionally, the most abundant molluscs were representatives of the bivalve family Mytilidae, followed by the gastropod families *Cerithiidae* and *Neritidae*.

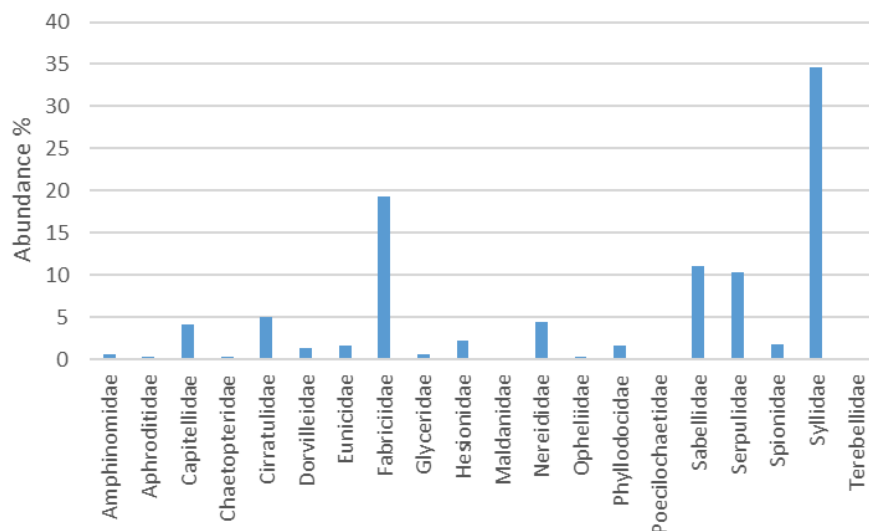


Figure 3.20 Relative abundances of Annelida recorded across the control sites in Atzikiari Bay.

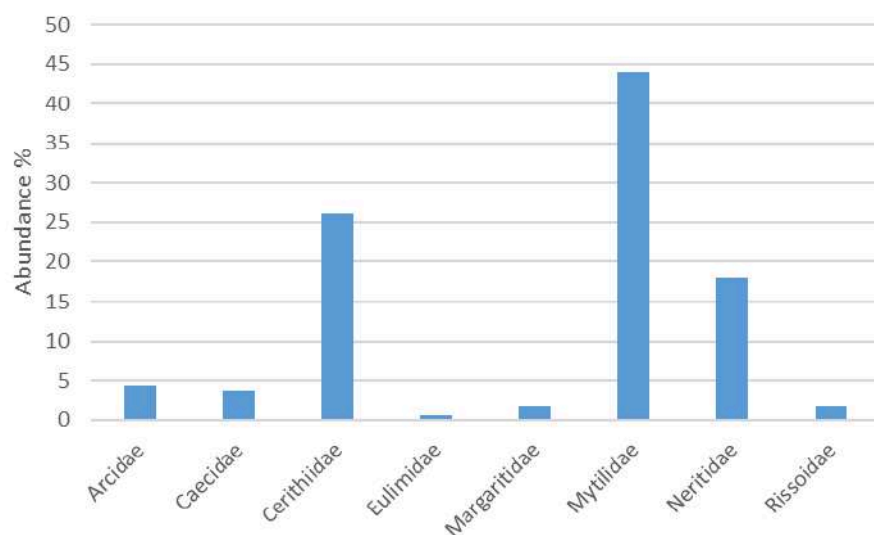


Figure 3.21 Relative abundances of Mollusca recorded across the control sites in Atzikiari Bay.

Also, individuals from 2 Families of Cnidaria and from 7 Families of bryozoans (Fig. 3.22) were recorded on the *P. oceanica* meadow canopy. Both families of cnidarians exhibited similar abundances, while the most abundant Bryozoans were representatives of the family *Alcyonidiidae*, followed by the families *Crisiidae*, *Electridae* and *Lichenoporidae*.

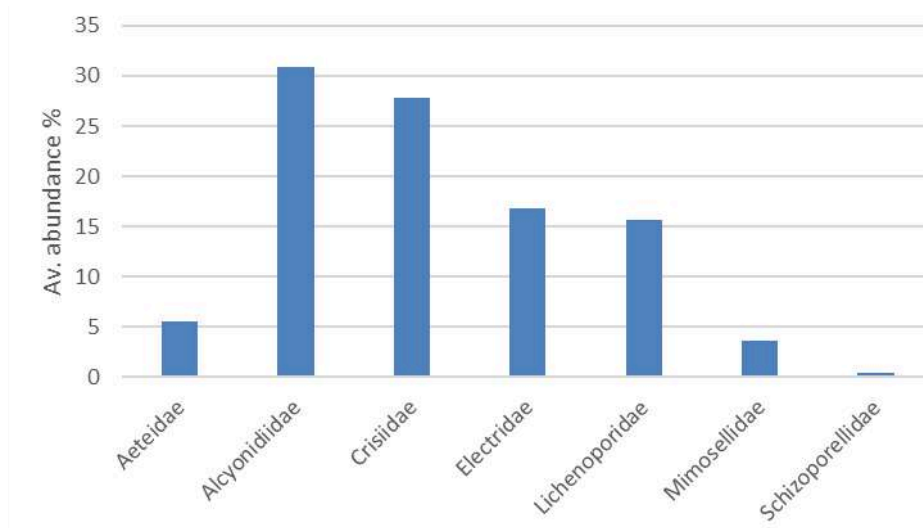


Figure 3.22 Average abundances of Bryozoan families recorded across the control sites in Atzikiari Bay.

3.3.3.4 Endofauna

Two groups were analyzed in the matte samples, Annelids and Molluscs. 23 families of annelids were recorded in the dead matte of the impacted (ST2) and 15 in the living matte of the control *P. oceanica* meadow (ST5) (Annex 4, Table 9.13 and 9.14). In the dead matte the most abundant annelids were representatives of the family Syllidae, followed by the families Fabriciidae, Sabellidae and Nereididae, while in the living matte the most abundant annelids were representatives of the family Syllidae, followed by the families Capitellidae and Sabellidae (Fig. 3.23). Also, 6 families of molluscs were recorded in the dead matte of the impacted area of the site and 11 in the living matte of the *P. oceanica* meadow. In the dead and living matte the most abundant molluscs were representatives of the gastropod family Cerithiidae. Bivalves of the Lucinidae family were present only in the dead matte, while the bilvalves of the Mytilidae family were only present in the *living P. oceanica* matte (Fig. 3.24).

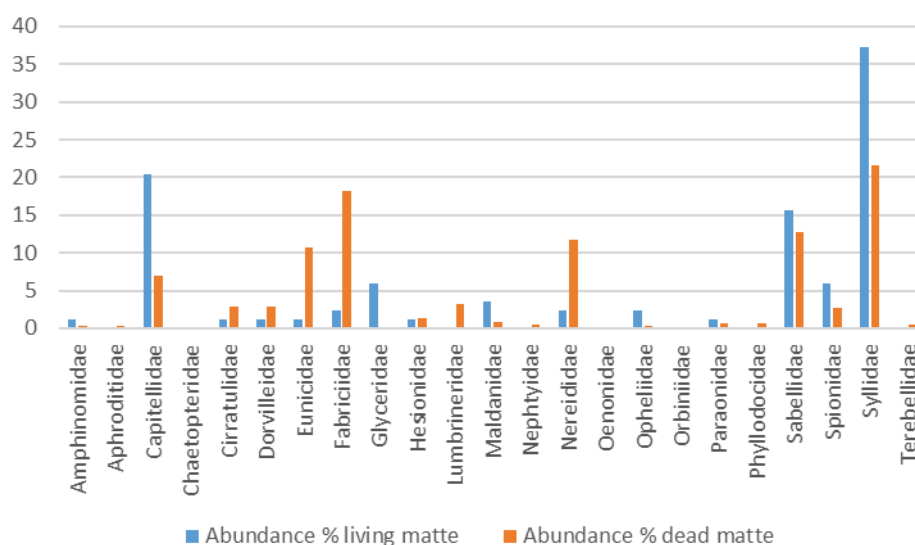


Figure 3.23 Relative abundances of Annelida families recorded in living and dead *P. oceanica* matte across the impacted and

control sites, respectively, in Atzikiari Bay.

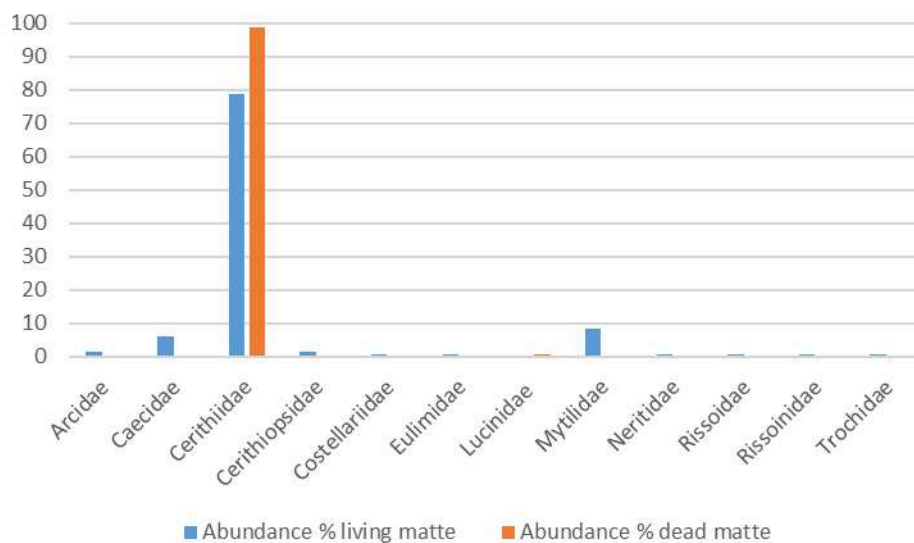


Figure 3.24 Relative abundances of Mollusca families in living and dead *P. oceanica* matte across the impacted and control sites, respectively, in Atzikiari Bay.

ANOSIM & MDS analysis

The analysis of similarities (ANOSIM) performed on the endofauna data produced a sample statistic (Global R) = 0.815 with a significance level of 10 %. The multi-dimensional scaling analysis (MDS) visualizes the differences recorded in the endofauna dataset (Fig. 3.25). ANOSIM analysis indicated that the endofauna communities did not differ significantly between the living and the dead matte, however we can see a distinction between the communities between the living and dead matte.

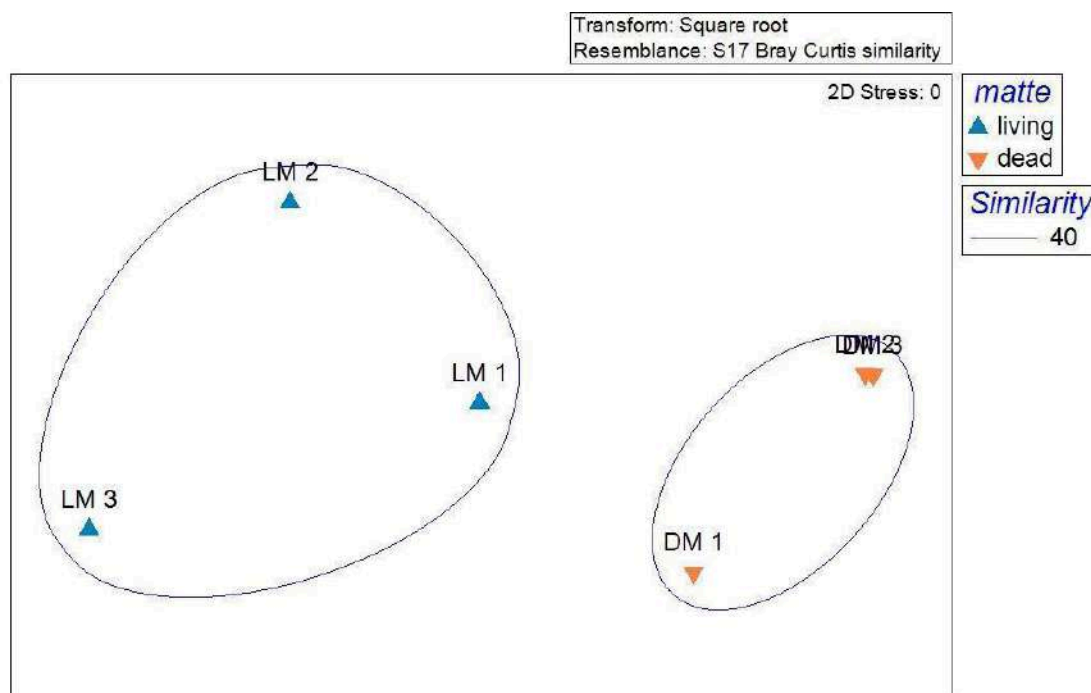




Figure 3.25 Non-metric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarity illustrates differences in benthic macroinvertebrate community composition between the dead matte in ST2 and the living matte in ST5. Points represent individual transects, grouped by sampling station. Ellipses show 40 % confidence limits around group centroids. The stress value of 0.00 indicates a good ordination fit in two dimensions.

3.3.4 Blue Carbon

3.3.4.1 Vegetative carbon pool

Shoot density varied among stations, with values approximately 4-fold higher in the control than in the impacted area (Table 3.11). Similarly, above- and below-ground biomass were almost 5-fold and 7-fold, respectively, higher in the control compared to the impacted area. Litter biomass showed the opposite pattern, being around 3-fold higher in the impacted area. Epiphyte biomass was almost 4-times higher in the control, while epiphyte load showed the opposite pattern, being higher at the impacted area. As ST1 represents a complete loss of *P. oceanica*, all related measurements are absent.

The mean living stock (kg C m^{-2}) was 0.07 ± 0.02 at ST3, 0.13 at ST2, and 0.50 ± 0.07 at ST5. Litter stock (kg C m^{-2}) was 0.05 ± 0.02 at ST2 and 0.02 at ST5 (Fig. 3.26). As ST1 represents a complete loss of *P. oceanica*, neither living nor litter stock was recorded.

Table 3.11 Shoot density, above and below-ground biomass, litter biomass, epiphyte biomass and load across stations.

Variable	Impacted			Control
	ST1	ST2	ST3	ST5
Cover	Complete loss	Sparse	Sparse	Continuous
Density (shoots m ⁻²)	0	180 ± 29	142 ± 17	695 ± 69
Above ground biomass (g DW m ⁻²)	0	192.4 ± 22.6	118.3 ± 28.4	714.1 ± 187.7
Below ground biomass (g DW m ⁻²)	0	111.3 ± 17.1	53.8 ± 18.5	578.7 ± 59.3
Epiphyte biomass (g m ⁻²)	0	10.0 ± 2.8	5.6 ± 1.8	29.2 ± 14.2
Epiphyte load (g g ⁻¹ leaf)	0	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.01
Litter biomass (g DW m ⁻²)	0	140.6 ± 55.2	-	53.5 ± 16.2

3.3.4.2 Sedimentary carbon pool

The mean C_{org} stock in the top meter of sediment was 1.5 times higher in the impacted area (26.6 ± 1.3 kg m⁻² at ST1, 19.8 ± 2.9 kg m⁻² at ST2, and 23.1 ± 2.4 kg m⁻² at ST3) than in the control (15.3 ± 3.0 kg m⁻² at ST5), with an overall mean of 21.2 ± 4.9 kg m⁻². One-way ANOVA revealed significant differences in C_{org} stocks among stations ($p < 0.01$). Post hoc Tukey HSD tests showed that C_{org} stocks at ST1 were significantly higher than those at ST2 ($p < 0.05$) and ST5 ($p < 0.01$). ST3 also had significantly higher C_{org} stocks than ST5 ($p < 0.05$) but did not differ significantly from ST1 or ST2.

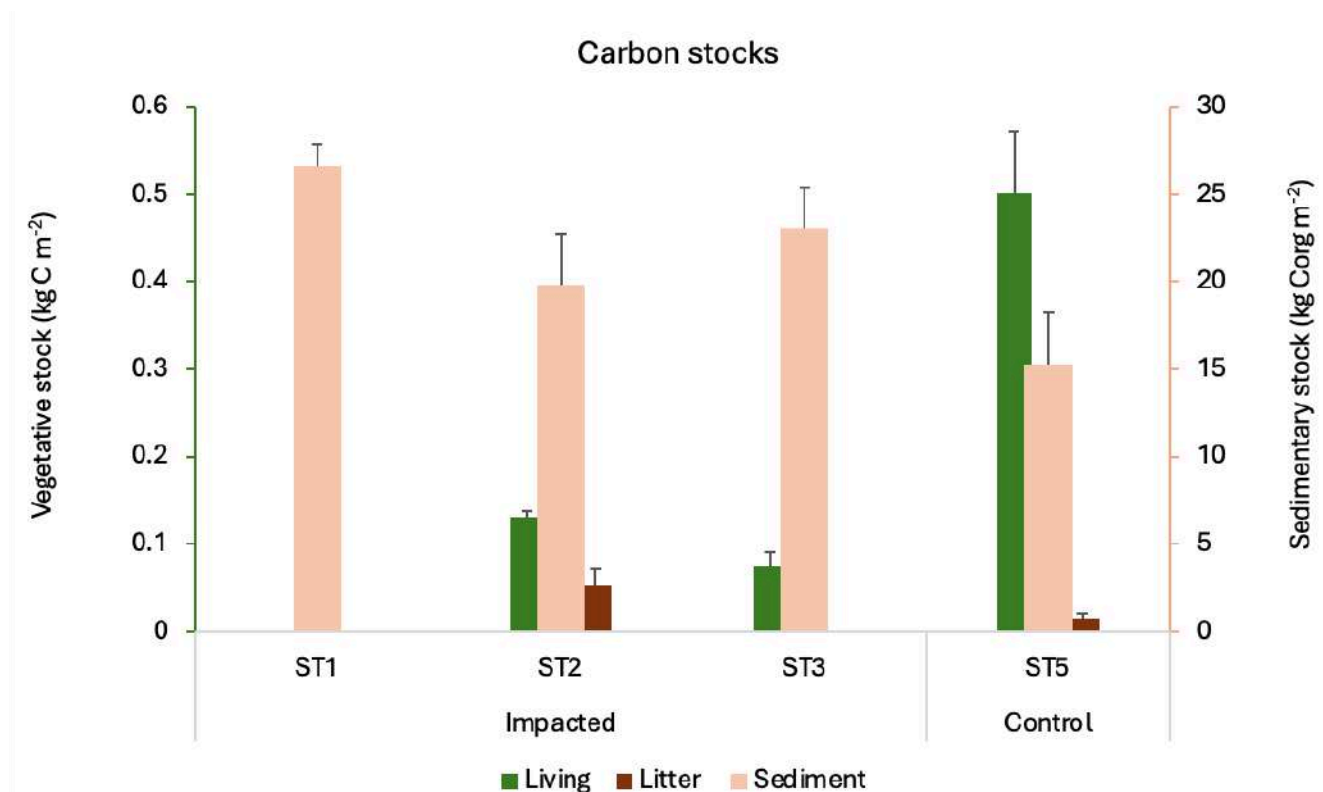


Figure 3.26 Mean vegetative and sedimentary stocks of carbon (kg C m^{-2}) across stations.

The mean short-term (100 years) C_{org} accumulation rate across stations ranged from 26.7 to 48.8 $\text{g m}^{-2} \text{y}^{-1}$. The long-term C_{org} accumulation rate range was 29.2 - 38.7 $\text{g m}^{-2} \text{y}^{-1}$ across stations in the last 500 years, and 27.2 ± 4.1 to 34.2 ± 3.3 in the last 1,000 years (Table 3.12). No significant differences were found among stations for short- or long-term C_{org} accumulation rate.

Table 3.12 Mean $\pm$ SD per station for sediment accretion rates and carbon organic accumulation rates.

Variable	Period	Impacted			Control
		ST1	ST2	ST3	ST5
SAR (cm yr^{-1})	Short-term (last 100 years)		0.31 ± 0.14	0.10 ± 0.02	0.19 ± 0.02
	Long-term (last 500 years)	0.14		0.12	0.23
C_{org} acc. rate ($\text{g m}^{-2} \text{yr}^{-1}$)	Short-term (last 100 years)		48.8 ± 6.8	26.7 ± 10.7	44.1 ± 21.1
	Long-term (last 500 years)	38.7 ± 4.4		29.2 ± 5.1	32.5 ± 4.7
	Long-term (last 1,000 years)	34.2 ± 3.3		27.2 ± 4.1	

3.3.4.3 Sediment analysis

Sediment grain size distributions varied across stations (Fig. 3.27). Very coarse sand and gravel ranged from 8.6 % to 27.9 %, coarse sand ($> 0.5 - < 1 \text{ mm}$) from 1 % to 22 %, medium sand ($> 0.25 - < 0.5 \text{ mm}$) from 1.5 % to 20.2 %, fine sand ($> 0.063 - < 0.25 \text{ mm}$) from 10.2 % to 48 %, and silt/clay ($< 0.063 \text{ mm}$) from 19.7 % to 57.7 %. The greatest difference in sediment grain size was observed between ST1 and ST5. ST1 was dominated by finer sediments, with an average of 57.7 % silt/clay ($< 0.063 \text{ mm}$), which was 2.9 times higher than at ST5. In contrast, ST5 was characterized by coarser sediments, with an average of 27.9 % very coarse sand and gravel ($> 1 \text{ mm}$).

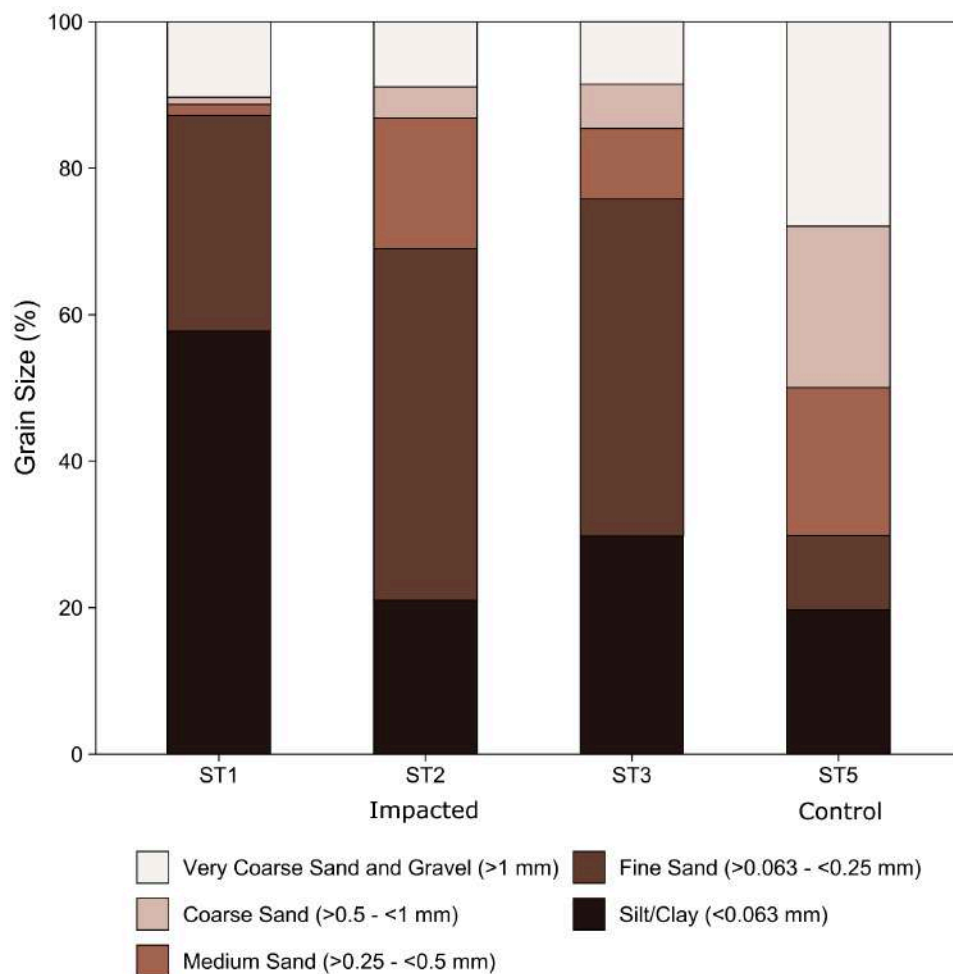


Figure 3.27 Mean cumulative grain size fractions (%) on the top meter of sediment at each site.

Mean DBD varied across stations ranging from 0.7 to 1 g cm⁻³, with the lowest values observed at ST1 and the highest at ST5 (Fig. 3.28A). Mean C_{org} content per station ranged from 2.3 % to 4.6 % dry weight (Fig. 3.28B). Mean δ¹³C values per station ranged from -18.3 ‰ to -16.8 ‰ (Fig. 3.28C).

3.3.4.4 Origin of sedimentary carbon

The contribution of organic carbon sources varied among stations (Fig. 3.29). The highest contribution of *P. oceanica* and associated epiphytes was measured at ST5 (64 ± 15%). The contribution of *P. oceanica* was lower in ST2 and ST3 (17 - 22 %), where other vegetative sources (epiphytes and macrophytes such as *H. stipulacea*, *C. nodosa*, and *Caulerpa sp.*) had the highest contribution (26 - 34 %). The highest contribution of other macrophytes was measured at ST1 (31 %), where *P. oceanica* was completely lost. The contribution of SPOM and terrestrial material was similar across the stations (23 - 30 % and 16 - 28 %, respectively).

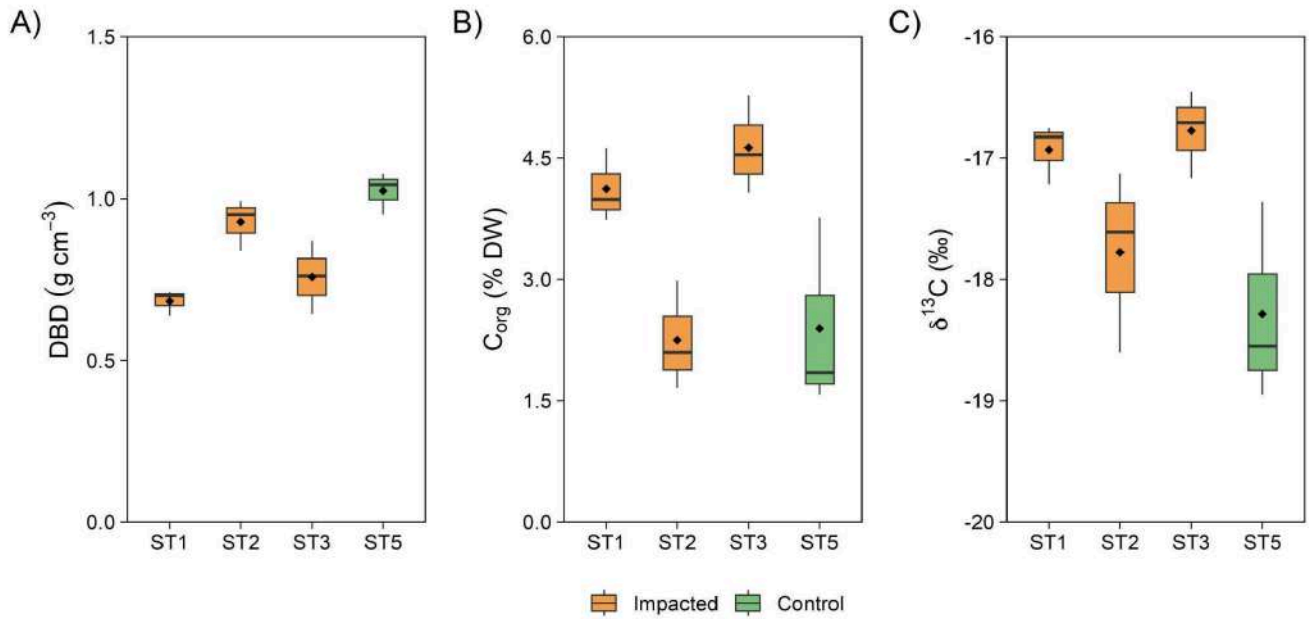


Figure 3.28 Boxplots of (A) dry bulk density (DBD) and (B) C_{org} content and (C) $\delta^{13}C$ composition. Each box encompasses the 25 % and 75 % quantiles, the horizontal dash indicates the median, and the rhombus indicates the mean.

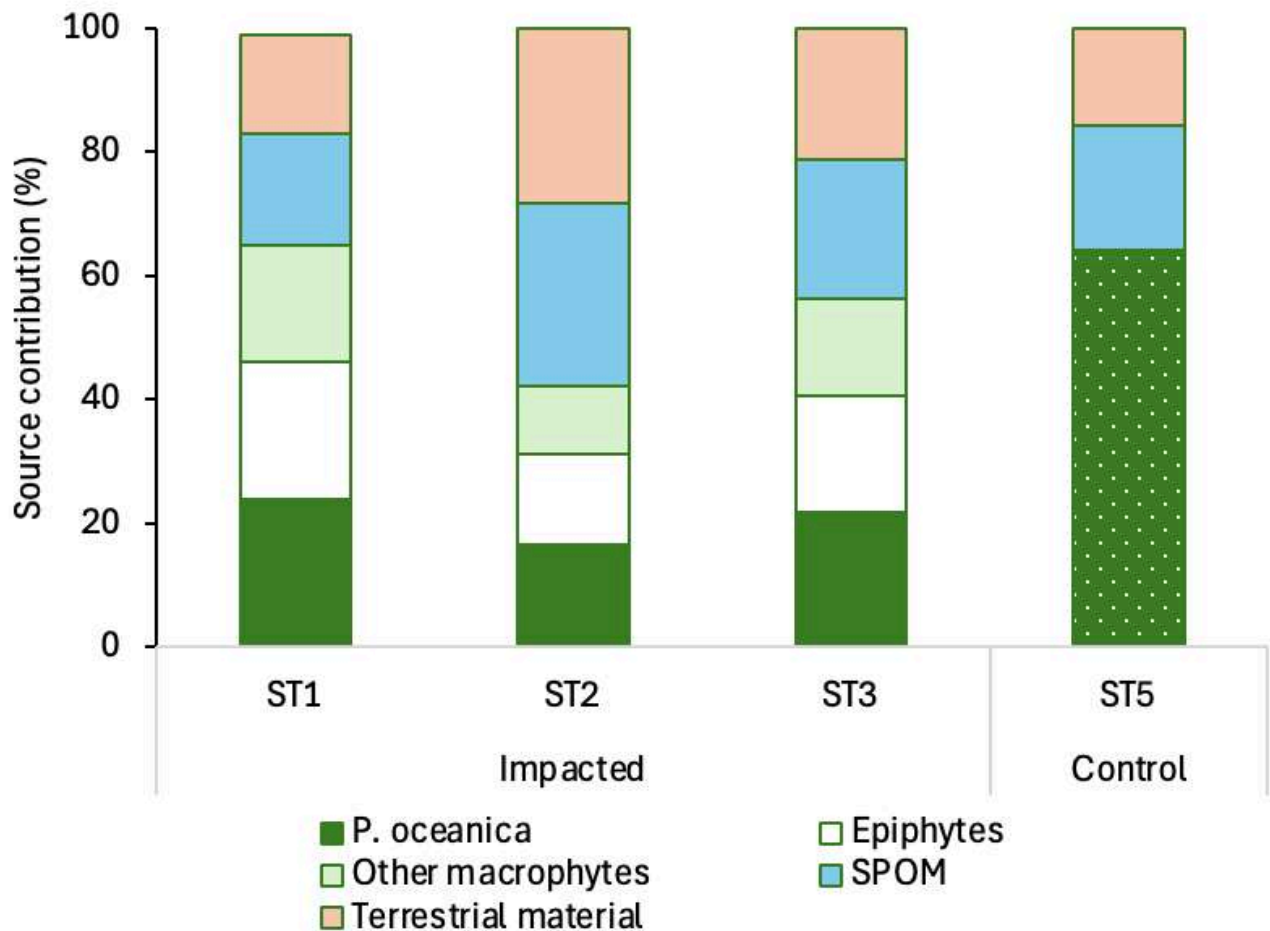


Figure 3.29 Contribution of different sources of carbon to the sedimentary carbon pool at each station. Note that the D.1.2.1Pilots Ecosystem Services Baseline

contribution of *P. oceanica* and epiphytes are combined.

The Generalized Additive Model (GAM) was fitted with a smooth term for depth ($s(\text{Depth})$), with an effective degree of freedom (edf) of 1.775, indicating a moderately non-linear relationship between depth and carbon stock. The model demonstrated significant predictive performance ($p = 0.031$), explaining 55.6% of the deviance in observed C_{org} stocks (adjusted $R^2 = 0.471$) despite the limited sample size ($n = 12$).

The GAM smooth term revealed a non-linear relationship between water depth and C_{org} stock (Fig. 3.30). Within the sampled depth range (6-12 m), carbon stocks initially showed a slight increase with depth, peaking around 8-9 m, followed by a decline toward deeper areas (Fig. 3.31).

The total C_{org} stock in the top meter of sediment across the Atzikiari bay was estimated at $1,285 \pm 89$ Mg C (mean $\pm$ SE), corresponding to a mapped area of 9.83 ha. This represents all areas in Atzikiari bay where *P. oceanica* currently exists or historically occurred (as indicated by dead matte), excluding bare sediment areas without past seagrass presence.

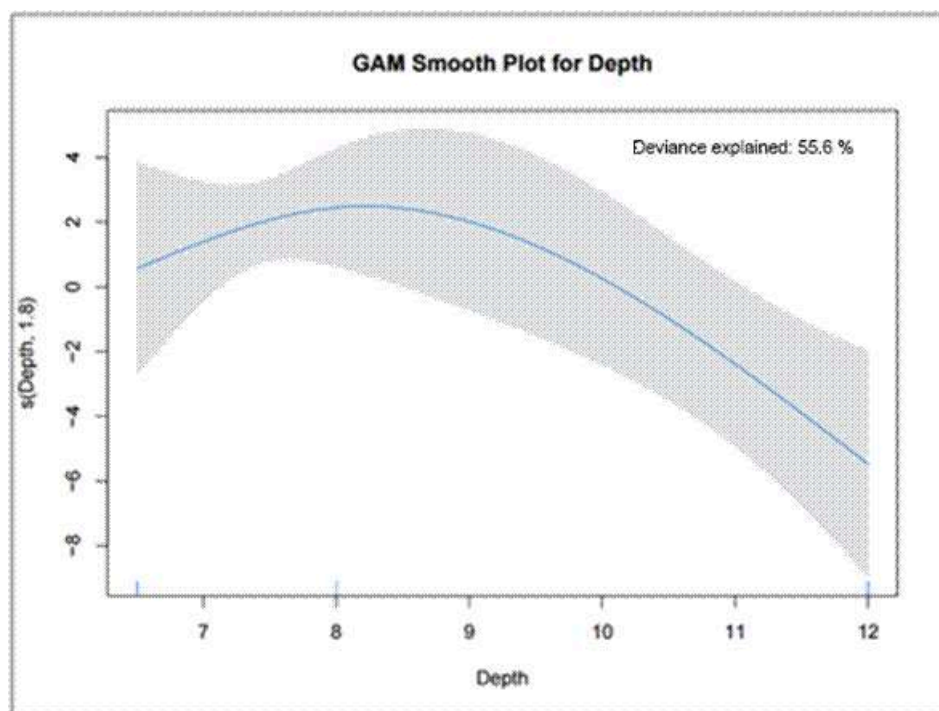


Fig. 3.30. GAM smooth term for the relationship between water depth and sedimentary C_{org} stock in Atzikiari bay.

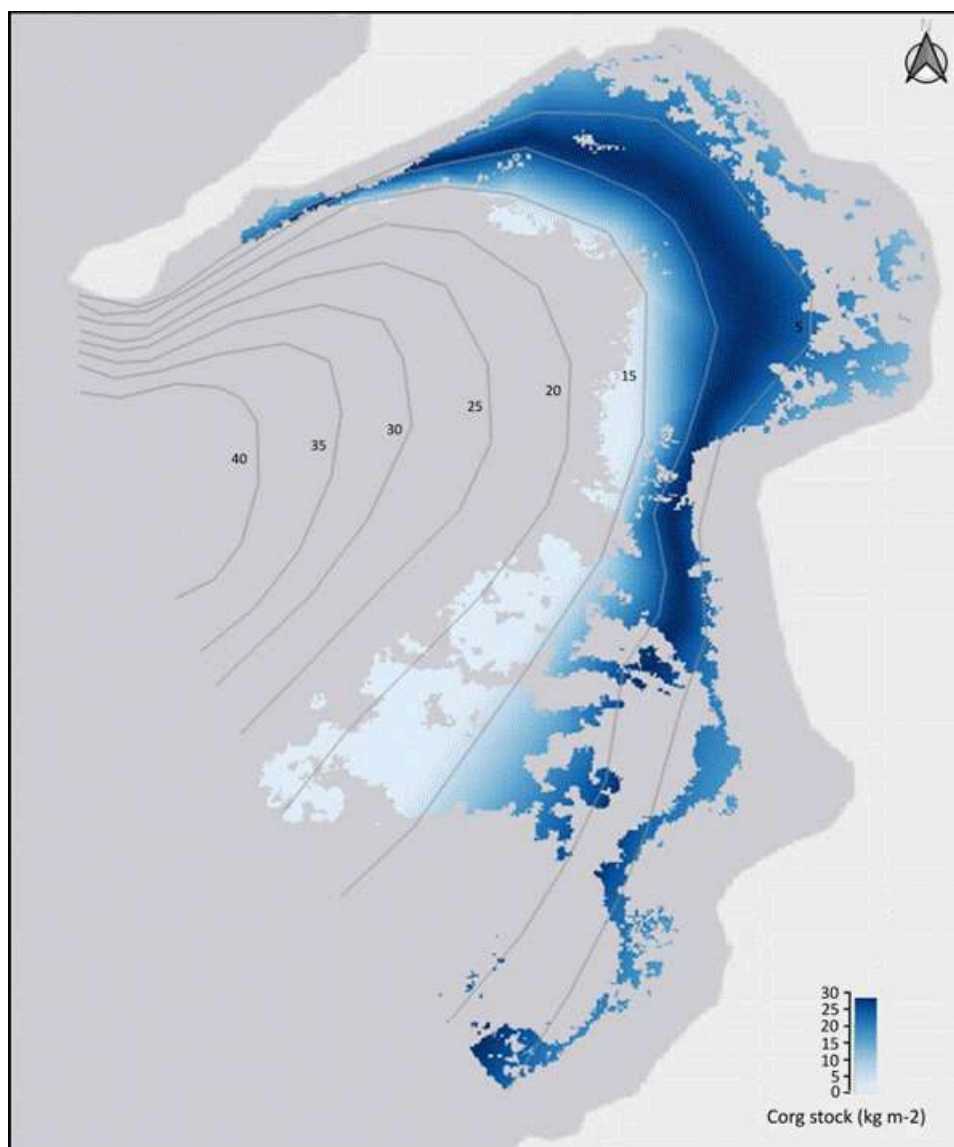


Fig. 3.31. Spatial distribution of predicted C_{org} stocks in the top meter of sediment within *Posidonia oceanica* meadow and dead matte areas in Atzikiari bay. Blue shading indicates predicted C_{org} stocks (kg C m^{-2}), with darker blue representing higher stocks. Grey contour lines show bathymetry (depth in meters).

3.4 Discussion

This study employed a multi-methodological approach, integrating high-resolution habitat mapping with multiple ecological indices, to provide critical insights into the extent and current ecological status of *P. oceanica* meadows in Atzikiari bay. The high-resolution orthophotomosaic analysis revealed extensive historical damage within the 36.2-ha study area. Healthy *P. oceanica* meadows occupy only 3 % (1.1 ha) of the mapped area, while dead matte covers 18 % (6.5 ha), indicating substantial past seagrass loss. Mixed *P. oceanica* meadows with dead matte represent an additional 6.1 % (2.2 ha), suggesting transitional zones between impacted and recovering areas. This spatial distribution pattern reflects the persistent impacts of fish farming activities that



operated within the bay. Fish farm operations involve the release of dissolved and particulate matter to the surrounding environment. Usually, the effect on the water column is limited, as dissolved nutrients are quickly dispersed or taken up by plankton (Pitta et al., 2009), but it is rather pronounced in the sediments, where excess particulate matter accumulates, boosting mineralisation and, thereby, decreasing oxygen availability to the benthic organisms (Kalantzi & Karakassis, 2006). It is well documented that fish farming in the vicinity of *P. oceanica* meadows causes extensive seagrass degradation (Pergent-Martini et al., 2006). Shading and high nutrient and organic matter discharges from the fish cages interfere with multiple physiological and biochemical processes of the seagrass (e.g., photosynthesis, metabolism), resulting in decreased shoot growth and enhanced mortality (Díaz-Almela et al., 2008). The effect of fish farms on seagrass is a function of their distance from the fish cages since the footprint of nutrient and organic matter enrichment decreases exponentially with distance from the farm along the prevailing currents (Holmer et al., 2007). In our case, the small patches inside the impacted area (ST1 - ST3) are situated in the direction of the prevailing currents of the area, and therefore, they have been receiving a larger amount of fish farm effluents, for the period the fish farm was operating, than the meadow of ST5. Available results show that *P. oceanica* shoot density decreases by 50 % in 3 - 26 months from the onset of fish farming, and complete seagrass loss occurs in 5 - 11 years (Holmer et al., 2008). Given that the fish farm operated for 21 years (1996 - 2017), fish farm effluents should have been the main cause of extensive seagrass loss in the impacted area, as opposed to higher seagrass cover found in ST5, where the distance between the meadow and the fish cages was greater and deeper water intervened between them.

The application of multiple complementary indices revealed spatially heterogeneous ecological conditions, consistent with a system in early-stage recovery. Structural indicators demonstrate contrasting conditions between sampling stations, with ST5 exhibiting "Good" ecological status according to WePOSI (EQR = 0.739) and "Good" conservation status based on CI, SI, and PSI indices, while ST4 shows "Moderate" ecological status (WePOSI EQR = 0.479) and "Poor" conservation status. The absence of species substitution (SI = 0 at both stations) and only low levels of phase shift suggest ecosystem stabilization rather than continued degradation.

The bad status based on the EBQI index calculated for Atzikiari bay reflects the regression of the *P. oceanica* meadow after the multiannual intensive aquaculture activity in the area. The condition of the meadow itself ranges from moderate to good with signs of recovery since the destructive aquaculture activity has ceased for 8 years now. For the rest of the functional groups described in the index to recover to satisfactory levels, the meadow has to expand to its former cover of the area and other human derived pressures such as overfishing have to also cease in the area. The discrepancy between WePOSI and EBQI assessments highlights different temporal scales of ecosystem recovery following disturbance cessation. The more favourable WePOSI classification reflects the relatively faster structural recovery of *P. oceanica* shoots and meadow structure observed in the eight years since aquaculture activities ended. In contrast, EBQI's bad rating captures the lag in recovery of higher trophic levels and associated biodiversity, which depend on both meadow expansion to former coverage levels and the establishment of complex habitat structure, processes requiring considerably longer timeframes.



The biodiversity survey of the Cretan pilot site highlights the strong association between the *P. oceanica* meadow and its resident biocommunity. The ichthyofauna within the meadow primarily comprises planktotrophic fish species, while the macrofauna assemblages are dominated by the bryozoan *Pentapora ottomulleriana*, which has a known preference to inhabit *P. oceanica* stems. As the condition of the meadow declines due to human-induced pressures, such as past aquaculture activities, significant ecological shifts are observed. The degradation of the seagrass meadow leads to a marked decrease in the abundance of planktotrophic fish, which struggle to be sustained in impacted meadows and are nearly absent in areas where the meadow is completely lost. This pattern suggests that a healthy *P. oceanica* meadow provides essential habitat and resources that cannot be replicated in degraded environments. Moreover, the presence of non-indigenous fish species appears to be closely linked to habitat degradation. In the control *P. oceanica* meadow, invasive species are present but remain a minor component of the fish community. However, as the habitat deteriorates, their numbers increase, with invasive species becoming dominant in terms of biomass over the dead matte. This trend suggests that these species, particularly the thermophilic goatfish *Parupeneus forsskali*, may possess opportunistic traits that allow them to thrive in altered conditions, potentially outcompeting native species.

A similar transformation is evident in macrofauna assemblages. The shift from a healthy *Posidonia*-associated community to an assemblage dominated by gastropod grazers in degraded areas indicates a fundamental restructuring of the benthic ecosystem. This shift suggests that as the seagrass canopy declines, species reliant on its structural complexity are replaced by generalist and opportunistic taxa that can exploit the changing conditions. Overall, these findings underscore the ecological importance of *P. oceanica* meadows as biodiversity hotspots in Mediterranean marine ecosystems. The loss of these habitats leads to profound shifts in community composition, favouring more resilient, opportunistic, and invasive species while reducing the presence of specialized native taxa. This transition could have cascading effects on ecosystem functionality, altering trophic interactions and habitat stability. (Apostolaki et al., 2007)

Data analysis showed that the wide seagrass loss in the pilot site resulted in higher variation in the vegetative than the sedimentary carbon stocks (coefficient of variation of 79 % and 23 %, respectively) across stations. The effect of disturbance on carbon storage in seagrass meadows depends on multiple factors, including the type of impact (i.e., distance and duration), and the compartment affected, i.e., the plant and/or the sediment (Fu et al., 2025; Spivak et al., 2019). Pressures such as eutrophication or pollution cause extensive loss in seagrass cover, but they can result in small changes in sedimentary stocks (Fu et al., 2025) because they do not cause physical damage. On the contrary, in cases of mechanical disturbance of the seabed, e.g. from mooring or anchoring, a severe loss of sedimentary carbon stocks is measured (Serrano et al., 2016). In our case, i.e. the fish farm operation, the seagrass canopy was severely impacted, reaching far lower biomass, but the superficial sediments were enriched from the fish farm discharges, yielding higher sedimentary carbon storage. Due to the spatial installation of the fish farming facility in relation to the natural distribution of the meadow in the area (i.e., water currents and depth), the fish farm discharges affected more the part of the meadow along the direction of the main water currents and at a smaller water depth, resulting in the highest loss of seagrass at ST1 - ST3,



although signs of canopy degradation are evident across the whole range of the seagrass distribution in the area, as shown by the structural and physiological data at ST4 (see previous section). Consequently, the 5 times lower living carbon stocks in the impacted area than at the control are due to the decrease in shoot density and biomass by 77 % and 82 %, respectively, there. Similarly, the 5-fold higher litter input measured in the patches, which were once closer to the fish farm, should be attributed to higher leaf turnover resulting in increased leaf shedding, as has been observed previously in fish farm-impacted meadows (Apostolaki et al., 2009). Nevertheless, in total, the vegetative carbon stocks contributed on average around 1.5 % (range 0.3 - 3.3 %) to the sedimentary carbon stocks, proving to be a negligible fraction of total carbon stored in seagrass meadows. It has been widely recognised that seagrass store carbon mainly in their sediments, as opposed to other vegetative ecosystems like forests (Jakobsson, 2008).

Storage of organic carbon in the seagrass sediments followed a decreasing trend from ST1 towards ST5, which should be attributed to the vicinity to the fish cages and the geomorphology of the pilot site. Modelling results have indeed predicted increase in carbon storage under eutrophication in the presence of strong seagrass-hydrodynamic positive feedback (Le Vilain et al., 2025, accepted). Stations inside the impacted area are physically closer to the once installed farm, meaning that they should have received primarily the material released from the farm. At the same time, they are situated in the most protected part of the bay, especially ST1, which receives the least hydrodynamic force, while ST5 is found in the most exposed area of the bay. As a result, the proportion of mud and fine sediments decreased going from ST1 to ST5. Finer sediments are known to be positively correlated with C_{org} storage (Mazarrasa et al., 2017b, 2021). This is also seen by the trends in short and long-term (500 and 1,000 years) accumulation rates across stations, which both show a decrease towards ST5. As the ^{210}Pb operates on short time scales, it provides a finer resolution of the recent sedimentation and carbon accumulation rate, contrary to ^{14}C which may miss recent changes. This explains why the CV of long-term accumulation rate is small (16 - 18%), whereas short-term is high (54 %) across stations. In fact, the change in accumulation over the recent past due to fish farm enrichment was better captured by ^{210}Pb . As the ^{14}C works for long-term assessments, the higher accumulation in the now impacted area should be attributed to the physical position of those stations, regardless of the influence of the fish farm derived organic matter. Greater depth of ST5 could also be held responsible for the lower storage, as water depth has been shown to negatively affect sedimentary C_{org} stocks in seagrass meadows (Masarassa et al., 2021; Serrano et al., 2014), although the depth threshold for reduction in *P. oceanica* stocks in Greece is below 15 m (Apostolaki et al. submitted), i.e., below the depth range of ST5.

However, carbon stored in the impacted area, although high in amount, derived mainly from allochthonous sources, accounting for 65 %, on average, of the organic matter pool. Underlying seagrass sediments are usually a mixture of autochthonous and allochthonous matter, where the contribution of seagrass itself varies depending on the species identity and environmental settings (Kennedy et al., 2010). In our case, the contribution of *P. oceanica* was, on average, 3 - 4 times lower in the impacted area than in the control. Furthermore, in the case of the impacted patches,



the contribution of epiphytes (14 % and 17 % at ST2 and ST3, respectively- data not shown) was almost as high as that of *P. oceanica* (17 % and 22 % at ST2 and ST3, respectively - data not shown). This was due to the higher epiphyte load on the small, low-weight leaves, as a result of the nutrient enrichment of the area (Apostolaki et al., 2011). Given that *P. oceanica* detritus is less susceptible to decomposition than that of epiphytes and other macrophytes, the carbon stored in the impacted area, although more in quantity, represents a more labile carbon pool (Mazarrasa et al., 2017a) and therefore a less permanent carbon sink. The time interval since the fish farm cessation and intense hydrodynamics in the bay are probably responsible for the restoration of the water column conditions, explaining eventually the similar contribution of SPOM in the superficial sedimentary C_{org} pool across stations. In fact, the superficial sediments in our study area have been shown to recover after 6 years (Koulouri et al., 2023). The fish feed is very enriched in carbon and nitrogen (48.1 % and 7.8 % DW, respectively (Holmer et al., 2007), suggesting that it gets rapidly decomposed once deposited in the underlying sediments (Holmer et al., 2007). As a result, it was not expected to detect significant carbon enrichment in the sediments at the time of sampling (i.e., already 7 years from the cessation of the fish farm).

Overall, mean C_{org} stock and long-term (500 yr) accumulation rate of this study ($21.2 \pm 4.9 \text{ kg m}^{-2}$ and $33.4 \pm 5.9 \text{ g m}^{-2} \text{ yr}^{-1}$, respectively) ranged higher than the mean calculated from an extended dataset from several meadows of the South Aegean Sea ($11.1 \pm 0.5 \text{ kg m}^{-2}$ and $25.3 \pm 2.2 \text{ g m}^{-2} \text{ yr}^{-1}$, respectively) (Apostolaki et al. submitted). Yet comparing our values with those measured in other meadows, even in those extending in the wider area of study, is problematic, as both biotic and abiotic factors acting in concert affect carbon storage (Kennedy et al., 2022). At short spatial scales, it is usually local factors, such as geomorphology or hydrodynamics, that determine the magnitude of storage in a given meadow. Our values should be considered the baseline of carbon storage in the area and the starting point to follow the possible increase in carbon accumulation as a result of the transplantation that initiated one year after collecting these cores in the frame of the project.

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4 Menorca, Spain

4.1 Study area

The study area was located at about 1 km south-west of Ciutadella, in the island of Menorca, Spain (39° 58.279'N, 3° 49.727'E; Fig. 4.1A). The site is characterized by rocky shorelines with extensive *P. oceanica* meadows located between 2 to > 30 m water depth. The seagrass meadows grow on medium to coarse sandy bottoms intertwined with coarse sandy patches and rocky reefs (Fig. 4.1B). The substrate in the area impacted by the construction of the desalination plant consisted of bare coarse sands with very few small and sparse clumps of *P. oceanica* across the relatively homogenous and even area of bare sand that was vegetated previous to the impact.

In 2010, the construction of a desalination plant to supply water to Ciutadella resulted in the loss of 1,808 m² of *P. oceanica* meadows near Cala Blanca, located at a depth of 20 m, owing to the direct impact of the construction of the tower to collect water (170 m²) and the incidental spill of bentonite during construction that decimated the surrounding meadows (1,638 m²; Fig. 4.1C and Fig. 4.1D). Although the desalination plant was built in 2010, desalination operations only started in 2019, and the plant has been operating at 30 % capacity since.

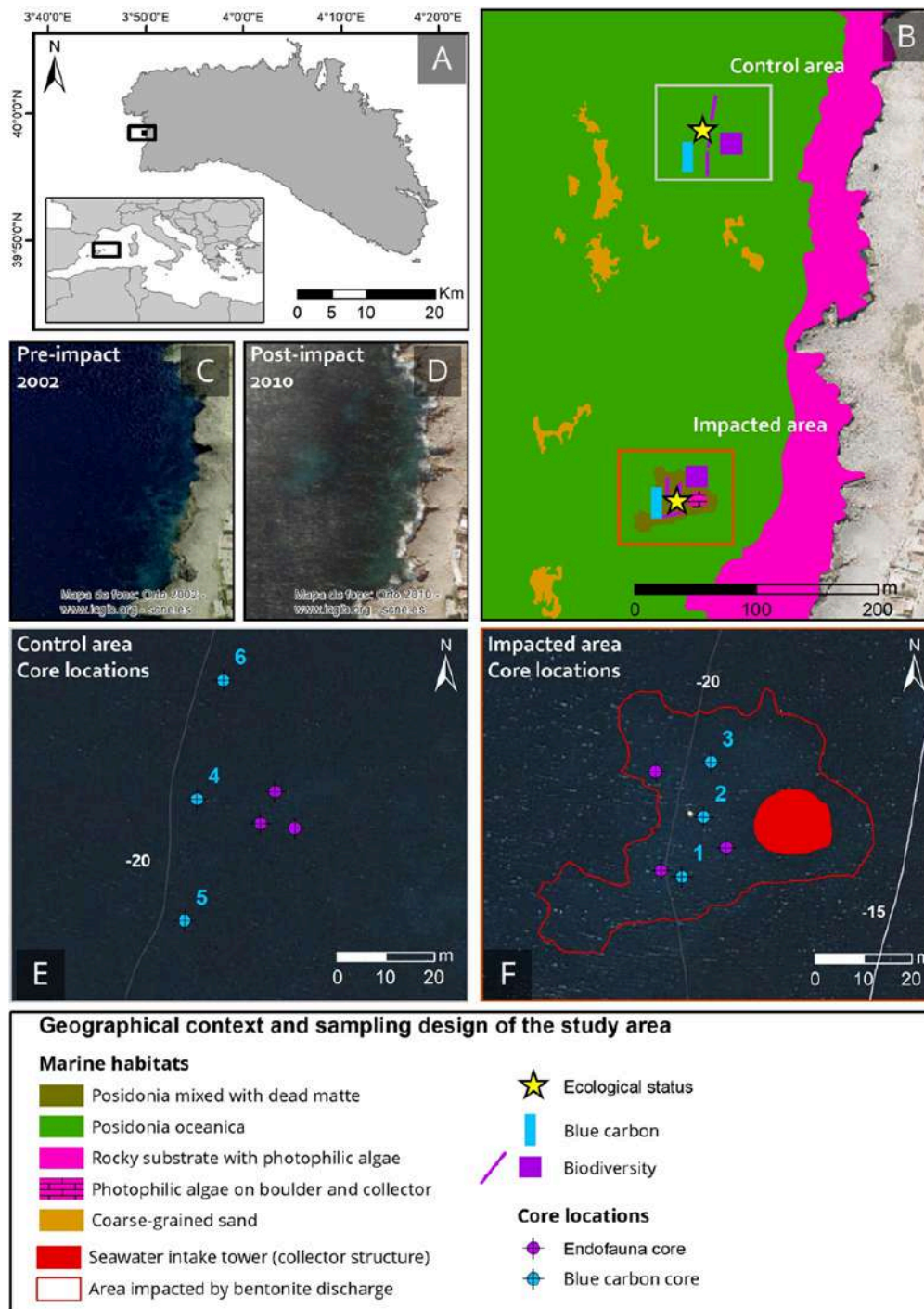


Figure 4.1 Geographical context and sampling design at the study area in Ciutadella (Menorca, Spain) to determine the impact of the construction of a desalination plant on the capacity of *P. oceanica* meadows to store Blue Carbon. (A) Study area in Cala Blanca, located in the Island of Menorca in the Western Mediterranean Basin. (B) Location of the sampling sites with impacted and control stations, which were located about 300 m within continuous *P. oceanica* meadows interspaced with coarse-grained sandy patches. Habitat maps in the region, which include *P. oceanica* meadows, rocky and algae substrates, and soil grain-size characteristics. (C) and (D) Satellite imagery from 2002 (predating the construction of the desalination plant) and 2010 (post-impact) showing the loss of *P. oceanica* meadows. (E) Sampling locations of seagrass biomass and soil cores within the control station, located about 30 m apart. (F) Sampling locations of seagrass biomass and soil cores within the impacted station, located about 20 m apart. The purple contour shows the area of *P. oceanica* lost, whereas the purple shaded area shows the location of the seawater collection tower built for the desalination plant. Maps created in ArcGIS.



4.2 Methods

4.2.1 Habitat mapping

The methodology followed to produce the habitat map was based on the existing cartography by MAGRAMA (2008) and its update by Julià et al. (2019). The cartography carried out in 2007 (MAGRAMA, 2008) covered the marine area of Menorca from the minimum navigable depth to the -50 m isobath or at least 1 km offshore. The spatial distribution of the communities was delineated through 259 towed video transects, spaced every 500 m and extending down to 40 m depth. Subsequently, sampling plots were established for quantitative characterization using substrate-specific methodologies: biometric measurements in *P. oceanica* and *C. nodosa* meadows, Van Veen grab sampling on soft bottoms, and scraping with photo-identification in rocky substrates with photophilic algae. A total of 440 bionomic samples were collected over two seasonal campaigns. In addition, 120 visual censuses of marine fauna and verification dives were conducted to characterize the benthic communities of the island.

Based on this cartography, made prior to the construction of the desalination intake tower, the perimeter margin of the degraded seagrass meadow was delineated by marking GPS points in the field and later complemented with orthophotographs acquired after construction, processed through Geographic Information System (GIS) software.

During the field surveys conducted for the present study, the various anthropogenic structures associated with the intake tower that condition the seabed typology were delineated. These structures included the intake tower with its surrounding protective rock reef, the concrete block signalling the tower, and the pipeline. Their dimensions were measured in situ using a measuring tape to establish their limits.

4.2.2 Ecological status

For the assessment of the conservation status of the area to be restored, the methodology described in Personnic et al. (2024) was adapted. The most relevant adaptation was focusing the survey at a depth of 20 m, as the impacted station targeted for restoration is located at this depth.

The study area was assessed in two stations within continuous *P. oceanica* meadows: one station impacted by the construction of the desalination plant (hereafter named 'impacted'), and another station located approximately 300 m north of the desalination plant that was not affected by its construction (hereafter named 'control') (Fig. 4.1B and Fig. 4.2). Surveys were conducted in April 2025 (Fig. 4.2) and in July 2025.

All parameters recommended by Personnic et al. (2024) were measured. Table 4.1 provides a summary of the parameters assessed, the methodologies employed for their measurement, and the number of replicates conducted.



Figure 4.2 Team of researchers from IME-OBSAM and IMEDEA departing from Cala Santandria (Ciutadella, Menorca) to carry out fieldwork as part of the April 2025 sampling campaign.

Table 4.1 Details of the parameters analysed for each Box in relation to Personnic et al. (2024). Methodology used and number of replicates.

Box nº	Functional Compartment	Parameter	In situ /Lab	Unit	Measurement Method	
1	<i>P. oceanica</i> roots and rhizomes	Growth of orthotropic rhizomes	Field & Lab	mm /yr ¹	Shoots	lepidochronology
2	<i>P. oceanica</i> leaves	Shoot density	Field	shoots /m ²	Quadrats	20 x 20cm quadrat
	<i>P. oceanica</i> leaves	Cover	Field	%	Vertical photographs	visual observation/photos
3 - 4	Multicellular Photosynthetic organisms (MPO), filter- and suspension-feeder s leaf epibiota	Biomass of leaf epibiota (only the two oldest external leaves)	Field & Lab	g DM /shoot	Shoots	DW of epibiota biomass -Filtered and dried (60 °C) during 24 h
5	Benthic filter-feeders (<i>Pinna nobilis</i>)	Density	Field	Individuals /100 m ²	Abundance of individuals	50 cm x 50 cm quadrat
6	Other benthic filter- and suspension-feeder s. High levels of organic matter in the water (HOM).	Density	Field	individuals /m ²	Abundance of individuals	50 cm x 50 cm quadrat
	Other benthic filter- and suspension-feeder s. Low levels of organic matter in the water (LOM)	Density	Field	Individuals /m ²	Abundance of individuals	50 cm x 50 cm quadrat
7	Litter Detritus	Biomass of litter detritus	Field & Lab	g DM /m ²	Leaves	10 cm x 10 cm quadrat - DW biomass (70 °C until constant weight)
8	Detritus Feeders (<i>Holothuria</i> sp.)	Density	Field	Individuals /10 m ²	Abundance of individuals	50 cm x 50 cm quadrat



9	Herbivores (Sea Urchins)	Density	Field	Individuals /m ²	Abundance of individuals	50 cm x 50 cm quadrat
	Herbivores (<i>Sarpa salpa</i>)	Grazing index	Field & Lab	%	Shoots	visual examination of leaves
10	Predatory Teleosts	Visual census	Field	kg teleosts WM /100 m ²	Transects	length 20-m / width 5-m
11	Piscivorous Teleosts	Visual census	Field	kg teleosts WM /100 m ²	Transects	length 20-m / width 5-m
12	Planktivorous Teleosts	Visual census	Field	kg teleosts WM /100 m ²	Transects	length 20-m / width 5-m
13	Sea Birds (<i>Gulosus aristotelis desmarestii</i> and <i>Pandion haliaetus</i>)	Distance to nearest nesting sites	Literature	km	-	measurement of distance

The following sections describe in greater detail the methodologies used for each Box.

Box 1: *P. oceanica* roots and rhizomes. Growth of orthotropic rhizomes (mm y⁻¹): To determine this parameter, 20 vertical roots were manually collected per study area during fieldwork and placed individually in plastic bags (Fig. 4.3C). The samples were kept frozen (-20 °C) until further processing.

The length of the rhizome corresponding to the last 8 dead leaf bases in 20 vertical (orthotropic) shoots of *P. oceanica* was measured in each of the extant seagrass patches within the impacted station where *P. oceanica* will be planted and in the control *P. oceanica* meadow located 300 m north of the impacted station. The rhizome lengths were multiplied by 1.5 as indicated by Personnic et al. (2014).

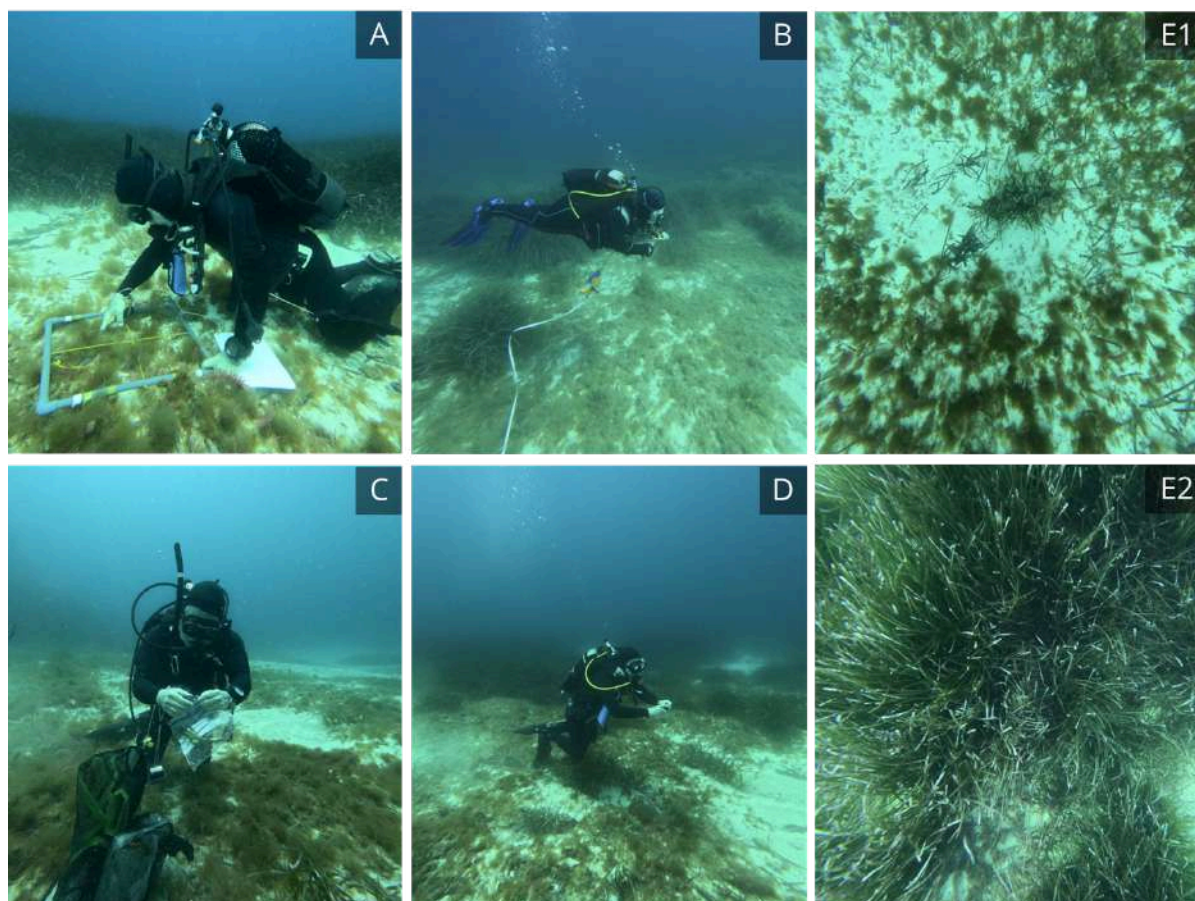


Figure 4.3 (A) Images showing the sampling procedures conducted in *P. oceanica* meadows. Census of filter- and suspension-feeders using 50 × 50 cm quadrats, (B) visual census of teleosts, (C) collection of seagrass shoots for herbivory index and growth of orthotropic rhizomes, (D) taking photographic quadrats for seagrass cover estimation and (E) photographic records of seagrass cover: (E1) impacted station (E2) control station.

Box 2. *Posidonia oceanica* Leaves:

Density (shoots m^{-2})

To estimate *P. oceanica* density, the number of shoots was counted in 20 randomly placed quadrats (20 × 20 cm) within both the impacted station and the control station.

Cover (%)

P. oceanica cover was estimated using vertical photographs taken by divers positioned 1 – 2 m above the seabed (Fig. 4.3D) in both the impacted (Fig. 4.3E1) and control stations (Fig. 4.3E2). The images were analysed to estimate percentage cover using the software ImageJ. A grid was superposed over each image and the number of cells with/without *P. oceanica* was counted and used to calculate the percentage cover relative to the total number of cells in the image.

Box 3–4. Multicellular Photosynthetic Organisms (MPO), Filter- and Suspension-feeders – Leaf Epibiota. Biomass (g DM mass shoot⁻¹): Epiphytes on the two oldest external leaves of 20 *P.*



oceanica shoots, collected from both the impacted and control stations, were scraped using a razor blade into a Petri dish containing deionized water. The samples were then filtered and dried at 60 °C for 24 hours to obtain dry mass.

Box 5. Benthic Filter-Feeders (*Pinna nobilis*). Density (individuals 100 m⁻²): The number of fan mussels (*Pinna nobilis*) was counted within 30 randomly placed quadrats (50 × 50 cm) in both the impacted and control stations.

Box 6. Other Benthic Filter- and Suspension-Feeders:

High Organic Matter (HOM) Indicators. Density (individuals m⁻²)

Organisms indicative of high levels of organic matter (HOM) — such as annelids (*Sabella spallanzanii*) and ascidians (*Phallusia mammillata*) — were counted on *P. oceanica* rhizomes within 30 quadrats (50 × 50 cm) randomly placed in the impacted (Fig. 4.3A) and control stations.

Low Organic Matter (LOM) Indicators. Density (individuals m⁻²)

Organisms indicative of low levels of organic matter (LOM) — including bryozoans, sponges, ascidians (*Halocynthia papillosa*), and echinoderms (*Antedon mediterranea*) — were similarly counted in 30 quadrats (50 × 50 cm) placed randomly in the same stations.

Box 7. Litter Detritus: Dead Leaves and Broken Rhizomes. Wrack Biomass (g dry weight m⁻²): These measures are commonly used for blue carbon assessments. A total of 10 seagrass wrack samples were collected — 5 in the impacted station and 5 in the control station — consisting of all dead biomass within five 10 × 10 cm quadrats per station. Only quadrats containing wrack were sampled. The material was dried at 70 °C until constant weight to estimate biomass (g DW m⁻²).

Box 8. Detritus Feeders 2 and 3 (*Holothuria* sp.). Density (individuals 10 m⁻²): The number of sea cucumbers (*Holothuria* sp.) was recorded within 30 randomly placed quadrats (50 × 50 cm) in the impacted and control stations.

Box 9. Herbivores 1

Paracentrotus lividus. Density (individuals m⁻²)

The number of sea urchins (*Paracentrotus lividus*) was counted within 30 quadrats (50 × 50 cm) randomly placed in the impacted (Fig. 4.3A) and control stations.

Grazing Index (% of leaves grazed)

Grazing pressure by *Sarpa salpa* was assessed by counting the number of intermediate and adult *P. oceanica* leaves with visible bite marks in 20 shoots collected from both the impacted and control stations. The grazing index was calculated as the percentage of leaves presenting bite scars (Fig. 4.3C).

Box 10–12. Teleosts (kg WM 100 m⁻²): Ichthyofauna biomass was estimated following a methodology based on visual censuses conducted in both study stations. Surveys were carried out between 10:00 and 16:00 using transects measuring 20 × 5 × 5 m (Fig. 4.3B). The length of the transects was adjusted from the 50 m recommended by Personnic et al. (2024) due to spatial

limitations within the impacted station. This adaptation ensured that all censuses could be conducted entirely within the impacted station, avoiding the edges and minimizing the influence of nearby structures—specifically the intake tower and its surrounding riprap, which could bias fish observations.

During each census, all individuals were identified to species level, counted, and assigned to size classes using 2 cm size intervals. A total of three replicates were conducted, with the number of replicates also adapted to the spatial limitations of the impacted station, for the same reasons that justified the adjustment of transect length.

Box 13. Seabirds (km): To determine the shortest distance to the nesting areas of *Gulosus aristotelis desmarestii* and *Pandion haliaetus*, bibliographic and cartographic information from monitoring studies of these two species was used (Sales et al., 2021; de Pablo, 2022). In the case of *G. aristotelis*, nesting sites are published, whereas for *P. haliaetus* they are not disclosed in order to protect their location, as only 4 – 5 breeding pairs nest in Menorca depending on the year.

4.2.3 Biodiversity

To assess biodiversity, different compartments were selected to represent the community: canopy, matte, water column, leaves, and rhizomes. For two of these compartments (rhizomes and water column), data obtained using the methodology described in the previous section (see 4.2.2. Ecological status) were applied. In the case of leaf-associated biodiversity, only the total biomass of leaf epifauna was quantified, as detailed in section 4.2.2 (Ecological status, Box 3 – 4), without species-level identification. Finally, for canopy and matte, dedicated sampling procedures distinct from those previously described were conducted specifically for this purpose and are detailed in the present section.

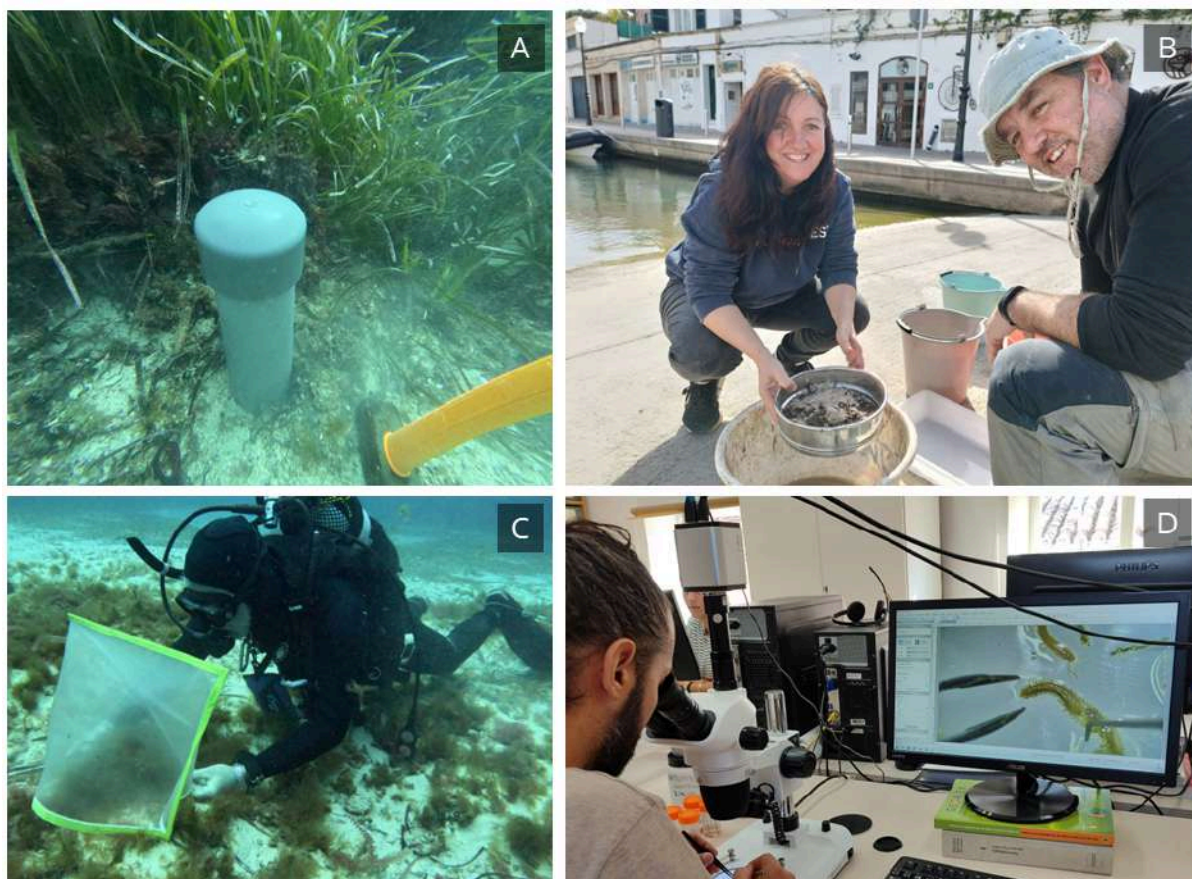


Figure 4.4 (A) Images of the fieldwork and laboratory work during the biodiversity assessment of the *P. oceanica* meadow. Sediment coring for endofauna analysis, (B) pre-sieve of the cores' contents in the harbour of Ciutadella, (C) sampling of epifauna by IMEDEA's researchers, (D) and taxonomic identification of endofauna samples at IME-OBSAM's laboratory.

4.2.3.1 Epifauna (canopy)

Three samples of epifauna (vagile organisms living in the seagrass and/or macroalgal canopy) were collected in each of three substrates: dead matte present inside the impacted station, *P. oceanica* patches present in the impacted station, and the control *P. oceanica* station located 300 m north of the impacted station. Epifauna was collected by placing a 500 μ m mesh bag open over a 625 cm² to 795 cm² surface of seabed, cutting with scissors all macrophytes enclosed by the bag and closing it (see Fig. 4.4C). Samples were transferred to seawater buckets and a mixture of sodium bicarbonate and tartaric acid was added to the water to reduce its oxygen content and promote the detachment of epifauna from the sample macrophytes. Seawater in the buckets was filtered and the epifauna stored in 70 % ethanol. Defaunated macrophytes were dried (60 °C) to estimate dry mass. Epifauna was separated into taxonomic groups under a dissecting microscope.

4.2.3.2 Endofauna (matte)

For the impacted and control stations, three independent sampling substations were established: C1, C2, C3 in the control station and I4, I5, I6 in the impacted station.

Sediment coring and sample volume. Sediment samples were collected by scuba using a cylindrical core of 13 cm diameter (sampling area = 132.7 cm²) and 20 cm depth (see Fig. 4.4A). Each core was extracted intact and handled carefully to prevent loss of fine material. Where substrate resistance precluded full penetration, the actual penetration depth was recorded. Per substation, sampled volumes ranged from 0.56 to 2.50 L, corresponding to 111.88 to 1,733.52 g wet weight. Samples were preserved in 70 % ethanol. The fraction of available sediment sampled at each substation (i.e., pseudo-replication within the station) ranged from 17.55 % to 50.65 % (see Fig. 4.5).

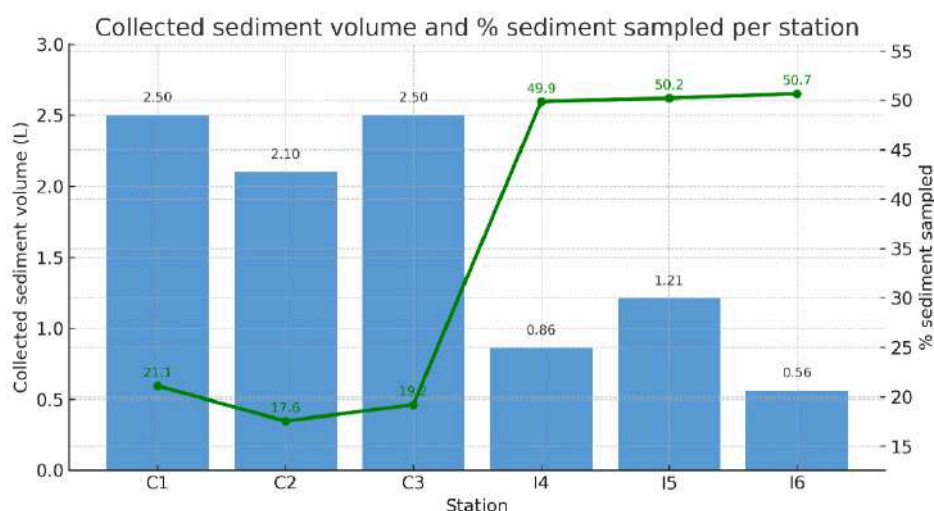


Figure 4.5 Initial in situ sediment volume collected (blue bars, in litres) and percentage of sediment used for the pseudo-replicates analysed after pre-sieving through a 1 mm mesh (green line) at each sampling substation. Volume values are from the field team, whereas the percentage corresponds to the subsampling performed in the laboratory.

The contents of each core were immediately pre-sieved through a stainless-steel circular mesh of 1 mm (see Fig. 4.4B). Only the fraction > 1 mm was retained, discarding all sediment and organisms below this size. This procedure removes the size range typically assigned to meiofauna (63 – 500 µm); consequently, the results obtained refer exclusively to the small macrofauna and mesofauna > 1 mm present in the sediment. Material retained on the sieve was placed in pre-labelled, airtight containers. Samples were fixed in 70 % ethanol and kept cool and dark until processing in the laboratory. Because porewater was initially present in the sediment, ethanol was renewed within 24 h to ensure the final preservation concentration and to improve morphological preservation of the organisms.

This protocol implies that the outcomes correspond solely to the > 1 mm small macrofauna/mesofauna fraction, excluding the component conventionally considered as meiofauna (63 – 500 µm). As a result, the diversity detected is partial with respect to the total benthic biodiversity of the sediment, with the absence of dominant meiofaunal groups such as nematodes, small harpacticoid copepods, or minute ostracods. Therefore, the data is not directly comparable with studies that use standard meiofaunal protocols, and conclusions should be interpreted within the frame of the > 1 mm sedimentary community. Despite this limitation, analyzing the > 1 mm fraction can yield relevant ecological information on functionally significant components of *P. oceanica* meadows and can contribute to management and environmental

monitoring when explicitly framed in this specific context.

Sample processing was conducted at the laboratory of IME-OBSAM. The pseudo-replicates corresponding to the > 1 mm fraction retained after pre-sieving were examined in their entirety under a Nexius Zoom stereomicroscope equipped with an Euromex HD-4K-rev.3 camera, which enabled detailed observation and photographic documentation of the organisms.

In the first phase, a complete gross sorting was performed with the aim of separating all items potentially representing living organisms, regardless of their vital status at the moment of sampling. At this stage, live and dead material were not yet discriminated; all items were recorded and labelled to ensure full traceability. Subsequently, each sample underwent a second, individualized re-inspection, during which the fraction of live organisms was separated from the fraction of dead remains (e.g., empty gastropod shells or cockle valves).

Following this second classification, the material was distributed into 5 ml tubes, grouping individuals by major taxonomic clades: Crustacea, Mollusca, Echinodermata, Annelida, and Brachiopoda (Fig. 4.6). This system facilitated subsequent taxonomic identification and long-term storage of the biological material.

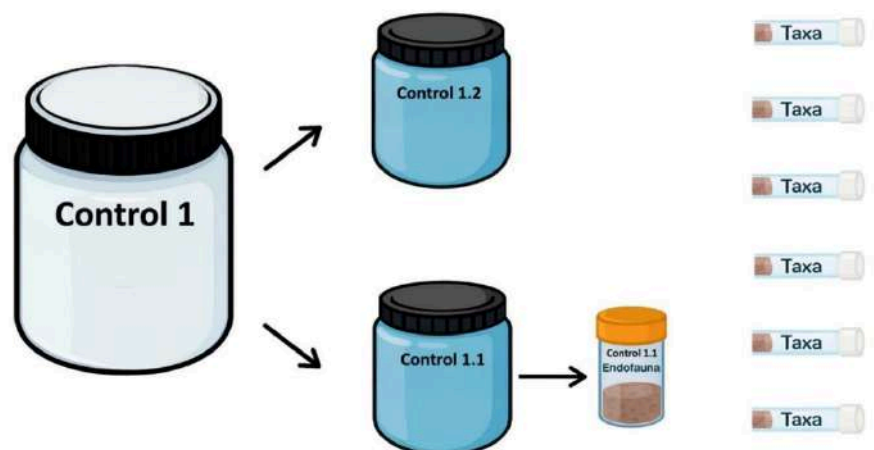


Figure 4.6 Schematic of the endofauna sample-processing procedure. The initial samples, collected in the field using sediment cores and preserved in 70 % ethanol, are divided into two sub-samples. From one sub-sample, the fauna eligible for inclusion in the study are extracted (vials with orange caps). From this material, organisms are separated into major taxonomic groups (Crustacea, Mollusca, Echinodermata, Annelida, and Brachiopoda). This initial classification enables the subsequent identification of individuals to species level, using reference literature and databases.

Taxonomic identification of the recovered organisms was carried out on the material previously sorted into major groups (Crustacea, Mollusca, Echinodermata, Annelida, and Brachiopoda) after the laboratory processing phase. To ensure maximum accuracy and consistency in the nomenclature used, the World Register of Marine Species (WoRMS; <https://www.marinespecies.org/index.php>) was adopted as the primary reference platform, which allowed validation of each taxon's placement and the updating of names according to international standards.

As support for identification, we used reference photographs and group-specific resources, D.1.2.1Pilots Ecosystem Services Baseline



together with a bibliographic search of the main identification keys at group and family level and, in some cases, down to species. Additionally, the Global Biodiversity Information Facility (GBIF; <https://www.gbif.org/>) and iNaturalist (<https://www.inaturalist.org/>) were consulted to identify the species most common in the study area, so that determinations were made within the local biogeographic context. This process enabled determination of the organisms present in the samples at the highest feasible taxonomic resolution, while ensuring traceability and consistency of taxonomic information with scientific literature and the cited reference databases.

After taxonomic validation and trophic guild coding, each taxon was classified into one of four habitat-affinity categories that reflect its typical position relative to the sediment column: Obligate infaunal (burrowing or tube-dwelling forms living within soft sediments), Sediment-associated (epibenthic or interstitial organisms occurring on/at the sediment surface but not strict infauna), Epifaunal (organisms living on leaves, algal turf, hard substrata or as surface crawlers), and Indeterminate (insufficient trait information at the reported taxonomic resolution). The assignment combined family-level trait knowledge with genus-level overrides where relevant (e.g., *Naticidae* = obligate infaunal; *Caprellidae*, *Rissoidae*, *Tricolia* = epifaunal; *Onuphidae*, *Lumbrineridae*, *Spionidae* and *Lucinidae* = obligate infaunal; *Haminoeidae*, *Pyramidellidae*, *Raphitomidae* and *Cystiscidae* = sediment-associated; *Arcidae*, *Pectinidae*, *Carditidae* and *Chamidae* = epifaunal). Ambiguous records were conservatively set to Indeterminate unless higher-level traits were unequivocal. For transparency, a brief affinity notes field documents the rationale for each assignment. Subsequent biodiversity and multivariate analyses for “sediment endofauna” were defined a priori to include Obligate infaunal + Sediment-associated categories, with Epifaunal retained for sensitivity analyses in the appendix.

Regarding the sampling design and analytical procedure used to quantify the diversity of the infauna associated with *P. oceanica* meadows, we describe the key indicators—total abundance (N), richness (S), Shannon diversity (H'), Pielou's evenness (J'), Simpson diversity ($1-D$), Hill numbers ($q = 1$, $q = 2$), and Margalef's index—together with interpretation criteria. We then compare the status of the impacted station and the control station using non-parametric tests (Mann-Whitney) and effect sizes (Cliff's delta), complementing, where appropriate, with compositional dissimilarity (Bray-Curtis) and an integrated reading of α - and γ -diversity.

- Total abundance (N): the total count of individuals found in a sample.
- Richness (S): the number of distinct types (species or taxa). Two sites can hold the same number of individuals, yet the one with more types has higher richness.
- Shannon diversity (H'): integrates how many types are present and how evenly their abundances are distributed. High values indicate many species with similar abundances; low values indicate dominance by a few species.
- Evenness (Pielou's J'): ranges from 0 to 1 and reflects how equal abundances are among species. Values near 1 denote an absence of strong dominance.
- Simpson diversity ($1-D$) and Inverse Simpson: measures of dominance. High values indicate well-balanced communities without highly dominant species.

- Hill numbers ($q = 1$ and $q = 2$): express diversity as an effective number of equally common species. For example, a value of 20 means the community behaves as if it comprised 20 equally abundant species.
- Margalef's index (d): compares richness while accounting for sample size. Useful when total individuals (N) differ markedly among samples.
- Bray–Curtis dissimilarity (between zones): measures how different two samples are in composition and abundance (0 = identical; 1 = completely different).

Notes on use and interpretation

Effort dependence: Richness (S) and Margalef's index depend on total individuals (N). Evenness versus dominance: Shannon and Pielou's J' capture overall evenness; Simpson/Inverse Simpson and Hill $q = 2$ are more sensitive to common or dominant species. Effective species: Hill numbers ($q = 0, 1, 2$) translate diversity into intuitive equivalents and are directly comparable across zones. Diversity scales: α -diversity (per sample/replicate), γ -diversity (pooled per zone), and β -diversity (difference/complementarity between sets; e.g., Bray–Curtis).

4.2.3.3 Statistical comparison

Mann–Whitney U test: non-parametric test used to compare indicators between the impacted station and the control station at the replicate scale. Effect size (Cliff's delta): values near ± 1 indicate large effects, whereas values near 0 indicate little or no stochastic dominance.

4.2.4 Blue Carbon

This study was conducted in the established impacted and control stations in Cala Blanca (Fig. 4.7A and Fig. 4.7B) in November 2024 (Fig. 4.8). At both stations, sediment cores, seagrass vegetative biomass, seagrass wrack (hereafter referred to as litter) samples, and estimates of meadow cover and shoot density were obtained (Fig. 4.1E and Fig. 4.1F).

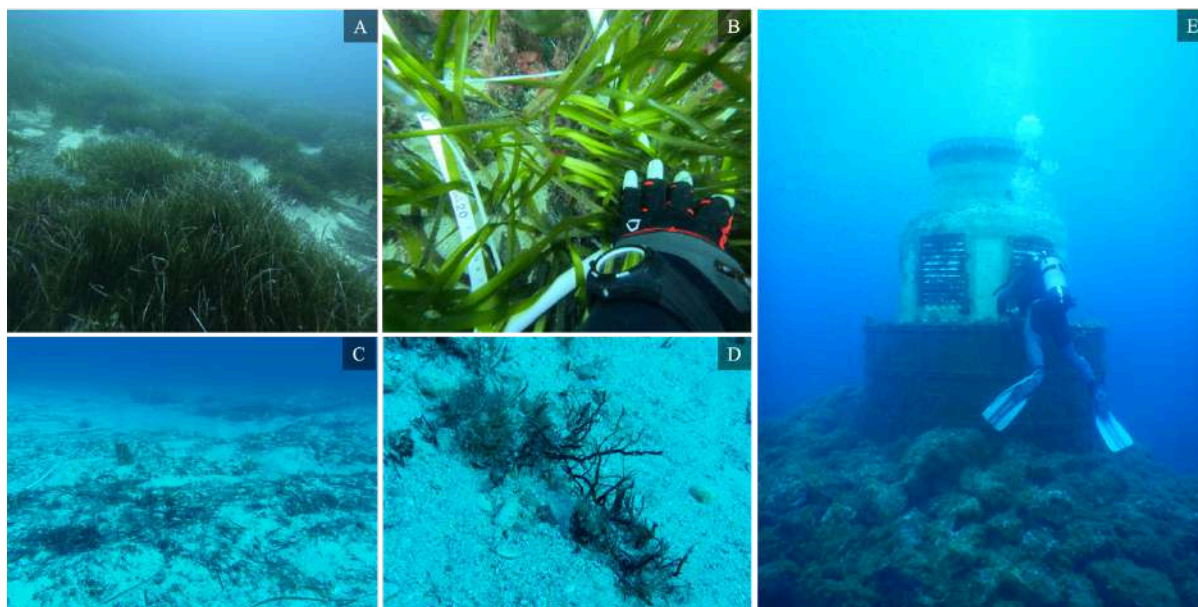


Figure 4.7 (A and B) Images showing the sampling locations of seagrass meadow structure, including biomass, wrack, shoot density and % cover, and sediment cores within the (C and D) control and impacted stations. (E) Seawater intake tower built for the desalination plant.



Figure 4.8 (A) team of researchers from IME-OBSAM and CEAB-CSIC ready to depart for the sampling mission from the Ciutadella harbour (Menorca). (B) cutting down the PVC pipe to cap the core above the sediment surface prior to transport.

Three replicate sediment cores were sampled at 20 m water depth within the control station of mono-specific seagrass *P. oceanica* meadows using manual percussion and rotation (PVC pipe with an inner diameter of 80 mm; Table 4.2) upon refusal. Three additional replicate sediment cores were sampled also upon refusal at 20 m water depth within the impacted station at 10 - 20 m west of the seawater collection tower built for the desalination plant. A rea bar (1.5 m in length) was used to test the thickness of sediment deposits in the study stations, which ranged from 0 to 45 cm, and 40 to 70 cm in the impacted and control stations, respectively. The total length of the PVC core barrel used, the empty space inside the barrel before retrieval, the length of barrel outside

the sediment before retrieval, and the length of the retrieved seagrass sediment core were recorded to correct the core lengths for compression effects. The whole cores were capped above the sediment surface and sealed at both ends before processing in the laboratory within 24 hours. All variables studied here are referenced to the corrected, uncompressed depths (in the text, Figures and Tables).



Table 4.2 Details of the sediment cores sampled, including the GPS position, substrate type (bare sand, dead *P. oceanica* matte, and *P. oceanica*), treatment (impacted and control), water depth, the total length of sediment recovered, and the % compression during coring operations.

Core ID	Latitude	Longitude	Substrate type	Treatment	Water depth (m)	Total sediment recovered (cm decompressed)	Compression (%)
ART_1	39° 58.279'N	3° 49.727'E	Bare sand	Impacted	20.4	33	0.0
ART_2	39° 58.288'N	3° 49.731'E	Dead matte	Impacted	19.8	40	21.1
ART_3	39° 58.296'N	3° 49.736'E	Dead matte	Impacted	19.7	34	5.9
ART_4	39° 58.451'N	3° 49.745'E	<i>P. oceanica</i>	Control	19.8	59	10.5
ART_5	39° 58.443'N	3° 49.742'E	<i>P. oceanica</i>	Control	19.9	41	13.2
ART_6	39° 58.463'N	3° 49.750'E	<i>P. oceanica</i>	Control	19.4	64	10.4

The cores were opened lengthwise using a circular saw to expose the sediments (Fig. 4.9), and cores ART_1 and ART_2 (impacted station), and cores ART_4 and ART_5 (control station) were cut into 5 cm-thick slices, whereas cores ART_3 (impacted station) and ART_6 (control station) were cut at 1 cm-thick slices. The samples were placed in zip block bags (Fig. 4.10B) and dried at 70 °C until constant weight to determine the dry bulk density (DBD; g DW cm⁻³). All sediment samples were divided in two subsamples by quartering, with one subsample milled in an agate mortar for sedimentary organic carbon (C_{org}) content and $\delta^{13}\text{C}$ analyses, and the other subsample kept bulk for sediment grain-size analyses. Sedimentary C_{org} and $\delta^{13}\text{C}$ analyses were run in all samples from cores ART_1, ART_2, ART_4 and ART_5, and in alternate samples of cores ART_3 and ART_6. For the analyses of sedimentary C_{org} and $\delta^{13}\text{C}$, 1 g of ground sample was acidified with 4 % HCl to remove inorganic carbon, centrifuged (3,400 revolutions per minute, for 5 min), and the supernatant with acid residues was carefully removed by pipette, avoiding resuspension. The sample was then washed with Milli-Q water, centrifuged and the supernatant removed. The residual samples were re-dried and then encapsulated for analyses using a FlashEA1112 (ThermoFinnigan) coupled to a MAT253 (ThermoFinnigan) at the Unidade de Técnicas Instrumentais de Análise (UTIA) at the University of Coruña, Spain. The content of C_{org} was calculated for the bulk (pre-acidified) samples. Carbon isotope ratios are expressed as δ values in parts per thousand (‰) relative to the Vienna PeeDee Belemnite standard. Replicate assays and standards indicated measurement errors of 0.5 % for C_{org} content and 0.15 ‰ for $\delta^{13}\text{C}$.

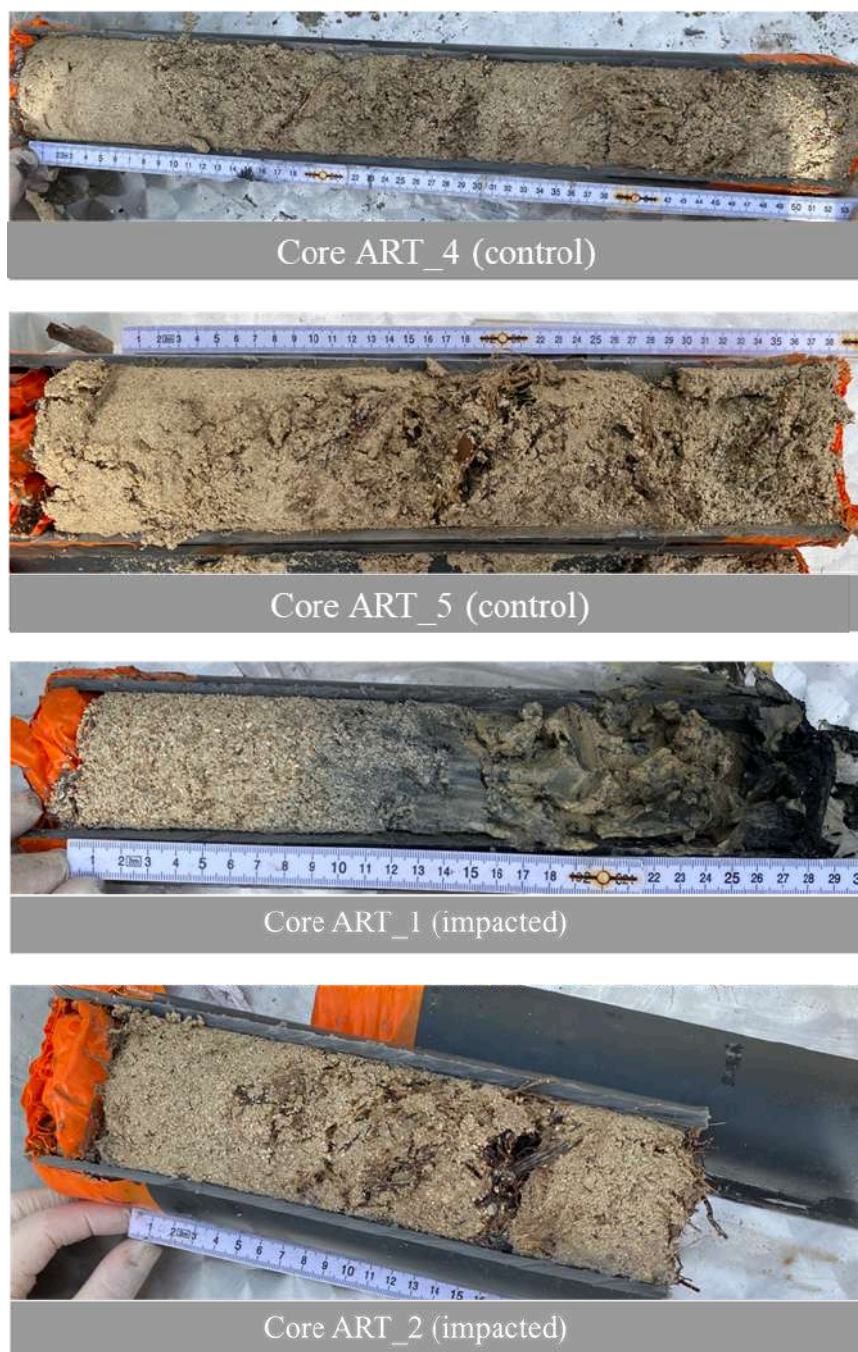


Figure 4.9 Images of the opened cores ART_4 and ART_5 collected at the control station (seagrass area) and cores ART_1 and ART_2 sampled at the impacted station.



Figure 4.10 (A) Seagrass *P. oceanica* plants including living above- and below-ground tissues being prepared for biomass estimates. (B) Sliced sediment core samples inside plastic zip-block bags ready.

Sediment grain-size analyses were run in all samples from all cores. For sediment grain-size analyses, a Mastersizer 2000-Malvern laser-diffraction particle analyser was used following sieving (1 mm) and digestion of < 1 mm sediment samples with 30 % hydrogen peroxide. Grain size fractions were categorized following Wentworth scale: clay and silt particles (< 0.063 mm), very fine sand (> 0.063 < 0.125 mm), fine sand (> 0.125 < 0.25 mm), medium sand (> 0.25 < 0.5 mm), coarse sand (> 0.5 < 1 mm), and very coarse sand plus gravel (> 1 mm; Wentworth, 1922).

The sediment cores ART_3 and ART_6 were dated by means of ^{210}Pb (last decades) and ^{14}C radiocarbon (last centuries). Concentrations of ^{210}Pb (Fig. 4.11) in the upper 21 cm were determined by alpha spectrometry through the measurement of its granddaughter ^{210}Po using Passivated Implanted Planar Silicon, PIPS detectors (CANBERRA, Meriden, CT, USA, Mod. PD-450.18 A.M), assuming radioactive equilibrium between the two radionuclides. After alpha spectrometry, selected samples were analysed for ^{226}Ra by ultra-low background liquid scintillation counting (Quantulus 1220, Wallac Oy, Finland). The concentration profile of excess ^{210}Pb was determined by subtraction of ^{226}Ra from total ^{210}Pb concentrations along the core.

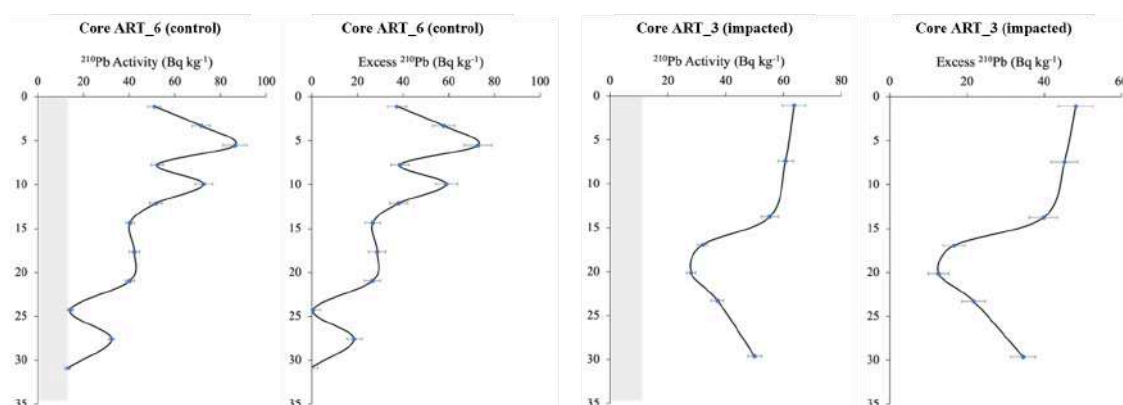


Figure 4.11 ^{210}Pb total activity and excess ^{210}Pb (Bq kg^{-1}) with sediment depth in cores ART_6 (control) and ART_3 (impacted). The shaded grey area indicates the supported ^{210}Pb activity. It was only possible to obtain age-depth models in core ART_6 by averaging concentrations over the surface mixed layer (top five data).

The surface total ^{210}Pb specific activities in core ART_3 (impacted station) were 64 Bq kg^{-1} that fluctuated with depth without any clear decreasing trend (Fig. 4.11). Nearly constant surface ^{210}Pb



concentrations in the upper 13 cm followed by lows and highs in total ^{210}Pb concentrations suggest intense downcore mixing of the sediment profile, or the accumulation of reworked sediment that lack excess ^{210}Pb (Arias-Ortiz et al., 2018). ^{226}Ra (or supported ^{210}Pb) in section 27 - 28 was found to be $15 \pm 2 \text{ Bq kg}^{-1}$. Although excess ^{210}Pb was present throughout all analysed sections with depth, indicating recent ($< 100 \text{ yr}$) sediment deposition, no age model could be applied due to intense downcore mixing (Krishnaswamy et al., 1971; Sanchez-Cabeza et al., 1998). On the contrary, core ART_6 from the control station showed surface total ^{210}Pb specific activities around 50 Bq kg^{-1} , with a subsurface peak of 86 Bq kg^{-1} at 5 cm and a decrease then after. ^{226}Ra (or supported ^{210}Pb) measured at 28 cm was $14 \pm 3 \text{ Bq kg}^{-1}$ and agreed with total ^{210}Pb specific activities measured at 22 and 28 cm. We applied the CF:CS model to estimate apparent average mass accumulation rates, by calculating the weighted average of grouped samples affected by mixing, specifically, the first 5 cm, and then 6 - 9 cm, and determined the weighted excess ^{210}Pb specific activity for each group before applying the CF:CS model. The mass accumulation rate was estimated at $0.70 \pm 0.12 \text{ g cm}^{-2} \text{ yr}^{-1}$. These estimates should be considered upper limits given the extent of mixing.

A total of eight radiocarbon ^{14}C analyses were conducted in cores ART_3 (impacted station) and ART_6 (control station) at the Yale University following standard procedures (Stuiver and Pollack, 1977). Samples consisted of pooled sheaths of *P. oceanica* debris, which were rinsed three times in ultrapure MQ water to remove fine sediment particles, inspected under a stereomicroscope for absence of attached reworked materials, and dried at 70°C to a constant weight before radiocarbon dating. All the ^{14}C results indicated that the samples dated were modern (younger than ~ 600 years) and therefore, it was not possible to calibrate the ^{14}C ages using post-bomb curves and thereby, it was not possible to obtain robust age-depth models based on calibrated ^{14}C ages using conventional approaches (Table 4.3). Although it might be possible to obtain ^{14}C -based age-depth models using post-bomb calibration curves, it was not attempted owing to the matching scales of ^{14}C and ^{210}Pb ages and thereby, only ^{210}Pb data was used to produce age depth models when possible.



Table 4.3 Raw radiocarbon results obtained from ^{14}C dating of *P. oceanica* sheaths along cores ART_3 (impacted) and ART_6 (control).

Yale Uni code	Core ID	Treatment	Depth (cm decompressed)	Radiocarbon age	
				Year	Error ($\pm$)
66081.1.1	ART_3	Impacted	1	-413	51
66082.1.1	ART_3	Impacted	16	-461	49
66083.1.1	ART_3	Impacted	24	-668	50
66084.1.1	ART_3	Impacted	33	-547	50
66085.1.1	ART_6	Control	1	-392	48
66086.1.1	ART_6	Control	21	330	52
66087.1.1	ART_6	Control	33	361	53
66088.1.1	ART_6	Control	56	356	52

A total of 34 seagrass whole plants, including living above- and below-ground biomass, were sampled within the control station of *P. oceanica* meadow (N = 28), and the meadows neighbouring the seagrass lost area at the impacted station (N = 6) for estimating seagrass vegetative biomass, organic carbon (C_{org}) content, and stable carbon isotope composition ($\delta^{13}\text{C}$) in vegetative biomass (Figure 4.10A). Whole seagrass plant samples were vigorously shaken in seawater to remove attached organic and inorganic particles, and particles further detached by rubbing the plants by hand prior to drying at 70 °C until constant weight (dry weight, DW) to estimate above- and below-ground biomass (g DW). Epiphytes within three replicate individual whole seagrass plants from each impacted and control stations were scrapped using a glass slide and dried at 70 °C until constant weight, milled until fine powder using an electric mill, and encapsulated for C_{org} and $\delta^{13}\text{C}$ analyses using a FlashEA1112 (ThermoFinnigan) coupled to a MAT253 (ThermoFinnigan) at the UTIA at the University of Coruña, Spain. The content of C_{org} was calculated for the bulk seagrass vegetative samples including above- and below-ground biomass. The $\delta^{13}\text{C}$ ratios are expressed as δ values in parts per thousand (‰) relative to the Vienna PeeDee Belemnite standard. Replicate assays and standards (USGS 40, USGS 41a, NBS 22, USGS 24 and Acentanilida) indicated measurement errors of 0.5 % for C_{org} content and 0.15 ‰ for $\delta^{13}\text{C}$.

Seagrass cover (%) and shoot density (number of shoots m^{-2}) were assessed along a randomly deployed 30 m transect at the impacted and control stations, with eight measurements every 4 m along each transect. The shoot density estimates (number of shoots per area) were obtained with a 20 x 20 cm quadrat, whereas four 20 x 20 cm measurements within 40 x 40 cm quadrats were used to estimate % seagrass cover and % litter cover.

A total of 10 seagrass litter samples were collected in the impacted (N = 5) and control (N = 5) stations, consisting of all dead biomass present within five units of 10 x 10 cm quadrats. Quadrats were randomly placed, but only those containing litter were sampled. The litter samples were dried at 70 °C until constant weight to estimate litter biomass (g DW litter m^{-2}).

The C_{org} density (g C_{org} cm^{-3}) was calculated for each sediment depth in each core by multiplying the sediment DBD (g cm^{-3}) by the C_{org} concentration (%; Table 4.17). For sediment depths where



C_{org} content (%) was not analysed, we extrapolated the % C_{org} (i.e. by averaging the % C_{org} between above and below sediment core depths) and multiplied the % C_{org} by the DBD to obtain C_{org} density. To allow direct comparisons among locations, the sedimentary C_{org} standing stocks per unit area (cumulative stocks; $Mg\ C_{org}\ ha^{-1}$) were standardized to 40 cm-thick deposits. The total sediment depth sampled was higher than 40 cm in four cores out of six cores sampled and therefore, no extrapolation was required for these cores. However, the sedimentary C_{org} stocks in 40 cm-thick sediment deposits were inferred in two cores (from 33 to 40 cm in core ART_1, and from 34 to 40 cm in core ART_4) by extrapolating linearly integrated values of C_{org} content (cumulative C_{org} stock; $g\ C_{org}\ cm^{-2}$) with depth. Sedimentary C_{org} accumulation rates (expressed in $g\ C_{org}\ m^{-2}\ yr^{-1}$) for the last decades were estimated using ^{210}Pb age-depth models in core ART_6 (Table 4.17).

The seagrass shoot density and % cover data were averaged across the measurements conducted within each control and impacted stations and converted to m^2 . The seagrass biomass data (g DW per plant) were combined with the shoot density and % C_{org} content in biomass data to obtain estimates of seagrass vegetative biomass and vegetative C_{org} stocks per unit area. The litter biomass data per unit area was multiplied by the C_{org} content (%) in seagrass biomass to obtain the litter C_{org} stocks per unit area.

Analyses to test for differences in the variables studied between impacted and control stations were performed using General Linear Model (probability distribution: gaussian; link function: identity) procedures in SPSS v. 14.0 (Table 4.17). General Linear Models were used to test for differences in DBD ($g\ cm^{-3}$), sedimentary organic carbon content (%), sedimentary organic carbon density ($mg\ C_{org}\ cm^{-3}$), sedimentary C_{org} stocks ($Mg\ C_{org}\ ha^{-1}$ in 40 cm-thick sediments), $\delta^{13}C$ signatures of sedimentary organic carbon (‰), sediment grain size fractions (%) $< 0.063\ mm$, $> 0.063 < 0.125\ mm$, $> 0.125 < 0.25\ mm$, $> 0.25 < 0.5\ mm$, and $> 0.5 < 1\ mm$, and $> 1\ mm$ (%), seagrass shoot density (number of shoots m^{-2}), seagrass vegetative biomass ($g\ DW\ m^{-2}$), C_{org} content in seagrass vegetative biomass (%), seagrass vegetative biomass C_{org} content ($g\ C_{org}\ m^{-2}$), seagrass cover (%), litter cover (%), litter biomass ($g\ DW\ m^{-2}$), C_{org} content in seagrass litter (%), litter biomass C_{org} content ($g\ C_{org}\ m^{-2}$), and $\delta^{13}C$ signatures of vegetative biomass (‰) between impacted and control stations.

4.3 Results

4.3.1 Habitat mapping

The benthic habitats of the restoration area were characterized by a rocky bottom fringe colonized by photophilic algae down to a depth of approximately 8 m, followed by a continuous *P. oceanica* meadow extending from the rocky coastal margin to around 34 m depth. This seagrass belt encircled nearly the entire coastline of the island, only interrupted by drainage channels associated with coves and bays. Beyond the *Posidonia* meadow, sandy bottoms occurred, hosting maërl communities.

Within the specific restoration zone, the mapped habitats included dead *Posidonia* matte interspersed with scattered patches of regenerating seagrass (1,742.4 m²), rocky bottoms corresponding mainly to the riprap surrounding the intake tower (161 and 9.7 m²), sandy areas produced by abrasion from the buoy chain and rope that had removed the dead matte substrate (48.7 m²), and the pipeline (3.6 m²) (Fig. 4.12 and Table 4.4).

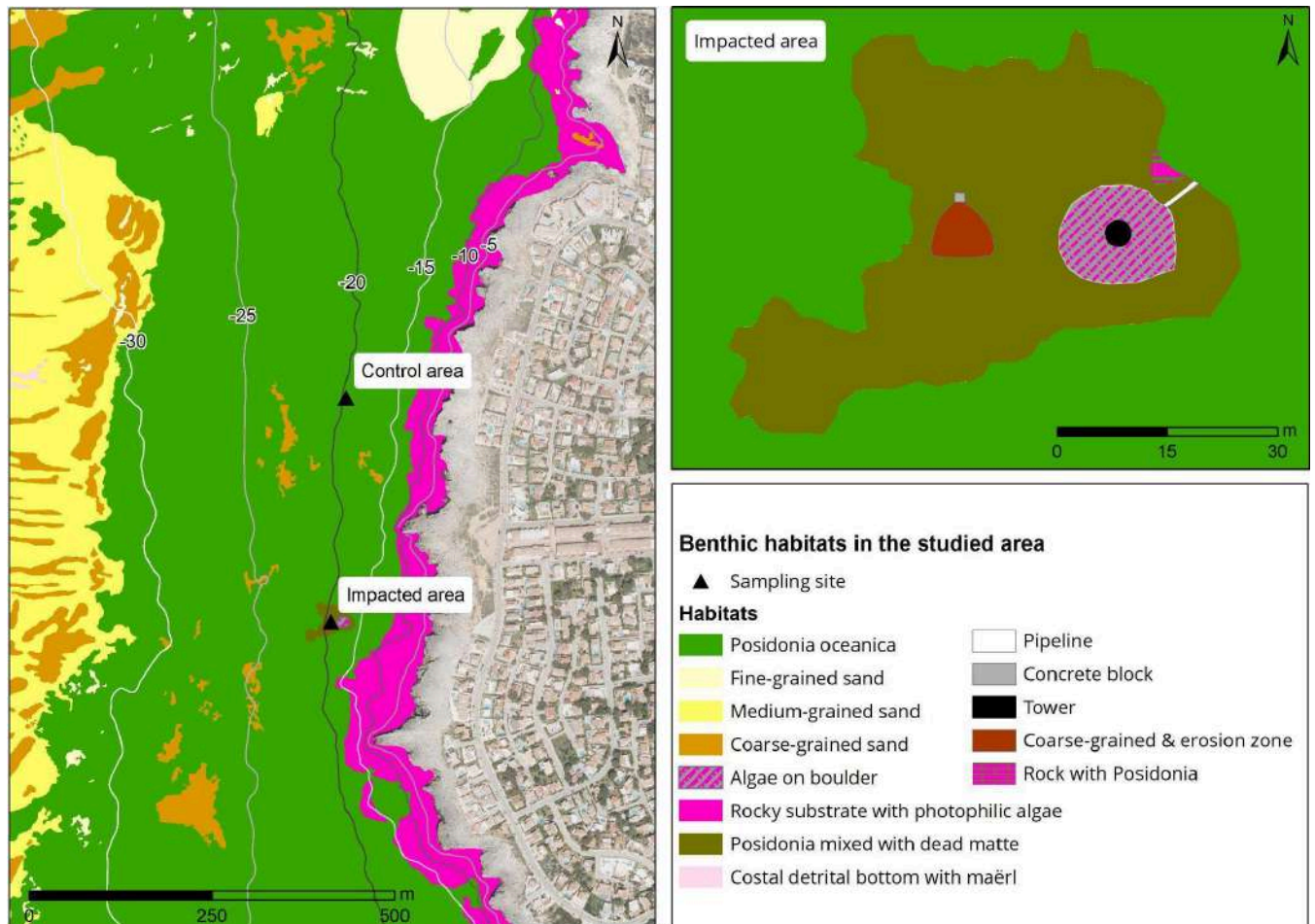


Figure 4.12 Benthic habitats within the study area and the surrounding region, including elements of the restoration zone that may influence plantation activities, such as the intake tower, pipeline, and the degraded area caused by the chain and the existing buoy mooring.

Table 4.4 Surface area of each bottom type in the restoration zone in square meters.

Bottom type	Area (m ²)
Dead <i>Posidonia</i> matte with scattered patches	1742.4
Riprap & algae on boulder	161.0
Concrete block	1.9
Medium-grained sand	48.7
Rock with <i>Posidonia</i>	11.6
Tower	9.7
Pipeline	3.6

In addition to habitat mapping and confirming the presence of dead *Posidonia* matte, it should be highlighted that before restoration works commence, the contracted company will carry out a detailed cartographic survey of the area. This survey will assess substrate thickness, as field observations showed strong variability across the restoration site, with shallow rock outcrops in some sectors that preclude deep staking. Therefore, the most suitable dead matte areas will be selected to ensure secure anchorage of transplanted rhizomes. The restoration strategy will not reduce the overall number of fragments but will instead maintain their total amount while increasing planting density in the most favourable areas.

4.3.2 Ecological status

The conservation status was assessed following the density-based classification system for *Posidonia oceanica* meadows proposed by Pergent et al. (1995), later revised by Pergent-Martini et al. (1999) and UNEP/MAP-RAC/SPA (2015). The updated framework defines five categories of conservation status—very good, good, moderate, poor, and bad—based on mean shoot density at a given depth. The thresholds applied in this report correspond to those published in the *Guidelines for the Standardization of Mapping and Monitoring Methods of Marine Magnoliophyta in the Mediterranean* (UNEP/MAP-RAC/SPA, 2015).

In the case of Menorca, the control station is classified as being in very good condition, with a global shoot density (shoot density adjusted by percent cover) of 556.45 shoots/m². This category corresponds to values above 366 shoots/m² at 20 metres depth. In contrast, the impacted station is classified as bad, with a global shoot density of 56.60 shoots/m², falling within the range defined by densities below 82 shoots/m² at the same depth.

Other parameters contributing to the assessment of the conservation status, following the references cited above, indicate that the lower depth limit of both stations is in good condition, as it is stable and shows no presence of dead matte. The two stations are located very close to each other, and the conditions at the depth limit are considered to be similar.

As a reference for the study area, the ecological status of the coastal water body—according to

the Water Framework Directive—is Good, which applies to the water body that includes both the control and impacted stations (Santandreu, M.M. et al., 2019).

The following section introduces the results of the EBQI assessment. In this section, the individual results for each box are first presented, followed by the calculation of the EBQI index for the impacted and control stations. Subsequently, the individual results for each box and the number of replicates performed are shown (Table 4.5).

Table 4.5 Results (mean $\pm$ standard deviation) of each functional compartment and parameter. Values are presented for the impacted and control stations, with the number of replicates (N) specified for each.

Box n°	Functional compartment and Parameter	Impacted station	N	Control station	N
1	<i>P. oceanica</i> roots and rhizomes. Growth of orthotropic rhizomes (mm y ⁻¹)	9.5 $\pm$ 5.0	19	7.6 $\pm$ 2.4	23
2	<i>P. oceanica</i> leaves. Density (shoots m ⁻²)	514 $\pm$ 127	20	630 $\pm$ 106	20
	<i>P. oceanica</i> leaves. Cover (%)	11 $\pm$ 18	53	88 $\pm$ 11	50
3-4	Multicellular Photosynthetic organisms (MPO), filter- and suspension-feeders leaf epibiota. Biomass (g DM shoot ⁻¹)	0.049 $\pm$ 0.018	19	0.041 $\pm$ 0.023	23
5	Benthic filter-feeders (<i>Pinna nobilis</i>). Density (individuals 100 m ⁻²)	0	30	0	29
6	Other benthic filter- and suspension-feeders. High levels of organic matter in the water (HOM) (individuals m ⁻²)	0	30	1.38 $\pm$ 2.68	29
	Other benthic filter- and suspension-feeders. Low levels of organic matter in the water (LOM) (individuals m ⁻²)	0	30	7.03 $\pm$ 9.66	29
7	Litter detritus: dead leaves and broken rhizomes	4.75 $\pm$ 4	2	6.09 $\pm$ 1.365	3
8	Detritus feeders (<i>Holothuria</i> sp.) (individuals 10 m ⁻²)	16 $\pm$ 22.5	30	0	29
9	Herbivores. <i>Paracentrotus lividus</i> (individuals m ⁻²)	0	30	0	29
	Herbivores. Grazing index (% leaves)	2.3 $\pm$ 7.1	30	0.7 $\pm$ 3.5	29
10	Predatory teleosts, cephalopods and seastars (individuals 100 m ⁻²)	0.13 $\pm$ 0.02	3	0.11 $\pm$ 0.17	3
11	Piscivorous teleosts (individuals 100 m ⁻²)	0	3	0.0004 $\pm$ 0.001	3
12	Zooplankton feeders (individuals 100 m ⁻²)	0.01 $\pm$ 0.01	3	0.06 $\pm$ 0.03	3
13	Sea birds (<i>Gulosus aristotelis desmarestii</i>) (km)	1.10	1	1.30	1
	Sea birds (<i>Pandion haliaetus</i>) (km)	13.46	1	13.16	1

For orthotropic rhizome growth (box 1), the mean growth in the impacted station was 9.5 mm yr⁻¹ compared to 7.6 mm yr⁻¹ in the control station. This indicates that rhizomes in the impacted station exhibit higher growth rates, probably related to the younger age of the shoots and their response to the need for colonizing available space showing a growth form intermediate between horizontal and vertical (Molenaar et al. 1993; Marbà & Duarte, 1998; Piazzzi et al., 1998). Conversely, in the control station, rhizome growth was more moderate and stable throughout the



year, which may be associated with the more mature and stable state of the meadow.

Regarding *P. oceanica* shoot density (box 2), no major differences were observed between control and impacted stations (630 and 514 shoots m⁻², respectively), although the impacted station exhibited a lower density. However, seagrass cover showed a much greater contrast between stations, with only 11 % of the impacted station being covered compared to 88 % of the control station. This highlights the structural degradation of the meadow associated with the bentonite discharge.

The relatively high shoot density observed in the impacted station is explained by the sampling methodology, as samples were randomly taken but only in patches where seagrass was present. This leads to an overestimation of shoot density, since unvegetated patches are not accounted for. Therefore, by calculating the global density (density × cover) for each area, a more realistic approximation is obtained: 56.54 shoots m⁻² in the impacted station and 554.5 shoots m⁻² in the control station. According to the classification proposed by Pergent et al. (1995) and UNEP/MAP-RAC/SPA (2015) for assessing seagrass conservation status based on global density at 20 m depth, the impacted station is classified as being in “bad” condition, whereas the control station is classified as in “very good” condition. These results indicate that, although live shoots remain in the impacted station, their distribution is fragmented and patchy. When density is considered alone, the sampling design, which favoured areas of maximum shoot density, fails to reflect the true condition of the meadow. In contrast, the control meadow is homogeneous and dense.

For epibiotic biomass (box 3 – 4), values were low in both stations and did not differ substantially: 0.049 g shoot⁻¹ in the impacted station versus 0.041 g shoot⁻¹ in the control station. The similarity in values may be explained by the overall comparable environmental conditions in the study area and by the fact that sampling targeted sites where seagrass was present; thus, the lower meadow cover in the impacted station did not strongly affect results. Prado et al. (2008) showed that epiphyte load increases significantly under nutrient enrichment conditions. The low epibiont biomass in our study suggests that waters in the area remain oligotrophic, with no evidence of nutrient enrichment from the bentonite discharge that could have promoted epiphyte proliferation.

No individuals of *Pinna nobilis* were detected in either the impacted or control stations (box 5). This outcome is consistent with the situation throughout the Balearic Islands and Western Mediterranean, where the species has suffered mass mortality since 2016. Before the crisis, the average density of *P. nobilis* in Menorca was 2.7 ind. 100 m⁻², with local maxima of 13.3 ind. 100 m⁻² (Álvarez et al., 2018). This population, monitored since 2014, was almost exterminated during the summer of 2017, leaving only two surviving individuals detected in 2018 across the island. Therefore, the current absence of *P. nobilis* cannot be attributed to the direct impact of the desalination plant, and this indicator loses its diagnostic value for the assessment of the project.

For other benthic filter- and suspension-feeders (box 6), no individuals were recorded in the impacted station, while low densities were detected in the control station (1.4 ind. m⁻² for HOM and 7.0 ind. M⁻² for LOM). This pattern reflects habitat simplification due to reduced seagrass



cover in the impacted station and corresponds to the "gray zone" described by Airolidi et al. (2008), where habitat loss and simplification lead to reduced biodiversity and deterioration of essential ecological functions. Thus, this parameter may serve as a reliable indicator of meadow stability in the control station.

The results for litter accumulation (box 7) showed values of 183 g m^{-2} in the control station and 65 g m^{-2} in the impacted station. Despite variability, these differences suggest that the better-conserved meadow maintains a more developed detrital compartment with greater carbon storage capacity, compared to the impacted station. This pattern is consistent with Romero et al. (1992), who demonstrated that decomposition rates, nutrient retention, and litter mineralization are determined by meadow cover and stability. In other words, a meadow with higher litter accumulation provides a more robust carbon and nutrient sink, an essential ecosystem service that is impaired in the impacted station.

For holothurians (box 8), 16 individuals per 10 m^2 were recorded in the impacted station, while no individuals were found in the control station. This pattern may be explained by the greater sandy substrate availability in the impacted station due to lower seagrass cover, which provides suitable habitat for these deposit-feeding echinoderms. Thus, the higher abundance of holothurians in the impacted station does not reflect better ecological status, but rather a functional shift from seagrass meadow habitat to unvegetated sandy areas. No sea urchins (*Paracentrotus lividus*) were found in either station, although the herbivory index indicated some grazing activity, particularly in the impacted station (2.3 %, box 9). This suggests that other herbivores, such as the fish *Sarpa salpa*, may be contributing to seagrass leaf consumption.

Densities of mobile predators (fish, cephalopods, and asteroids, box 10) were very low and similar in both stations ($0.13 \text{ ind. } 100 \text{ m}^{-2}$ in the impacted station vs. $0.11 \text{ ind. } 100 \text{ m}^{-2}$ in the control station). This indicates that predator assemblages associated with the meadow are scarce regardless of the local impact of the desalination plant. Such low abundance may be linked to overfishing and the broader degradation of coastal habitats.

Indicators of mean abundance for different trophic groups of ichthyofauna (piscivores, zooplanktivores, and omnivores; boxes 11 – 12) showed either absence or minimal presence in the impacted station, whereas the control station, characterized by stable *P. oceanica* beds, supported higher ichthyofauna abundances across all trophic categories. Nevertheless, in both stations low biomass values are observed (Table 4.5), mainly because the individuals recorded were of small size. The results obtained for both stations suggest a high fishing pressure in the region, as shown in Cardona et al. (2002).

Finally, the functional compartment associated with the seabird community, defined as the distance between the impacted and control stations and the nearest known nesting sites of *Gulosus aristotelis desmarestii* and *Pandion haliaetus*, differed between species. For *G. aristotelis*, the closest colony is located south of both stations, at 1.1 km from the impacted station and 1.3 km from the control station. In the case of *P. haliaetus*, the distance is considerably greater, with the nearest nest located approximately 13.46 km from the control station and 13.16 km from the impacted station.

4.3.2.1 EBQI index

Parameter values of the different functional compartments assessed were used to assign an ecosystem status grade (S) following the criteria provided in Table 1 of Personnic et al. (2014). The relative weighting (W) of each functional compartment is also indicated in that same reference table. Similarly, the Confidence Index (CI) of the status of each functional compartment was determined according to Table 4.1 in Personnic et al. (2014). Since all parameter values were obtained from recent and direct field sampling, the CI of all functional compartments was set to 4.

The results of the EBQI assessment, including the application of these criteria, are presented in Table 4.6 of the present study, which shows the EBQI calculations for both the impacted station and the control station.

Table 4.6 Results of the ecological status assessment using the EBQI index in impacted and control stations. For each functional compartment, the following are shown: assigned weight (W), status (S), contribution to the index (CI), status score (W×S), and CI score (W×CI). The maximum possible score (W×Smax) is also indicated. Final EBQI values are provided in the last row of the table.

Box n°	Functional compartment	Weighting (W)	Status (S)	CI	Impacted station			Control station			
					Status grade (W×S)	CI grade (W×CI)	W×Smax	Status (S)	CI	Status grade (W×S)	CI grade (W×CI)
1	<i>P. oceanica</i> roots and rhizomes. Growth of orthotropic rhizomes	3	4	4	12	12	12	2	4	6	12
2	<i>P. oceanica</i> leaves	5	2	4	10	20	20	4	4	20	20
	Density	5	4	4	20	20	20	4	4	20	20
	Cover	5	0	4	0	20	20	4	4	20	20
3 - 4	Multicellular Photosynthetic organisms (MPO), filter- and suspension-feeders leaf epibiota.	4	1	4	4	16	16	1	4	4	12
5	Benthic filter-feeders (<i>Pinna nobilis</i>)	2	0	4	0	8	4	0	4	0	20
6	Other benthic filter- and suspension-feeders. HOM, LOM	2	2	4	4	8	4	2.5	4	5	20
	HOM	2	4	4	8	8	8	2	4	6	20
	LOM	2	0	4	0	8	8	3	4	4	16
7	Litter detritus: dead leaves and broken rhizomes	2	4	3	8	6	4	4	3	8	8
8	Detritus feeders. <i>Holothuria</i> sp.	2	2	4	4	8	8	1	4	2	8
9	Herbivores	5	1	4	5	20	20	1	4	5	8



	<i>P. lividus</i>	5	1	4	5	20	20	1	4	5	8
	Grazing index. <i>S. salpa</i>	5	1	4	5	20	20	1	4	5	6
10	Predatory teleosts, cephalopods and seastars	5	0	3	0	15	20	0	3	0	8
11	Piscivorous teleosts	5	0	3	0	15	20	0	3	0	20
12	Planktivorous teleosts	3	0	3	0	9	12	0	3	0	20
	Zooplankton feeders	3	0	3	0	9	12	0	3	0	20
	Omnivores	3	0	3	0	9	12	0	3	0	15
10 - 12	All teleosts	3	0	3	0	9	12	0	3	0	15
13	Seabirds	1	2,5	4	2,5	4	4	2,5	4	2,5	9
	<i>G. aristotelis</i>	1	4	4	4	4	4	4	4	4	9
	<i>P. haliaetus</i>	1	1	4	1	4	4	1	4	1	9
EBQI		2.9					3.1				

According to the classification proposed by Personnic et al. (2014), both study stations fall within the “Bad” ecological status category (EBQI < 3.5). The impacted station scored 2.9, while the control station reached 3.1. Although the control station presents a slightly higher value, this difference of only 0.2 points is not sufficient to place it in a higher status class. Given their close spatial proximity, both meadows are likely subjected to similar anthropogenic pressures, with the exception of the bentonite spill, which represents the unique disturbance accounting for the lower score of the impacted station. The negative effect of this accident is most evident in *P. oceanica* structural descriptors, particularly cover, which contributed to the reduced EBQI value. Nevertheless, in the impacted station, patches of shoots exhibited relatively high density and enhanced growth, reflecting the presence of younger shoots compared to those in the control station.

In both stations, the teleost fish compartments scored zero, revealing a severe depletion of ichthyofauna stocks. This pattern is consistent with observations in rocky reef ecosystems: Thibaut et al. (2017) applied an ecosystem-based index to rocky habitats—including four sites in Menorca—and found that areas outside marine reserves only reached a “moderate” status, while reserve sites attained a “high” ecological status. Their study also highlighted that Menorcan rocky habitats displayed good structural condition but poor ichthyofauna populations, a pattern attributed to fishing pressure. Similarly, in the seagrass meadows assessed here, ichthyofauna-related compartments provided the lowest scores, underscoring their sensitivity and their major contribution to determining the overall EBQI value.

In summary, while the control station (EBQI = 3.1) maintains slightly better structural integrity than the impacted station (EBQI = 2.9), both seagrass meadows are classified as being in Bad ecological status. These results suggest that a localized disturbance such as a bentonite spill can exacerbate degradation in ecosystems already under pressure, while consistently low ichthyofauna biomass confirms that the loss of upper trophic levels is a widespread feature across Mediterranean

coastal habitats.

4.3.3 Biodiversity

In this section, we present results for each habitat compartment, with the exception of epiphytes, for which only total epiphytic biomass was obtained. For the matte and canopy compartments, additional analyses were conducted and are described herein. Table 4.7 summarizes biodiversity metrics by compartment and study area. Because methodologies and sampling units differ among compartments, we did not compute a single value per study area; instead, each compartment was analyzed independently. Nevertheless, in the control station we recorded 44 species and 488 individuals across all compartments, whereas the impacted station recorded 36 species and 262 individuals.

Table 4.7 Summary of biodiversity metrics by habitat compartment. For each compartment, mean richness per station (Species Richness) and mean abundance per station (N_{mean}) expressed as mean $\pm$ SD based on the compartment's sampling unit; and Shannon diversity (H') and species evenness were calculated from pooled abundance data for each compartment.

		Matte (100 m²) (N=3)	Epifauna (100 cm²) (N= 3)	Ichthyofauna (m²) (N=3)	Epibenthic macrofauna (m²) (N= 30 impacted and N=29 control station)
		taxonomic groups			
Impacte d station	N_{mean}	9.29 $\pm$ 3.72	9.39 $\pm$ 6.44	0.12 $\pm$ 0.06	1.6 $\pm$ 2.2
	Species	8.3 $\pm$ 1.5	-	3.33 $\pm$ 2.08	0.36 $\pm$ 0.49
	Richness				
	Species	0.95	-	0.763	-
	Evenness				
Control station	H'	1.98	-	1.37	-
	N_{mean}	8.79 $\pm$ 3.13	12.11 $\pm$ 8.79	0.55 $\pm$ 0.42	1.8 $\pm$ 2.4
	Species	7.3 $\pm$ 2.5	-	5.33 $\pm$ 1.53	1.14 $\pm$ 0.95
	Richness				
	Species	0.95	-	0.483	0.688
	Evenness				
	H'	1.86	-	1.11	1.58

The compartment-wise assessment showed consistent differences between stations. In the matte, mean abundance was similar between stations, while the impacted station displayed slightly higher richness and Shannon diversity. In the canopy epifauna, where organisms were classified to taxonomic groups, diversity indices were not calculated, but the control station showed higher mean abundance. In the ichthyofauna, the control station exhibited higher richness and mean abundance, although with lower Shannon diversity, indicating greater numerical dominance by a few taxa. The strongest contrast occurred in the epibenthic macrofauna, where the impacted station contained only a single taxon, whereas the control station supported a notably richer and more diverse assemblage. Overall, richness and abundance tended to be higher in the control station across most compartments, with epibenthic macrofauna standing out as particularly



sensitive to the disturbance.

4.3.3.1 Ichthyofauna (water column)

The impacted station exhibits a slightly higher Shannon diversity ($H' = 1.37$) and higher Simpson's diversity ($1-D = 0.67$) than the control station ($H' = 1.11$; $1-D = 0.50$), indicating a more even distribution of individuals among species. This pattern is corroborated by Pielou's evenness ($J' = 0.76$ in the impacted station vs 0.48 in the control station). By contrast, species richness is greater in the control station (Margalef's $d = 1.76$) than in the impacted station ($d = 1.38$) (Table 4.8). Overall, these results suggest that observations of juvenile schools of *Spicara smaris* in the control station strongly influenced the diversity metrics; however, such shoals are highly mobile and less substrate-affiliated than other surveyed species, which should be considered when interpreting these indices.

Table 4.8 Number of individuals per species recorded in each transect (replicates 1–3) at the impacted and control stations. For each transect we report the total number of taxonomic groups (S) and total individuals (N), together with Shannon diversity (H'), Simpson dominance ($1-D$), Pielou evenness (J') and Margalef richness (d).

Station		Impacted station			Control station		
Replicate		1	2	3	1	2	3
Piscivor teleost	<i>Trachinotus ovatus</i>	0	0	0	2	0	0
Plantivor omnivor	<i>Oblada melanura</i>	0	0	0	0	0	1
Plantivor zooplankton	<i>Chromis chromis</i>	3	1	0	14	7	3
	<i>Spicara smaris</i>	0	0	0	9	75	30
Predador teleost	<i>Coris julis</i>	6	6	7	1	3	1
	<i>Diplodus annularis</i>	0	0	0	0	8	0
	<i>Diplodus vulgaris</i>	3	3	0	1	0	0
	<i>Mullus surmuletus</i>	6	0	0	0	0	0
	<i>Spondyliosoma cantharus</i>	0	1	0	0	0	0
	<i>Serranus scriba</i>	1	0	0	0	1	0
	<i>Symphodus tinca</i>	0	0	0	0	1	0
	<i>Thalassoma pavo</i>	0	0	0	0	8	0
Number of species per transect, S		5	4	1	5	7	4
Total number of individuals per transect; N		19	11	7	27	103	35
Shannon diversity, H'		1.466	1.121	0	1.144	1.004	0.546
Simpson dominance, $1-D$		0.748	0.612	0	0.612	0.452	0.256
Pielou equity, J'		0.911	0.809	0	0.711	0.516	0.394
Margalef richness, d		1.358	1.251	0	1.214	1.295	0.844

Differences among indices were small, and no significant between-station differences were detected for any metric (Mood's median test: all $p = 1$; for N, $\chi^2 = 2.67$, $df = 1$, $p = 0.102$) (Table 4.9). Only a tendency toward higher per-sample abundance was observed in the control station (median N: 35 vs 11); the remaining indices (S, H' , $1-D$, J' , d) showed very similar medians. The small number of replicates limits statistical power; moreover, as noted earlier, the reduced spatial

extent of the impacted station constrained achievable transect replication.

Table 4.9 Medians of the total abundance, and of the different diversity indexes of the three habitats studied, and results of the median tests to assess differences among them which, if significant, are indicated in bold.

Index	Impacted median	Control median	Chi-square	d.f.	P
S	4	5	0	1	1
N	11	35	2.67	1	0.10
H'	1.12	1.00	0	1	1
1-D	0.61	0.45	0	1	1
J'	0.81	0.52	0	1	1
d	1.25	1.21	0	1	1

4.3.3.2 Epibenthic macrofauna (rhizomes)

Epibenthic macrofauna, those invertebrates living over the seafloor or epibiotic on *P. oceanica* rhizomes, was very different between the impacted and control stations. Sea urchins were not present in any of the two stations while sea cucumbers (*Holothuria* sp.) were found in the impacted station only (16 ± 22.5 individuals per 10 m^{-2} , $N = 30$). The rhizomes of *P. oceanica* had no epibiotic macrofauna while 10 taxa of annelida, bryozoan and sponges were present in the rhizomes of *P. oceanica* in the control station (Table 4.10).

Table 4.10 Epibenthic macrofauna associated with *Posidonia oceanica* rhizomes in the control station. Values are expressed as total abundance (individuals m^{-2} , mean ± 1 SD, $N = 29$). No epibiotic macrofauna was recorded on rhizomes in the impacted station.

	Total abundance, individuals m^{-2}
	mean ± 1 SD, $N=29$
Annelida: <i>Sabella pavonina</i>	0.35 ± 0.67
Bryozoan: <i>Reteporella grimaldi</i>	0.07 ± 0.26
Bryozoan: <i>Calpensia nobilis</i>	1.07 ± 1.81
Bryozoan: unknown white species	0.03
Poriphera: <i>Crambe crambe</i>	0.27 ± 0.59
Poriphera: <i>Clathrina</i> sp	0.10
Poriphera: <i>Crella elegans</i>	0.03
Poriphera: unknown orange species	0.03
Poriphera: unknown gray species	0.03
Poriphera: unknown bluish black species	0.10

The higher abundance of sea cucumbers in the impacted station may be due to the higher extension of sediment devoid of vegetation while the absence of epibiotic macrofauna on *P. oceanica* rhizomes might be caused by sediment erosion and burial/unburial cycles that the rhizomes might be exposed to in the impacted station. Higher stability of sediment in the control station would facilitate the establishment of epibiotic organisms on *P. oceanica* rhizomes.

4.3.3.3 Epifauna (canopy)

The number of taxonomic groups of vagile organisms living in the impacted *P. oceanica* canopy



was higher than those living in macroalgae present in dead matte or in control *P. oceanica* (Tables 4.11 and 4.12). Total abundance tended to be also higher in the impacted *P. oceanica* canopy than in the other two habitats, but differences were not statistically significant. Shannon diversity, Simpson dominance and Pielou equity indexes were not different among habitats, but Margalef richness was highest in the impacted *P. oceanica* canopy (Tables 4.11 and 4.12).

Table 4.11 Number of individuals of each taxonomic group in each of the epifaunal samples, and total number of taxonomic groups (S), and individuals (N) per sample, Shannon diversity (H'), (1-D) Simpson dominance, Pielou equity (J') and Margalef richness (d) indexes calculated for each sample. The biomass (g) of macrophytes (*P. oceanica* leaves and/or macroalgae) of each sample is also included.

Station	Impacted station						Control station		
Habitat	<i>P. oceanica</i>			Dead Matte			<i>P. oceanica</i>		
Replicate	1	2	3	4	5	6	7	8	9
Taxonomic group									
Pycnogonida	1	0	1	0	1	0	0	0	2
Acari	4	3	1	0	0	0	10	4	3
Ostracoda	2	2	0	1	0	1	0	0	0
Copepoda	24	76	9	0	6	4	78	22	23
Caprellidae	3	11	9	5	10	2	2	1	3
Gammaridae	2	15	9	0	12	0	25	1	0
Mysidacea	0	2	0	0	0	1	1	0	1
Isopoda	0	2	5	1	3	0	0	0	1
Bivalvia	2	0	14	0	14	9	0	0	1
Gastropoda	6	2	72	3	29	35	11	6	4
Echinoidea	0	0	3	0	2	0	0	0	2
Nematoda	8	19	4	0	0	2	2	0	0
Polychaeta	8	21	22	6	19	10	10	6	8
Number of taxonomic groups per sample, S	10	10	11	5	9	8	8	6	10
Total number of individuals per sample; N	60	153	149	16	96	64	139	40	48
Shannon diversity, H'	1.87	1.60	1.72	1.39	1.87	1.42	1.37	1.31	1.71
Simpson dominance, 1-D	0.80	0.71	0.73	0.77	0.83	0.66	0.64	0.66	0.74
Pielou equity, J'	0.81	0.69	0.72	0.86	0.85	0.68	0.66	0.73	0.74
Margalef richness, d	2.20	1.79	2.00	1.44	1.75	1.68	1.42	1.36	2.32
Macrophyte biomass per sample, g	7.31	13.96	6.01	4.08	10.31	4.66	26.92	15.88	16.59

Table 4.12 Medians of the number of taxonomic groups, total abundance, and of the different diversity indexes of the three habitats studied, and results of the median tests to assess differences among them which, if significant, are indicated in bold.

	Impacted <i>P. oceanica</i>	Impacted Dead Matte	Control <i>P. oceanica</i>	Median test
Number of taxonomic groups per sample, S	10	8	8	Chi-square=6.3, d.f.=2, P=0.04
Total number of individuals per sample, N	149	64	48	Chi-square=0.9, d.f.=2, P=0.64
Shannon diversity, H'	1.72	1.41	1.37	Chi-square=0.9, d.f.=2, P=0.64
Simpson dominance, 1-D	0.72	0.77	0.66	Chi-square=0.9, d.f.=2, P=0.64
Pielou equity, J'	0.72	0.85	0.73	Chi-square=0.9, d.f.=2, P=0.64
Margalef richness, d	1.99	1.68	1.42	Chi-square=6.3, d.f.=2, P=0.04

Non-metric multidimensional scaling (MDS) of Bray-Curtis similarities among samples detects no significant differences among habitats (Fig. 4.13), which is further confirmed by ANOSIM analysis (Global R statistic = 0.317, P = 5 %) and no significant pairwise comparisons (Table 4.13).

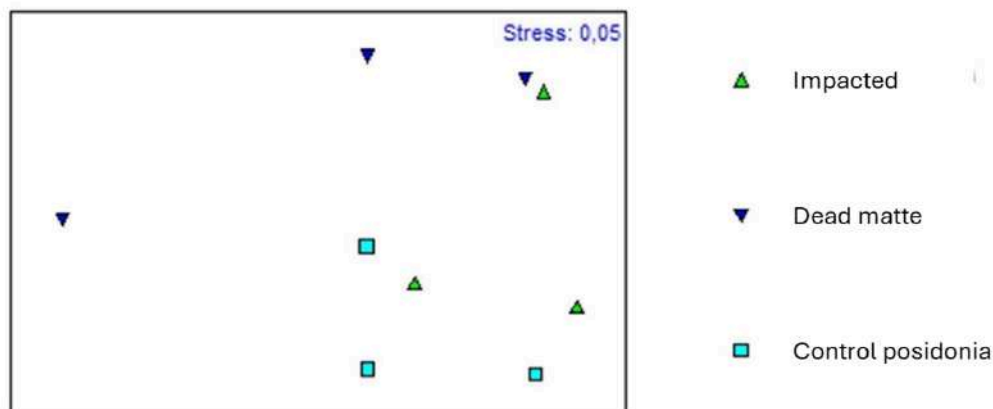


Figure 4.13 Non-metric multidimensional scaling of similarities (Bray-Curtis) among epifaunal communities.

Table 4.13 Pairwise comparisons of differences in epifaunal communities after ANOSIM analysis.

Groups	R Statistic	Significance level, %
Impacted <i>P. oceanica</i> vs Dead Matte	0.259	20
Impacted vs Control <i>P. oceanica</i>	0.185	30
Dead Matte vs Control <i>P. oceanica</i>	0.481	10

The overall results show that the epifaunal community in the *P. oceanica* canopy in the small meadow patches present in the impacted station has a higher number of taxonomic groups and total abundance than the epifaunal communities in macroalgae established in dead matte in the impacted station and the *P. oceanica* canopy in the control station. This result is unexpected



considering that macrophyte biomass of the habitats inside the impacted station is lower than that in the control *P. oceanica* (Table 4.12). Although the Margalef richness index is also higher in the impacted *P. oceanica* canopy, the other diversity indexes (Shannon diversity, Simpson dominance, Pielou equity) and the multivariate comparison of the epifaunal communities (MDS, ANOSIM) suggest that the epifaunal communities in the three habitats are, however, similar. The structure associated with macrophytes in the impacted station might provide enough habitat for epifauna as compared with the control *P. oceanica* station. Epifaunal communities might then be considered recovered in the impacted station. Further sampling will be necessary to confirm this suggestion. It is possible that the high level in the taxonomic hierarchy of the groups analyzed might have prevented the detection of differences in community structure among habitats.

4.3.3.4 Endofauna (matte)

Taxonomic composition of the community (sediment endofauna)

In the initial exploratory analysis, we described the full set of organisms recovered from the cores, including epibenthic and epiphytic forms (65 species and 375 individuals). For the formal biodiversity assessment, we refined the scope to the sediment endofauna, defined as taxa that are obligate infaunal or sediment-associated (i.e., living within, or on/at, the sediment surface). This decision removes epifaunal components (e.g., small gastropods on leaves and detrital surfaces) that can confound density and diversity patterns when the focus is the fauna of the sediment compartment. Habitat affinity was assigned at family/genus level using trait information; ambiguous cases were kept conservative.

Across all cores, the habitat-affinity filter (obligate infaunal + sediment-associated taxa) yielded 24 taxa and 72 individuals distributed among four major phyla: Mollusca, Annelida, Echinodermata, and Arthropoda. In terms of pooled abundance, Mollusca was the most represented group (62.5 % of individuals), followed by Annelida (19.4 %), Echinodermata (13.9 %), and Arthropoda (4.2 %). Molluscs also contributed the largest share of taxa (11 of 24), with annelids close behind (10 taxa), while echinoderms and arthropods were present with fewer lineages.

This composition is consistent with what is expected for the sediment compartment of *P. oceanica* meadows: a fauna dominated by burrowing and surface-associated molluscs (e.g., infaunal bivalves and naticid gastropods) and polychaete annelids typical of soft bottoms (deposit-feeders and infaunal predators), complemented by small infaunal echinoids and a minor arthropod component. The emphasis on sediment-dwelling forms reflects the analytical scope adopted here and contrasts with whole-assemblage descriptions that include epiphytic/epibenthic species living on leaves or detrital mats.

Station-level details (impacted vs control) are presented in subsequent sections; here we note simply that the phylum-level picture is broadly similar between meadows once restricted to the sediment endofauna, with differences emerging mainly in richness and dominance patterns addressed elsewhere in the report.

Control station community (endofauna)

In the control station, the endofauna pooled across the three replicates comprised 12 taxa and 35 individuals (Table 4.14). The assemblage was dominated taxonomically and numerically by Mollusca, with Annelida as the secondary contributor, and Echinodermata and Arthropoda present at lower abundance. Within molluscs, the set was typified by infaunal bivalves (e.g., Lucinidae, *Ctena decussata*, *Lucinella divaricata*; Cardiidae, *Parvicardium exiguum*; Veneridae, *Venus verrucosa*) and infaunal/sediment-associated gastropods, notably naticids (e.g., *Euspira fusca*, *Notocochlis dillwynii*) and burrowing/opisthobranch forms (e.g., *Haminoea hydatis*). Polychaetes characteristic of soft sediments were consistently represented (e.g., Capitellidae *Notomastus* spp.; Onuphidae *Aponuphis bilineata*; Lumbrineridae *Lumbrineriopsis cf. paradoxa*; Spionidae *Laonice cirrata*), together with infaunal echinoids (e.g., *Echinocyamus pusillus*, *Psammechinus microtuberculatus*) and a minor contingent of sediment-associated peracarid crustaceans. Overall, the phylum-level picture in the control meadow station reflects a classic soft-bottom infaunal mix in *Posidonia* sediments, where burrowers and surface-associated infauna predominate and no single taxon dominates the pooled counts.

Impacted station community (endofauna)

In the impacted station, the endofauna pooled across replicates comprised 16 taxa and 37 individuals (Table 4.14). As in the control station, Mollusca provided the largest share of taxa and individuals, with Annelida forming the next most important component and Echinodermata present at low abundance; Arthropoda remained marginal. Relative to the control meadow station, the impacted sediment assemblage showed a broader taxonomic spread within polychaetes (e.g., Glyceridae *Glycera cf. tessellata*; Goniadidae *Goniada maculata* alongside Capitellidae, Onuphidae, Spionidae) and maintained the core of infaunal bivalves and naticid gastropods. In practical terms, this translates into a more evenly shared contribution among infaunal molluscs and polychaetes when samples are pooled, consistent with the lower pooled dominance we reported for diversity indices in the sediment-only dataset.



Table 4.14 Endofauna (> 1 mm) identified from the sediments of *P. oceanica* meadows at 20 m depth. For each taxon, the table reports the taxonomic classification (Phylum, Class, Order, Family, Genus, Species/Authority), the assigned trophic guild, and the number of individuals counted in each replicate for the two stations: impacted (I4, I5, I6) and control (C1, C2, C3).

Taxa	Trophic guild	Impacted			Control		
		I4	I5	I6	C1	C2	C3
Annelida							
Polychaeta							
Capitellida							
Capitellidae							
<i>Notomastus</i> sp1	Deposit feeders	1	0	0	0	0	0
<i>Notomastus</i> sp2	Deposit feeders	0	0	1	0	0	0
Eunicida							
Eunicidae							
<i>Eunice</i> cf. <i>vittata</i> (Delle Chiaje, 1828)	Carnivores/omnivores	0	0	1	0	0	0
<i>Lysidice</i> cf. <i>ninetta</i> Audouin & H. Milne Edwards, 1833	Carnivores/omnivores	1	0	0	0	0	0
<i>Lysidice unicornis</i> (Grube, 1840)	Carnivores/omnivores	3	0	0	0	0	0
Lumbrineridae							
<i>Lumbrineriopsis</i> cf. <i>paradoxa</i> (Saint-Joseph, 1888)	Carnivores/omnivores	0	0	0	1	0	0
Onuphidae							
<i>Aponuphis bilineata</i> (Baird, 1870)	Carnivores/omnivores	1	1	1	0	0	0
Phyllodocida							
Glyceridae							
<i>Glycera</i> cf. <i>tesselata</i> Grube, 1863	Carnivores/omnivores	0	1	0	0	0	0
Goniadidae							
<i>Goniada maculata</i> Örsted, 1843	Carnivores/omnivores	0	0	0	1	0	0
Spionida							
Spionidae							
<i>Laonice cirrata</i> (M. Sars, 1851)	Deposit feeders	0	0	0	1	0	0
Arthropoda							
Malacostraca							
Amphipoda							
Phoxocephalidae							
<i>Harpinia</i> sp1	Deposit feeders / detritivores	0	0	2	0	0	0
Isopoda							
Anthuridae							
<i>Mesanthura romulea</i> Poore & Lew Ton, 1986	Deposit feeders / detritivores	1	0	0	0	0	0
Echinodermata							
Echinoidea							
Echinolampadacea							
Fibulariidae							
<i>Echinocyamus pusillus</i> (O. F. Muller, 1776)	Deposit feeders / detritivores	0	2	1	3	1	3
Mollusca							
Bivalvia							
Cardiida							
Cardiidae							
<i>Parvicardium exiguum</i> (Gmelin, 1791)	Suspension /filter feeders	0	1	1	0	0	0
Semelidae							
<i>Moerella donacina</i> (Linnaeus, 1758)	Mixt (Suspension / Deposit feeders)	3	0	1	1	0	0
Lucinida							
Lucinidae							
<i>Ctena decussata</i> (O. G. Costa, 1829)	Deposit feeders	3	1	1	2	2	2

Taxa	Trophic guild	Impacted			Control		
		I4	I5	I6	C1	C2	C3
<i>Lucinella divaricata</i> (Linnaeus, 1758)	Deposit feeders	4	3	0	0	0	0
Venerida							
Veneridae							
<i>Venus verrucosa</i> Linnaeus, 1758	Suspension/filter feeders	0	0	0	0	0	1
Gastropoda							
Cephalaspidea							
Haminoeidae							
<i>Haminoea hydatis</i> (Linnaeus, 1758)	Deposit feeders	1	1	0	0	0	0
Littorinimorpha							
Naticidae							
<i>Euspira fusca</i> (Blainville, 1825)	Carnivores/omnivores	0	0	0	1	2	2
<i>Notocochlis dillwynii</i> (Payraudeau, 1826)	Carnivores/omnivores	0	0	0	1	1	1
Neogastropoda							
Cystiscidae							
<i>Gibberula miliaria</i> (Linnaeus, 1758)	Carnivores/omnivores	0	0	0	3	1	3
Raphitomidae							
<i>Raphitoma cf. histrix</i> Bellardi, 1847	Carnivores/omnivores	1	0	0	0	0	1
Pyramidellida							
Pyramidellidae							
<i>Odostomella doliolum</i> (R. A. Philippi, 1844)	Carnivores/omnivores	0	0	0	1	0	0
abundance (n°)		18	10	9	15	7	13
species (n°)		10	7	8	10	5	7



Figure 4.14 Taxonomic identifications of polychaetes (Annelida: Polychaeta) from *P. oceanica* sediment cores. From left to right, top to bottom: (A) Capitellida, Capitellidae: *Notomastus* sp., (B) Eunicida, Eunicidae: *Eunice* cf. *vittata* (Delle Chiaje, 1828); (C) *Lysidice* cf. *ninetta* (Audouin & H. Milne Edwards, 1833); (D) *Lysidice unicornis* (Grube, 1840). (E) Eunicida, Lumbrineridae: *Lumbrineriopsis* cf. *paradoxa* (Saint-Joseph, 1888). (F) Eunicida, Onuphidae: *Aponuphis bilineata* (Baird, 1870). (G) Phyllodocida, Glyceridae: *Glycera* cf. *tessellata* (Grube, 1863). (H) Phyllodocida, Goniadidae: *Goniada maculata* Örsted, 1843. (I) Spionida, Spionidae: *Laonice cirrata* (M. Sars, 1851). Note: the qualifier *cf.* indicates a tentative identification ("affinity to"). White scale bar = 2 mm.



Figure 4.15 Taxonomic identifications of crustaceans (Arthropoda: Peracarida + Leptostraca) and echinoids (Echinodermata: Echinoidea) from *P. oceanica* sediment cores (sediment endofauna subset). From left to right, top to bottom : (A) Anthuridae, *Mesanthura romulea* Poore & Lew Ton, 1986; (B) Nebaliidae, *Nebalia* cf. *bipes* (Fabricius, 1780); (C) Phoxocephalidae, *Harpinia* sp.; (D) Fibulariidae, *Echinocyamus pusillus* (O. F. Müller, 1776); (E) Parechinidae, *Psammechinus microtuberculatus* (Blainville, 1825). Note: cf. denotes tentative identification. White scale bar = 2 mm.

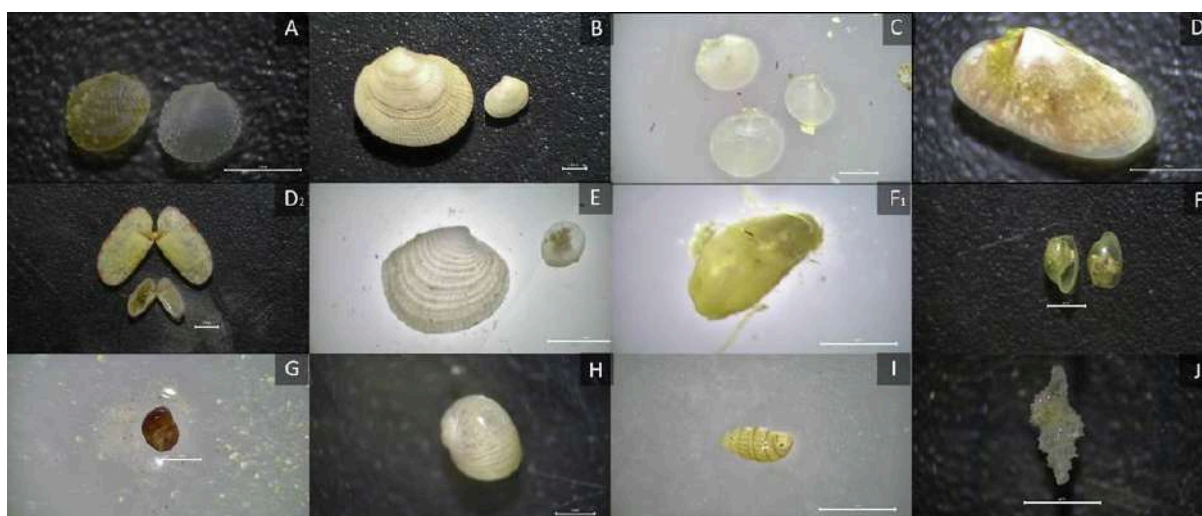


Figure 4.16 Taxonomic identifications of Mollusca (Mollusca: Bivalvia and Gastropoda) from *P. oceanica* sediment cores (sediment endofauna subset). From left to right, top to bottom: (A) Cardidae, *Parvicardium exiguum* (Gmelin, 1791); (B) Lucinidae, *Ctena decussata* (O. G. Costa, 1829); (C) Lucinidae, *Lucinella divaricata* (Linnaeus, 1758); (D) Semelidae, *Moerella donacina* (Linnaeus, 1758); (E) Veneridae, *Venus verrucosa* Linnaeus, 1758. (F) Haminoeidae, *Haminoea hydatis* (Linnaeus, 1758); (G) Naticidae, *Euspira fusca* (Blainville, 1825); (H) Naticidae, *Notocochlis dillwynii* (Payraudeau, 1826); (I) Pyramidellidae, *Odostomella doliolum* (R. A. Philippi, 1844); (J) Raphitomidae, *Raphitoma* cf. *hystrix* Bellardi, 1847. Note: cf. denotes tentative identification. White scale bar = 2 mm.

Contrast with the full-assemblage view

When the full assemblage (including epibenthic/epiphytic taxa on *Posidonia* leaves and detrital



mats) retained into the cores is considered, the community appears strongly mollusc-dominated (particularly by small epiphytic gastropods) and total observed richness is much higher (e.g., 65 species and 357 individuals across all samples in the exploratory pass). By contrast, restricting the scope to the sediment endofauna (obligate infaunal + sediment-associated) yields a truer picture of the sediment compartment: totals decrease (24 taxa, 72 individuals, all samples combined), gastropod guilds tied to leaf canopies largely disappear, and the balance between burrowing bivalves, infaunal gastropods, and polychaetes becomes clearer. This separation of scopes is therefore informative rather than contradictory: the full assemblage highlights the canopy pathway (epiphyte–detritus–epifauna), while the sediment endofauna isolates infaunal processes (deposit feeding, bioturbation, infaunal predation) that are most diagnostic of conditions within the sediment.

Diversity of the endofauna in P. oceanica meadows

At the replicate (α) scale, both meadow stations exhibit a broadly comparable community structure within the endofauna. Abundance and richness per core are of similar magnitude between stations, and the main diversity/evenness metrics—Shannon (H'), Pielou's evenness (J'), Simpson's complement ($1-D$), and the Hill numbers (q_1 , q_2)—show only slight, non-systematic differences. In practical terms, cores from both the impacted and control stations tend to contain a similar mix of taxa with no strong dominance at the individual-replicate level. The Margalef index follows the same pattern, indicating that richness adjusted for sample size does not diverge markedly between stations when each core is considered independently.

When the three replicates per station are pooled (γ scale), a clearer contrast emerges. The impacted station presents a more even and taxonomically richer infaunal assemblage in the sediment compartment, reflected by higher H' , higher $1-D$, and higher Hill numbers (q_1 and q_2), alongside a higher Margalef. Together, these signals point to lower aggregated dominance and a more balanced distribution of individuals among taxa when the impacted meadow station is viewed as a whole. By contrast, the control station shows a slightly more concentrated composition at the pooled scale, suggesting that a small subset of common infaunal taxa accounts for a larger share of the total.

Interpretation and cautions. At the α scale, the absence of strong contrasts is consistent with the limited replication ($n = 3$ per station) and the natural variability of soft-sediment cores. At the γ scale, the pattern of higher evenness and effective diversity in the impacted station should be read as an aggregate property (i.e., how replicates complement each other), not as evidence of uniformly higher diversity in every core. Because richness and Margalef depend on total N , strict comparisons of S are best supported by rarefaction or coverage-based standardization. Overall, the results indicate similar core-level structure between meadows, with the impacted station expressing greater pooled evenness and effective diversity in the endofauna. (Quantitative values are reported in the accompanying Table 4.15).

Table 4.15 Diversity taxonomic indices for the endofauna (> 1 mm) in P. oceanica sediments at 20 m depth (habitat-affinity filter: obligate infaunal + sediment-associated). For each replicate in the impacted station (I4–I6) and control station (C1–C3), the table reports: N (total individuals), S (taxon richness), Shannon H' , Pielou's evenness J' , Simpson's $1-D$, Hill q_1 , Hill q_2 and



Margalef. Species abundances are computed at the species level per core. The table also includes station means (average across replicates) and pooled values per station (y-diversity; sums across the three replicates). Pooled rows yield $n=1$ per zone and are therefore descriptive rather than inferential.

Sample	Station	N	S	H	J	1-D	Hill q1	Hill q2	Margalef
I4	Impacted	18	10	2.03	0.93	0.85	7.64	6.75	2.77
I5	Impacted	10	7	1.83	0.94	0.82	6.26	5.56	2.61
I6	Impacted	9	8	2.04	0.98	0.86	7.72	7.36	3.19
C1	Control	15	10	2.18	0.95	0.87	8.81	7.76	3.32
C2	Control	7	5	1.55	0.96	0.78	4.71	4.45	2.06
C3	Control	13	7	1.84	0.95	0.83	6.33	5.83	2.34
means	Impacted	12.3	8.3	1.97	0.95	0.85	7.20	6.56	2.85
means	Control	11.7	7.3	1.86	0.95	0.83	6.62	6.01	2.57
pooled	Impacted	37	16	2.53	0.91	0.90	12.59	10.29	4.15
pooled	Control	35	12	2.15	0.86	0.86	8.55	7	3.09

Trophic structure and functional diversity (endofauna)

All identified taxa were assigned to trophic guilds based on feeding mode—deposit feeders (detritivores), suspension/filter feeders, carnivores/omnivores, and a small set of mixed feeders—following a trait-based, literature-informed scheme. Analyses here refer to the sediment endofauna (> 1 mm; obligate infaunal + sediment-associated), so epiphytic herbivores tied to the *Posidonia* canopy are largely excluded by design.

Overall pattern. The infaunal assemblage is clearly dominated by deposit feeders, consistent with a system in which particulate organic matter (POM) and leaf-litter detritus are key energy inputs to the sediment compartment. Suspension/filter feeders form the second contribution, primarily infaunal bivalves; carnivores/omnivores (notably polychaete predators and opportunists) provide an additional, smaller pathway; and mixed feeders occur at low frequency. This partitioning indicates that detrital processing and bioturbation underpin the functioning of the sediment community, with secondary fluxes via filtration and predation.

When considered per core (α -scale), the guild profiles of the impacted and control stations are broadly similar, with variability typical of soft-sediment samples. When the three replicates are pooled by station (γ -scale), a clearer contrast emerges: the control station shows a stronger preponderance of deposit feeders, whereas the impacted station exhibits a more balanced trophic profile, with a relatively larger share of suspension feeders and carnivores/omnivores. In other words, the control station concentrates biomass in a single dominant feeding mode, while the impacted station spreads individuals more evenly across several modes.

Functional diversity metrics computed on guild proportions (i.e., Shannon for guild composition and Pielou's evenness) point to higher functional evenness in the impacted station, reflecting the more even partitioning among deposit feeding, filtration, and predatory/omnivorous pathways. The control station shows lower functional evenness because the detritivore guild dominates the pooled profile. These outcomes do not imply higher core-level diversity at the impacted station; rather, they indicate that, in aggregate, its infaunal community is less dominated by a single



trophic role.

In a well-conserved *P. oceanica* meadow, sustained leaf production and litterfall fuel abundant detritus in the sediment, favouring specialised deposit feeders that can become numerically dominant. Where meadow condition is degraded, a reduced, less continuous detrital subsidy likely constrains strict detritivores, allowing filter feeders and generalist predators/omnivores to gain relative prominence—even if total infaunal abundance is similar or lower. In short, the control station supports more fauna overall concentrated in a detritivore pathway, whereas the impacted station exhibits slightly higher functional evenness within the endofauna.

Table 4.16 Diversity indices for the endofauna (> 1 mm) in P. oceanica sediments at 20 m depth (habitat-affinity filter: obligate infaunal + sediment-associated). For each replicate in the impacted station (I4–I6) and control station (C1–C3), the table reports: N (total individuals), G (functional guilds), Shannon H', Pielou's evenness J', Simpson's 1–D, Hill q1, Hill q2 and Margalef. Species abundances are computed at the species level per core. The table also includes station means (average across replicates) and pooled values per station (γ-diversity; sums across the three replicates). Pooled rows yield n=1 per station and are therefore descriptive rather than inferential.

Sample	Station	N	G	H	J	1-D	Hill q1	Hill q2
I4	impacted	18	5	1.336	0.83	0.691	3.804	3.24
I5	impacted	10	4	1.221	0.88	0.66	3.389	2.941
I6	impacted	9	6	1.735	0.968	0.815	5.67	5.4
C1	control	15	5	1.459	0.907	0.738	4.304	3.814
C2	control	7	3	0.956	0.87	0.571	2.6	2.333
C3	control	13	4	1.157	0.835	0.627	3.18	2.683
means	impacted	12.3	5	1.431	0.893	0.722	4.288	3.86
means	control	11.6	4	1.191	0.87	0.645	3.362	2.943
pooled	impacted	37	7	1.613	0.829	0.748	5.018	3.968
pooled	control	35	6	1.361	0.76	0.679	3.901	3.117

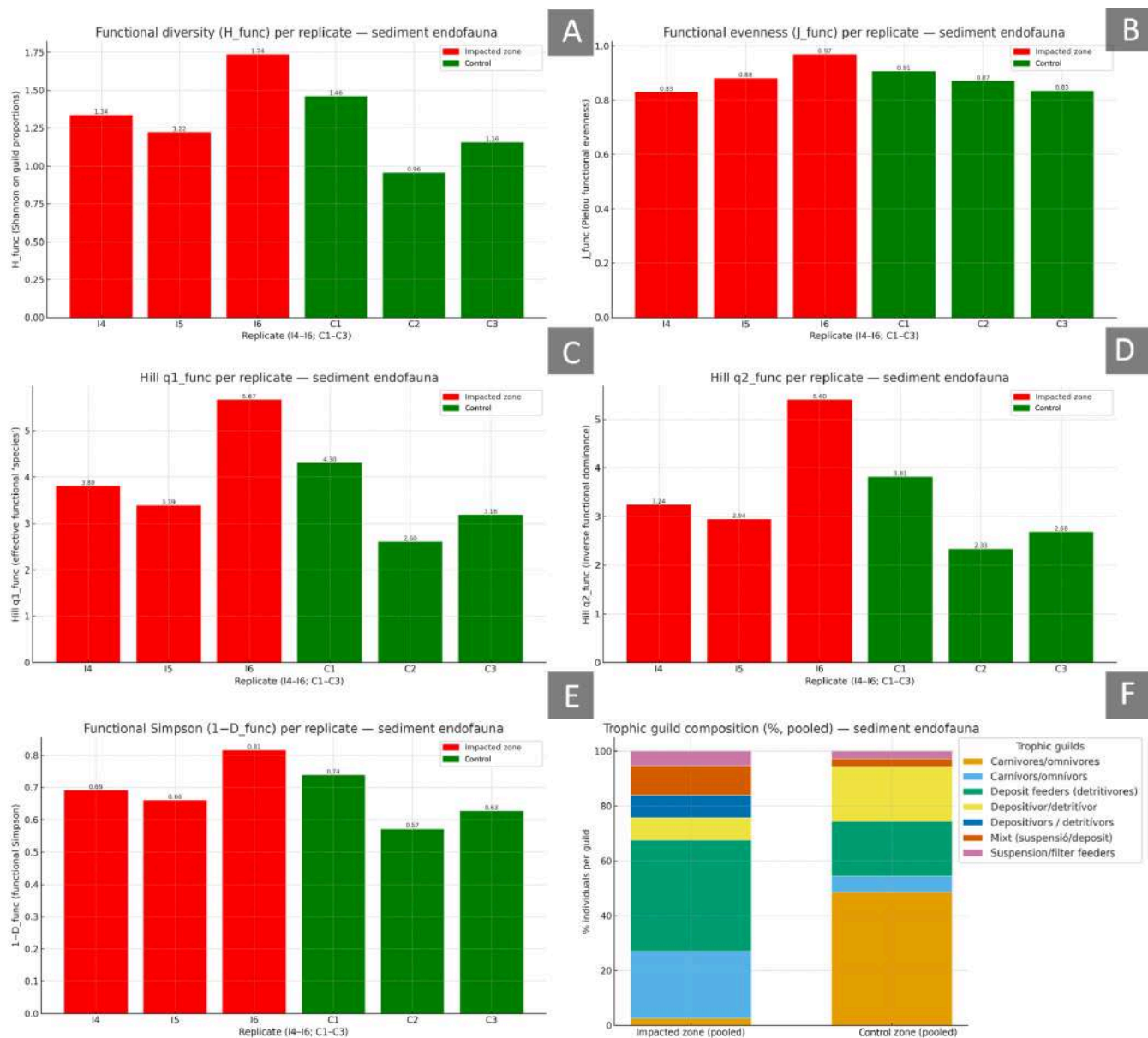


Figure 4.17 Functional diversity of the endofauna (> 1 mm) in *P. oceanica* meadows (panels A–F). (A) Functional diversity H_{func} (Shannon on trophic-guild proportions) per replicate; (B) functional evenness J_{func} (Pielou on guild proportions) per replicate; (C) Hill $q1_{\text{func}}$ ($= e^{H_{\text{func}}}$) per replicate; (D) Hill $q2_{\text{func}}$ ($= 1/D_{\text{func}}$) per replicate; (E) functional Simpson $1-D_{\text{func}}$ per replicate; (F) pooled trophic guild composition (%) by station. Replicates are labelled I4–I6 (impacted station, red) and C1–C3 (control station, green). The legend lists all guilds present in the dataset (e.g., Deposit feeders [detritivores], Suspension/filter feeders, Carnivores/omnivores, Mixed feeders). All indices are computed on the endofauna subset (obligate infaunal + sediment-associated) at core level (panels A–E); pooled bars (panel F) aggregate the three replicates per station.

Core-level structure of the endofauna (> 1 mm) is very similar between stations; abundance, richness, Shannon, Simpson, Hill numbers and evenness show only small, non-systematic differences. When the three replicates are aggregated by station, the impacted station shows higher richness, evenness and effective diversity (higher H' , J' , $1-D$, Hill q_1 – q_2 , Margalef) with similar total N , indicating lower dominance and a more balanced taxonomic profile.

The control station is detritivore-dominated, whereas the impacted station exhibits a more even mix of deposit feeders, suspension/filter feeders, and carnivores/omnivores—thus higher functional evenness in the sediment compartment.

Sustained detrital inputs in well-conserved *Posidonia* beds favour high detritivore dominance; under impact, reduced/less stable detrital subsidy limits strict detritivores and elevates other guilds' share, producing a more even functional mosaic.

4.3.4 Blue Carbon

The *P. oceanica* meadows within the control station exhibited an apparent good health condition, with low epiphyte load and exuberant canopies (Fig. 4.7A and 4.7B). The living meadow was characterized by interspersed sandy channels surrounded by mounts of seagrass ranging from 0.5 to 1 m in height, which were likely caused by the highly hydrodynamic conditions prevailing at the station. On the contrary, meadows surrounding the impacted station showed signs of deterioration, including lower density and cover compared to the meadows at the control station. The presence of sparse *P. oceanica* shoots and clumps within the impacted station indicate signs of seagrass recovery following the disturbance in 2010.

The seagrass *P. oceanica* meadows within the region of study are characterized by high hydrodynamics that shaped multiple sandy areas across the habitat, with seagrasses growing in up to 1 m high mounts along the sides of sandy areas. The meadows at the control station had higher structure than the meadows surrounding the impacted station, with up to 2.5-fold higher shoot density and biomass ($P < 0.01$ and < 0.001 , respectively), 8-fold higher % cover and up to 3-fold lower litter cover and biomass ($P < 0.001$ in both cases; Table 4.17 and Table 4.18). The $\delta^{13}\text{C}$ signatures of seagrass vegetative biomass were similar between impacted and control stations ($P > 0.05$). The sediments underneath the control meadow station were up to 1.5-fold thicker than those at the impacted station. All above suggests erosion of the sediments within the impacted station and the legacy of the disturbance 15 years after linked to the absence of seagrasses within the impacted station and the relatively poor structure of the meadows surrounding it compared to the control station.



Table 4.17 Count (N), Mean and Standard Deviation (SD) of dry bulk density (g cm^{-3}), sedimentary organic carbon content (C_{org} %), sedimentary organic carbon density ($\text{mg C}_{\text{org}} \text{cm}^{-3}$), sedimentary organic carbon stocks in the top 40 cm of sediments ($\text{Mg C}_{\text{org}} \text{ha}^{-1}$), $\delta^{13}\text{C}$ of sedimentary organic carbon (‰), sediment grain-size fractions (%) $< 0.063 \text{ mm}$, $> 0.063 < 0.125 \text{ mm}$, $> 0.125 < 0.25 \text{ mm}$, $> 0.25 < 0.5 \text{ mm}$, and $> 0.5 < 1 \text{ mm}$, and $> 1 \text{ mm}$ (%), shoot density (number of shoots m^{-2}), seagrass vegetative biomass (g DW m^{-2}), seagrass vegetative biomass C_{org} content (in % and $\text{g C}_{\text{org}} \text{m}^{-2}$), seagrass cover (%), litter cover (%), litter biomass (g DW m^{-2}), and litter biomass C_{org} content ($\text{g C}_{\text{org}} \text{m}^{-2}$), and $\delta^{13}\text{C}$ signatures of vegetative biomass (‰). *The % in litter was assumed to be the same for each control and impacted stations.

Variable	Treatment	N	Mean	SD
Dry bulk density (g DW cm^{-3})	Control	77	1.00	0.13
	Impacted	45	1.01	0.21
Sedimentary C_{org} content (%)	Control	41	0.80	0.68
	Impacted	27	0.69	0.32
Sedimentary C_{org} density ($\text{mg C}_{\text{org}} \text{cm}^{-3}$)	control	41	9.44	9.59
	Impacted	27	7.88	5.17
Sedimentary C_{org} stocks in top 40 cm	Control	3	21.94	4.83
	Impacted	3	26.08	3.89
$\delta^{13}\text{C}$ of sedimentary C_{org} (‰)	Control	37	-15.89	3.67
	Impacted	27	-11.33	5.04
$< 0.063 \text{ mm}$ (%)	Control	77	2.07	1.47
	Impacted	45	5.97	16.59
$> 0.063 < 0.125 \text{ mm}$ (%)	Control	77	3.04	1.79
	Impacted	45	2.40	3.41
$> 0.125 < 0.25 \text{ mm}$ (%)	Control	77	9.88	4.55
	Impacted	45	5.14	3.30
$> 0.25 < 0.5 \text{ mm}$ (%)	Control	77	17.82	7.92
	Impacted	45	13.53	9.09
$> 0.5 < 1 \text{ mm}$ (%)	Control	77	16.86	6.62
	Impacted	45	15.41	11.39
$> 1 \text{ mm}$ (%)	Control	77	50.33	19.40
	Impacted	45	57.54	27.37
Shoot density (number of shoots m^{-2})	Control	8	556	73
	Impacted	8	341	161
Seagrass vegetative biomass (g DW m^{-2})	Control	28	4500	75
	Impacted	6	2029	162
Seagrass vegetative biomass C_{org} (%)	Control	28	37.8	0.1
	Impacted	6	40.4	1.2
Seagrass vegetative biomass C_{org} ($\text{g C}_{\text{org}} \text{m}^{-2}$)	Control	28	1.701	75
	Impacted	6	820	163
Seagrass cover (%)	Control	32	32.5	31.1
	Impacted	32	4.0	9.3
Litter cover (%)	Control	8	10.5	1.6
	Impacted	4	41.4	6.3
Litter biomass (g DW m^{-2})	Control	3	6089	1,365
	Impacted	2	4756	4
Litter biomass C_{org} ($\text{g C}_{\text{org}} \text{m}^{-2}$)	Control	3	2302	1,365
	Impacted	2	1921	5.1
$\delta^{13}\text{C}$ signatures of biomass (‰)	Control	3	-15.4	0.6
	Impacted		-15.9	0.4



Table 4.18 Results of the Generalized Linear Models (GLM) testing for significant effects of desalination impact (control and impacted stations) on dry bulk density (g cm^{-3}), sedimentary organic carbon content (C_{org} %), sedimentary organic carbon density ($\text{mg C}_{\text{org}} \text{cm}^{-3}$), sedimentary organic carbon stocks in the top 40 cm of sediments ($\text{Mg C}_{\text{org}} \text{ha}^{-1}$), $\delta^{13}\text{C}$ of sedimentary organic carbon (‰), sediment grain-size fractions (%) $< 0.063 \text{ mm}$, $> 0.063 < 0.125 \text{ mm}$, $> 0.125 < 0.25 \text{ mm}$, $> 0.25 < 0.5 \text{ mm}$, and $> 0.5 < 1 \text{ mm}$, and $> 1 \text{ mm}$ (%), shoot density (number of shoots m^{-2}), seagrass vegetative biomass (g DW m^{-2}), seagrass vegetative biomass C_{org} content (in % and $\text{g C}_{\text{org}} \text{m}^{-2}$), seagrass cover (%), litter cover (%), litter biomass (g DW m^{-2}), and litter biomass C_{org} content ($\text{g C}_{\text{org}} \text{m}^{-2}$), and $\delta^{13}\text{C}$ signatures of biomass (‰). Bold values indicate significant interactions.

Variable	Estimate	Error	t value	P value
Dry bulk density (g DW cm^{-3})	-0.008	0.031	-0.268	0.79
Sedimentary C_{org} content (%)	0.110	0.140	0.784	0.44
Sedimentary C_{org} density ($\text{mg C}_{\text{org}} \text{cm}^{-3}$)	1.560	2.017	0.773	0.44
Sedimentary C_{org} stocks in top 40 cm ($\text{Mg C}_{\text{org}} \text{ha}^{-1}$)	-4.143	3.581	-1.157	0.31
$\delta^{13}\text{C}$ of sedimentary C_{org} (‰)	-4.562	1.088	-4.192	< 0.001
$< 0.063 \text{ mm}$ (%)	-3.901	1.897	-2.056	< 0.05
$> 0.063 < 0.125 \text{ mm}$ (%)	0.646	0.47	1.374	0.17
$> 0.125 < 0.25 \text{ mm}$ (%)	4.742	0.775	6.116	< 0.001
$> 0.25 < 0.5 \text{ mm}$ (%)	4.289	1.570	2.731	< 0.01
$> 0.5 < 1 \text{ mm}$ (%)	1.443	1.628	0.886	0.38
$> 1 \text{ mm}$ (%)	-7.219	4.250	-1.698	0.09
Shoot density (number of shoots m^{-2})	4.378	1.456	2.659	< 0.01
Seagrass vegetative biomass (g DW m^{-2})	4.867	0.875	3.838	< 0.001
Seagrass vegetative biomass C_{org} (%)	-4.821	1.023	-4.321	< 0.001
Seagrass vegetative biomass C_{org} ($\text{g C}_{\text{org}} \text{m}^{-2}$)	4.581	1.178	3.949	< 0.001
Seagrass cover (%)	4.391	0.965	4.185	< 0.001
Litter cover (%)	4.744	1.046	4.387	< 0.001
Litter biomass (g DW m^{-2})	-4.633	0.899	-3.798	< 0.001
Litter biomass C_{org} ($\text{g C}_{\text{org}} \text{m}^{-2}$)	4.243	0.937	3.733	< 0.001
$\delta^{13}\text{C}$ signatures of biomass (‰)	1.655	0.912	4.064	0.42

4.3.4.1 Soil

The DBD in 40 cm-thick sediments in both control and impacted stations was similar (mean $\pm$ SD; 1.00 ± 0.13 and $1.01 \pm 0.21 \text{ g cm}^{-3}$, respectively; $P > 0.05$, Table 4.17 and Table 4.18). Similarly, the sedimentary C_{org} concentration was not significantly different between control and impacted stations ($0.80 \pm 0.68 \%$ and $0.69 \pm 0.32 \%$, respectively; and 9.44 ± 9.59 and $7.88 \pm 5.17 \text{ mg C}_{\text{org}} \text{cm}^{-3}$; $P > 0.05$). Finally, sedimentary C_{org} stocks within the top 40 cm were also similar between control and impacted stations (21.9 ± 4.83 and $26.1 \pm 3.9 \text{ Mg C}_{\text{org}} \text{ha}^{-1}$, respectively; $P > 0.05$; Fig. 4.18). The presence of rock underneath the top 20 to 60 cm of soil precluded collecting longer sediment cores at both the control and impacted stations.

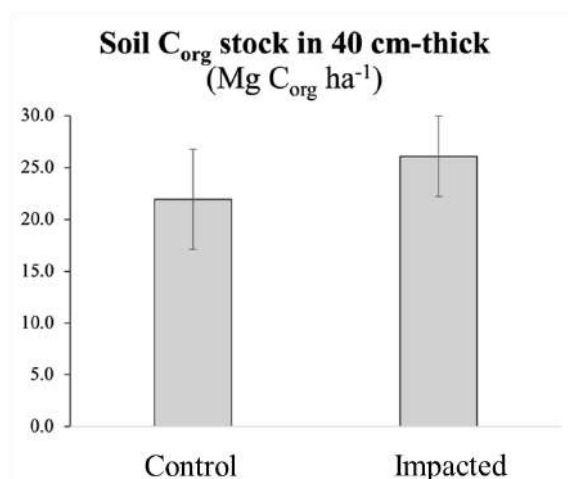


Figure 4.18 Mean $\pm$ SD sedimentary organic carbon stocks in the top 40 cm of sediments (Mg C_{org} ha⁻¹) in the control and impacted stations studied.

The $\delta^{13}C$ values of sedimentary organic matter in control meadows were significantly lower than at the impacted station (-15.9 ± 3.7 ‰ and -11.3 ± 5.0 ‰, respectively; $P < 0.001$, Table 4.17 and Table 4.18). Sediments in both the control and impacted stations were mainly constituted of gravel (50 ± 19 % and 57 ± 27 %; $P > 0.05$), with a relatively low abundance of silt and clay in the control station (2 ± 1 %; $P < 0.05$) compared to the impacted station (6 ± 17 %). The content of fine and medium sands was significantly higher at the control station (10 ± 4 and 18 ± 8 %, respectively) compared to the impacted station (5 ± 3 % and 13 ± 9 %, respectively; $P < 0.01$ and > 0.01 , respectively).

The downcore trends in DBD at the control station were relatively constant from topsoil until cm 30, and increased thereafter, whereas at the impacted station followed either increasing or decreasing trends downcore trends depending on the replicate core studied (Fig. 4.19). Sedimentary C_{org} content (% and mg cm⁻³) decreased down to cm 40, increased from cm 40 to 55 and decreased then after (Fig. 4.19).

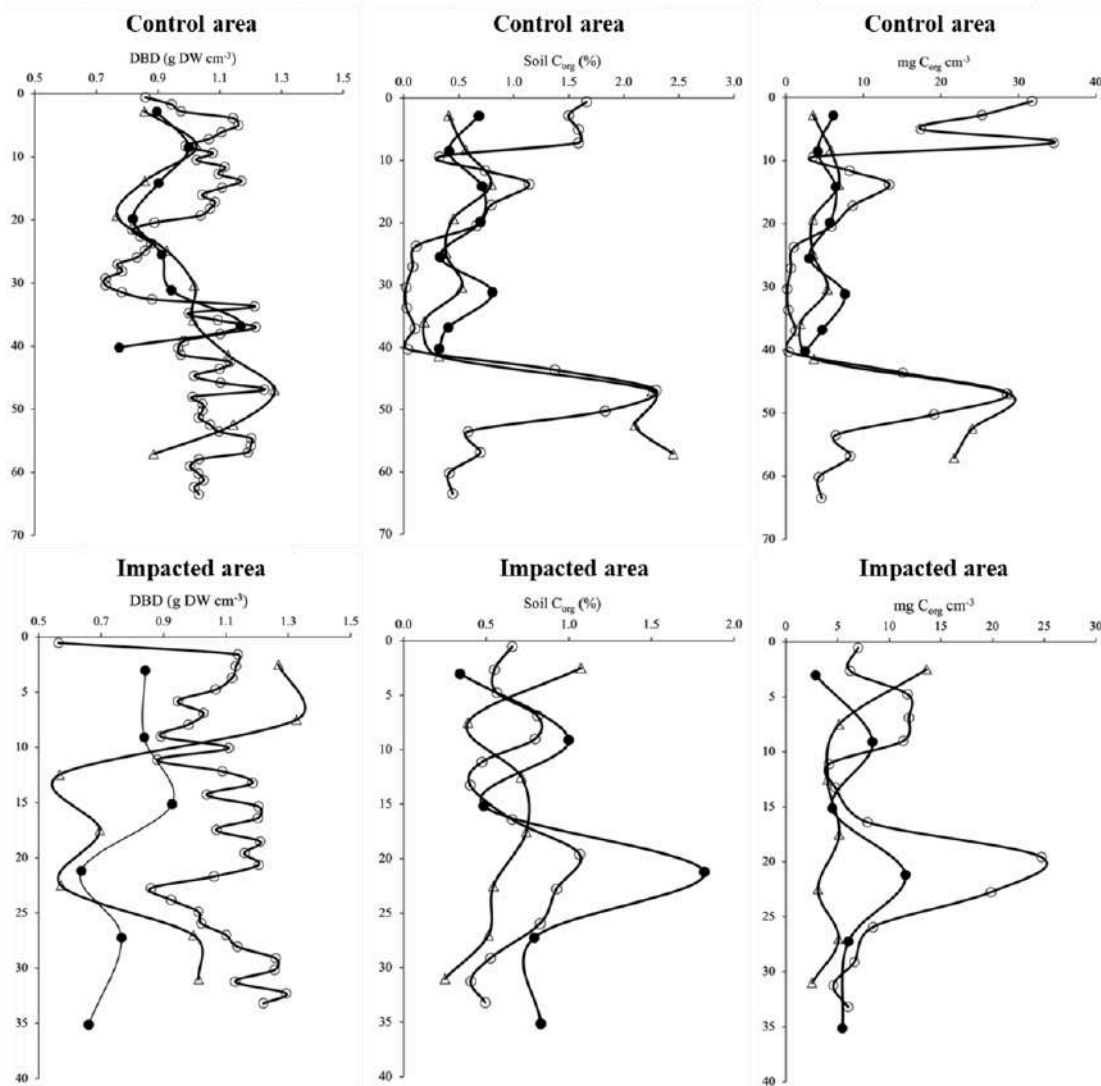


Figure 4.19 Changes in sediment dry bulk density (DBD; g DW cm⁻³), sedimentary organic carbon content (% and mg C_{org} cm⁻³) along the sediment cores collected in the control (ART_4 represented with a triangle, ART_5 represented with a dark circle, and ART_6 represented with a clear circle) and impacted stations (ART_1 represented with a triangle, ART_2 represented with a dark circle, and ART_3 represented with a clear circle).

The $\delta^{13}\text{C}$ values of sedimentary organic matter remained relatively stable downcore at the control station, whereas showed an overall decreasing trend with wide oscillations at the impacted station (Fig. 4.20). The content of clay and silt, very fine sand, and fine sand showed an overall increase downcore at both the control and impacted stations but were highly variable among replicates (Fig. 4.21). The content of medium and coarse sands increased downcore whereas very coarse sand plus gravel decreased downcore at the control station, whereas these variables remained relatively constant at the impacted station. However, clear differences were observed among replicate cores at both control and impacted stations that made comparisons difficult.

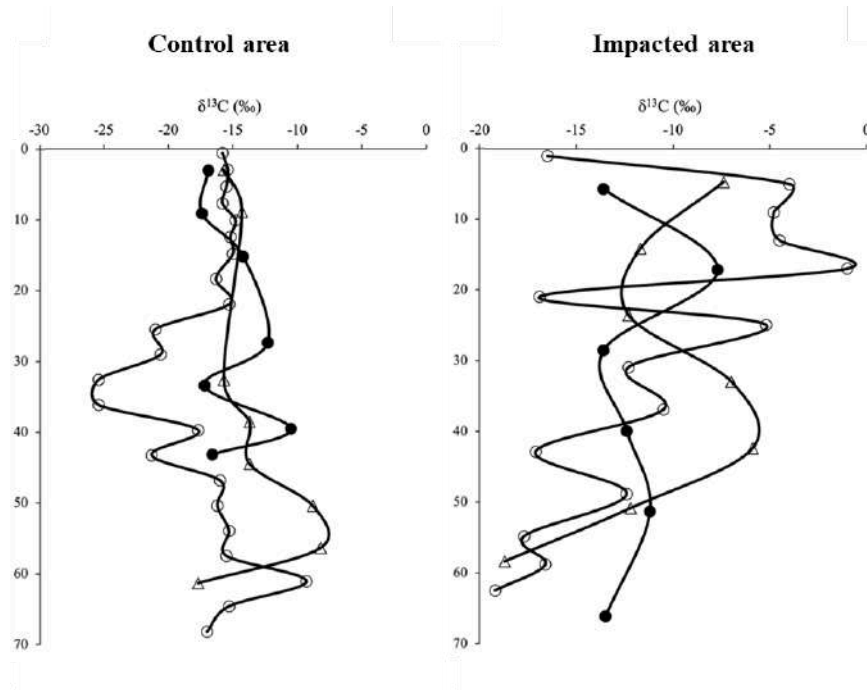


Figure 4.20 Downcore changes in the $\delta^{13}\text{C}$ signatures of sedimentary organic carbon content in the sediment cores collected in the control (ART_4 represented with a triangle, ART_5 represented with a dark circle, and ART_6 represented with a clear circle) and impacted stations (ART_1 represented with a triangle, ART_2 represented with a dark circle, and ART_3 represented with a clear circle).

The sediment accretion rates in core ART_6 (control) over the last century were estimated at $0.64 \pm 0.11 \text{ cm yr}^{-1}$. In 40 cm-thick sediment deposits and over about 26 years of accumulation, seagrass meadows accumulated $1.05 \pm 6.13 \text{ Mg C}_{\text{org}} \text{ ha}^{-1} \text{ yr}^{-1}$, acknowledging that the mixed ^{210}Pb profile likely resulted in overestimating C_{org} accumulation rates (Fig. 4.11). It was not possible to obtain age-depth models using conventional approaches based on ^{14}C ages, and the use of post-bomb for the calibration of the modern ages obtained was dismissed owing to the overlap between the periods covered by ^{210}Pb and ^{14}C ages.

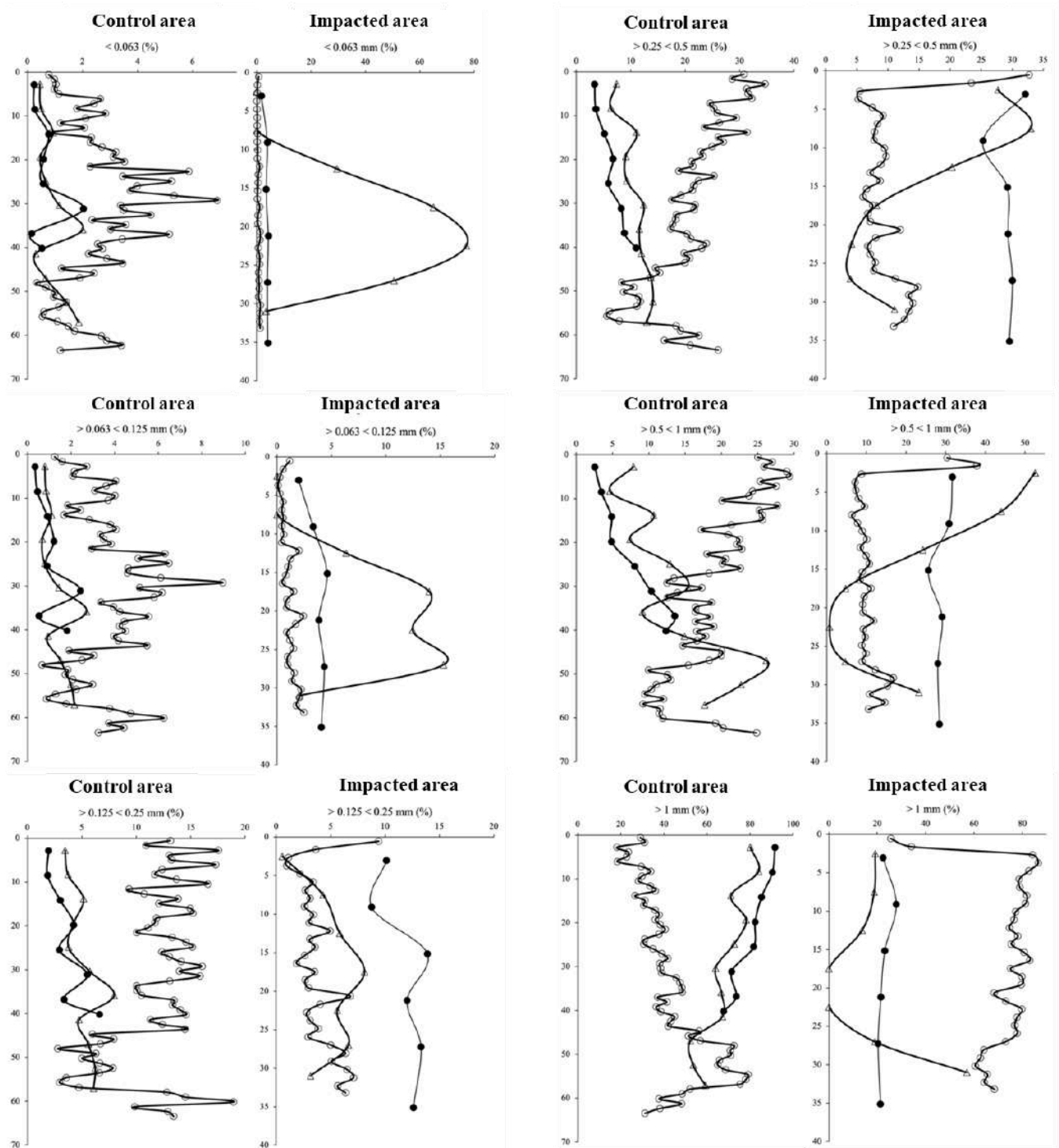


Figure 4.21 Changes in the sediment grain-size fractions (< 0.063 mm, $> 0.063 < 0.125$ mm, $> 0.125 < 0.25$ mm, $> 0.25 < 0.5$ mm, and $> 0.5 < 1$ mm, and > 1 mm; %) along the sediment cores collected in the control (ART_4 represented with a triangle, ART_5 represented with a dark circle, and ART_6 represented with a clear circle) and impacted stations (ART_1 represented with a triangle, ART_2 represented with a dark circle, and ART_3 represented with a clear circle).

4.3.4.2 Biomass

All variables studied related to meadow structure were significantly higher at the control station compared to the meadows adjacent to the impacted station (Table 4.17 and Table 4.18). Shoot density and seagrass vegetative biomass were up to 2-fold higher in the control station (556 ± 73

shoots m^{-2} and $4,500 \pm 75 \text{ g DW m}^{-2}$) compared to the impacted station (341 ± 161 shoots m^{-2} and $2,029 \pm 162 \text{ g DW m}^{-2}$; $P < 0.01$), whereas seagrass cover was more than 8-fold higher at the control station ($32.5 \pm 31.1 \%$) compared to the impacted station $4.0 \pm 9.3 \%$; $P < 0.001$; Fig. 4.22 and Fig. 4.23). Seagrass vegetative biomass C_{org} content (%) was significantly higher at the impacted station ($40.4 \pm 1.2 \%$) compared to the control station ($37.8 \pm 0.1 \%$; $P < 0.001$), whereas seagrass vegetative biomass C_{org} stocks were 2-fold higher in the control station compared to the impacted station ($1,701 \pm 75$ and $820 \pm 163 \text{ g } C_{\text{org}} \text{ m}^{-2}$; $P < 0.001$). The $\delta^{13}\text{C}$ values of seagrass vegetative biomass were similar between control and impacted stations ($-15.4 \pm 0.6 \text{ ‰}$, and $-15.9 \pm 0.4 \text{ ‰}$, respectively; $P > 0.05$, Fig. 4.23).

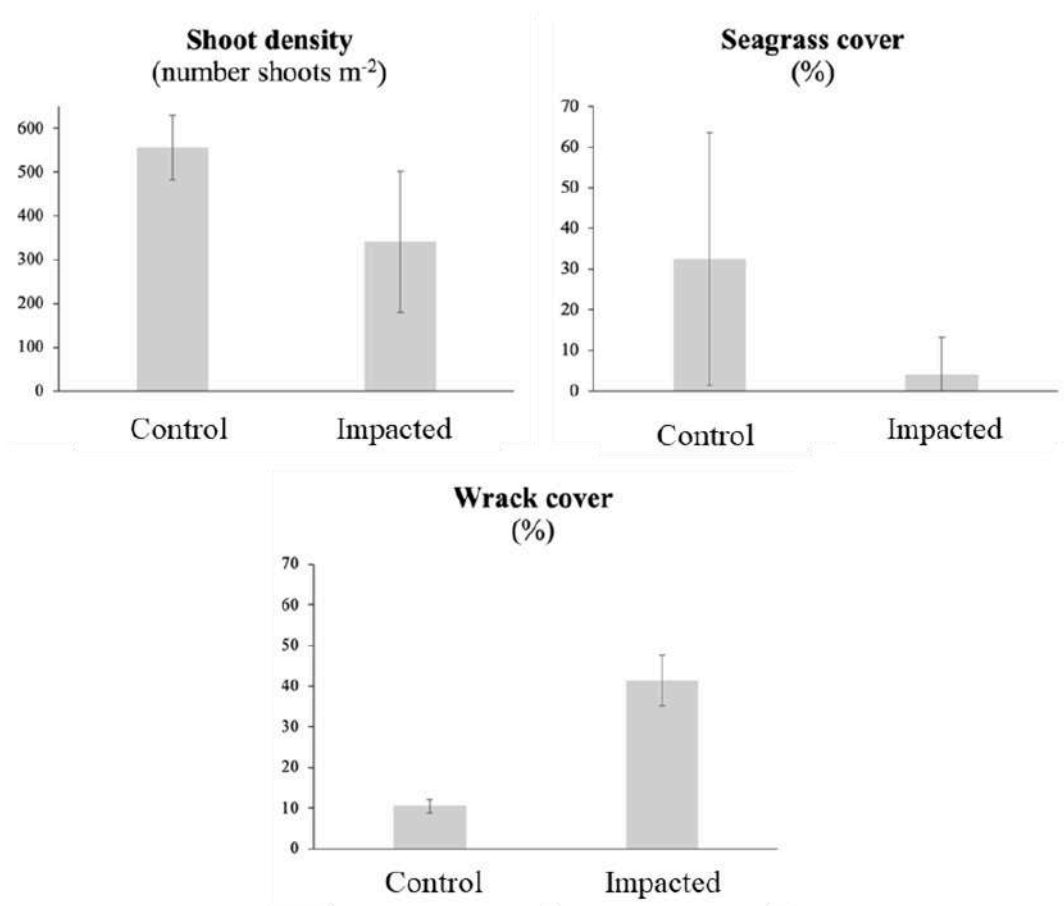


Figure 4.22 Mean $\pm$ SD shoot density (number of shoots m^{-2}), seagrass cover (%) and litter cover (%) in the control and impacted stations studied. Shoot density and seagrass cover at the impacted station were measured in the meadows adjacent to the lost area, whereas litter cover was estimated within the bare but previously vegetated area.

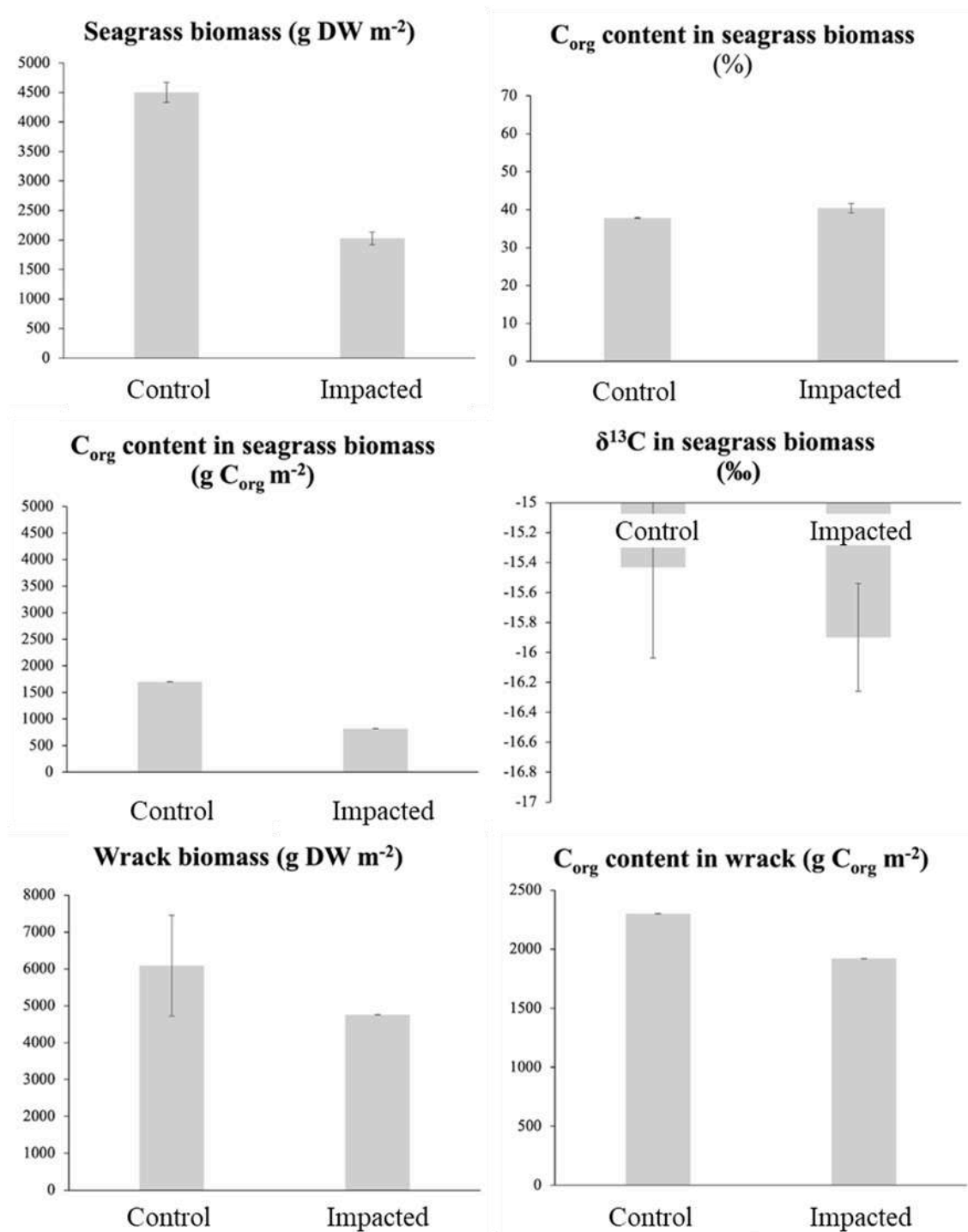


Figure 4.23 Mean $\pm$ SD seagrass vegetative biomass ($g\ DW\ m^{-2}$), C_{org} content in seagrass vegetative biomass (in % and $g\ C_{org}\ m^{-2}$), organic carbon content in seagrass vegetative biomass, $\delta^{13}C$ signatures of seagrass vegetative biomass, litter biomass ($g\ DW\ m^{-2}$) and C_{org} content in litter ($g\ C_{org}\ m^{-2}$) in the control and impacted stations studied.

4.3.4.3 Litter

Litter cover was 3-fold higher in the impacted ($41 \pm 6.3\ %$) than in the control stations ($10.5 \pm 1.6\ %$; $P < 0.001$) compared to the impacted station ($341 \pm 161\ shoots\ m^{-2}$ and $2,029 \pm 109\ g\ DW\ m^{-2}$; $P < 0.01$, Fig. 4.22 and Figure 4.23), whereas litter biomass and litter C_{org} stocks were significantly higher at the control station ($6,089 \pm 1,365\ g\ DW\ m^{-2}$ and $2,302 \pm 1,365\ g\ C_{org}\ m^{-2}$) compared to the



impacted station ($4,756 \pm 4 \text{ g DW m}^{-2}$ and $1,921 \pm 5.1 \text{ g C}_{\text{org}} \text{ m}^{-2}$, respectively; $P < 0.001$; Tables 4.17 and 4.18).

4.4 Discussion

The construction of the seawater intake tower and associated infrastructure of the desalination plant in Cala Blanca (Ciutadella, Menorca), together with the incidental bentonite spill in 2010, resulted in the loss of seagrass meadows ($1,638 \text{ m}^2$) and the deterioration of surrounding meadows (Fig. 4.1C–D, Fig. 4.6A–D, Fig. 4.22, Fig. 4.23). Accordingly, this study establishes the baseline for the planned restoration.

The integrated assessment of the *P. oceanica* meadow at the impacted and control stations confirms that the system is in a compromised ecological state, with clear differences in meadow structure and some functional components, yet with recovery potential, as this baseline has provided relevant information to design the restoration methodology.

The ecological status and structural integrity of the study area, evaluated with the EBQI index, place both sites in the “poor” category ($\text{EBQI} < 3.5$), with slightly higher values at the control station (3.1) than at the impacted station (2.9). This difference is too small to change class and reflects that both meadows share regional anthropogenic pressures; however, the bentonite spill explains the additional penalty at the impacted station, especially via structural descriptors (cover) that drive the index downward. Although shoot density measured within vegetated patches is relatively similar (514 vs $630 \text{ shoots m}^{-2}$), cover differs markedly (11% vs 88%), and the calculation of global density (density $\times$ cover) evidences a highly fragmented meadow at the impacted station (≈ 57 vs $555 \text{ shoots m}^{-2}$; “poor” vs “very good” at 20 m depth). This pattern—“apparently adequate shoot density but very low cover”—is consistent with habitat loss and spatial discontinuity, in line with the known impact history.

The absence of suspension/filter feeders at the impacted station ($\text{HOM} = 0$; $\text{LOM} = 0$), versus low but non-zero densities at the control station, aligns with the habitat simplification characteristic of the “gray zone” (Airoldi et al., 2008): loss of complexity $\rightarrow$ loss of biodiversity and key functions. Wrack (dead leaves) is greater at the control station, consistent with a more stable detrital supply and, therefore, greater capacity for carbon and nutrient storage; at the impacted station, lower cover limits this compartment. The dominance of holothurians ($16 \text{ ind } 10 \text{ m}^{-2}$) at the impacted station, absent at the control station, suggests a functional shift toward open sandy substrates rather than an improvement in status. Finally, the ichthyofauna compartments (boxes 10 – 12) score zero at both stations; this is a regional pattern attributed to fishing pressure that depresses upper trophic levels and drags down the EBQI, even in structurally good meadows.

The physical and spatial characteristics of the restoration area (very small surface, mosaic of patches with ecotones near rocky substrate and infrastructure) and the study depth (20 m) constrained protocol application and required adapting the EBQI to the reality of the Menorca pilot. This entailed design adjustments by station and to accommodate diving tasks at greater



depth (e.g., shorter ichthyofauna transects and fewer replicates within the impacted polygon; 0.25 m² quadrats instead of 1 m² to reduce bottom time) and, consequently, Confidence Index (CI) adjustments to reflect the true robustness of affected boxes. For interpretation, EBQI should be used as a relative indicator between the impacted and control stations and not extrapolated beyond the pilot.

In terms of biodiversity, the balance of total richness and abundance shows a richer community at the control station (44 species and 488 individuals) than at the impacted station (36 and 262), with marked differences in rhizome epifauna ($S_{total} = 10$ and $H' = 1.56$ at the control station vs $S_{total} = 1$ and $H' = 0$ at the impacted station), indicating ecosystem degradation at the impacted station. In the water column, the control station shows higher richness and abundance but lower evenness (dominance by few taxa, e.g., juveniles of *Spicara smaris*), which calls for cautious interpretation and strengthened replication in future surveys. In the matte (dead mat) and canopy, differences are subtler; in the canopy, H' is similar between stations despite higher abundance at the control station.

With respect to blue carbon, the results obtained showed that apparently the construction of the desalination plant did not reduce the sedimentary C_{org} stocks within the top 40 cm sediment per unit area (Fig. 4.18), rather altered its composition (Fig. 4.20). Although a direct cause-effect relationship can be established between the construction of desalination plant and seagrass loss, and even with the deterioration of surrounding meadows, it was not possible to estimate the thickness of seagrass-associated sediments eroded following seagrass loss and associated emissions following the disturbance, rather a change in the composition of sedimentary C_{org} stocks based on sedimentary $\delta^{13}C$ signatures. It appears that the seawater intake tower was built on top of a rocky outcrop and that the substrate under the impacted meadow station was less thick than that at the control station, which together with the apparent lower sediment elevation at the impacted station compared to the surrounding meadows suggests that sediment erosion occurred. However, the amount of sediments eroded upon meadow loss remains unknown and not possible to estimate accurately with the datasets obtained.

The comparison of downcore profiles of biogeochemical proxies between control and impacted stations did not allow determining the thickness of sediments eroded, in part due to the high variability of profiles among replicate cores (Fig. 4.19 and 4.20). However, it seems likely that a portion of the seagrass mat within the impacted station has been eroded and replaced by bentonite and reworked sand, likely resulting in greenhouse gas emissions. In addition, the downcore biogeochemical patterns in the sediments from the impacted station also suggest that the sediments within the impacted station have been altered. These include the mixed ^{210}Pb profile, wide oscillations in DBD, up to 1.5-fold thicker sediment in the control station, and unusually high $\delta^{13}C$ signatures of sedimentary C_{org} and silt and clay contents that are most likely indicative of the presence of bentonite, in particular at sediment depths ranging from 10 to 30 cm (Fig. 4.11, 4.19, 4.20, and 4.21). The complexity and associated uncertainties and assumptions linked to the use of retrospective approaches to estimate sedimentary C_{org} erosion (e.g., ^{210}Pb , comparison of downcore trends in biogeochemical sediment characteristics; Arias-Ortiz et al., 2018; Serrano et al., 2021) precluded their use in our case study. Therefore, further studies are

required aiming at unequivocally link seagrass loss with negative sedimentary C_{org} fluxes that could be used for holistic carbon crediting under IPPC or emerging blue carbon accounting methodologies (Hiraishi et al., 2014; Comte et al., 2024). The sedimentary $\delta^{13}C$ signatures at the control station indicate a relatively large contribution of seagrass organic matter to the sedimentary C_{org} pool, whereas the sedimentary $\delta^{13}C$ signatures at the impacted station were largely influenced by the presence of bentonite (Fig. 4.19). Although current carbon crediting methodologies apply a discount in carbon gains due to the presence of allochthonous C_{org} , emerging scientific evidence suggests that most (if not all) allochthonous sedimentary C_{org} in blue carbon projects should be accounted for as additional (Houston et al., 2025). Nevertheless, the amount of allochthonous sedimentary C_{org} at the study areas seems negligible, likely linked to the absence of major run-off from rivers, the open coastline and oligotrophic water along the western coastline of Menorca.

On the other hand, it seems plausible to establish a direct cause-effect relationship between the construction of the desalination plant and the loss of seagrass vegetative biomass within the directly impacted area, and thereby greenhouse gas emissions from seagrass biomass loss can be estimated. Although the legacy of impacts of the desalination plant are still tangible within the surrounding meadows 15 years after the disturbance (i.e., lower shoot density, cover, vegetative biomass and vegetative biomass C_{org} stocks; Fig. 4.22 and Fig. 4.23), the link between the decreased structure of meadows surrounding the impacted station and the construction of the desalination plant is less clear and difficult to ascertain under carbon crediting methodologies. Although the meadows from the control station had up to 2.5-fold higher shoot density and biomass, and 8-fold higher % cover than the meadows surrounding the impacted station, linking carbon benefits beyond the boundaries of the blue carbon project activities planned (i.e., restore a portion of lost meadows within the impacted area) may not be feasible.

The other carbon pool that could be accounted for in carbon crediting schemes is the litter carbon pool, which was up to 3-fold higher within the impacted station than in the control station (Fig. 4.23). Thus, the results obtained showed that sandy patches within the control station accumulate higher litter biomass, whereas litter was more widespread but less abundant in the open space within the bare impacted station, showcasing how fragmented landscapes and environmental conditions shape the accumulation of litter (Fig. 4.22). It seems likely that the litter in the impacted station originated from nearby meadows rather than produced in situ by the small and sparse plants growing. Litter within *P. oceanica* meadows exhibits high seasonal variability and therefore, further studies encompassing larger areas and sampling effort, and seasonal variability should be conducted to obtain more robust estimates of the litter C_{org} pool and drivers.

The shape of seagrass meadows within the control station consists of up to 1 m tall seagrass mounts in between sandy channels, which together with the coarser nature of the sediments suggests that the study region at the west coast of Menorca is regularly subjected to hydrodynamic energy. The fact that the study region is not a depositional environment likely explains the relatively low sedimentary C_{org} stocks within the top 40 cm observed ($22 \pm 5 \text{ Mg } C_{org} \text{ ha}^{-1}$) compared to other regions within the Balearic Islands ($> 60 \text{ Mg } C_{org} \text{ ha}^{-1}$; Mazarrasa et al., 2017) or within geomorphological settings that offer protection from strong winds and swell in



Mediterranean Sea ($150 \pm 3 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$; adapted from Serrano et al., 2016). On the contrary, sedimentary C_{org} sequestration rates at the control station studied ($1.05 \pm 6.13 \text{ Mg C}_{\text{org}} \text{ ha}^{-1} \text{ yr}^{-1}$) were one order of magnitude higher than previous estimates in the Balearic Islands (ranging from 0.09 to 0.5 $\text{Mg C}_{\text{org}} \text{ ha}^{-1} \text{ yr}^{-1}$; Mazarrasa et al., 2017) but similar to estimates across the Mediterranean Sea ($0.84 \pm 0.20 \text{ Mg C}_{\text{org}} \text{ ha}^{-1} \text{ yr}^{-1}$; Serrano et al., 2016). The fact that only one core was dated together with the mixing of ^{210}Pb profile that likely resulted in an overestimate of sediment accumulation rates precludes further discussion around the relatively high sedimentary C_{org} accumulation rates obtained.

Notwithstanding, seagrass meadows at the study area did not accumulate thick organic-rich sediments compared to other regions in the Mediterranean where seagrass mats can reach more than 6 meters in thickness (Serrano et al., 2012). The presence of a rocky substrate underneath the meadows at the west coast of Menorca together with the young age of the sediment deposits (40 cm- thick; < 100 years) suggests that either the meadows only established recently within the area or that the sediment deposits are dynamic and did not build up with a coherent chronological sequence. Further studies that aim at obtaining sediment age-depth models along a coastal-offshore gradient and within a broader area are encouraged to determine the age of the meadows studied, and their sedimentary C_{org} sequestration capacity.

Although the presence of sparse *P. oceanica* shoots and clumps within the impacted station suggest limited physiological and ecological impairment 15 years after the disturbance and some potential for natural recovery, the high silt-clay content (e.g., bentonite) likely constrains both natural and assisted recolonization. *P. oceanica* is better adapted to sandy substrates and typically fails to persist in mud-rich sediments due to reduced pore-water exchange and O_2 diffusion, which can lead to hypoxia and enhanced sulfate reduction, with negative effects on growth and survival (Piñeiro-Juncal et al., 2022; Nelson, 2009; Folmer et al., 2012). Accordingly, restoration should concentrate planting in sectors with sufficiently thick, stable matte and secure anchorage, prioritizing sandy or mixed sands over bentonite-rich muds, and use high-resolution mapping to guide patch selection. In parallel, it recommended (i) side-scan sonar coupled with multibeam/INTERFEROMETRIC bathymetry to map fine-scale bed-elevation changes in the lost-meadow area relative to surrounding meadows and derive eroded matte thickness and associated carbon emissions; (ii) installation of Surface Elevation Tables (SETs/RSETs) in both impacted and control stations to track in situ vertical sediment dynamics and validate acoustic estimates; and (iii) targeted biogeochemical analyses (e.g., grain size, mineralogy/XRD, $\delta^{13}\text{C}$, C:N, porewater chemistry) to confirm bentonite presence and diagnose geochemical constraints that should inform restoration methods. Although, it depends on the capability and the resources that we have, or we can obtain for it.

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5 Capo Testa-Punta Falcone, Italy

5.1 Study area

The Capo Testa – Punta Falcone MPA is located in northern Sardinia, Italy (Fig. 5.1). Established by the Ministerial Decree in 2018, it is entirely managed by the Municipality of Santa Teresa Gallura (SS) and divided into four zones of different protection levels. The MPA also includes the marine SCI and SPA site “da Capo Testa all’Isola Rossa” (ITB013052).

The area is characterized by an extensive *Posidonia oceanica* meadow of exceptional natural and conservation value, recognized as a priority habitat under the EU Habitats Directive (92/43/EEC). Covering approximately 294.47 ha, the meadow is generally in good conservation status. However, in the eastern part of the MPA, unregulated boat anchoring has caused significant fragmentation. In particular, MPA mapping data collected in 2021 and dedicated underwater surveys carried out in 2023, identified degraded areas in La Marmorata bay, where the *Posidonia oceanica* meadow shows the presence of dead matte patches (Fig. 5.2).

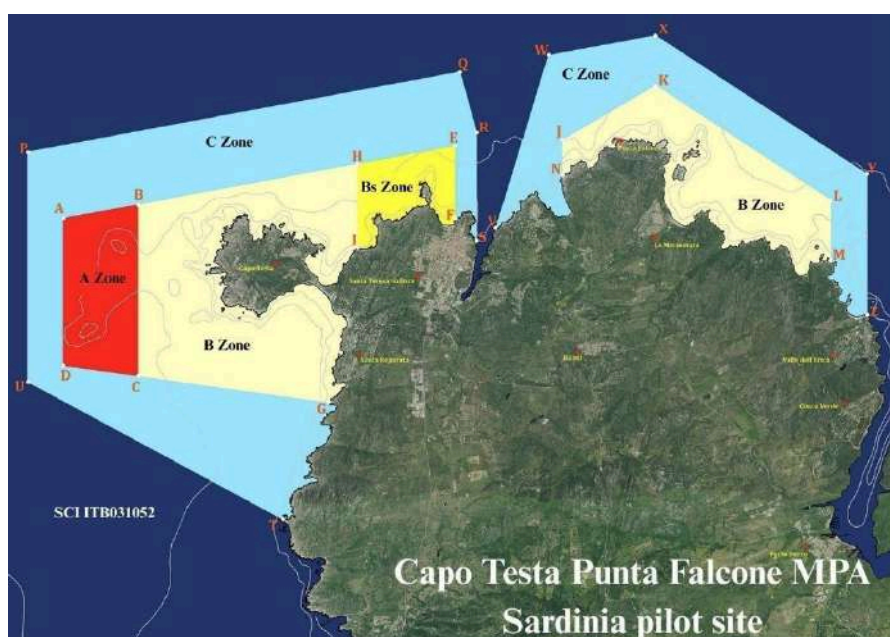


Figure 5.1 Map of the Sardinia pilot site: Capo Testa-Punta Falcone MPA.

5.2 Methods

5.2.1 Habitat mapping

The baseline for habitat mapping was the cartographic dataset of the MPA produced by Aera in 2021, which covered the entire marine protected area. This mapping highlighted the extension of the meadow in the MPA several zones where *Posidonia oceanica* meadows showed signs of damage or fragmentation (Fig. 5.2).

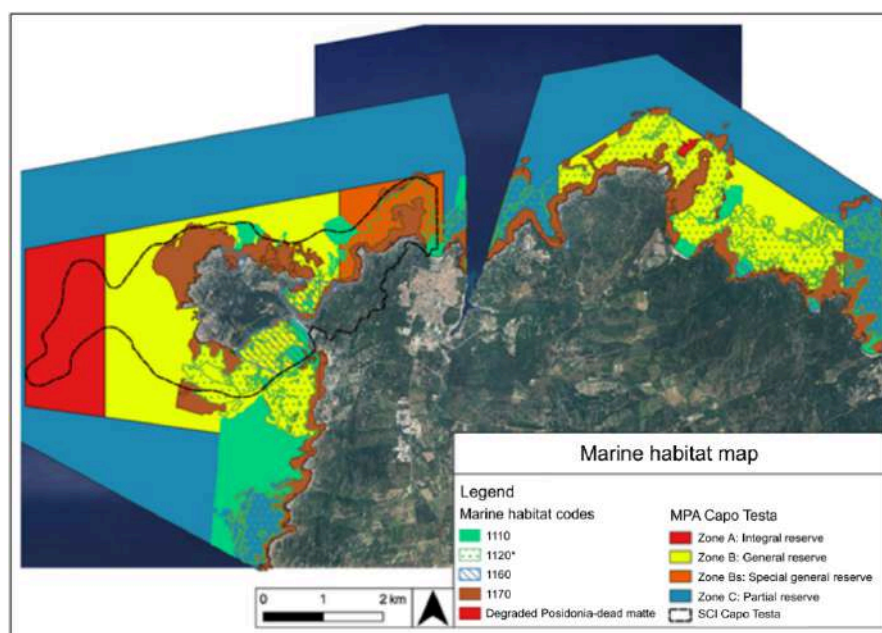


Figure 5.2 Biocenotic map of the MPA

Building on these data, targeted underwater SCUBA surveys were carried out in March 2023 across the whole La Marmorata Bay. Surveys were performed along linear transects and complemented by systematic photo collection. Depth data were recorded with dive computers, while the precise coordinates of both control, named control, and impacted meadows were collected using GPS devices. These surveys provided a more accurate characterization of the meadow and a clearer definition and localization of impacted patches. In particular, impacted areas were tested with the same metal pegs later used for transplantation, in order to verify both substrate type and thickness. The extent of dead matte areas was measured using a measuring wheel. This process enabled the identification of the most suitable area for restoration, as well as the evaluation of ecosystem services in both areas.

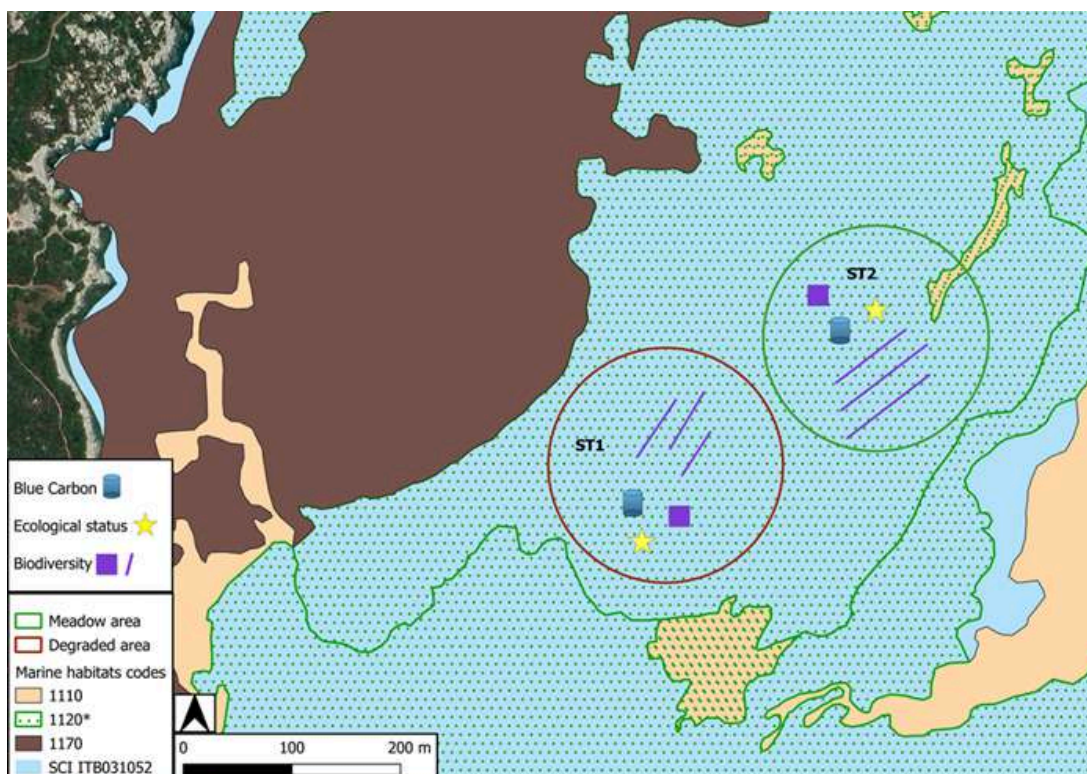
5.2.2 Ecological status

The first baseline of the ecological status of the *Posidonia oceanica* meadow was established in

2021 through the application of the *Posidonia oceanica* Rapid Easy Index (PREI) (Gobert et al., 2009; Pergent et al., 2015). This index, widely recognized within the framework of the EU Water Framework Directive, integrates structural and functional parameters such as shoot density, leaf biometry and epiphytic load, thus providing a standardized tool for the ecological quality assessment of seagrass meadows across the Mediterranean. The analysis of the data collected in 2021 showed an average shoot density of approximately 500 shoots m⁻², allowing the meadow to be classified as dense, according to the five density classes proposed by Giraud (1977). Based on the PREI, the meadow was classified as having a Good ecological status, despite some critical issues identified in certain areas, including La Marmorata, which is subject to strong anchoring pressure. These 2021 data constitute the first historical dataset available for the area and are here considered as a reference baseline.

During the ARTEMIS project, the conservation status of the two areas, “impacted” (ST1) and “control” (ST2), was assessed following the methodology proposed by Personnic et al. (2014) through the application of the EBQI index, specifically aimed at evaluating the ecological status of the *Posidonia oceanica* meadow. The EBQI index integrates data collected and calculated both for the assessment of biodiversity and for the evaluation of the health, structure, and functioning of *Posidonia oceanica* meadows and their associated biotic communities.

Parameters recommended by Personnic et al. (2014) were measured in the two areas, one characterised by a fragmented *Posidonia oceanica* meadow with low cover and presence of several dead matte patches (impacted area - ST1), whereas the other is characterised by high cover and a better state of conservation (control area - ST2) (Fig. 5.3). In both sites, sampling was carried out in the central part of the meadow (about 15 m deep) and along the upper limit (about 5 meters deep).



D.1.2.1Pilots Ecosystem Services Baseline

Figure 5.3 Monitoring sampling sites: impacted (ST1) and control (ST2)

At the central part of *Posidonia oceanica* meadows of both stations (about 20m deep) the following sampling activities and data collection were performed:

- a) **Visual coverage** a) was estimated by a photographic method with 30 photos taken at about 5 metres above the bottom. Pictures of the meadow were analysed through the ImageJ software to evaluate the percentage of seabed covered by the plants.
- b) **Meadow density** was estimated by counting the number of shoots within a square frame of 0.16 m², with 20 random replicates. The density of the meadows was expressed as the number of shoots per m².
- c) **Shoot collection:** 30 *P. oceanica* shoots were sampled for laboratory analyses.
- d) **Litter detritus mass** was collected by hand within 5 random 0.4 m² plots.
- e) ***Pinna nobilis* density** a) was estimated along 20 transects, each 10 m long and 1 m wide.
- f) **Teleosts** were counted within 10 linear transects, each 50 m long and 5 m wide; the total length (to the nearest 2 cm) and the number of individuals per species were recorded. The Specific Relative Diversity Index (SRDI) of teleosts was evaluated as the mean number of species per transect. Teleosts were categorized as predatory, piscivorous, omnivorous and planktivorous and the wet biomass (WM) of each group was estimated as:

$$WM\ 100\ m^{-2} = (\text{individuals counted}) * a * (\text{average size} * b) / 1000$$

where a and b are species-specific growth conversion parameters.

At the upper limit of the *P. oceanica* meadows (about 5 m deep), the following sampling activities and data collection were performed:

- g) **Quadrat surveys:** a) 30 quadrats of 1 m² each were randomly positioned to count the number of *Paracentrotus lividus*, *Holoturia* spp. and filter and suspension-feeders other than *P. nobilis*. For colonial species, the number of colonies was taken into account, while for non-colonial species, the number of individuals was counted; only individuals and colonies over 5 cm in diameter and/or height were considered. Filter and suspension-feeders other than *P. nobilis* were categorized as indicators of high level of organic matter in the water (HOM; e.g. *Sabella spallanzani*, *Phallusia mammillata*, *P. fumigata*, Didemnidae) or low level of organic matter (LOM; e.g. bryozoans, sponges, *Halocynthia papillosa*, *Antedon mediterranea*); the number of individuals of each category was calculated for each quadrat.
- h) **Shoot collection:** a) 30 *Posidonia oceanica* shoots were sampled for laboratory analyses.

5.2.2.1 Laboratory activities

Lepidochronological analysis was performed on the *Posidonia oceanica* shoots sampled in the central part of the meadow at about 15 m; the growth rate of vertical rhizomes was expressed in



cm year⁻¹, the biomass production was expressed in g year⁻¹. Epiphyte biomass was estimated on the two outermost (older) leaves of each shoot. The entire surface of the leaves was scraped, and the epiphytes were dried in an oven at 50 °C for 48 hours before being weighed. The litter detritus and the leaves of all shoots were also dried at 50 °C in an oven to constant weight and then weighed. The percentage of intermediate and adult leaves of shoots collected at the upper limit of the meadows exhibiting bite scars due to *S. salpa* was evaluated. Finally, all sessile epiphyte organisms on shoots (leaves and rhizomes) collected at the upper limit of the meadows were identified.

5.2.2.2 EQBI Index calculation

Following Personnic et al. (2014), the data obtained for each considered variable were categorized into five groups, and a value (Status) ranging from 0 to 4 was assigned to each category. Each status value was then multiplied for its relative weight (from 1 to 5). The sum of all values obtained through this procedure was multiplied by 10 and divided by the reference value (168). The resulting ratios were assigned to one of the five ecological quality classes according to Personnic et al. (2014): bad (EBQI < 3.5), poor (3.5 > EBQI < 4.5), moderate (4.5 > EBQI < 6), good (6.0 > EBQI < 7.5) and high (EBQI > 7.5).

5.2.3 Biodiversity

To evaluate biodiversity, data were collected from teleost visual census, density of *Pinna nobilis*, and invertebrate abundance. In addition to the parameters included in the EBQI protocol, matte and canopy samplings were performed to further assess epifauna, endofauna, epibenthic organisms' abundance and enrich the species list.

As with the other analyses, sampling was carried out in both stations (Fig. 5.3). A total of 12 samples were collected, equally divided between matte and canopy, using two different tools, corer and quadrat. All samples were collected randomly within each site, starting from the central entry point and maintaining a minimum distance of 5 m between replicates.

For matte sampling, a corer (Fig. 5.4) specifically designed for this study (15 cm diameter, 40 cm length) was used to extract samples (15 × 20 cm). Three replicates were taken from the control (ST2) and three from the impacted one (ST1). The corer was inserted into the matte substrate and rotated; when necessary, a serrated knife was employed to cut rhizomes and roots to facilitate extraction.



Figure 5.4 Matte sampling using a corer (15 cm diameter, 40 cm length) designed for this study.

For canopy sampling, three replicates were collected in each station, control (ST2) and impacted (ST1) using a 20 × 20 cm quadrat and enclosing the above-ground material in small-mesh bags.

5.2.3.1 Laboratory activities

All samples collected in the field were fixed in alcohol, stored in portables and transported to the laboratory for analysis. Once in the laboratory, samples were subsequently washed through a 0.5 mm mesh sieve for taxonomic identification and abundance estimation. Sorting performed under a stereomicroscope, and taxa were identified to species level whenever possible (Fig. 5.5). The abundance of mobile taxa was expressed as the number of individuals, while the abundance of sessile taxa was assessed based on their orthogonal projection and expressed in cm².



Figure 5.5 Laboratory activities during the sorting process under a stereomicroscope.

For the assessment of biodiversity associated with *Posidonia oceanica* meadows, the following parameters were considered: total abundance and species richness for epifauna, epibenthic organism, endofauna and for fish. Biodiversity indices were calculated, including Shannon diversity (H'), Pielou's evenness (J'), and Margalef's index (d), to describe overall diversity, the evenness of abundance distribution, and richness standardized to sample size, respectively. To

assess differences in benthic community composition, a PERMANOVA (Permutational Multivariate Analysis of Variance) was applied on Bray–Curtis dissimilarity matrices based on relative abundance data of mobile fauna and sessile organisms. In the case of sessile organisms, the analysis was complemented by the SIMPER (Similarity Percentage analysis) test, in order to identify the taxa contributing most to the dissimilarity between areas.

5.2.4 Blue Carbon

5.2.4.1 Study Sites and Sampling Design

This study was conducted in October 2024 at two stations, the same for EBQI assessment and biodiversity (Fig. 5.6). Both stations were located at approximately 20 m depth.

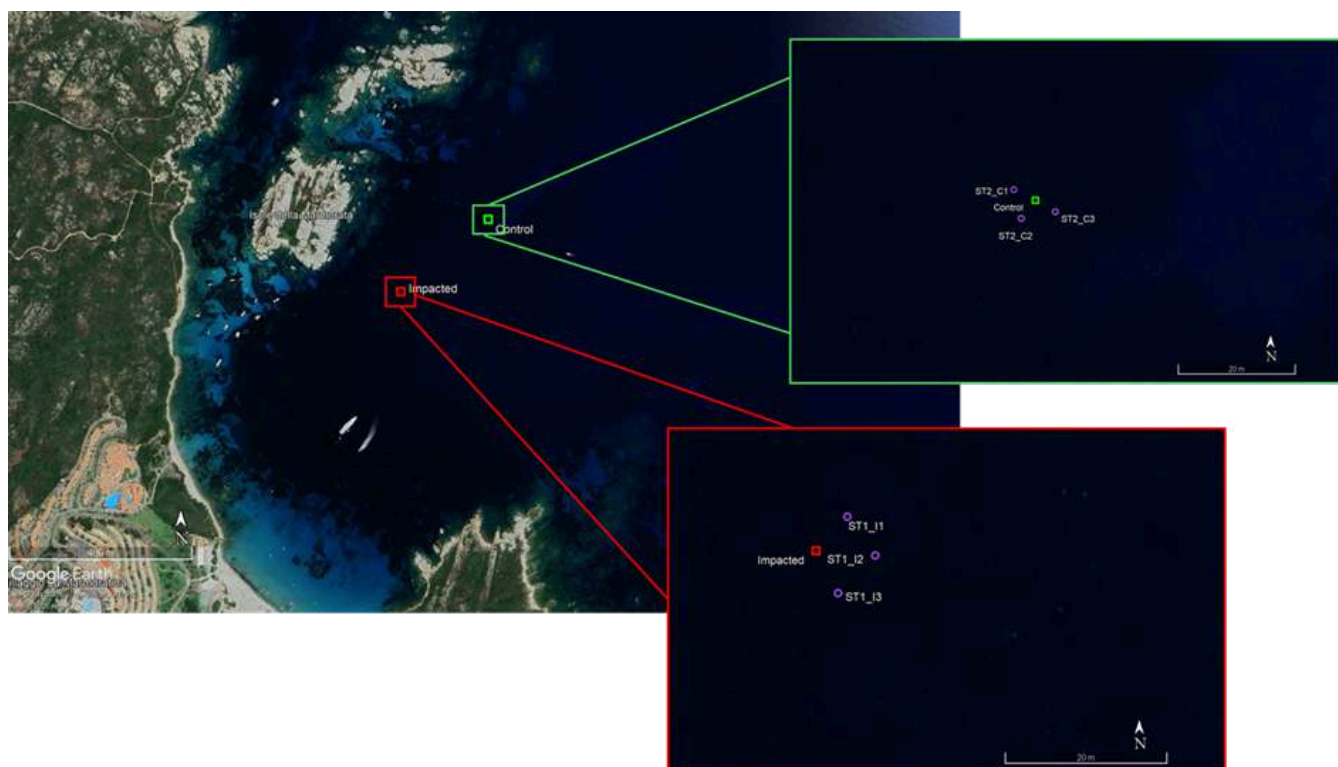


Figure 5.6 BC sampling stations: control ST2 (in green) and impacted ST1 (in red).

5.2.4.2 Sedimentary pool

Sedimentary pool sampling was performed using PVC corers (1.5 m in length, with a 1 m effective core capacity; 75 mm diameter), serrated at the lower edge to facilitate penetration into the sediment, and fitted with caps at both ends to ensure sample integrity during retrieval (Fig. 5.7). A total of six cores were collected, three from the control (ST2) and three from the impacted station (ST1). Core sampling was carried out by OTS-certified divers under the supervision and coordination of experienced marine biologists and scientific diving operators. The core sampling

was conducted from a central reference point, shifting approximately 10 m between consecutive sampling points in both study sites.

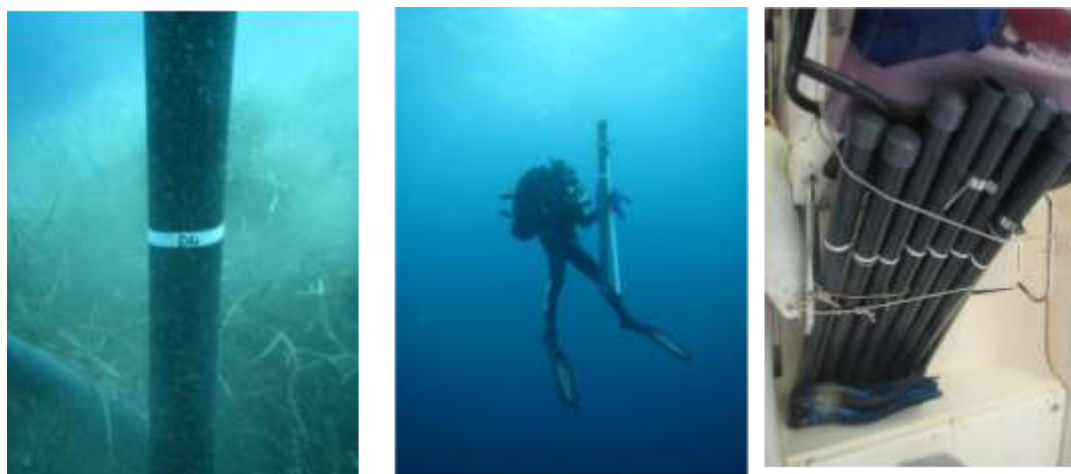


Figure 5.7 Matte sampling using PVC corers (1.5 m in length and 75 mm diameter).

5.2.4.3 Sample Handling and Laboratory Analyses

Immediately after collection, cores were sealed, labelled, and transported under controlled conditions to an accredited laboratory for processing and analysis. All laboratory analyses were performed by an accredited facility to ensure compliance with quality standards and reproducibility of results. Laboratory activities consisted of analytical procedures conducted on a variable number of sediment subsamples (layers) obtained from the stratification of the cores (1 m maximum length), with three cores from the impacted area (ST1) and three from the control area (ST2). For clarity, cores labelled with the prefix “I” refer to samples collected in the ST1, while those labelled with the prefix “C” refer to samples collected in ST2. Prior to sectioning, the cores were first air-dried and then split longitudinally using a circular saw to expose the sediment profile.

In the laboratory, cores were sectioned and analysed as follows.

5.2.4.4 Analytical Analyses

Cores intended for analytical determinations (BC Index: TC, IC, TOC, and sediment characterization) were sectioned as follows:

- Samples I2, I3, C1, and C2:
 - every 5 cm down to 50 cm
 - every 10 cm thereafter to the end of the core length
 - additionally at any visible change in color, texture, or other sediment features
- Samples I1 and C3:

- o every 3 cm down to 25 cm
- o every 5 cm from 25 to 50 cm
- o every 10 cm thereafter to the end of the core length
- o additionally at any visible change in color, texture, or other sediment features

For the analytical determinations, standardized laboratory methods were applied, as summarized in Table 5.1.

Table 5.1 Parameters and analytical methods applied

Parameter	Analytical Method
Macroscopic description (color, odor, presence of concretions, natural or anthropogenic residues)	ASTM D4979:2019
Gravel > 2 mm	ASTM D422
Sand 2 mm > x > 0.063 mm	ASTM D422 + ISO 13320:2020
Silt 0.063 mm > x > 0.004 mm / Clay < 0.004 mm	ISO 13320:2020
Total Carbon (TC)	UNI EN 13137:2002, Method B
Inorganic Carbon (IC)	UNI EN 13137:2002, Method B
Total Organic Carbon (TOC)	UNI EN 13137:2002, Method B

The sediment C_{org} density ($mg C_{org} cm^{-3}$) was calculated for each sediment depth in each core by multiplying the soil DBD ($g cm^{-3}$, estimated by granulometry and water content) by the C_{org} concentration (%; Table 5.13). The Organic carbon content of a sample was detected and then calculated using the relationship between % LOI and % Corg for the Seagrasses (% LOI > 0.2) ecosystem, $\% C_{org} = 0.43 * \% LOI - 0.33$. To allow direct comparisons among locations, the sedimentary C_{org} stocks ($Mg C_{org} ha^{-1}$) were calculated as the cumulative carbon mass in 40 cm-thick deposits and standardised to 1 m of sediment thickness.. Sediment C_{org} accumulation rates (expressed in $g C_{org} m^{-2} yr^{-1}$) for the last decades were estimated using ^{210}Pb age-depth models in core I3 and C3 (Table 5.13).

5.2.4.5 Radiometric Analyses

Radiometric analyses (^{210}Pb dating) were conducted on two cores, one from the impacted area and one from the control one. These cores were sectioned every 1 cm down to 25 cm depth, resulting in a total of 50 layers (25 per site). Radiometric dating (^{210}Pb) was carried out using alpha spectrometry of the granddaughter ^{210}Po isotope to determine supported ^{210}Pb and to establish sediment accumulation rates and chronological profiles. For the radiometric determinations, standardized laboratory methods were applied, as summarized in Table 5.2.

Table 5.2 Parameters and methods applied

Parameter	Method
^{210}Po	Alpha spectrometry using Si detectors

Table 5.3 Details of the soil cores sampled and analyses

Core ID	Meadow area	Latitude	Longitude	Tot. soil recovered	Analysis
I1	Impacted	41°15'06.70"N	9°14'26.90"E	58	C_{tot}
I2	Impacted	41°15'06.70"N	9°14'26.90"E	54	C_{tot}
I3	Impacted	41°15'06.70"N	9°14'26.90"E	49	$C_{\text{tot}} + ^{210}\text{Pb}$
C1	Control	41°15'12.10"N	9°14'35.40"E	42	C_{tot}
C2	Control	41°15'12.10"N	9°14'35.40"E	44	C_{tot}
C3	Control	41°15'12.10"N	9°14'35.40"E	52	$C_{\text{tot}} + ^{210}\text{Pb}$

Once sectioned, the subsamples were placed in hermetically sealed, pre-labelled bags and allocated to their respective laboratory analyses (Fig. 5.8).



Figure 5.8 Laboratory activities during the sorting process.

5.2.4.6 Vegetative pools

To quantify the vegetative pool of *Posidonia oceanica* meadows, both living and litter biomass were sampled within the two stations, control and impacted. A total of 60 *P. oceanica* shoots (30 per station), were sampled to quantify living above and below ground biomass, while a total of 10 litter samples (5 per station), were collected at a depth of 15 m. The collected samples were used to estimate plant biomass and organic carbon (Corg) content. Following Scanu et al. (2022), we applied a conversion factor for living biomass $F_c = 0.34$ to estimate organic carbon content from

seagrass dry biomass; this value is derived from established literature on *P. oceanica* elemental composition (Duarte 1990; Fourqurean et al. 2012). The same conversion approach was used for litter biomass, allowing the estimation of carbon content (kg) for the given plot size (m²).

5.3 Results

5.3.1 Habitat mapping

The habitat mapping of the MPA identified the presence of the following habitats:

- **1110**: sandbanks
- **1120***: *P. oceanica* meadow
- **1160**: Large shallow inlets and bays
- **1170**: reef (rocky seabed)

The *Posidonia oceanica* habitat (1120*) covers an area of approximately 294.47 ha. Within this habitat, three impacted areas described as dead matte were detected, covering a total surface of around 7.08 ha. Two of these impacted areas are located within La Marmorata bay and served as the starting point for the underwater surveys, which were then progressively extended to the surroundings until the most suitable restoration site was identified and selected (Fig. 5.9).

The selected site is characterized by a large patch of dead matte substrate, extending over approximately 200 m² at a depth of 20 – 25 m. This site was also used for the evaluation of ecosystem services, together with a control area identified within the bay and characterized by a control *Posidonia oceanica* meadow. The control station (ST2) is located at a similar depth to the impacted area (ST1) and shows a higher percentage of leaf cover.

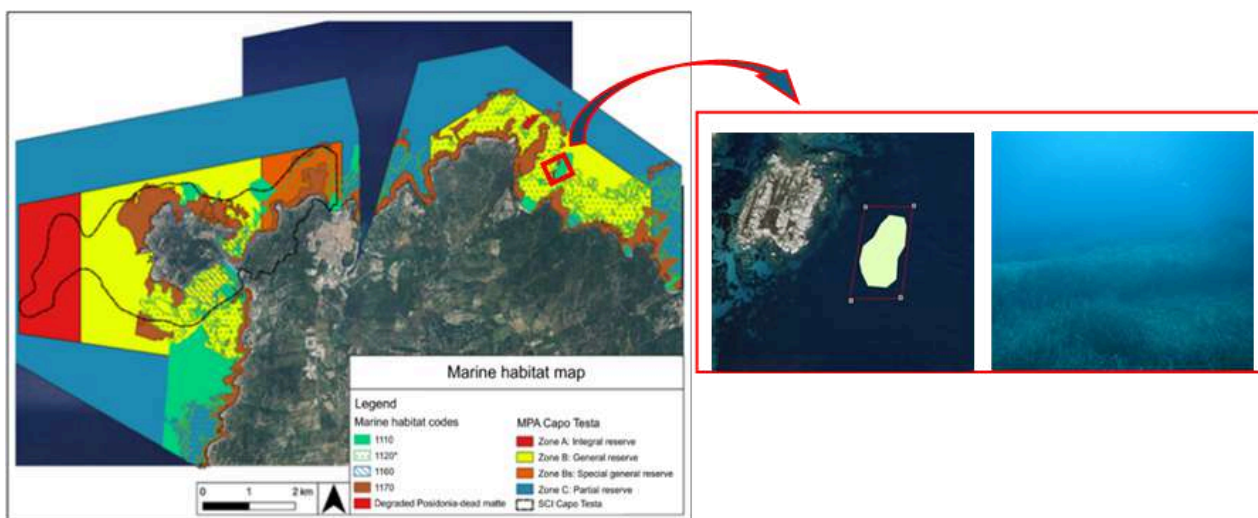


Figure 5.9 Localization of the degraded site identified as suitable for transplantation.

5.3.2 Ecological status

5.3.2.1 EBQI - results

The two studied areas, both developed on matte, showed clear different structures. The percentage cover resulted higher in the control area (94.2 ± 0.6 , mean $\pm$ SE, $n = 30$, Fig. 5.10) than in the impacted one (40.3 ± 4.2 , Fig. 5.11). On the contrary, the density was similar, with 290.6 ± 1.8 shoots m^{-2} in the control and 263.7 ± 1.5 in the impacted area. Wide deposits of dead leaves were present in all cleared areas (Fig. 5.12) resulting in very high litter biomass in both areas. In contrast, the epiphyte biomass was very low in both. No individuals of *Pinna nobilis* were found in the studied areas.

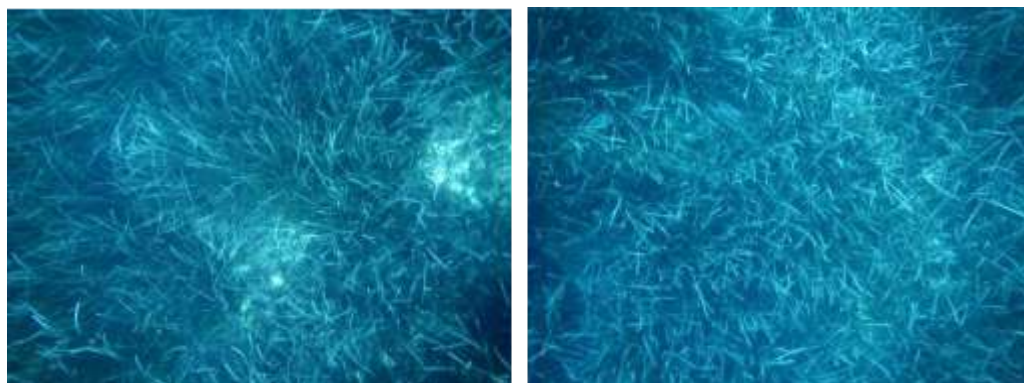


Figure 5.10 Images of the control area (ST2)

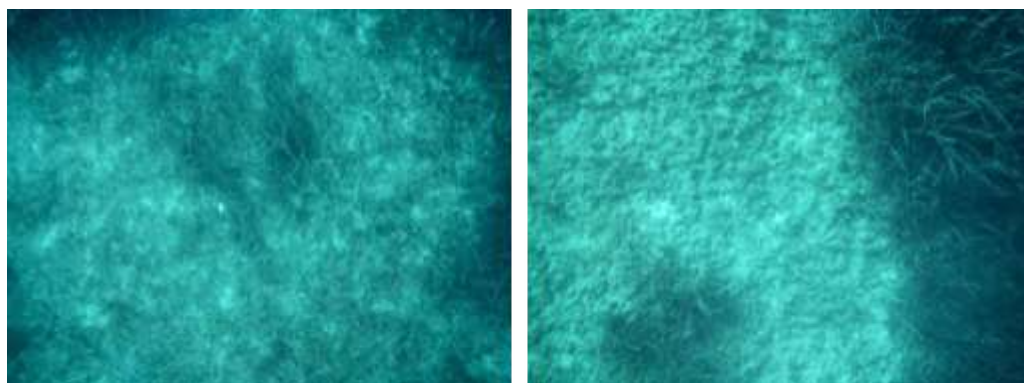


Figure 5.11 Images of the impacted area (ST1)



Figure 5.12 Deposits of dead leaves within the meadow

The fish assemblages were quite poor, as only thirteen species of teleost were found in total, with eleven species found per each area (Table 5.4). The most common species were *Chromis chromis*, *Spicara smaris*, *Serranus scriba*, *Coris julis*, *Diplodus annularis*, *D. vulgaris* and *Spondyllosoma cantharus*. *Paracentrotus lividus* and *Holoturia* spp. were common at the upper limit of the control station, while were rare at the impacted one. *Halocynthia papillosa* was the only suspension-feeder detected in the survey.

Table 5.4 Species composition and mean abundance ($\pm$ SE) per transect of teleosts assemblages

TAXA	Control (ST2)	Impacted (ST1)
Omnivorous teleosts		
<i>Boops boops</i>	5.9 $\pm$ 3.2	0.0
<i>Oblada melanura</i>	0	0.2 $\pm$ 0.2
Piscivorous teleosts		
<i>Serranus cabrilla</i>	0	0.2 $\pm$ 0.1
<i>Serranus scriba</i>	0.5 $\pm$ 0.3	0.3 $\pm$ 0.1
Predatory teleosts		
<i>Coris julis</i>	4.8 $\pm$ 1.6	4.9 $\pm$ 1.0
<i>Diplodus annularis</i>	1.3 $\pm$ 0.4	1.6 $\pm$ 0.4
<i>Diplodus vulgaris</i>	0.6 $\pm$ 0.3	1.0 $\pm$ 0.6
<i>Spondyllosoma cantharus</i>	1.8 $\pm$ 0.7	0.5 $\pm$ 0.2
<i>Symphodus doderleini</i>	0.1 $\pm$ 0.1	0
<i>Symphodus tinca</i>	0.4 $\pm$ 0.2	0
Zooplankton feeders		
<i>Chromis chromis</i>	180.4 $\pm$ 47.9	139.5 $\pm$ 49.4
<i>Sardina pilchardus</i>	22.0 $\pm$ 21.1	92.4 $\pm$ 59.8
<i>Spicara smaris</i>	18.4 $\pm$ 12.3	12.5 $\pm$ 9.4

Values of EBQI descriptors are reported in Table 5.5. The biomass of leaves was 1.44 $\pm$ 0.11 g shoot⁻¹ in the control and 0.96 $\pm$ 0.07 g shoot⁻¹ in the impacted station. The biomass production of rhizomes was 0.12 $\pm$ 0.01 g shoot⁻¹ in the control station and 0.08 $\pm$ 0.01 g shoot⁻¹ in the impacted one (Table 5.6). Values of EBQI were quite low in both stations (2.97 and 4.31 in impacted and

control station, respectively) which consequently resulted in bad status the impacted station and in poor status the control one (Table 5.7). The Confidence Index was equal to the reference as all data were obtained from the survey.

Table 5.5 Values (mean $\pm$ SE) of EBQI descriptors of the two studied meadows

Descriptor	Control (ST2)	Impacted (ST1)
Growth of rhizomes (cm y ⁻¹)	10.6 $\pm$ 0.5	6.3 $\pm$ 0.4
Shoot density (shoots m ⁻²)	290.6 $\pm$ 1.8	263.7 $\pm$ 1.5
Meadow cover (%)	94.2 $\pm$ 0.6	40.3 $\pm$ 4.2
Biomass of leaf epiphytes (g shoot ⁻¹)	0.016 $\pm$ 0.003	0.018 $\pm$ 0.002
Density of <i>Pinna nobilis</i> (individuals m ⁻²)	0	0
Density of HOM (individuals m ⁻²)	0	0
Density of LOM (individuals m ⁻²)	0	0.06 $\pm$ 0.05
Litter detritus (g m ⁻²)	6179.3 $\pm$ 741.0	4045.6 $\pm$ 524.5
Density of <i>Holoturia</i> spp (individuals m ⁻²)	1.1 $\pm$ 0.2	0.03 $\pm$ 0.00
Density of <i>Paracentrotus lividus</i> (individuals m ⁻²)	0.6 $\pm$ 0.1	0.03 $\pm$ 0.00
Grazing index (% leaves)	2.6	3.3
Predatory teleosts (kg 100 m ⁻²)	284.8 $\pm$ 57.9	205.6 $\pm$ 46.5
Piscivorous teleosts (kg 100 m ⁻²)	14.0 $\pm$ 11.8	11.6 $\pm$ 7.1
Planktivorous teleosts (kg 100 m ⁻²)	442.1 $\pm$ 122.8	671.0 $\pm$ 349.6
Onnivorous teleosts (kg 100 m ⁻²)	494.7 $\pm$ 295.1	7.4 $\pm$ 7.1
SRDI	5.2$\pm$0.6	4.2$\pm$0.5

Table 5.6 Additional variables of the two studied meadows (mean $\pm$ SE)

Descriptor	Control (ST2)	Impacted (ST1)
Biomass of rhizomes (g y ⁻¹)	0.12 $\pm$ 0.01	0.08 $\pm$ 0.01
Biomass of leaves (g shoot ⁻¹)	1.44 $\pm$ 0.11	0.96 $\pm$ 0.07

Table 5.7 Status grade of the mean functional compartment and values of EBQI index



Functional compartment	Status		Weight		Status grade	
	Impacted (ST1)	Control (ST2)			Impacted (ST1)	Control (ST2)
Growth of rhizomes	2	4	3	3	6	12
Shoot density	2	2				
Meadow cover	1	4				
Meadow	1.5	3	5	5	7.5	15
Biomass of leaf epiphytes	2	2	4	4	8	8
Density of <i>Pinna nobilis</i>	0	0	2	2	0	0
Density of HOM	4	4				
Density of LOM	0	0				
Filter feeders	2	2	2	2	4	4
Litter detritus	4	4	2	2	8	8
Density of <i>Holothuria</i> spp	1	4	2	2	2	8
Density of <i>Paracentrotus lividus</i>	3	1				
Grazing index	1	1				
Herbivorous	2	2	5	5	10	10
Predatory teleosts	0	0	5	5	0	0
Piscivorous teleosts	0	0	5	5	0	0
Planktivorous teleosts	0	0				
Onnivorous teleosts	0	0				
Planktivorous-Onnivorous teleosts	0	0	3	3	0	0
SRDI	1	2	3	3	3	6
<i>Phalacrocorax</i> spp.	3	3				
<i>Pandion haliaetus</i>	0	0				
Sea birds	1.5	1.5	1	1	1.5	1.5
Sum of status grades					56	66.5
Sum x 10					560	665
Reference value					168	168
EBQI					2.97	4.31

A total of 36 epiphyte species were found on the upper limit shoots, 16 macroalgae and 20 sessile animals (Table 5.8). The most common organisms on leaves were the Rhodophyta *Hydrolithon farinosum*, *Chondria mairei* and *Ceramium diaphanum* and the Bryozoa *Aetea truncata* and *Electra posidoniae*. The most common organisms on rhizomes were the Rhodophyta *Acrosorium ciliolatum*, *Acrothamnion preissii* and *Peyssonnelia rubra*, the Foraminifera *Miniacina miniacea*, the Cnidaria *Aglaophenia harpago* and the Bryozoa *Chlidonia pyriformis*, *Chorizopora brongniartii*, *Fenestrulina malusii* and *Cradoscrupocellaria reptans*.

Table 5.8 Species composition of epiphyte assemblages



Taxa	Control (ST2)		Impacted (ST1)	
	leaves	rhizomes	leaves	rhizomes
Heterokontophyta				
<i>Dictyota linearis</i> (C. Agardh) Greville	X		X	
<i>Sphacelaria cirrosa</i> (Roth) C. Agardh	X			X
Rhodophyta				
<i>Acrosorium ciliolatum</i> (Harvey) Kylin		X		X
<i>Acrothamnion preissii</i> (Sonder) Wollaston		X		X
<i>Apoglossum ruscifolium</i> (Turner) J. Agardh		X		X
<i>Ceramium diaphanum</i> (Lighfoot) Roth	X		X	
<i>Chondria mairei</i> G. Feldmann	X		X	
<i>Crouania attenuata</i> (C. Agardh) J. Agardh	X		X	
<i>Dasya ocellata</i> (Grateloup) Harvey	X		X	
<i>Jania virgata</i> (Zanardini) Montagne		X		X
<i>Herposiphonia secunda</i> (C. Agardh) Ambronn	X		X	
<i>Hydrolithon farinosum</i> (Lamouroux) Penrose	X	X	X	X
<i>Laurencia minuta</i> Vandermeulen, Garbary & Guiry	X		X	
<i>Peyssonnelia rubra</i> (Greville) J. Agardh		X		X
<i>Vertebrata fruticulosa</i> (Wulfen) Kuntze	X		X	
<i>Womersleyella setacea</i> (Hollenberg) R.E. Norris		X		X
Foraminifera				
<i>Miniacina miniae</i> (Pallas, 1766)		X		X
Cnidaria				
<i>Aglaophenia harpago</i> Von Schenk, 1965		X		X
<i>Tridentata perpusilla</i> (Stechow, 1919)			X	
Annelida				
<i>Spirorbis spirorbis</i> (Linnaeus, 1758)	X	X	X	X
Bryozoa				
<i>Aetea truncata</i> (Landsborough, 1852)	X	X	X	X
<i>Amathia lendigera</i> (Linnaeus, 1761)	X			
<i>Beania hirtissima</i> (Heller, 1867)				X
<i>Celleporina caminata</i> (Waters, 1879)		X		
<i>Chlidonia pyriformis</i> (Bertoloni, 1810)		X		X
<i>Chorizopora brongniartii</i> (Audouin, 1826)		X		X
<i>Cribrilaria radiata</i> (Moll, 1803)		X		
<i>Crisia fistulosa</i> (Heller, 1867)		X		
<i>Electra posidoniae</i> Gautier, 1954	X		X	
<i>Fenestrulina malusii</i> (Audouin, 1826)		X		X
<i>Patinella radiata</i> (Audouin, 1826)	X			X
<i>Pherusella tubulosa</i> (Ellis & Solander, 1876)		X		
<i>Scrupocellaria scruposa</i> Busk, 1851		X		X
<i>Cradoscrupocellaria reptans</i> (Linnaeus, 1758)				X
<i>Tubicellopora magnicostata</i> (Barroso 1919)		X		
Tunicata				
<i>Halocynthia papillosa</i> (Linnaeus, 1767)				X

5.3.3 Biodiversity

Macrozoobenthos analyses revealed clear differences between matte and canopy samples. Canopy samples contained both sessile and mobile organisms, whereas only mobile organisms were found in matte samples. Sessile assemblages in the canopy included both macroalgae and macrozoobenthic organisms. In total, 54 macroalgae species were identified (8 Heterokontophyta, 6 Chlorophyta, and 40 Rhodophyta) together with 29 macrozoobenthic species (1 Foraminifera, 1 Porifera, 4 Cnidaria, 1 Annelida, 19 Bryozoa, and 3 Chordata). Additionally, a total of 71 mobile macrozoobenthic taxa (29 Annelida, 22 Arthropoda, 16 Mollusca, 2 Sipuncula, and 2

Echinodermata) were found across both areas.

Multivariate analysis showed that no significant differences were detected in the composition of mobile fauna between the two areas (PERMANOVA: Pseudo-F = 1.117; $p = 0.384$). In contrast, for sessile organisms, PERMANOVA revealed significant differences between the impacted and control stations (Pseudo-F = 3.769; $p = 0.044$). The SIMPER test identified the taxa contributing most to the dissimilarity between the two stations. Among them, *Peyssonnelia rubra*, *Amathia lendigera*, and *Scropucellaria scrupea* accounted for a substantial contribution, along with other taxa such as *Hydrolithon farinosum*, *Obelia geniculata*, and *Flabellia petiolata*. Regarding leaf epiphytes, no clear pattern was observed, whereas for rhizome-associated taxa in the control area, a higher occurrence of species typical of more sciaphilous conditions (*Peyssonnelia spp.*, *Scropucellaria spp.*, *Margaretta cereoides*, *Flabellia petiolata*, *Miniacina miniacina*) was detected, consistent with a more developed canopy compared to the impacted area.

Comparison between impacted and control stations - CANOPY

Epibenthic assemblages exhibited comparable overall species richness (65 species in the impacted vs. 67 in the control station), yet their structure and cover differed substantially (Table 5.9). Rhodophyta dominated in both conditions (32 species), confirming their central role in the community. In the control station, Bryozoa, Cnidaria, and Porifera were slightly more represented, indicating a more complex and stable sessile assemblage, whereas Heterokontophyta were more abundant in the impacted canopy, reflecting the opportunistic behaviour of several brown algae under degraded conditions. Ascidiaceans (Chordata) also appeared slightly more common in the impacted station, possibly due to their capacity for rapid colonization of newly available substrates following disturbance. In terms of total cover, the control station exhibited higher mean values (275.1 ± 31.9 SE 18.4) compared to the impacted one (256.9 ± 42.7 SE 24.7), with lower variability, suggesting that pristine conditions support greater and more stable biomass. Overall, these findings highlight how disturbance does not necessarily reduce sessile species richness, but it significantly reshapes community composition and cover. In the impacted station, the enhanced edge effect and the loss of the canopy alter habitat characteristics, leading to a decline of species typically associated with *Posidonia* and adapted to shaded conditions (e.g., sciaphilous fauna), while favouring less specialized, more light-demanding taxa such as photophilic brown algae.

Table 5.9 List of epibenthic organisms in the canopy. Abundance is expressed as cm²



PHYLUM	SPECIE	AUTHOR	Impacted (ST1)			Control (ST2)		
			I1	I2	I3	C1	C2	C3
Heterokontophyta	Cutleria multifida (sporofito)	(Turner) Greville	1,2	2	1,6	0	0,4	0
Heterokontophyta	Dictyopteris polypodioides	(A.P.De Candolle) J.V. Lamouroux	0	0	0	0	0	0
Heterokontophyta	Dictyota spp.		4,8	4,4	2,4	0,4	0,8	6,8
Heterokontophyta	Halopteris filicina	(Grateloup) Kützing	14	6,8	6,8	1,2	3,2	4,4
Heterokontophyta	Nereia filiformis	(J. Agardh) Zanardini	0	0	0,4	0	0	0
Heterokontophyta	Sphacelaria cirrosa	(Roth) C. Agardh	1,2	3,2	3,6	1,6	2	5,6
Heterokontophyta	Sphacelaria pluma	Zanardini	0	0,4	0	0,4	0	0
Heterokontophyta	Zanardinia typus	(Nardo) P. C. Silva	10	0	0	0	0	0
Chlorophyta	Anadyomene stellata	(Wulfen) C. Agardh	0	0	0	0	0	0,8
Chlorophyta	Caulerpa cylindracea	Sonder	0	0	2	0	0	0
Chlorophyta	Cladophora prolifera	(Roth) Kützing	0,8	0	2,4	0,8	0	0,8
Chlorophyta	Flabellia petiolata	(Turra) Nizamuddin	4	26	8	2	38	0
Chlorophyta	Halimeda tuna	(J. Ellis et Solander) J.V. Lamouroux	0	0	0	0	6	0
Chlorophyta	Valonia macrophysa	Kützing	0,4	0,4	0,4	0,4	0,4	0,8
Rhodophyta	Acrosorium ciliatum	(Havey) Kylin	0	0	0	7,6	0	0
Rhodophyta	Acrothamnion preissii	(Sonder) Wollaston	10	3,6	5,2	9,2	4,8	8,4
Rhodophyta	Anthithamnion cruciatum	(C. Agardh) Nägeli	0,4	0,4	0,4	0,4	0,4	0,4
Rhodophyta	Apoglossum ruscifolium	(Turner) J. Agardh	0,4	0	0,4	0,4	0,4	0,4
Rhodophyta	Botryocladia botryoides	(Wulfen) Feldmann	0,8	2	4,8	2,4	6	1,2
Rhodophyta	Carradoriella elongata	(Hudson) Savoie & G.W.Saunders	0	0,8	0	0	0	0
Rhodophyta	Ceramium diaphanum	(Lighthfoot) Roth	6,4	18	6,8	1,2	1,6	4,8
Rhodophyta	Ceramium flaccidum	H.E. Petersen (H.E. Petersen) G. Furnari et Serio	0,4	0,4	0	0	0	0
Rhodophyta	Ceramothamnion codii	H.Richards	0,4	0,8	0	0	0	0,8
Rhodophyta	Champia parvula	(C.Agardh) Harvey	0	0	0	0	0	0,4
Rhodophyta	Chondria capillaris	(Hudson) M.J. Wynne	0,8	4,4	0,8	0	0,4	0,8
Rhodophyta	Chondria mairei	Feldmann-Mazoyer	0,4	2	1,2	3,6	4,8	4
Rhodophyta	Contarinia squamariae	(Meneghini) Denizot	0	2,4	0	0	0,4	0
Rhodophyta	Crouania attenuata	(C.Agardh) J.Agardh	0,4	0,4	0,8	0,4	0	0,8
Rhodophyta	Dasya ocellata	(Grateloup) Harvey	0,4	1,2	1,2	0	0	0,4
Rhodophyta	Dasya rigidula	J. Ellis et Solander	0,4	0,4	0,4	0	0,4	0,8
Rhodophyta	Dipterosiphonia rigens	(Schousboe) Falkenberg	0	0,4	0,4	0,4	0,4	0
Rhodophyta	ErythroGLOSSUM sandrianum	(Kützing) Kylin	0	0	0	0	0	0,4
Rhodophyta	Eupogodon planus	(Kützing) Ardissonne	0	0,8	2,4	0,4	0,4	0,4
Rhodophyta	Feldmannophycus raissiae	(Feldmann et Feldmann-Mazoyer) H. Augier et Bo	1,6	0	4	0	0,8	1,2
Rhodophyta	Gloiocladia furcata	(C. Agardh) J. Agardh	0	0	0	0,4	0	0,8
Rhodophyta	Herposiphonia secunda	(C. Agardh) Ambronn	0,8	0,8	0,8	0	0,8	0,4
Rhodophyta	Heterosiphonia crispella	(C. Agardh) M.J. Wynne	0	0	0	0	0,4	1,6
Rhodophyta	Hydrolithon farinosum	(J.V. Lamouroux) Penrose & Y.M.Chamberlain	90	80	90	60	60	90
Rhodophyta	Irvinea chiajeana	(Meneghini) Cormaci, Alongi & G.Furnari	0,8	0,8	1,2	1,2	2	0
Rhodophyta	Jania virgata	(Zanardini) Garbary et H.W. Johansen	0,8	1,2	0,4	0	0,4	4,8
Rhodophyta	Laurencia chondrioides	Bergesen	12,8	12	7,6	1,6	6	3,6
Rhodophyta	Lomentaria chylodadiella	Funk	0,4	0	0,8	0	0	0
Rhodophyta	Meredithia microphylla	(J.Agardh) J.Agardh	0	0	0	0	2	0
Rhodophyta	Monosporus pedunculatus	(J. Agardh) J. Agardh	0,4	1,2	0	0	0	0
Rhodophyta	Osmundaea pelagosae	(Schiffner) F.W. Nam	0	3,6	0	2	1,2	0
Rhodophyta	Peyssonnelia rubra	(J. Agardh) J. Agardh	16	30	10	36	70	16
Rhodophyta	Plocamium cartilagineum	(Linnaeus) P.S. Dixon	0,4	0	0	0	0	0
Rhodophyta	Polysiphonia perforans	Cormaci, G. Furnari, Pizzuto et Serio	0	0	0	0	0	0
Rhodophyta	Pterothamnion plumula	(J. Ellis) Nägeli	0,4	0	0	0	0	0
Rhodophyta	Spermothamnion flabellatum	Bornet	0	0	0	0	1,6	0
Rhodophyta	Spyridia filamentosa	(Wulfen) Harvey	0,4	0,8	0,4	0	0	0
Rhodophyta	Vertebrata subulifera	(C.Agardh) Kuntze	0,8	8,8	0,8	0,4	0,4	4,4
Rhodophyta	Womersleyella setacea	(Hollenberg) R.E. Norris	0	0,4	0	1,2	0,8	0,8
Rhodophyta	Wrangelia penicillata	(C. Agardh) C. Agardh	0	0	1,6	0	0	0,4
Foraminifera	Miniacina miniacina	(Pallas, 1766)	12	2	6	14	10	6,4
Porifera	Chondrilla nucula	Schmidt, 1862	0	0	0	0	0	3
Cnidaria	Aglaophenia harpago	Von Schenk, 1965	0	2	0	14	2	8
Cnidaria	Obelia geniculata	(Linnaeus, 1758)	1,2	8	4	34	16	10
Cnidaria	Sertularia cfr. distans	(Lamouroux, 1816)	0	0	0	2,4	0	0
Cnidaria	Sertularia perpusilla	Stechow, 1919	6	10	10	20	14	8
Annelida	Spirorbis spirorbis	(Linnaeus, 1758)	0,4	0,4	0,8	0,4	0,4	0,4
Bryozoa	Aetea truncata	(Landsborough, 1852)	3,2	3,2	7,2	2	2	0,8
Bryozoa	Amathia lendigera	(Linnaeus, 1761)	18	38	18	1,2	2	1,2
Bryozoa	Annectocyma cfr. major	(Johnston, 1847)	0,8	0	0	2,4	0,8	0,4
Bryozoa	Beania hirtissima	(Heller, 1867)	0,8	0	0,4	2,8	0	0,8
Bryozoa	Beania magellanica	(Busk, 1852)	0	0	0	1,2	0	0
Bryozoa	Celleporina sp.		0	0	0	0,8	0	0
Bryozoa	Chartella papyrea	(Pallas, 1766)	0	14	2	0	0	0
Bryozoa	Chlidonia pyriformis	(Bertoloni, 1810)	0,4	0	0,4	0,8	0	0,4
Bryozoa	Chorizopora brongniartii	(Audouin, 1826)	1	0,8	0,9	1,5	0,9	0,8
Bryozoa	Crisia cfr. denticulata	(Lamack, 1816)	0	0,4	0	0,4	0,4	0,8
Bryozoa	Electra posidoniae	Gautier, 1954	1,2	0	0	1,6	2	0,4
Bryozoa	Fenestrulina malusii	(Audouin, 1826)	0,5	0,4	0,2	0,5	0,6	0,2
Bryozoa	Margaretta cereoides	(Ellis et Solander, 1786)	0	0	0	12	6	4
Bryozoa	Mimosella gracilis	Hincks, 1851	0	0	6	0	0	0
Bryozoa	Patinella radiata	(Audouin, 1826)	0,4	0,4	0	0,4	0,4	0
Bryozoa	Pentapora ottomuellieriana		0,5	0,8	0,9	6	2,5	1
Bryozoa	Pherusella tubulosa	(Ellis & Solander, 1786)	2	0,8	1,2	5,2	0	0
Bryozoa	Scrupocellaria scruposa	Busk, 1851	0,8	1,2	0	22	20	20
Bryozoa	Scrupocellaria sp.		0	0	0	2	0	1,2
Chordata	Botryllus schlosseri	(Pallas, 1776)	0	0	4	0	0	0
Chordata	Didemnum sp.		0	0	0	4	2	4
Chordata	Phallusia mamillata	(Cuvier, 1815)	0	3	0	0	0	0
Totale cm²			232,4	306,2	232	287,2	299,2	239

For epibenthic organisms of the canopy, the mean biodiversity indices were similar between the
D.1.2.1Pilots Ecosystem Services Baseline



impacted and control stations. Species richness was 48.3 ± 1.15 and 49.3 ± 1.52 , respectively, while total abundance was slightly higher in the control (275 ± 31.7) compared to the impacted one (256.7 ± 42.7). The Shannon index (H') showed nearly equivalent values (8.55 ± 0.23 vs 8.62 ± 0.46), as did Pielou's evenness (J') (0.686 ± 0.034 vs 0.701 ± 0.029). The Margalef index (d) also highlighted minimal differences (2.66 ± 0.13 in the impacted station and 2.73 ± 0.11 in the control one). Overall, these results indicate that sessile canopy communities are largely comparable between the two conditions, with a slight tendency toward higher richness and evenness in the control area. Considering epibenthic macrofauna and macroalgae separately, some differences emerge between the impacted and control areas. For macrofauna, the control area shows higher values of abundance ($2550 \text{ cm}^2 \text{ m}^{-2}$ vs. $1633 \text{ cm}^2 \text{ m}^{-2}$) and species richness (20.3 vs. 15.7), together with a higher Shannon diversity index, indicating a richer and more diverse community. However, the impacted area also displays relatively good evenness (0.72), despite generally lower values. Epibenthic macroalgae shows a different trend: the Impacted area presents higher species richness (32.7 vs. 29) and a higher Shannon index (2.14 vs. 1.94), suggesting a more structured macroalgal community, whereas the Meadow, despite showing similar abundance, exhibits slightly lower values of diversity and evenness.

Epifauna showed clear differences in abundance and composition. Mean abundance was almost twice as high in the control station (61.0 ± 30.3 SE 17.5 for sample and 1525 Ind/m²) compared to the impacted one (35.7 ± 20.3 SE 11.7 and 891 Ind/m²). Species richness was also higher in the control (50 species) than in the impacted one (42 species). Annelids represented the most diverse group in both conditions (21–22 species), but mollusks and sipunculans were markedly more represented in the control (13 and 2 species, respectively, vs. 5 and 0 in the impacted one), indicating a more structured and diversified community. Conversely, arthropods were relatively more abundant in the impacted station (15 vs. 12 species), suggesting that some taxa may tolerate or even take advantage of impacted conditions. (Table 5.10).

Overall, these first results highlight that disturbance probably not only reduces the abundance of mobile fauna but also reshapes their taxonomic balance, with a decline of species typically associated with the *Posidonia* canopy and an increase of taxa less specialized for this habitat.

Table 5.10 List of epifauna in the canopy. Abundance is expressed as the number of individuals.



PHYLUM	SPECIE	AUTHOR	Impacted (ST1)			Control (ST2)		
			I1	I2	I3	C1	C2	C3
Annelida	Amphitrite sp.		0	0	0	0	1	0
Annelida	Chrysopetulum debile	(Grube, 1855)	1	2	0	1	0	2
Annelida	Eumida sanguinea	(Orsted, 1843)	2	1	0	0	0	2
Annelida	Eunice vittata	(Delle Chiaje, 1828)	5	1	0	1	0	3
Annelida	Glycera tessellata	Grube, 1863	2	1	1	0	0	2
Annelida	Goniada maculata	Oersted, 1844	0	0	2	0	0	0
Annelida	Haplosyllis spongicola	(Grube, 1855)	0	0	0	0	2	0
Annelida	Harmothoe imbricata	(Linnaeus, 1767)	2	1	0	0	1	3
Annelida	Lumbrineriopsis paradoxa	(Saint-Joseph, 1888)	0	1	0	0	0	2
Annelida	Lysidice ninetta	Audouin & Milne-Edwards, 1833	0	1	1	0	2	1
Annelida	Lysidice unicornis	(Grube, 1840)	0	0	0	0	1	0
Annelida	Melinna palmata	Grube, 1860	1	0	0	0	0	0
Annelida	Nephtys hombergii	Savigny, 1818	0	0	0	0	1	0
Annelida	Notomastus sp.		0	0	1	0	0	0
Annelida	Paradiopatra calliopae	Arvanitidis & Koukouras, 1997	0	0	0	0	1	0
Annelida	Perinereis cultrifera	(Grube, 1840)	0	0	0	0	0	2
Annelida	Pholoe inornata	Johnston, 1839	0	2	0	0	0	1
Annelida	Platynereis dumerilii	(Audouin & Milne-Edwards, 1833)	1	2	0	1	0	0
Annelida	Polvophthalmus pictus	(Dujardin, 1839)	6	2	0	4	2	2
Annelida	Pontogenia chrysocoma	(Baird, 1865)	0	2	0	0	0	0
Annelida	Pseudopotamilla reniformis	(Bruguière, 1789)	0	0	0	1	0	5
Annelida	Serpula vermicularis	Linnaeus, 1767	0	1	0	0	0	0
Annelida	Sphaerosyllis hystrix	Claparède, 1863	2	4	0	1	1	4
Annelida	Syllidae ind.		1	0	1	0	0	2
Annelida	Syllis alternata	Moore, 1908	5	3	1	10	4	18
Annelida	Syllis garciai	Campoy, 1982	7	2	0	4	2	3
Annelida	Syllis variegata	Grube, 1860	0	0	1	0	0	1
Annelida	Vermiliopsis labiata	(O. G. Costa, 1861)	1	0	0	0	0	0
Arthropoda	Achaeus cranchii	Leach, 1817	0	1	0	0	0	1
Arthropoda	Alpheus macrocheles	(Hailstone, 1835)	0	0	0	0	1	1
Arthropoda	Athanas sp.		0	0	2	0	0	0
Arthropoda	Coboldus nitior	Krapp-Schickel, 1974	0	0	0	1	0	0
Arthropoda	Cymodoce truncata	Cymodoce truncata	0	1	0	0	1	0
Arthropoda	Dexamine spiniventris	(A. Costa, 1853)	1	0	0	0	1	0
Arthropoda	Eurydice sp.	Leach, 1815	0	0	0	0	1	0
Arthropoda	Galathea intermedia	Lilljeborg, 1851	0	2	0	0	2	1
Arthropoda	Gammaropsis sp.		1	1	0	0	0	1
Arthropoda	Harpinia agna	Boeck, 1876	0	0	0	0	0	0
Arthropoda	Leptocheirus guttatus	(Grube, 1864)	3	0	1	0	1	3
Arthropoda	Leucothoe sp.		0	1	0	0	0	0
Arthropoda	Lysianassa pilicornis	Heller, 1866	0	0	1	0	3	2
Arthropoda	Macropodia sp.		0	1	0	0	0	0
Arthropoda	Melphidippella macra	(Norman, 1869)	1	0	0	0	0	0
Arthropoda	Metaphoxus fultoni	(Scott, 1890)	0	1	0	0	0	0
Arthropoda	Paragnathia formica	(Hesse, 1864)	1	1	0	0	0	0
Arthropoda	Pereionotus testudo	(Montagu, 1808)	0	1	0	0	0	0
Arthropoda	Pseudoprotella phasma	(Montagu, 1804)	1	0	0	0	0	0
Arthropoda	Pycnogonida ind.		0	0	0	2	0	0
Arthropoda	Stenetrium armatum	Haswell, 1881	0	0	0	3	0	1
Mollusca	Acanthochitona fascicularis	(Linnaeus, 1767)	0	2	0	0	1	1
Mollusca	Alvania lineata	Risso, 1826	0	0	0	0	3	0
Mollusca	Ascobulla fragilis	(Jeffreys, 1856)	0	1	0	0	0	0
Mollusca	Ctena decussata	(O. G. Costa, 1829)	0	0	0	0	0	0
Mollusca	Flexopecten flexuosus	(Poli, 1795)	1	0	1	0	0	1
Mollusca	Gibberula miliaria	(Linnaeus, 1758)	0	0	0	1	0	1
Mollusca	Glans trapezia	(Linnaeus, 1767)	0	0	0	1	0	0
Mollusca	Gouldia minima	(Montagu, 1803)	0	0	0	0	0	0
Mollusca	Hiatella arctica	(Linnaeus, 1767)	0	0	0	1	0	1
Mollusca	Ischnochiton rissoi	(Payraudeau, 1826)	0	0	0	0	0	1
Mollusca	Loripinus fragilis	(Philippi, 1836)	0	0	0	2	0	0
Mollusca	Muricopsis cristata	(Brocchi, 1814)	0	0	0	1	0	0
Mollusca	Musculus discors	(Linnaeus, 1767)	4	2	0	4	3	9
Mollusca	Rissoa sp.		0	0	0	0	0	2
Mollusca	Striarca lactea	(Linnaeus, 1758)	1	1	0	3	3	9
Mollusca	Tetrarca tetragona	(Poli, 1795)	0	0	0	0	2	5
Sipuncula	Aspidosiphon muelleri muelleri	Diesing, 1851	0	0	0	1	0	0
Sipuncula	Golfingia (Golfingia) vulgaris vulgaris	(Blainville, 1827)	0	0	0	0	2	2
Echino dermata	Amphipholis squamata	(Delle Chiaje, 1828)	2	0	0	1	1	1
Total No. of individuals			52	42	13	44	43	96

Comparison between impacted and control stations – MATTE

Endofauna assemblages exhibited very low abundances in both conditions, with mean values of 2.0 ± 1.0 SE 0.58 individuals in the impacted station and 2.3 ± 0.6 SE 0.33 in the control one. Species richness was similarly low, with five species recorded in each station (Table 5.11). The

taxonomic composition, however, showed some differences: annelids and mollusks were found in the impacted area, while annelids, arthropods and echinoderms were present in the control one.

Table 5.11 List of endofauna organisms in the matte. Abundance is expressed as number of individuals.

PHYLUM	SPECIE	AUTHOR	Impacted (ST1)			Control (ST2)		
			I1	I2	I3	C1	C2	C3
Annelida	<i>Aponuphis bilineata</i>	(Baird, 1870)	0	0	0	0	1	0
Annelida	<i>Eunice vittata</i>	(Delle Chiaie, 1828)	0	0	1	0	0	0
Annelida	<i>Glycera tessellata</i>	Grube, 1863	0	0	0	0	0	1
Annelida	<i>Perinereis cultrifera</i>	(Grube, 1840)	0	0	1	0	0	0
Annelida	<i>Platynereis dumerilii</i>	(Audouin & Milne-Edwards, 1833)	0	0	1	0	0	0
Arthropoda	<i>Harpinia agna</i>	Boeck, 1876	0	0	0	0	0	1
Arthropoda	<i>Periculodes longimanus</i>	(Bate & Westwood, 1868)	0	0	0	0	0	1
Mollusca	<i>Ctena decussata</i>	(O. G. Costa, 1829)	1	1	0	0	0	0
Mollusca	<i>Gouldia minima</i>	(Montagu, 1803)	0	1	0	0	0	0
Echinodermata	<i>Echinocyamus pusillus</i>	(O. F. Miller, 1776)	0	0	0	2	1	0
Total No. of individuals			1	2	3	2	2	3

For the endofauna, index values were very low and similar between the two conditions. Species richness was 2 in both areas, with mean abundance slightly higher in the control station (2.3 ± 0.6) compared to the degraded one (2.0 ± 1.0). The Margalef index (d) showed modest values without clear differences (1.63 ± 0.27 in the impacted area; 1.09 ± 0.96 in the control one). Pielou's evenness (J') remained maximal (1.0) in both conditions, while the Shannon index (H') exhibited nearly identical values (0.60 ± 0.56 in both areas). Overall, the mobile fauna community of the matte appears poor and highly simplified, with no significant differences between the impacted and control areas.

Ictiofauna - results

For the fish assemblages, mean values showed only minor differences between areas. Species richness was slightly higher in the control station (5.3 ± 0.54) compared to the impacted one (4.3 ± 0.47), while total abundance for transect (173 ± 134.7 vs 164.8 ± 200.2) and the abundance for m^2 (0.65 in impacted and 0.69 in the control) was comparable. The Shannon index (H') displayed similar values (0.69 ± 0.12 vs 0.82 ± 0.14). Pielou's evenness (J') was higher in the impacted area (0.56 ± 0.34) compared to the control (0.41 ± 0.28), suggesting a slightly more uniform distribution of abundances. The Margalef index (d) showed close values between the two conditions (0.91 ± 0.29 in the control station and 0.89 ± 0.49 in the impacted one). Overall, fish assemblages appeared largely comparable, with minor differences in richness and evenness, but no clear pattern between the impacted and control areas.

5.3.4 Blue Carbon

5.3.4.1 Sedimentary pool

The mean sediment C_{org} stocks in top 40 cm ($Mg C_{org} ha^{-1}$) of sediment was comparable between

the impacted station (297 ± 41 at ST1, 269 ± 38 at ST2, and 309 ± 45 at ST3) and the control one (average 305 ± 42 from ST1-2-3), with an overall mean of 291 ± 41 (Fig. 5.13) in the top 40 cm. The mean value of sediment C_{org} stock in the two areas has been reported at theoric depth of 1 meter, resulting in 751 ± 105 and 717 ± 101 $Mg C_{org} ha^{-1}$ in control and impacted area, respectively. One-way ANOVA revealed no significant differences in C_{org} stocks among stations ($p < 0.01$), mainly related to no significant differences in C_{org} content in the sediments (32 and 37 n. samples at control and impacted area, respectively).

The Dry Bulk Density (DBD) in 40 cm-thick sediments in both control and impacted areas has been estimated on the basis of the granulometry pattern and water content. The assumption of $1.4 g DW cm^{-3}$ for DBD in all the samples has been made after comparison to literature data. Indeed, the measured C_{org} concentration was not significantly different between control and impacted areas ($7.22 \pm 1.25 \%$ and $6.88 \pm 1.33 \%$, respectively; and 72.5 ± 7.2 and 74.9 ± 7.7 $mg C_{org} cm^{-3}$; $P > 0.05$).

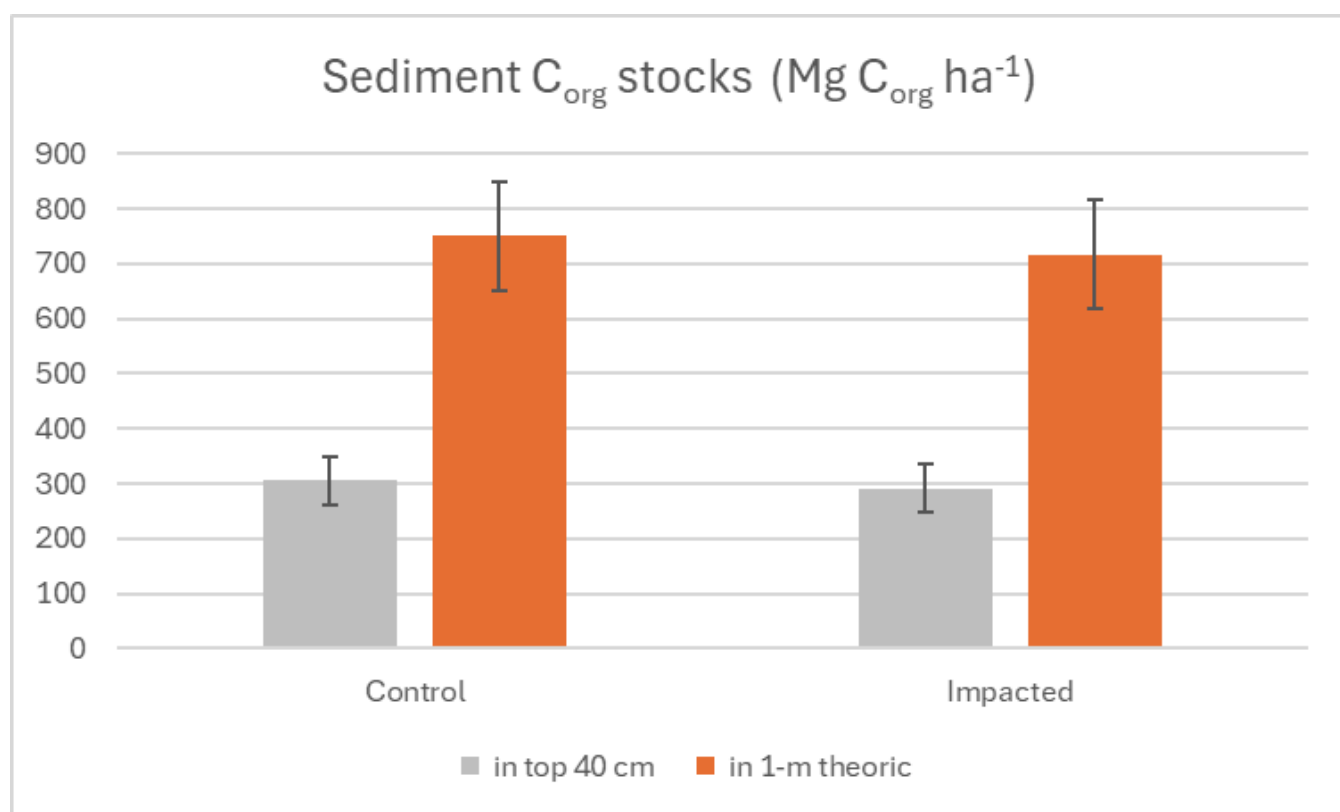


Figure 5.13 Mean $\pm$ SD organic carbon stocks in the top 40 cm of sediment and in 1-m theoric depth ($Mg C_{org} ha^{-1}$) in the control and impacted areas.

The short-term (100 years) C_{org} accumulation rate across areas ranged from 516 to 706 $g m^{-2} y^{-1}$ without a significant difference between impacted and control areas (Table 5.12). The control area shows a higher SAR, suggesting faster sediment deposition over the past century; on the other hand, the overlapping uncertainty ranges ($0.46\text{--}0.90$ vs $0.66\text{--}1.28$ $cm yr^{-1}$) suggest that while P4 tends to accumulate more sediment, the difference might not be statistically strong — it indicates a trend rather than a clearly significant contrast.

Table 5.12 Mean $\pm$ SD per station for sediment accretion rates and carbon organic accumulation rates.

Variable	Period	Impacted area	Control area
		I3	C3
SAR (cm yr ⁻¹)	Short-term (last 100 years)	0.68 $\pm$ 0.22	0.97 $\pm$ 0.31
C _{org} acc. rate (g C _{org} m ⁻² yr ⁻¹)	Short-term (last 100 years)	516 $\pm$ 174	706 $\pm$ 236

Table 5.13 Count (N), Mean and Standard Deviation (SD) of dry bulk density (g cm⁻³), sediment organic carbon content (C_{org} %), sediment organic carbon density (mg C_{org} cm⁻³), sediment organic carbon stocks in the top 40 cm of sediment and in 1-m theoric (Mg C_{org} ha⁻¹), sediment grain-size fractions (%) < 0.063 mm, > 0.063 < 0.125 mm, > 0.125 < 0.25 mm, > 0.25 < 0.5 mm, and > 0.5 < 1 mm, and > 1 mm (%).

Variable	Treatment	N	Mean	SD
Dry bulk density (g DW cm ⁻³)	Control	estimated	1,4	-
	Impacted	estimated	1,4	-
Sediment C _{org} content (%)	Control	32	5,2	0,2
	Impacted	37	5,3	0,3
Sediment C _{org} density (mg C _{org} cm ⁻³)	Control	32	72,5	7,2
	Impacted	37	74,9	7,7
Sediment C _{org} stocks in top 40 cm (Mg C _{org} ha ⁻¹)	Control	3	305	43
	Impacted	3	292	41
Sediment C _{org} stocks in 1-m theoric (Mg C _{org} ha ⁻¹)	Control	3	751	105
	Impacted	3	717	101
< 0.063 mm (%)	Control	32	30,07	3,43



	<i>Impacted</i>	37	25,59	3,51
> 0.063 < 0.125 mm (%)	<i>Control</i>	32	25,16	3,05
	<i>Impacted</i>	37	23,14	2,37
> 0.125 < 0.25 mm (%)	<i>Control</i>	32	31,52	1,66
	<i>Impacted</i>	37	31,80	2,39
> 0.25 < 0.5 mm (%)	<i>Control</i>	32	11.57	6.27
	<i>Impacted</i>	37	15.62	5.72
> 0.5 < 1 mm (%)	<i>Control</i>	32	1,68	1,65
	<i>Impacted</i>	37	3,85	3,20
> 1 mm (%)	<i>Control</i>	32	0	0
	<i>Impacted</i>	37	0	0

The vertical profiles of Corg content along the cores reveal a gradual decline with depth, from values above 6 % in the upper 10 cm to around 3 – 4 % below 40 cm, consistent with reduced organic input and early diagenetic mineralization in deeper layers. This pattern aligns with typical vertical attenuation observed in Mediterranean *Posidonia oceanica* sediments (Mateo et al., 1997; Serrano et al., 2016).

The mean sediment carbon stock in the top 40 cm ($\approx 297 \pm 41 \text{ Mg C}_{\text{org}} \text{ ha}^{-1}$) falls within the range reported for well-preserved Mediterranean meadows (250 – 350 $\text{Mg C}_{\text{org}} \text{ ha}^{-1}$; Fourqurean et al., 2012; Mazarrasa et al., 2021). Despite moderate mechanical disturbance, the impacted site maintained comparable stock levels, likely due to localized sediment redeposition and short-term sedimentation processes confirmed by ^{210}Pb dating.

The radiometric age-depth models indicate recent sediment accumulation rates of 0.7–1.0 cm yr^{-1} , corresponding to carbon burial rates of 0.5–0.7 $\text{kg C m}^{-2} \text{ yr}^{-1}$. These values describe the active depositional layer for approximately the last century, confirming that both control and impacted meadows remain significant short-term carbon sinks under current hydrodynamic and ecological conditions.

5.3.4.2 Sediment analysis

Sediment grain size distribution shows a clear textural shift (Fig. 5.14): the impacted site has lost part of its fine fraction ($<125\ \mu\text{m}$) and is enriched in coarse sand ($>250\ \mu\text{m}$). So, both areas are dominated by sand (65–75%), but differ in fine content: control sediments are silty sand while impacted sediments can be classified as slightly silty medium sand (Folk ternary classification). Indeed, the control area shows higher fine content (30–25 %) compared to the impacted area (26–23 %). This indicates finer sediment texture and more silt-rich material in the control area. Both areas are very similar Medium sand fraction (125–250 μm) (around 31 %), suggesting the core of the sediment distribution remains around medium sand. The impacted area contains more coarse material ($>250\ \mu\text{m}$) — 15.6 % in the 250–600 μm range and 3.9 % in the 600 μm class vs 11.6 % and 1.7 % in the control area.

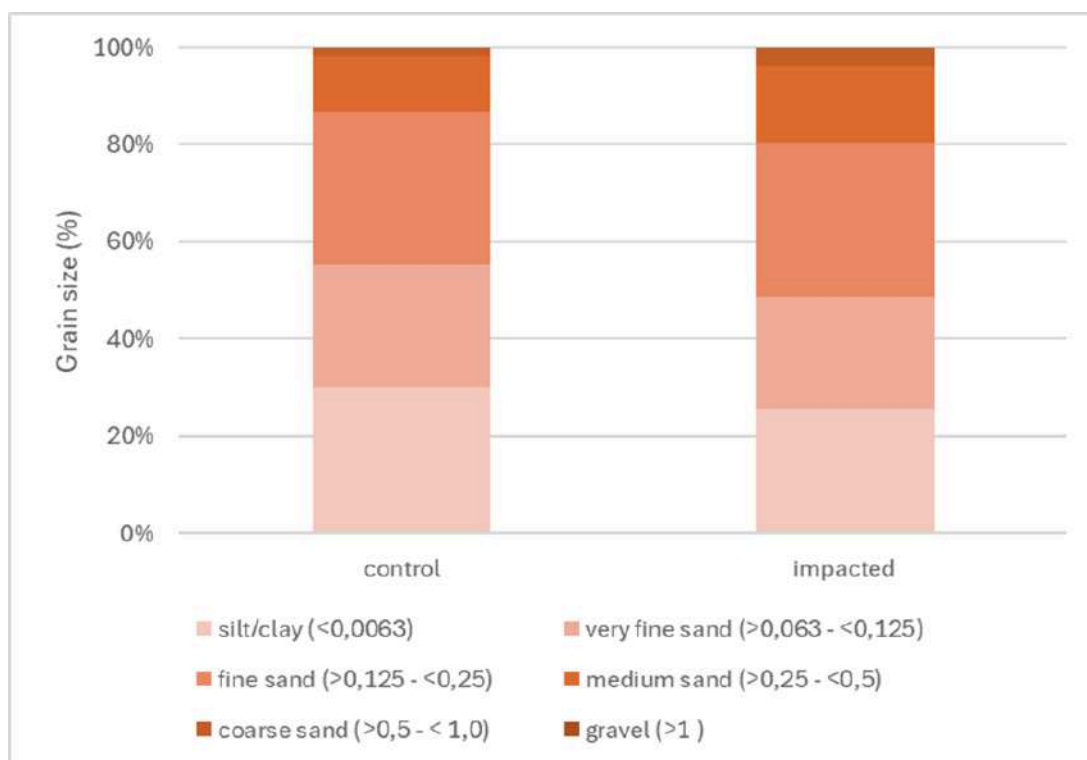


Figure 5.14 Mean cumulative grain size fractions (%) on the top meter of sediment at each site.

5.3.4.3 Vegetative carbon pools

The analysis of living leaf biomass revealed differences between impacted and control areas (Tab 5.14). In the impacted area, mean living leaf biomass was $0,25 \pm 0,11\ \text{Kg DW m}^{-2}$, corresponding to a mean organic carbon (C_{org}) content of $0,08 \pm 0,04\ \text{Kg } C_{\text{org}}\ \text{m}^{-2}$ ($84.4 \pm 36.9\ \text{g } C_{\text{org}}\ \text{m}^{-2}$). In contrast, shoots from the control station showed higher values, with a mean leaf biomass of $0,42 \pm 0,19\ \text{Kg DW m}^{-2}$ corresponding C_{org} content of $0,14 \pm 0,6\ \text{Kg } C_{\text{org}}\ \text{m}^{-2}$ ($142.4 \pm 63.9\ \text{g } C_{\text{org}}\ \text{m}^{-2}$), respectively.

These results indicate that plants from the control support greater biomass and carbon storage capacity, while impacted areas exhibit reduced values and higher variability, reflecting the impact of habitat disturbance on seagrass productivity and carbon sequestration potential.

The mean litter C_{org} stock ($kg C_{org} m^{-2}$) was 2.1 ± 0.2 , and 1.3 ± 0.1 in the control and impacted area, respectively, recording a half reduction of litter stock in the impacted area compared to the control one.

Table 5.14 Count (N), Mean and Standard Deviation (SD) of shoot density (number of shoots m^{-2}), living leaf biomass (Kg DW m^{-2}), living leaf stock C_{org} ($kg C_{org} m^{-2}$), Litter stock C_{org} ($kg C_{org} m^{-2}$) and seagrass cover (%).

Variable	Treatment	N	Mean	SD
Shoot density (number of shoots m^{-2})	Control	20	290,6	8,3
	Impacted	20	263,75	7,0
Living leaf biomass (Kg DW m^{-2})	Control	30	0,42	0,19
	Impacted	30	0,25	0,11
Living leaf stock C_{org} ($kg C_{org} m^{-2}$)	Control	30	0,14	0,6
	Impacted	30	0,08	0,04
Litter stock C_{org} ($kg C_{org} m^{-2}$)	Control	5	2.1	0.2
	Impacted	5	1.3	0.1
Seagrass cover (%)	Control	30	94,2	3,5
	Impacted	30	40,3	23,36

When expressed relative to the sedimentary pool, the vegetative carbon stock of the *Posidonia oceanica* meadow represents less than 2 % of the total carbon stored within the top 40 cm of substrate, confirming that belowground sediment constitutes the dominant carbon reservoir. Nevertheless, living biomass and litter play a crucial role in sustaining continuous organic matter inputs to the sediment.

The approximately two-fold reduction in leaf and litter carbon observed at the impacted site (0.08 vs 0.14 $kg C_{org} m^{-2}$ and 1.3 vs 2.1 $kg C_{org} m^{-2}$, respectively) suggests a decrease in net primary production and detritus retention. Lower litter accumulation implies accelerated decomposition and export of particulate organic carbon, potentially reducing short-term sequestration efficiency.

These findings are consistent with other Mediterranean meadows under moderate anthropogenic

stress, where a decline in canopy density and litter persistence leads to weakened coupling between vegetative productivity and sedimentary burial (Serrano et al., 2014; Mazarrasa et al., 2021). The results thus underscore the importance of protecting aboveground structure to maintain both ecological functionality and long-term blue-carbon performance.

5.4 Discussion

Main conclusions EBQI and Biodiversity

The two studied meadows showed an evident difference in structure, principally due to the low coverage in the impacted area. Other compartments also varied between meadows, such as the density of suspension feeders and the growth rate of rhizomes. EBQI consequently differed between the two stations but was generally low. This pattern could be related to the sampling period (fall) which is not the suitable season to study *Posidonia oceanica* meadows. In fact, several variables could be deeply influenced by the season; in fall, for example, litter is very abundant due to the dead leaves detached by the plant at the end of summer and both epiphytes and teleost assemblages may be different and poorer than in summer.

Epiphyte assemblages showed a structure typical of the habitat (Piazzi et al. 2016), with a dominance of Rhodophyta and Bryozoa. The Rhodophyta *Acrothamnion preissii* was the only alien species detected in the survey.

The comparison between impacted and control areas showed that species richness remained similar across sites, but both sessile and mobile assemblages were affected in terms of composition and abundance. In the canopy, sessile organisms displayed shifts towards opportunistic brown algae in the impacted area, while bryozoans, cnidarians, and sponges were more frequent in the control area. Total cover of the canopy was also higher and more stable in the control area, confirming the importance of habitat integrity in supporting sessile biomass.

Mobile assemblages mirrored these patterns: higher abundance and richness were recorded in the canopy of meadow station, with mollusks and sipunculans more represented, whereas arthropods prevailed in the disturbed canopy. In the matte, endofauna was scarce in both conditions, and differences were mainly taxonomic, with mollusks restricted to the degraded matte, and arthropods and echinoderms occurring only in the meadow one. However, the overall low abundance of organisms limits the robustness of comparisons between conditions. This scarcity may be influenced by low oxygen availability and possible methodological bias.

Overall, our results highlight that disturbance does not necessarily reduce species numbers but reshapes community structure, reducing typical meadow assemblages and favoring less specialized organisms. The canopy plays a key role in sustaining both sessile and mobile diversity, emphasizing the need to preserve intact *Posidonia oceanica* meadows to maintain their ecological

complexity and biodiversity.

Blue Carbon

With respect to blue carbon, the results obtained at Capo Testa – Punta Falcone MPA (northern Sardinia, Italy) indicate that the apparent human disturbance within the impacted area did not significantly reduce the organic carbon (C_{org}) stocks within the upper 40 cm of sediment, but rather modified their textural composition. The mean sediment C_{org} stock was comparable between impacted ($292 \pm 41 \text{ Mg } C_{org} \text{ ha}^{-1}$) and control ($305 \pm 43 \text{ Mg } C_{org} \text{ ha}^{-1}$) stations (Fig. 5.13). The one-way ANOVA confirmed that differences in C_{org} stocks between sites were not statistically significant ($p > 0.05$), consistent with the similar C_{org} content in sediments ($5.3 \pm 0.3 \%$ vs $5.2 \pm 0.2 \%$).

The $\delta^{13}\text{C}$ signal was not available for this dataset; however, the sediment grain-size distributions reveal clear textural shifts between impacted and control areas (Fig. 5.14). Impacted sites exhibit a marked loss of fine particles ($<125 \mu\text{m}$) and enrichment in coarse fractions ($>250 \mu\text{m}$), moving the sediment classification from silty sand (control) to slightly silty medium sand (impacted), according to Folk's ternary system. This trend suggests an alteration of depositional dynamics and hydrodynamic exposure, potentially driven by mechanical disturbance or reduced seagrass coverage. The higher proportion of coarse sand in impacted areas (15.6 % in the 250–500 μm class vs 11.6 % in the reference) points to increased resuspension and sediment winnowing, which may have reduced the retention of organic matter despite stable carbon concentrations.

The short-term sediment accumulation rates (SAR) derived from ^{210}Pb profiles ranged from $0.68 \pm 0.22 \text{ cm yr}^{-1}$ in the impacted site to $0.97 \pm 0.31 \text{ cm yr}^{-1}$ in the control one, suggesting that although both sites maintain active sedimentation, the control meadow tends to accrete more rapidly. The overlapping uncertainty ranges ($0.46\text{--}0.90$ vs $0.66\text{--}1.28 \text{ cm yr}^{-1}$) indicate that this difference may represent a trend rather than a statistically robust contrast. The corresponding C_{org} accumulation rates (706 ± 236 vs $516 \pm 174 \text{ g } C_{org} \text{ m}^{-2} \text{ yr}^{-1}$) are within the upper range of reported Mediterranean seagrass blue carbon systems, reflecting relatively high productivity and carbon burial potential in both conditions.

Regarding the vegetative carbon pool, both living and litter components showed higher values in the control area compared to the impacted area. In the control meadow mean living leaf biomass reached $0.14 \pm 0.06 \text{ Kg } C_{org} \text{ m}^{-2}$, while litter stocks of $2.1 \pm 0.2 \text{ kg C m}^{-2}$. In contrast, the impacted area showed reduced values $0.08 \pm 0.04 \text{ Kg } C_{org} \text{ m}^{-2}$ and mean litter stock of $1.3 \pm 0.1 \text{ kg C m}^{-2}$ in the impacted area. This pattern reflects a clear degradation of plant structure and productivity in disturbed plots, likely reducing both primary production and belowground carbon inputs. The roughly twofold difference in litter stocks further supports a decrease in organic detritus accumulation and stabilization following disturbance.

The analysis of leaf biomass confirms the clear differences in the vegetative carbon pool between the impacted and control areas. Seagrass shoots from the impacted site exhibited significantly lower biomass and carbon content compared to those from the control area, suggesting a decline

in plant productivity and carbon storage capacity under disturbance conditions. The mean leaf biomass and C_{org} content per shoot were approximately 34% lower in the impacted area, while areal estimates of biomass and carbon stocks were nearly halved. This reduction, together with the higher variability observed in the impacted site, indicates that environmental stressors—such as physical disturbance or sediment resuspension—may have compromised seagrass density and growth. Conversely, the higher and more consistent values recorded in the control area reflect healthier and more stable meadow conditions, with enhanced potential for carbon sequestration and long-term blue carbon storage.

Overall, the Capo Testa Punta Falcone MPA results suggest that the disturbance has not substantially reduced sedimentary carbon storage per unit area within the top 40 cm but has altered the physical texture and vegetative dynamics of the system. The loss of fine-grained sediment and lower plant biomass indicates decreased capacity for long-term carbon retention, even if short-term stocks remain comparable. The combined evidence points toward a shift in the sedimentary and ecological equilibrium, where hydrodynamic energy and reduced seagrass cover drive coarser substrate composition and potentially enhance post-disturbance erosion processes, with yet limited measurable losses in stored carbon due to compensatory sedimentation and spatial heterogeneity.

The carbon stocks and accumulation rates measured in Capo Testa–Punta Falcone are within the higher range of values reported for Mediterranean *Posidonia oceanica* meadows (typically 150–400 Mg C ha⁻¹ and 300–700 g C m⁻² yr⁻¹; Serrano et al., 2014; Mazarrasa et al., 2021). This confirms the site's high carbon storage potential despite localized physical degradation. The absence of statistically significant differences in sedimentary carbon stocks between the impacted and control areas indicates that the disturbance has not yet produced measurable losses in total sedimentary carbon mass. However, the observed coarsening of sediments and reduction in vegetative biomass suggest a functional weakening of the carbon sink mechanism: reduced canopy density and lower detritus accumulation likely impair long-term carbon burial efficiency and increase susceptibility to erosion.

The combined evidence highlights a transitional condition where carbon stocks are still substantial, but their stability and resilience are declining. In this context, restoration actions such as *Posidonia* transplantation and anchoring control could effectively enhance vegetative productivity and sediment stabilization, preventing future carbon losses. The measured SAR and C_{org} accumulation rates provide an empirical baseline for monitoring the effectiveness of these interventions over time.

From an ecosystem-service perspective, maintaining sediment integrity and canopy structure in this MPA safeguards not only carbon sequestration but also habitat complexity, biodiversity support, and water-quality regulation. The Capo Testa–Punta Falcone case therefore demonstrates the dual benefit of blue-carbon conservation and habitat restoration, reinforcing the contribution of Mediterranean MPAs to climate-mitigation targets under the ARTEMIS framework.

5.5 References

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6 Monfalcone, Italy

6.1 Study area

The Monfalcone pilot site lies geographically in the Panzano Bay, located in the northeastern part of the Gulf of Trieste, along the coast of Friuli Venezia Giulia, and is bordered by Punta Sdobba to the east, near the mouth of the Timavo River (Fig. 6.1). The bay covers an area with significant bathymetric variability, with depths ranging considerably depending on the location. The coastal area, primarily shallow, has depths varying from 1 m in the inner zones (with emergent conditions at low tide due to the presence of sandy shoal) to approximately 5 - 6 m in the more distant waters.

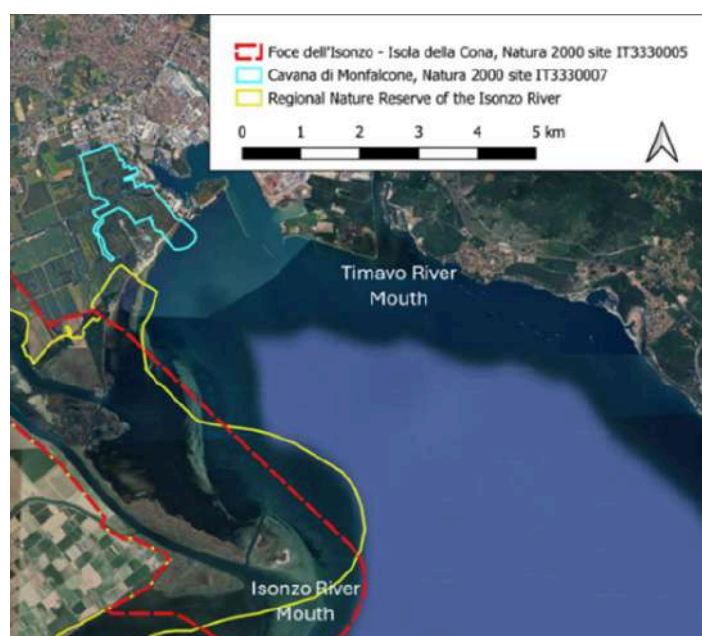


Figure 6.1 Geographical framework of Panzano Bay with references to Natura 2000 sites and regional reserves.

This marine habitat is of ecological interest as it hosts extensive seagrass meadows (*Cymodocea nodosa* and species of the genera *Zostera* and *Ruppia*), which play a crucial role in maintaining the ecological balance of the bay. This is also an area of great conservation value and a part of the Natura 2000 network. In fact, two Natura 2000 sites are located inside Panzano Bay: SAC (Special Conservation Area) IT3330007 – “Cavana di Monfalcone” and SPA/SAC (Special Area of Conservation/Special Protection Area) IT3330005 – “Foce dell'Isonzo – Isola della Cona” sites. These sites are crucial for the conservation of marine habitats, including sandy seabed and muddy bottoms, which host marine seagrass meadows. Part of the western marine sector of the bay that included the SPA/SAC IT3330005 – “Foce dell'Isonzo – Isola della Cona” overlaps with the “Foce dell'Isonzo Regional Nature Reserve”.

The SAC “Cavana di Monfalcone” extends over a surface of 133 ha (12 % are marine ones), in the transition area between the flat land and the Adriatic Sea. In the marine part of the site, the

following habitats are present: 1) **habitat 1110** (Sandbanks which are slightly covered by shallow sea water all the time - an area of 15 ha) and 2) **habitat 1140** (Mudflats and sandflats not covered by seawater at low tide - 3 ha).

The SAC/SPA “Foce dell’Isonzo-Isola della Cona” extends over a surface of 2,668 ha, 40 % of which is marine. It is located along the last stretch of the Isonzo River, and it largely coincides with the “Foce dell’Isonzo Regional Nature Reserve”. The marine part of the site covers about 1,100 ha of shallow water with relevant extensions of seagrass meadows. The following habitats are present: 1) **habitat 1110** (an area of more than 1200 ha) and 2) **habitat 1140** (almost 70 ha).

6.2 Methods

6.2.1 Habitat mapping

Considering Monfalcone pilot site, both present habitats (1110 and 1140) inside and outside the two Natura 2000 sites are characterized by a vast presence of marine seagrasses. Historical records show that *Zostera marina* and *Zostera noltei* once grew in Panzano Bay. In the early 20th century, *Z. marina* formed wide meadows, and it was still common in the 1930s but declined sharply by the 1970s, probably due to worsening environmental conditions and disease caused by a myxomycete pathogen (*Labyrinthula macrocystis*). *Z. noltei* was always more limited in distribution and never dominant in the area. Since the 1990s, neither species has been regularly observed in Panzano Bay, where *Cymodocea nodosa* has gradually become the main and most stable seagrass (Gianni et al., 2025).

The seagrass meadows in Panzano Bay are complex ecosystems that provide essential ecological services, such as water quality improvement, stabilization of sandy substrates, and the creation of habitats for numerous marine species. Recent mapping confirms the presence of the following seagrass species:

- ***Cymodocea nodosa***: the dominant species in the bay, particularly in areas with brackish waters and shallow depths.
- ***Zostera noltei***: typically present in shallower, brackish zones.
- ***Ruppia* spp.**: a genus that thrives in waters with variable salinity, particularly near the mouths of the Isonzo River.

The seagrass meadows are spread over a large area, but their density varies depending on environmental conditions such as sediment type, salinity, and water depth. The most extensive meadows of *Cymodocea nodosa* are found in shallower waters, while *Zostera noltei* and *Ruppia* spp. are concentrated in more brackish areas. The first detailed scientific information on the colonized areas and the seagrass species of the meadows in the Bay of Panzano dates to about a decade ago. To date, three maps have been produced showing the distribution of marine seagrasses,

each with a different level of detail, sometimes more precise on the marine side but less specific in the western sectors beyond the sandy shoals, where shallow depths require a specialized survey methodology (navigation with shallow-draft boats and only under optimal tidal conditions).

The first detailed mapping was carried out between 2014 and 2015 by the Autonomous Region of Friuli Venezia Giulia in collaboration with the University of Trieste (S. Ciriaco, personal communication). A second mapping was carried out in 2019 within the Interreg IT-CH SASPAS project (<https://programming14-20.italy-croatia.eu/web/saspas>). Specifically, this was an updated survey that verified the boundaries of the previous study with partial investigations of the shallower areas behind the emerging sandy shoals. The third and last mapping, carried out in 2024 as part of the Interreg IT-CH BIOPRESSADRIA project (<https://www.italy-croatia.eu/web/biopressadria>), included detailed surveys both in marine areas down to depths of 5 m and in shallow zones behind the emerging sandy shoals, where freshwater inflows from the Isonzo River. To complete the knowledge framework, additional surveys were conducted in the seabed near the mouth of the Timavo River, east of the port of Monfalcone.

6.2.2 Ecological status

The initial project plan proposed the use of the Ecosystem-based Quality Index (EBQI – Personnic et al., 2014), originally developed for *Posidonia oceanica*. However, since data on other ecosystem elements were incomplete and reference values needed recalibrating, EBQI was not considered applicable for *Cymodocea nodosa*. As a result, indices specifically designed for *C. nodosa* meadows were considered, which, while effective in assessing meadow condition, do not account for broader ecosystem dynamics. Several of them, such as CymoSkew and CYMOX, were excluded because key parameters were missing from the available dataset.

The MediSkew index and the Pressure Index for Seagrass Meadows (PISM) were subsequently considered, as both have been successfully applied to multiple *C. nodosa* meadows along the northern Adriatic coast, providing robust and comparable estimates of ecological quality for classifying ecological status (five-class WFD) and environmental status (two-class MSFD) (Orlando-Bonaca, 2015).

Although the 2019 baseline survey under the Interreg SASPAS project provided the first detailed assessment of *C. nodosa* meadows in Panzano Bay (Interreg SASPAS, 2019), its simplified sampling design did not fully meet the requirements of the MediSkew index. For this analysis, the data were reorganized to support the index's implementation and guide the selection of stations and parameters for long-term monitoring at the Monfalcone pilot site. The original survey offered valuable insights into meadow condition, associated habitats, and key species, and helped identify suitable areas for transplanting and anchorage buoy placement. The meadows showed dense and uniform coverage, except in intertidal zones exposed during medium-low tides.

Follow-up monitoring campaigns were carried out in September 2020, April 2021, and September

2021, focusing on the same three zones (each with three stations) originally surveyed in 2019 (Interreg SASPAS, 2022) (Fig. 6.2 and Fig. 6.3). Zone 1, located within and adjacent to the “Cavana di Monfalcone” Special Area of Conservation (SAC), is subject to moderate anchoring pressure from medium-sized sailboats, which affects *C. nodosa* meadows. Zones 2 and 3, situated near and within the SPA-SAC “Foce dell’Isonzo – Isola della Cona” area, host mixed meadows: *C. nodosa* with *Zostera noltei* in Zone 2, and *C. nodosa* with *Zostera marina* in Zone 3. Sampling was stratified by depth to capture variability between deeper, light-limited stations and shallower fringes where vegetation develops more freely.

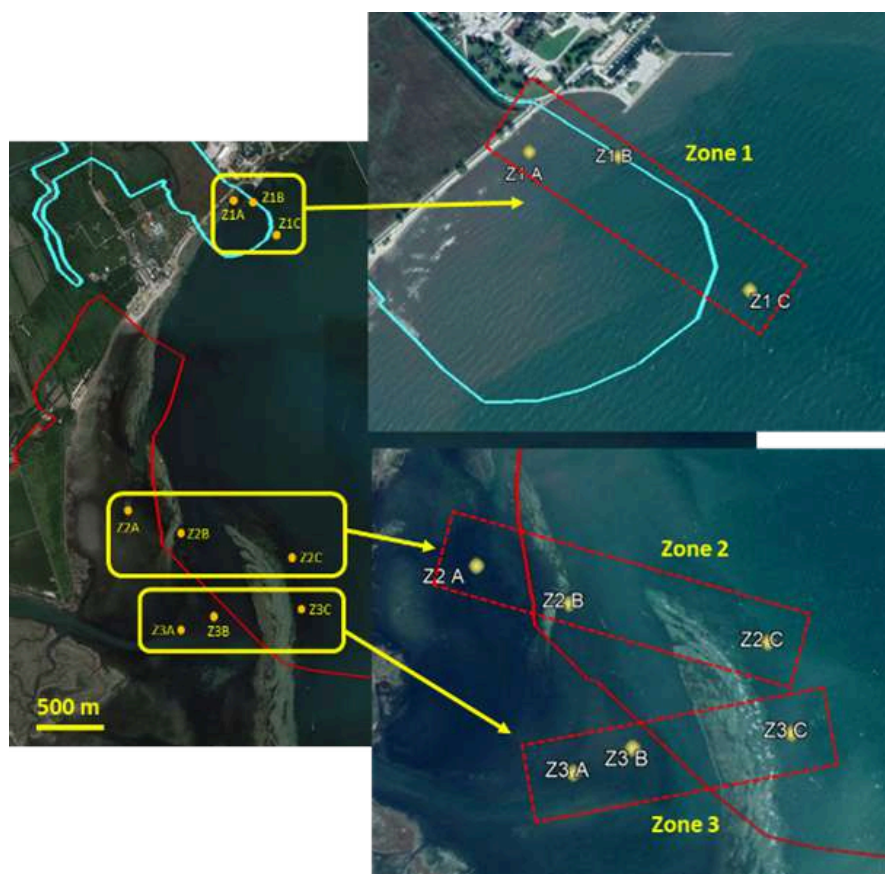


Figure 6.2 Bay of Panzano: map showing the sampling stations at “Cavana di Monfalcone” (Zone 1) and “Foce dell’Isonzo – Isola della Cona” (Zones 2 and 3) (Interreg SASPAS, 2019, modified).



Figure 6.3 SASPAS monitoring campaigns: shoot density, cover percentage and meadows continuity measurements (Interreg

SASPAS, 2019, modified).

6.2.2.1 MediSkew Index

Based on data availability and ecological objectives, the MediSkew index (Orlando-Bonaca et al., 2015) was selected as a structural indicator to assess the condition of *Cymodocea nodosa* meadows in Panzano Bay. This index uses leaf length measurements to detect changes in the shape of their distribution, helping to identify habitat variability and signs of stress or recovery in *C. nodosa* meadows.

This index quantifies canopy structural integrity by analyzing the distribution of photosynthetically active leaf lengths, using two components:

- The deviation from a reference median (Md_RC);
- The skewness (G) of the log-transformed leaf-length distribution.

The formula applied is: $\text{MediSkew} = ((\text{Md_area} - \text{Md_RC}) / A + |G| / 2) / 2$, where:

- Md_area = median of log-transformed leaf lengths in the sampled station.
- Md_RC = reference median from a station classified in good ecological condition.
- G = skewness of the log-transformed distribution.
- A = a normalization constant, chosen as a round number close to the maximum observed median difference among all stations.

The data used for the MediSkew analysis were collected in 2019 at six stations throughout Panzano Bay (Fig. 6.4), including:

- Three SASPAS monitoring stations: St.Z1B, St.Z2C, and St.Z3C (monitored from 2019 to 2021).
- Three additional stations: St.1, St.4, and St.5, included in May 2019 survey to increase the spatial coverage of the structural assessment. They were monitored only during this initial campaign, which helped define the final configuration of the SASPAS monitoring network. These stations were later excluded from the selected network and are therefore part of a dataset not formally published within the project.

Bootstrap resampling (5,000 repetitions per station) was performed to verify that log-transformed leaf lengths were well-sampled and to assess median stability. Confidence intervals (95%) were narrow and close to the medians, indicating reliable estimates at all six stations.

Spatial Distribution and Pressure Assessment

The spatial layout of the sampling stations was designed to characterize the environmental heterogeneity of Panzano Bay (Fig. 6.4):

- Site 1: includes St.1 and St.Z1B in the northern sector, adjacent to a relatively undisturbed

shoreline.

- Site 2: includes St.Z2C and St.Z3C in the central-southern sector, near mooring areas for small boats.
- Site 3: includes St.4 and St.5, located near the southern entrance of the bay, and exposed to stronger tidal flows, freshwater inflows from the Isonzo River sediment, resuspension, and boating activity.



Figure 6.4 Panzano Bay: map showing the three sites and the six stations considered for the MediSkew index (marine seagrass meadows highlighted in green).

To identify the most suitable reference station for MediSkew, the Pressure Index for Seagrass Meadows (PISM) was applied to the six stations surveyed in 2019. Additional spatial data from the SASPAS GIS platform (webgis.saspas.eu) were used to refine the assessment of human pressures, including boat density, port and harbour locations, fishing and mariculture activities, and bathing areas, discharge points, and other anthropogenic structures (Figs. 6.5–6.8).

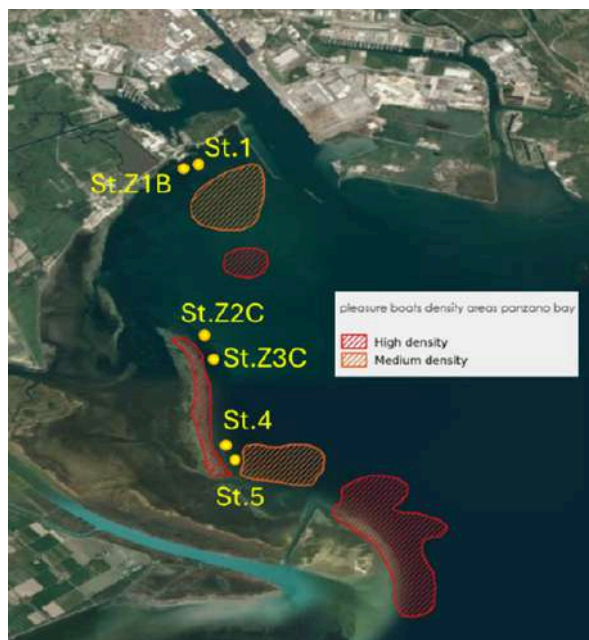


Figure 6.5 Bay of Panzano: Pleasure boats density areas in the Panzano Bay and the six stations considered for the MediSkew index (from webgis.saspas.eu, modified).



Figure 6.6 Bay of Panzano: Port/harbours distribution in the Panzano Bay and the six stations considered for the MediSkew index (from webgis.saspas.eu, modified).

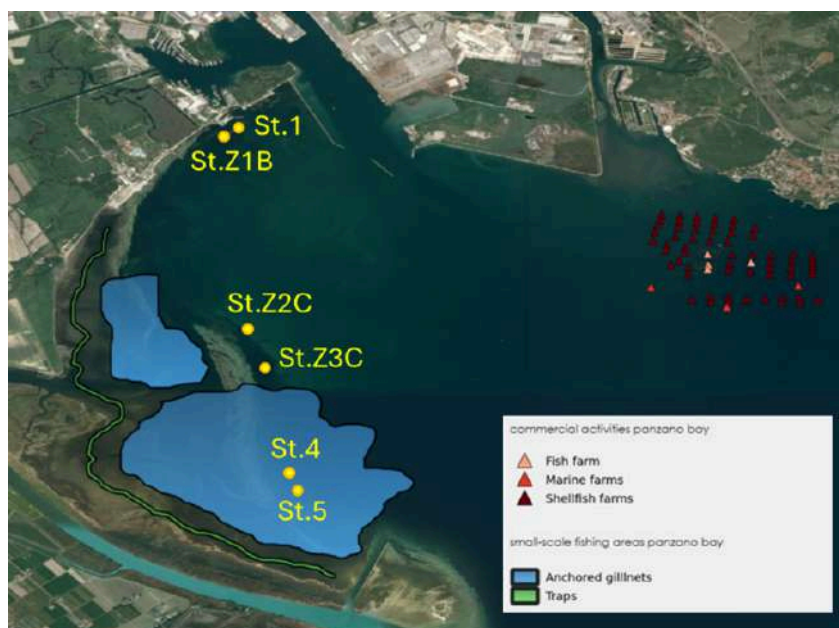


Figure 6.7 Bay of Panzano: Mussel and fish farming, and small-scale fishing areas and the six stations considered for the MediSkew index (from webgis.saspas.eu, modified).



Figure 6.8 Bay of Panzano: Bathing water, kitesurf areas, artificial structures, urban water treatment plant and discharge points distribution in the Panzano Bay and the six stations considered for the MediSkew index (from webgis.saspas.eu, modified).

Furthermore, the "Navigation Routes" category within the PISM framework was also refined to better capture the gradient of boating activity and its ecological implications. The revised scale includes:

- 0: Occasional navigation and anchoring of small boats.
- 1: Dense navigation and anchoring of leisure boats.

- 2: Navigation and anchoring of larger leisure boats.
- 3: n/a

To improve the accuracy of pressure assessment, the coastline of Panzano Bay was classified as concave, and a correction factor of 1.25 was applied to the total pressure scores. This adjustment reflects the tendency of concave shores to trap and intensify human impacts due to their shape. The method follows the approach described by Flo et al. (2011), who showed that these coastal configurations are more exposed to the build-up of pressures linked to human activities.

The adjusted PISM values ranged from 6.25 at St.1, St.Z1B, St.Z2C, and St.Z3C to 7.5 at St.4 and St.5, indicating a moderately higher level of pressure in the southern sector of the bay (Table 6.1).

Table 6.1 Cumulative and adjusted PISM scores for the six monitoring stations in Panzano Bay.

	St.1	Z1B	Z2C	Z3C	St.4	St.5
Artificial coastline	1	1	0	0	0	0
Direct leaching	2	2	2	2	3	3
Mariculture	0	0	0	0	0	0
Rainwater discharges	1	1	1	1	1	1
Industrial wastewater discharges	1	1	1	1	1	1
Navigation Routes	0	0	1	1	1	1
SUM	5	5	5	5	6	6
Confinement (concave coastlines)	x 1.25	x 1.25	x 1.25	x 1.25	x 1.25	x 1.25
Total pressures	6.25	6.25	6.25	6.25	7.5	7.5

This enhanced classification allows for more precise differentiation of boating-related impacts, which are known to affect seagrass canopy structure through mechanical disturbance, sediment resuspension, and shading. As a result, the updated PISM framework offers a more ecologically meaningful basis for interpreting MediSkew values.

6.2.3 Biodiversity

The Ecosystem-based Quality Index (EBQI), developed for *Posidonia oceanica*, was found unsuitable for *Cymodocea nodosa* due to insufficient data on other ecosystem components and the need for full recalibration (see paragraph 6.2.2). To fill these data gaps and enable a

comprehensive assessment of biodiversity-related ecosystem services, targeted data collection was carried out for key biological groups (benthic invertebrates, macroalgae, epiphytic algae, ichthyofauna, and others), literature values were reviewed, and, following the approach used for the MediSkew index (see paragraph 6.2.2.1), previously unpublished data from the SASPAS project were analysed (Interreg SASPAS, 2019). These efforts enabled the calculation of an alternative ecological index (M-AMBI for benthic invertebrates), supporting an integrated assessment of meadow condition and ecosystem health. For the macroalgal and fish communities, it was not possible to apply indices based on the Ecological Quality Ratio (EQR) as required by the Water Framework Directive (WFD), because the available dataset was mostly qualitative and lacked the quantitative information on species abundance and composition needed to calculate the required metrics.

6.2.4 Blue carbon

Although the full application of the standardized methodologies outlined in the Coastal Blue Carbon Manual (Howard *et. al.*, 2014) proves challenging, especially due to data limitations specific to *Cymodocea nodosa*, a comprehensive estimation of organic carbon (C_{org}) stocks was carried out by integrating both published and unpublished literature with data from previous targeted field surveys within the bay as planned in other Projects. In this way it was possible to estimate the C_{org} content of Vegetative Carbon Pools (Living biomass and Detrital matter) and Sedimentary Carbon Pools.

6.3 Results

6.3.1 Habitat mapping

Below is a description of the three marine seagrass mappings of Panzano Bay, referring to the areas covered, and the species recorded. The 2015 survey identified seagrass coverage of 750 ha, focused on undifferentiated meadows and attributed them exclusively to the species *Cymodocea nodosa* (Fig. 6.9). The maximum depth reached by marine seagrasses was at a bathymetry of 4 – 5 m towards the central sector of the Bay, while the shallowest plants were observed near the tidal zero limit, close to sandy shoals (not highlighted in Fig. 6.9).



Figure 6.9 Distribution of marine seagrasses: on the left 2015 survey (S. Ciriaco, personal communication) and, on the right, 2019 survey (Interreg SASPAS, 2019).

The second survey, conducted in 2019, was designed as an update to the previous mapping. It aimed to verify the presence and extent of previously reported patches and to identify any additional ones through specific surveys and the analysis of recent satellite and photographic images. The western sectors of the gulf were also examined to assess the possible presence of other species in areas behind the emerging sandy shoals. Unfortunately, this last verification was only partial, as a thorough inspection of the area would have required different timing and a specific authorization from the competent maritime authority (“Capitaneria di Porto”) for the use of a shallow-draft vessel. The survey confirmed seagrass coverage similar to the 2015 survey (approximately 750 ha) (Fig. 6.9). It also revealed the presence of additional seagrass species in the western sectors, particularly *Zostera marina* and *Zostera noltei*, in the areas targeted for monitoring activities planned within the SASPAS project (Zone 2 and Zone 3).

In September 2024, researchers working under the BIOPRESSADRIA Interreg Project carried out an extensive survey of the Panazano Bay’s seagrass meadows and recorded a total coverage of about 770 ha. *Cymodocea nodosa* accounted for the largest share (420 ha), followed by *Zostera noltei* (150 ha), *Ruppia* spp. (100 ha) and two types of mixed meadows, *Zostera noltei*–*Cymodocea nodosa* (30 ha) and *Zostera noltei*–*Ruppia* spp. (70 ha) (Fig. 6.10). The small *Zostera marina* meadow observed during the 2019 survey was no longer present, having already been replaced by a mixed meadow (*Zostera noltei*–*Cymodocea nodosa*) in 2014. This mapping, for the first time, aimed to detail as precisely as possible both the extent and the composition of the species present and confirmed the high biodiversity of the bay, with species distribution heavily influenced by seabed morphology, sediment types, and salinity gradients.

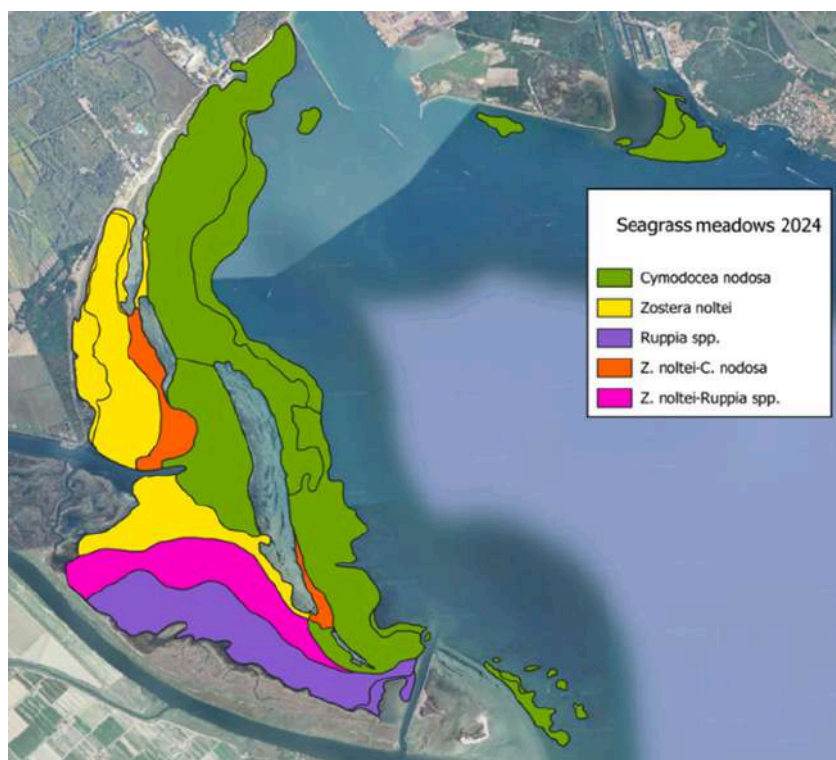


Figure 6.10 2024 Survey: distribution of marine seagrasses (Interreg BIOPRESSADRIA, 2025, modified).

Most meadows are in the western and northern areas of the bay, at depths of 4–5 m, while species dominance varies according to local conditions. In the northwest, *C. nodosa* forms dense meadows from the shore down to 2–3 m, especially between Panzano Beach and Marina Julia. Closer to shorelines influenced by freshwater, *Z. noltei* and mixed meadows are more common, while near the Isonzo River mouth, *Ruppia* spp. often forms single-species meadows that mix with *Z. noltei* offshore. In the northeast, near the Timavo River mouth, large *C. nodosa* meadows cover about 20 ha on medium-low seabeds.

When compared with the 2015 survey's undifferentiated mapping (which attributed 750 ha solely to *C. nodosa*), the 2024 results underscore a richer biodiversity and reveal how species distributions correlate with subtle environmental shifts (Table 6.2). Notably, seagrass beds have extended in medium-shallow zones near the Timavo River mouth, a detail missed in earlier data. The apparent decrease in *C. nodosa* coverage compared with the 2015 survey reflects the improved resolution and taxonomic differentiation of the 2024 analysis, which distinguished pure and mixed species meadows previously grouped under a single category. Thus, the lower cover value for this species does not indicate an ecological decline but rather a more accurate classification of meadows that were formerly recorded as monospecific.

According to Gianni et al. (2025), Panzano Bay is a stable and resilient area for *C. nodosa* meadows: its low-lying, sedimentary shores receive freshwater and nutrient inputs from the Isonzo River, which help maintain favourable conditions for seagrass growth, supporting primary productivity and mitigating environmental stressors. Unlike other parts of the Gulf of Trieste, where strong seagrass declines occurred between 2014 and 2018 (mainly near urbanized areas

such as Trieste and Miramare, with losses up to 89%), Panzano Bay showed no significant decline during this period. Meadows remained extensive and continuous, confirming the findings of the 2015 and 2019 surveys. Predictive modelling using Generalized Additive Models (GAMs) further confirmed the high suitability of Panzano for *C. nodosa* persistence, with the probability of occurrence consistently high, often exceeding 0.6.

From a management point of view, Panzano Bay can be considered a reference site for conservation and long-term monitoring. Its resilience to current pressures makes it an important benchmark for restoration efforts in more degraded parts of the Gulf of Trieste and a useful example for understanding how seagrass meadows persist in the northern Adriatic.

Special Areas of Conservation, such as “Cavana di Monfalcone” and “Foce dell’Isonzo – Isola della Cona”, also show stable ecological conditions, confirming the importance of this coastal sector for seagrass protection and ecosystem monitoring.

Table 6.2 Coverage values by species, in Panzano Bay in 2015 and 2024 maps.

	2015 Mapping	2024 Mapping
Species	ha	ha
<i>Cymodocea nodosa</i>	750	420
<i>Ruppia</i> spp.	-	100
<i>Zostera noltei</i>	-	150
<i>Z. noltei</i> - <i>C. nodosa</i>	-	30
<i>Z. noltei</i> - <i>Ruppia</i> spp.	-	70
Total	750	770

6.3.2 Ecological status

Throughout all campaigns (Interreg SASPAS, 2019 and 2022), *C. nodosa* maintained its dominance, forming continuous meadows, while the shallow fringes showed only patchy colonization. Percent cover and shoot density fluctuated naturally between bare substrate and fully developed meadows. Small increases in coverage at a shallow station and localized rises in shoot density at another highlighted small-scale variability, without indicating broader trends (Table 6.3).

Morphological measurements showed limited changes in sand bar dynamics, with only occasional coverage of existing patches. Seasonal timing and water depth affected plant morphology: leaves at deeper sites were longer, likely due to lower light availability. Seasonal variations in shoot

density and leaf length caused predictable changes in the Leaf Area Index (LAI), reflecting the typical phenological cycle of the species (Table 6.3).

Tabel 6.3 Value Ranges (2019–2021) of Cover %, Shoot Density (m^{-2}), Shoot length (cm) and Leaf Area Index (LAI).

Station	Species	Cover %	Shoot density (m^{-2})	Shoot length	LAI ⁽¹⁾
St.Z1A	<i>C. nodosa</i>	90-100	548-328	11.0-50.1	0.3-1.7
St.Z1B ⁽³⁾	<i>C. nodosa</i>	100	437-730	12.2-45.0	0.1-1.6
St.Z1C ⁽²⁾	-	-	-	-	-
St.Z2A	<i>Z. noltei</i>	75-100	1915-2357	9.3-40.6	0.3-1.7
St.Z2B	<i>C. nodosa</i>	95-100	578-719	16.2-58.6	0.2-2.1
St.Z2C ⁽³⁾	<i>C. nodosa</i>	100	639-798	8.9-70.8	0.1-2.6
St.Z3A	<i>Z. marina</i>	100	276-319	23.5-58.1	0.6-1.9
St.Z3B	<i>C. nodosa</i>	95-100	578-681	13.1-62.2	0.1-2.3
St.Z3C ⁽³⁾	<i>C. nodosa</i>	100	765-813	11.7-58.8	0.1-3.4

⁽¹⁾ The photosynthetically active surface (m^2/m^2)

⁽²⁾ Marine seagrasses were absent (bare seabed), although they were present just a few meters away.

⁽³⁾ Together with St.1, St.4, and St.5, also used for the application of the Mediskew index in 2025.

Mechanical disturbances were minor, mostly linked to fixed fishing nets recorded near Zones 2 and 3 in 2019 and 2020. Occasional anchoring by small recreational boats at depths of one to three meters caused only limited damage. The resilience of *C. nodosa* is mainly due to its fast branching and ability to recolonize disturbed areas, combined with the small size of the boats and the limited use of heavy anchor chains.

Monitoring results show that coastal morphology, small changes in elevation, from exposed tidal flats to shallow subtidal zones, and recreational boating pressures together influence meadow conditions, shaping both their structure and resilience. Despite these pressures, the seagrass meadows remained remarkably stable between 2019 and 2021. This detailed baseline provides a valuable reference for tracking future changes, whether expansion or decline, in the dynamic and resilient coastal ecosystem of Panzano Bay.

6.3.2.1 Results and Ecological Interpretation of the MediSkew Index application

MediSkew index was calculated using St.Z1B (in site 1) as the reference station, due to its low cumulative pressure (PISM value = 6.25) and the shortest median photosynthetic leaf length (7.8 cm), both indicating lower levels of impact compared with the other sites (Table 6.4).

Tabel 6.4 Summary of length measurements and absolute skewness ($|G|$) for the photosynthetically active portions of *Cymodocea nodosa* leaves collected from sampling stations in Panzano Bay.

Site	Station	Min length (cm)	Max length (cm)	Mean (cm)	Median (cm)	$ G $
1	St.1	1.8	17.4	9.3	9.2	1.21
	St.Z1B	1.7	15.2	7.9	7.8	0.98
2	St.Z2C	3.0	24.6	11.5	11.3	0.99
	St.Z3C	3.6	27.1	15.8	15.5	1.33
3	St.4	2.9	25.1	14.5	14.4	1.3
	St.5	3.2	26.1	14.0	13.8	0.83

The MediSkew index revealed a spatial gradient in structural integrity across the surveyed *Cymodocea nodosa* meadows in Panzano Bay (Table 6.5 and 6.6).

Tabel 6.5 Status Classification (Orlando-Bonaca et al., 2015)

Status Class	MediSkew Range
High	$0 \leq \text{MediSkew} < 0.2$
Good	$0.2 \leq \text{MediSkew} < 0.4$
Moderate	$0.4 \leq \text{MediSkew} < 0.6$
Poor	$0.6 \leq \text{MediSkew} < 0.8$
Bad	$0.8 \leq \text{MediSkew} \leq 1$

Tabel 6.5 MediSkew index values for each sampling station and site, with evaluations of Ecological Status (according to WFD) and Environmental Status (according to MSFD) for *Cymodocea nodosa* monitoring areas in the Panzano Bay.

Site	Station	Station MediSkew	Site MediSkew	Ecological status (WFD)	Environmental status (MSFD)
1	St.1	0.37	0.30	Good	Good/Achieved

	St.Z1B	0.24			
2	St.Z2C	0.42	0.57	Moderate	Not Good/
	St.Z3C	0.72			Not Achieved
3	St.4	0.65	0.57	Moderate	Not Good/
	St.5	0.50			Not Achieved

Considering the ecological status (as defined by WFD), “Good” values were recorded at site 1 (St.1 and St.Z1B), showing a mostly symmetrical leaf-length distribution around the reference median. This indicates minimal structural disturbance, a healthy canopy, and stable meadow dynamics under low ecological stress. The Environmental Status (MSFD - Marine Strategy Framework Directive) at these sites was also “Good/Achieved,” confirming that these areas experience low ecological pressure and generally favourable conditions.

At sites 2 (St.Z2C and St.Z3C) and 3 (St.4 and St.5), “Moderate” values were observed, reflecting partial deviations from the expected leaf-length distribution. These deviations likely result from environmental pressures such as reduced light, sedimentation, or local human impacts. Environmental Status at some of these stations was recorded as “Not Good/Not Achieved,” highlighting ongoing pressures that may affect meadow health. These moderate values may indicate early structural changes, acting as warning signs of potential functional degradation.

Overall, the gradient from “Good” values (site 1) to “Moderate” classifications (site 2 and site 3) at deeper or more impacted stations matches known environmental pressures in the area, such as light limitation and anchoring activity. This pattern shows the practical usefulness of the index for detecting early structural changes and supporting local management and conservation efforts.

6.3.3 Biodiversity

6.3.3.1 Benthic invertebrates

The most common mobile-bottom biocenotic units in the Gulf of Trieste are typically associated with muddy or muddy-detrital substrates. However, within the pilot site area, coastal sandy and sand-mud biocenoses are also present, particularly near Punta Sdobba (close to the mouth of the Isonzo River) and in Panzano Bay (Fig. 6.11) (Orel, 1988). These soft-bottom habitats support diverse macrozoobenthic communities whose ecological status has been evaluated using the M-AMBI index.

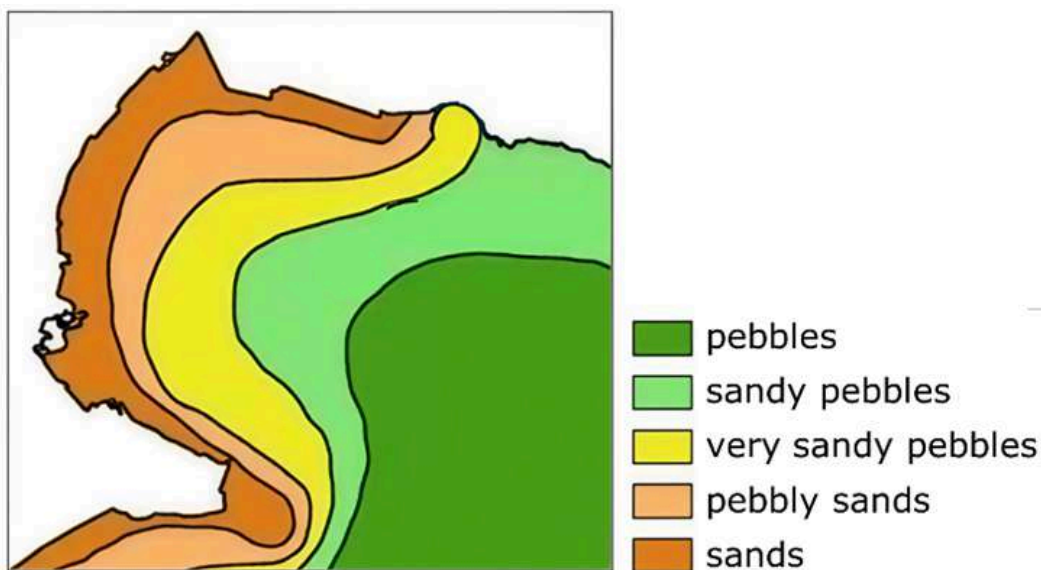


Figure 6.11 Bay of Panzano: sedimentological map (Bettoso et al., 2022, modified).

M-AMBI is a multimetric assessment tool developed for coastal waters, integrating the AZTI Marine Biotic Index (AMBI), which classifies benthic species based on their sensitivity to organic disturbance, with species diversity and richness, analyzed through multivariate techniques. Despite substantial anthropogenic pressures, including port activities, commercial and recreational fishing, tourism, boating, sewage discharges, and riverine input, the benthic macroinvertebrate communities of Panzano Bay consistently achieved an “High” ecological status between 2014 and 2019, according to M-AMBI assessments carried out under the EU Water Framework Directive. This evaluation, conducted at water body CE12 (station 121, Fig. 6.12), highlights the presence of healthy, well-structured benthic populations that correspond to reference conditions (Bettoso et al., 2022).



Figure 6.12 Localization of the CE12 water body (Panzano Bay) and its related monitoring stations (ARPA FVG - SOS Qualità

delle acque marine e di transizione www.arpa.fvg.it 04/03/2021).

In 2019, systematic sampling was conducted under the SASPAS project, with a focus on assessing marine benthic biodiversity, including both macroalgae and invertebrates (Interreg SASPAS, 2019). As with the MediSkew index calculation, data for M-AMBI evaluation were collected from six stations across Panzano Bay (see Fig. 6.4). Three of these (St.Z1B, St.Z2C, St.Z3C) served as long-term SASPAS monitoring sites (2019–2021), while the other three (St.1, St.4, St.5) were monitored only during the initial 2019 campaign and were not formally published within the SASPAS framework.

The sampling identified several benthic invertebrate taxa, confirming the rich biodiversity of the area (Table 6.7 and Fig. 13). Among the most common amphipods was *Gammarus insensibilis* (Epifauna). Decapods included *Carcinus aestuarii* and *Palaemon adspersus* (Epifauna). Isopods such as *Idotea spp.* and *Synischia hectica* (Epifauna) were also observed. Bivalve species included *Mytilus galloprovincialis*, *Modiolus barbatus* (Epibenthic macrofauna), and *Loripes orbiculatus* (Endofauna), while gastropods such as *Gibbula spp.*, *Bittium spp.*, and *Tritia spp.* (Epibenthic macrofauna) added to the diversity. Polychaetes such as *Platynereis dumerillii* (Epifauna/Endofauna) and serpulid polychaetes *Serpulidae*, *Pileolaria* genus, and *Spirorbidae* (Epibenthic macrofauna) were also found.



Figure 6.13 Images of some organisms identified during the surveys: on the left, the decapod crustacean *Diogenes pugilator*; on the right, the gastropod mollusc *Tritia nitida*.

Table 6.7 Taxonomic Composition of Macroinvertebrate Communities (Number of individuals per m²).

Faunal Group	Taxonomic group	Taxa	St.1	St.Z1B	St.Z2C	St.Z3C	St.4	St.5
Epibenthic macrofauna	Anthozoa	Actiniaria indet.	67		67	33		33
Epibenthic macrofauna		<i>Anemonia viridis</i> (Forsskål, 1775)	33	33		33		
Epifauna	Crustacea	<i>Dexamine spinosa</i> (Montagu, 1813)	33		33		33	
Epifauna	Amphipoda	<i>Gammarus insensibilis</i> Stock, 1966			67	33	67	33
Epifauna	Crustacea Decapoda	<i>Carcinus aestuarii</i> Nardo, 1847	67	133		67	33	
Epifauna		<i>Diogenes pugilator</i> (Roux, 1829)	33	33	33			
Epifauna		<i>Palaemon adspersus</i> Rathke, 1837			33	33	33	33
Endofauna		<i>Upogebia pusilla</i> (Petagna, 1792)	33			33		
Epifauna	Crustacea Isopoda	<i>Idotea</i> indet. Fabricius, 1798			33		33	
Epifauna		<i>Synischia hectica</i> (Pallas, 1772)				33		
Epibenthic macrofauna		<i>Anomia ephippium</i> Linnaeus, 1758	33	67	33			67
Epibenthic macrofauna		<i>Chama gryphoides</i> Linnaeus, 1758	33	100		33	33	
Endofauna		<i>Fabulina fabula</i> (Gmelin, 1791)	33		33	33		67
Endofauna	Mollusca Bivalvia	<i>Loripes orbiculatus</i> Poli, 1795	300	267	367	600	233	400
Endofauna		<i>Lucinella divaricata</i> Linnaeus, 1758		33	67		33	
Epibenthic macrofauna		<i>Modiolus barbatus</i> (Linnaeus, 1758)	133	167	33			33
Epibenthic macrofauna		<i>Mytilaster lineatus</i> (Gmelin, 1791)	33	33		67	33	
Epibenthic macrofauna		<i>Mytilus galloprovincialis</i> Lamarck, 1819	67	100	33			67
Epibenthic macrofauna		<i>Bittium reticulatum</i> (da Costa, 1778)	467	700	367	500	267	433
Epibenthic macrofauna		<i>Hexaplex trunculus</i> (Linnaeus, 1758)	33	100		33	67	
Epibenthic macrofauna		<i>Tritia nitida</i> (Jeffreys, 1867)	33		67	33	33	
Epibenthic macrofauna	Mollusca	<i>Pusillina lineolata</i> (Michaud, 1830)			67			
Epibenthic macrofauna	Gasteropoda	<i>Steromphala adriatica</i> (R. A. Philippi, 1844)	67	67	33		33	
Epibenthic macrofauna		<i>Steromphala albida</i> (Gmelin, 1791)	67	100	67	33	67	33
Epibenthic macrofauna		<i>Tricola pullus</i> (Linnaeus, 1758)			33	67	33	67
Epibenthic macrofauna		<i>Tritia neritea</i> (Linnaeus, 1758)	33	33		33		
Epifauna/Endofauna	Polychaeta	<i>Platynereis dumerilii</i> (Audouin & Milne Edwards, 1833)	33	67	100	33		33
Epibenthic macrofauna		<i>Pileolaria</i> indet. Claparède, 1868	600	700	433	467	267	367
Epibenthic macrofauna		Spirorbidae indet. Chamberlin, 1919	633	867	567	500	600	433
Epibenthic macrofauna		Serpulidae indet. Rafinesque, 1815	67	200	100	133	100	67

The composition of macroinvertebrate communities across the six stations, St.1, St.Z1B, St.Z2C, St.Z3C, St.4, and St.5, shows a clear predominance of taxa associated with low ecological disturbance. The widespread dominance of Group I (sensitive species) and Group II (indifferent or slightly tolerant species) indicates well-structured benthic assemblages typical of habitats with minimal organic enrichment (Fig. 6.14). These patterns are consistent with M-AMBI values corresponding to a “Good” ecological status (Table 6.8), where “High” ecological status is defined as $EQR > 0.81$, “Good” as $0.61 < EQR \leq 0.81$, and “Moderate” as $EQR \leq 0.61$.

Station St.5 is classified as “Moderate” despite the dominance of Groups I and II, likely due to localized environmental stressors that slightly reduce overall diversity and species richness compared to the other stations. Importantly, Group IV and V taxa, opportunistic species indicative of heavily disturbed conditions, were absent at all stations, further supporting the conclusion that soft-bottom habitats in Panzano Bay remain ecologically resilient.

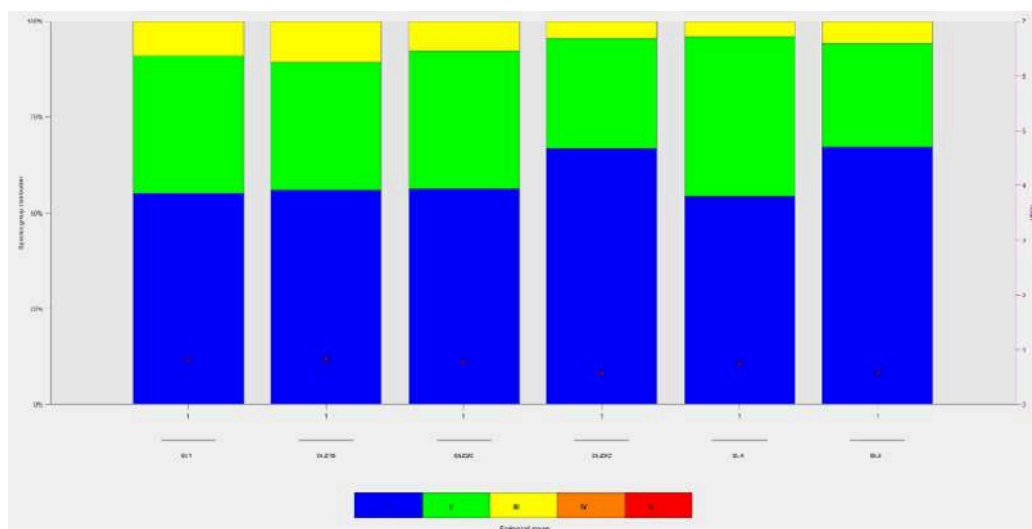


Figure 6.14 Proportional distribution of AMBI ecological groups across stations.

Table 6.8 Ecological Quality Metrics and M-AMBI classification across stations.

Stations	AMBI	Diversity	Richness	M-AMBI	Status
Bad	6	0	0	0	Bad
High	0.5	4.8	50	1	High
St.1	0.806	3.46	21	0.68	Good
St.Z1B	0.821	3.23	17	0.63	Good
St.Z2C	0.773	3.57	21	0.69	Good
St.Z3C	0.568	3.18	19	0.66	Good
St.4	0.750	3.13	16	0.62	Good
St.5	0.577	2.99	14	0.61	Moderate

The ecological relevance of M-AMBI in this context is further supported by the hydro-morphological characteristics of Panzano Bay. The entire arc of the bay is composed of fine sandy sediments with varying organic content depending on depth, distance from the shoreline, and proximity to freshwater inputs. This configuration influences the distribution patterns of *Cymodocea nodosa* and other seagrasses such as *Zostera noltei* and *Ruppia* genus, which colonize intertidal zones and areas near river mouths.

Although the Ecosystem-Based Quality Index (EBQI) developed for *Posidonia oceanica* is not directly applicable to *C. nodosa*, M-AMBI offers a meaningful ecological contribution by describing

the structure of endobenthic, and to some extent epibenthic, communities. *C. nodosa*, while not matching *Posidonia oceanica* in carbon storage capacity, plays a vital role in sediment stabilization through its dense belowground biomass, which exceeds its aboveground compartment. This root system promotes seabed consolidation, sediment deposition, and prevents resuspension, contributing to coastal protection against erosion.

Overall, the benthic macroinvertebrate communities in Panzano Bay reflect a generally favourable ecological status across all monitored stations. Benchmark conditions represent the two extremes of ecological quality: high-quality environments are characterized by low AMBI scores and high diversity and richness, whereas degraded habitats show the opposite. Most stations fell within intermediate or high ranges, reflecting balanced assemblages with low disturbance levels. The findings support the ecological integrity of the soft-bottom habitats in Panzano Bay, despite ongoing pressures such as port operations and urban runoff.

6.3.3.2 Macroalgae and epiphytic algae

Among the identified species during the SASPAS project monitoring (Interreg SASPAS, 2019), a significant portion is made up of algae, crucial to the health and functioning of the ecosystem. The green algae (Chlorophyta) are well-represented, with species such as *Blidingia ramifera*, *Bryopsis plumosa*, *Cladophora* spp., and *Ulva australis* being particularly common. Another group of algae, the brown algae (Ochrophyta), includes species like *Cystoseira compressa* and *Dictyota dichotoma*. The red algae (Rhodophyta), such as *Ceramium* spp., *Chondria capillaris*, *Gracilaria* spp., and *Hypnea spinella*, are also prominent in the bay. No filamentous macroalgal blooms or invasive algae were detected during any survey, and encrusting red calcareous algae (e.g., *Hydrolithon* and *Pneophyllum* genera) dominated the epiphytic community, an indicator of the good seagrass meadow health.



Figure 6.15 Images of some epiphytic algae identified during the surveys: on the left, *Ceramium* genus and, on the right, *Chondria capillaris*.



6.3.3.3 Ichthyofauna

Marine seagrass meadows are vital for sustaining diverse and thriving fish populations, offering key nursery habitats, refuge, and feeding zones for various fish species, including those of economic value. Among these fish, the most common ones belong to the families Syngnathidae (*Syngnathus* spp., *Nerophis ophidion*), Mugilidae (*Liza* spp., *Mugil cephalus*), Sparidae (*Sparus aurata*, *Lithognathus mormyrus*, *Diplodus* spp., *Pagellus erythrinus*), Blennidae (*Parablennius* spp.), Atherinidae (*Atherina boyeri*), Moronidae (*Dicentrarchus labrax*), Clupeidae (*Sprattus sprattus*, *Sardina pilchardus*), Engraulidae (*Engraulis encrasicolus*), Belonidae (*Belone belone*), Soleidae (*Solea* spp.) (Interreg SASPAS, 2019 - unpublished data).



Figure 6.16 Images of some specimens of gilthead seabream (*Sparus aurata*).

6.3.3.4 Other Key organisms

In 2019 and 2020, during the SASPAS monitoring campaigns conducted in the three designated zones (see Fig. 6.2) within Panzano Bay, the presence of a few *Pinna nobilis* specimens, both alive and dead, highlighted the ecological significance of the area and confirmed its exposure to the broader mortality crisis (Interreg SASPAS, 2019). Since 2016, *P. nobilis* populations across the Mediterranean have suffered catastrophic declines due to mass mortality events triggered by the protozoan *Haplosporidium pinnae*.



Figure 6.17 Images of *Pinna nobilis*.

6.3.4 Blue carbon

As mentioned before, by integrating both published and unpublished literature with data from previous targeted field surveys within the bay as planned in other Projects, it was possible to estimate the C_{org} content of Vegetative Carbon Pools and Sedimentary Carbon Pools.

6.3.4.1 Vegetative Carbon Pools

They are represented by 1) Living biomass, both aboveground (leaves and shoots, and epiphyte biomass) and below ground (rhizomes and roots) and 2) Detrital matter, consisting of senescent plant tissues and accumulated organic litter. Despite the difficulty in collecting precise biometric data for *Cymodocea nodosa*, established carbon content benchmarks from analogous Adriatic/Mediterranean ecosystems (Ros *et al.*, 2015) provide a solid foundation for our estimations. Field data further contribute to characterizing the structural density and spatial distribution of the meadows, enabling a tailored approach to quantifying the carbon stored in marine seagrasses in Panzano Bay. Based on the above, when considering the vegetative carbon pools, the estimated average organic carbon stocks are 0.127 kg/m^2 in *Cymodocea nodosa* meadows, 0.063 kg/m^2 in *Zostera noltei*, and 0.030 kg/m^2 in *Ruppia* spp. (Interreg SASPAS, 2022 and unpublished data).

6.3.4.2 Sedimentary Carbon Pools (the upper 20 cm of sediment)

In this analysis, the sediment beneath the seagrass canopy represents a critical reservoir for organic carbon. The focus is on the upper 20 cm of sediment, recognized as the most biologically active layer for carbon exchange and sequestration (Duarte *et al.*, 2011). Carbon concentration in



the bay's sediments exhibits clear spatial variation (Interreg SASPAS, 2022). Across the studied sites, the average total sedimentary carbon amounts to 73.0 mg/g, indicating a relatively consistent accumulation of carbon throughout the area. Organic carbon (Corg) comprises a substantial portion, with a bay-wide average of 7.65 mg/g. The highest concentrations of Corg are recorded in the southernmost area (Zone 3), reaching up to 10.4 mg/g, likely due to the influx of suspended sediments and nutrients from the Stella River, combined with localized hydrodynamic conditions that facilitate organic matter retention. When extrapolated across the upper 20 cm sediment layer, the estimated average organic carbon stocks are 2.034 kg/m² in *Cymodocea nodosa* meadows, 1.596 kg/m² in *Zostera noltei*, and 1.450 kg/m² in *Ruppia* spp. (Interreg SASPAS, 2022 and unpublished data).

Considering inputs from both vegetative and sedimentary carbon pools, the estimated average organic carbon stocks are 2.161 kg/m² in *Cymodocea nodosa* meadows, 1.659 kg/m² in *Zostera noltei*, and 1.480 kg/m² in *Ruppia* spp. (Interreg SASPAS, 2022 and unpublished data). These values align with the findings of Ros *et al.* (2015), who documented significantly higher carbon storage within *C. nodosa* meadows in Alfacs Bay (located in Catalonia, Spain, within the Ebro Delta Natural Park), reporting up to 2.482 kg C/m² in eutrophic, continuous meadows, and 0.899 kg C/m² in patchy, oligotrophic areas. The findings from both studies indicate that the structure of seagrass habitats and the availability of nutrients are key factors influencing carbon sequestration. This is consistent with the present analysis, which highlights how spatial variation and site-specific environmental conditions shape carbon dynamics. Together, they reinforce the essential role of seagrass ecosystems as efficient and dynamic blue carbon sinks throughout the Mediterranean region.

6.4 Discussion

Over the past decade, the seagrass meadows of Panzano Bay have experienced notable spatial and compositional changes, as revealed through successive mapping campaigns and ecological assessments. *C. nodosa* has consistently remained the dominant species, demonstrating remarkable structural stability and resilience across a range of environmental conditions. This resilience is partly due to its high regenerative capacity, as this species is known for rapid rhizome growth and efficient recolonization of disturbed areas, enabling quick recovery from physical disturbances such as anchoring and bottom-contact fishing.

At the same time, the increasing presence of *Z. noltei* and *Ruppia* genus, particularly in brackish and shallow areas, indicates an ongoing reorganization of seagrass species zonation, likely driven by salinity gradients, sediment dynamics, and freshwater inflows. The progressive refinement of

survey methodologies, combining remote sensing, field validation, and species-level classification, has enabled a more accurate understanding of habitat distribution and ecological status. The 2024 survey, in particular, yielded the most comprehensive dataset to date. It revealed previously undocumented meadow patches and provided new insights into spatial heterogeneity and ecosystem functioning.

Despite localized anthropogenic pressures, such as anchoring and fishing, the overall ecological condition of the seagrass beds remains stable. These meadows continue to support high biodiversity and contribute significantly to blue carbon sequestration, reinforcing their essential role within the northern Adriatic coastal ecosystems.

An integrated monitoring approach, combining historical data, targeted fieldwork, and complementary indices (MediSkew for canopy structure and M-AMBI for benthic condition), provided an evaluation of ecosystem status and service provision. Results revealed well-structured benthic assemblages dominated by sensitive taxa (AMBI Groups I-II), absence of opportunistic benthic species, and healthy epiphytic and macroalgal communities characterized by encrusting red algae, all consistent with good ecological quality. The ichthyofauna included several nursery and commercially relevant species, confirming the meadows' role in supporting fisheries-related ecosystem services.

Preliminary blue carbon assessments, integrating literature-derived conversion factors with local biometric and sediment data, showed measurable carbon storage within vegetative biomass and surface sediments, highlighting the meadows' carbon sequestration potential and resilience.

Based on the comprehensive data collected, the observed ecological trends, and reference benchmarks (when available), it is possible to delineate a set of conditions that may characterize a pristine environmental state of the Panzano Bay marine seagrass meadows. These reference parameters reflect a well-structured and resilient ecosystem, showing stability across biological, physical, and chemical indicators over time. Table 6.9 summarizes the key attributes defining this possible pristine baseline condition.

Table 6.9 Possible pristine reference conditions for Panzano Bay marine seagrass meadows.

Parameter	Reference value / description (pristine condition)	Notes / Source
Dominant species	<i>Cymodocea nodosa</i>	Stable dominance across monitoring years
Associated species	<i>Zostera noltei</i> , <i>Ruppia</i> spp. in brackish/shallow areas	Present in mixed assemblages
% Coverage	80 – 100 % in continuous pristine meadows	Ideally near continuous in pristine meadows



Parameter	Reference value / description (pristine condition)	Notes / Source
Shoot density (shoots/m ²)	400 – 1200 (<i>C. nodosa</i>)	Stable over time; marginal fluctuations
Leaf length (cm)	Longer leaves at deeper stations due to light limitation	Strong seasonal variation observed
Leaf Area Index (LAI)	Driven by seasonal shoot density and leaf length cycles	Predictable phenological oscillations
Seabed stability	Stable sandy/muddy substrates; limited sand bar dynamics	Occasional coverage of patches only
Epiphyte load	Dominance of encrusting red algae (genera <i>Hydrolithon</i> , <i>Pneophyllum</i>)	Indicator of good ecological status
Macroalgal blooms	None observed	No filamentous/invasive algae
Associated macrofauna	Diverse benthic invertebrate community (e.g., amphipods, decapods, bivalves, gastropods, polychaetes)	M-AMBI status: “High”
Ichthyofauna	Rich assemblage including Syngnathidae, Mugilidae, Sparidae, Moronidae, Soleidae	Nursery/refuge function confirmed
Key species presence	<i>Pinna nobilis</i> (few individuals)	Presence despite regional mortality event
Anthropogenic pressure	Minimal: small-boat anchoring, fixed nets; no heavy chain catenaries	Resilient due to rapid recolonization
Carbon stocks – vegetative	2.161 kg Corg/m ² (<i>C. nodosa</i>)	Estimates from local field data and literature
Carbon stocks – sedimentary (upper 20 cm)	7.65 mg/g Corg (bay-wide average)	Estimates from local field data and literature

The observed conditions at the Monfalcone pilot site generally align with many of these reference parameters, reinforcing its value as a baseline for biodiversity and carbon-oriented management. Overall, the site represents a valuable ecological reference within the ARTEMIS project. It expands

the project's geographic and ecological scope, supports the validation of adaptable monitoring protocols for seagrass meadows, and provides a pragmatic baseline for ecosystem and carbon-focused management, without being the target of active restoration efforts in the current phase.

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7 Main Takeaways

The results obtained across the pilot sites of the ARTEMIS project clearly show that *P. oceanica* meadows were deeply impacted by the local stressors acting at each pilot site (Atzikiari, Menorca, and Capo Testa-Punta Falcone). Although the management level, the environmental setting (i.e., water depth) and the sampling design (i.e., stations with different seagrass cover, controls) differed among the sites, the status of the *P. oceanica* ecosystem ranged from poor to bad across the three sites hosting *P. oceanica* meadows. On the contrary, the seagrass meadows at Monfalcone (*Cymodocea nodosa*, *Zostera noltei* and *Ruppia spp.*) remained relatively healthy despite the cumulative anthropogenic pressures at the area between 2019 and 2021, indicating that these species are more tolerant to disturbances (O'Brien et al., 2018).

Regardless of the stressor, the effect on *P. oceanica* meadows was more pronounced on the seagrass itself, rather than on the sediment. This was related to the nature of stressors in the case of Atzikiari bay and Ciutadella, i.e., fish farm discharges and bentonite spill during construction of desalination plant, respectively, that impacted mainly the integrity of the seagrass and less the integrity of the sediment. Even in the case of Capo Testa-Punta Falcone, where anchoring was identified as the main stressor, and, therefore, it was expected to affect sedimentary stocks significantly compared to the other pilots, the impact was more pronounced in the vegetation. Overall, the meadows had lower density (Fig. 7.1) and supported substantially low vegetative carbon stocks (1.5 - 4 times higher values at the control stations compared to the corresponding impacted stations across pilots, Fig. 7.2).

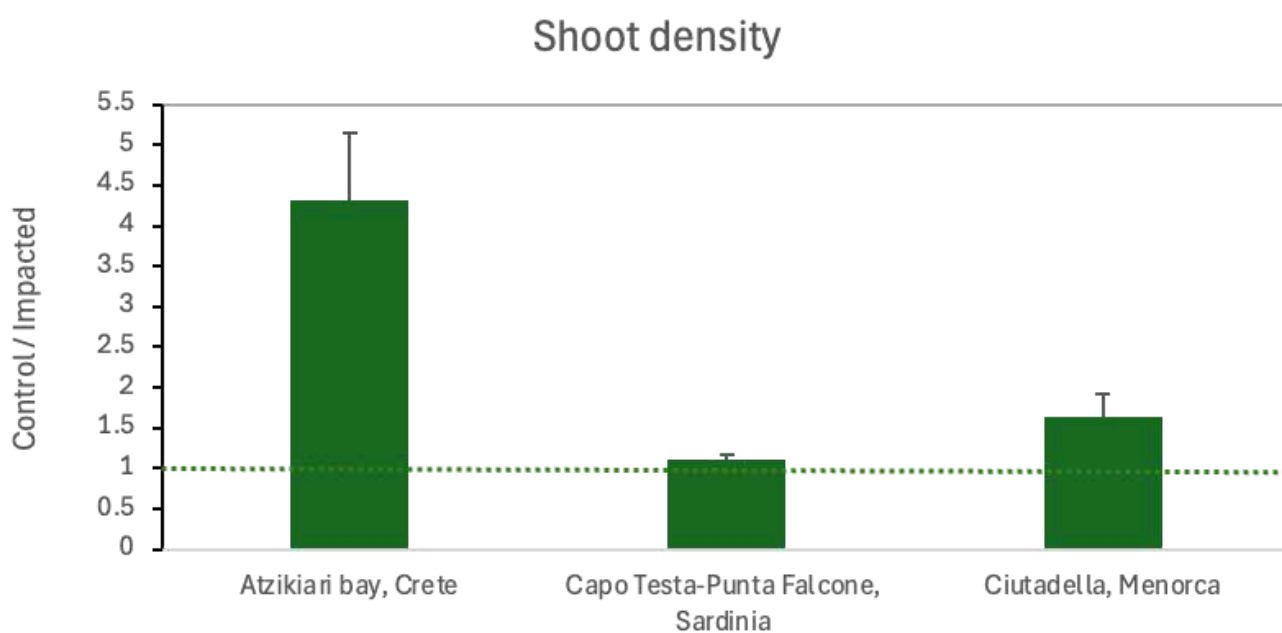


Figure 7.1. Relative shoot density ($\pm$ SE) between the control and impacted stations at each pilot site.

The $\delta^{13}\text{C}$ signals in seagrass and sediments in both Atzikiari bay and Ciutadella suggest a change in the composition of stored carbon towards a more labile nature as a result of the smaller seagrass contribution to it. The lack of substantial loss of stocks (Fig. 7.2) is probably because of the nature of the stressors (i.e. that do not cause physical damage to the habitat), at least for Atzikiari bay and Ciutadella. It could be also attributed to the time interval between seagrass degradation and our studies, which was too small to allow the detection of significant erosion, especially using standard blue carbon methodologies (i.e. stock measurements). This was particularly evident in the case of Capo Testa-Punta Falcone, where, although the sedimentary stocks did not differ among stations, the corresponding accumulation rates of carbon were higher at the control station. This suggests that the potential of the meadow to sequester carbon decreased, most probably as a result of lower leaf biomass due to anchoring. Finally, the lack of significant effect on the sedimentary stocks is because the multiple biogeochemical processes between the seagrass, the water column and the sediment affect the seagrass and sediment dynamics differently. Recently, a mechanistic stoichiometric model was developed and parameterised for *P. oceanica* based on empirical data (Le Vilain et al., 2025). The model coupled ecological and biogeochemical functioning to assess the effects of different stressors, including eutrophication and mechanical damage. The model showed that there is a cascading effect starting from the *P. oceanica* dynamics and leading to the abrupt loss of stored carbon, which sometimes involves tipping points that are harder to restore. However, the seagrass and biogeochemical processes occur at very different timescales, which may lead to significant time lags in the cascading effect from the seagrass to biogeochemical dynamics (Kooi & Poggiale, 2018), and the sedimentary carbon loss may appear more gradual over time. Therefore, restoring the meadows is imperative to avoid losing the carbon that is stored in their underlying sediments and enhance future storage.

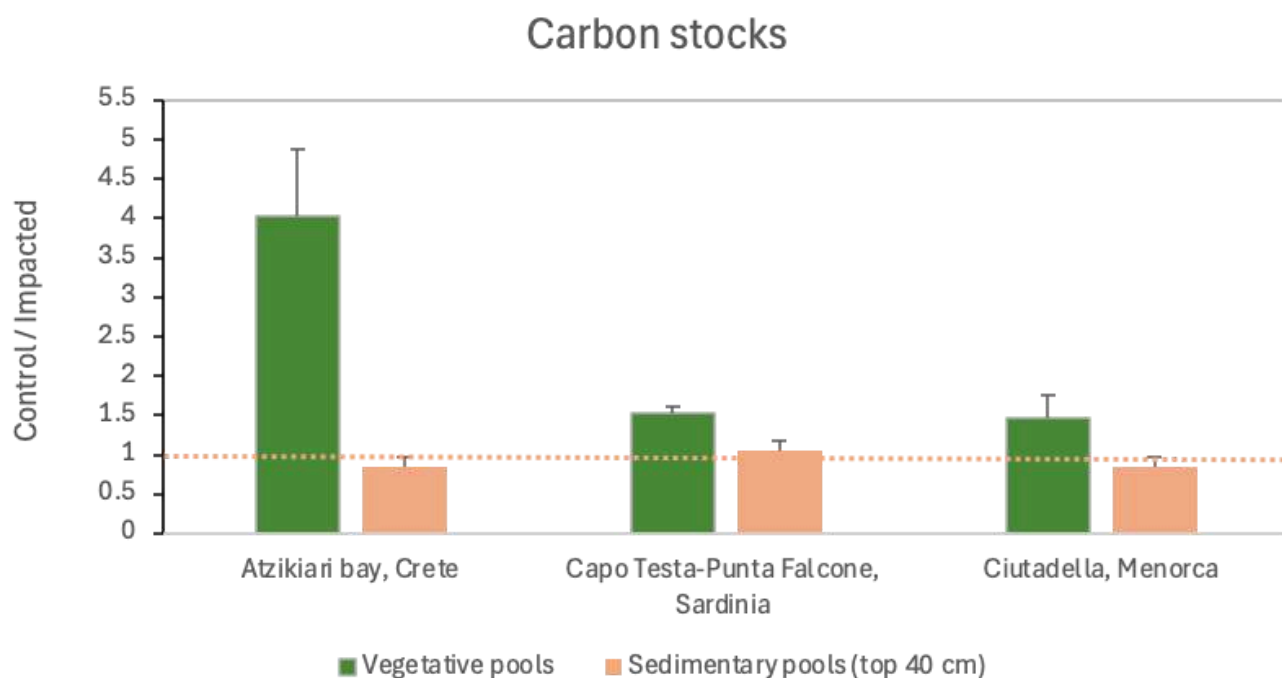


Figure 7.2. Relative vegetative and sedimentary carbon stocks ($\pm$ SE) between the control and impacted stations at each pilot site.

Across the three pilot sites where field biodiversity assessments were undertaken—Atzikiari (Crete), Ciutadella (Menorca), and Capo Testa-Punta Falcone (Sardinia)—the baseline surveys reveal consistent reductions in species richness and abundance in impacted areas relative to nearby control meadows, although the magnitude of these differences varies among biological compartments and local contexts. In Menorca, the control station supported 44 species and 488 individuals, compared with 36 species and 262 individuals in the impacted zone, indicating a broad decline across compartments. In Atzikiari, fish assemblages differed markedly between the dead matte and the seagrass meadow, with the impacted station showing the lowest total fish counts and a biomass dominated by non-indigenous species such as *Parupeneus forsskali*. In Sardinia, where the pilot site lies within a Marine Protected Area, biodiversity patterns were generally more consistent with those expected under protection —MPAs commonly support higher species richness and greater biomass of upper trophic levels (Guidetti & Sala 2007; Lester et al. 2009)— with comparatively richer communities recorded in the control meadow; nevertheless, impacted patches still exhibited reductions in several faunal groups. The synthesis of these results across compartments and sites (Fig. 7.3) highlights the contrasts in baseline biodiversity indicators among pilot areas, with the Sardinian MPA showing comparatively stronger values, while both Atzikiari and Menorca display more pronounced declines in impacted zones—particularly for ichthyofauna, which remains exceptionally low across all sites, including zero scores for teleost fish in Menorca’s EBQI assessment. These patterns align with well-documented ecological responses whereby seagrass degradation leads to simplified food webs, reduced faunal abundance, and community shifts toward opportunistic or stress-tolerant species (Duffy 2006; Orth et al. 2006). Taken together, the baseline results show that although

local pressures shape biodiversity differently at each site, the depletion of upper trophic levels and the reduced diversity in impacted zones represent shared regional trends that will serve as a key reference point for evaluating restoration outcomes.

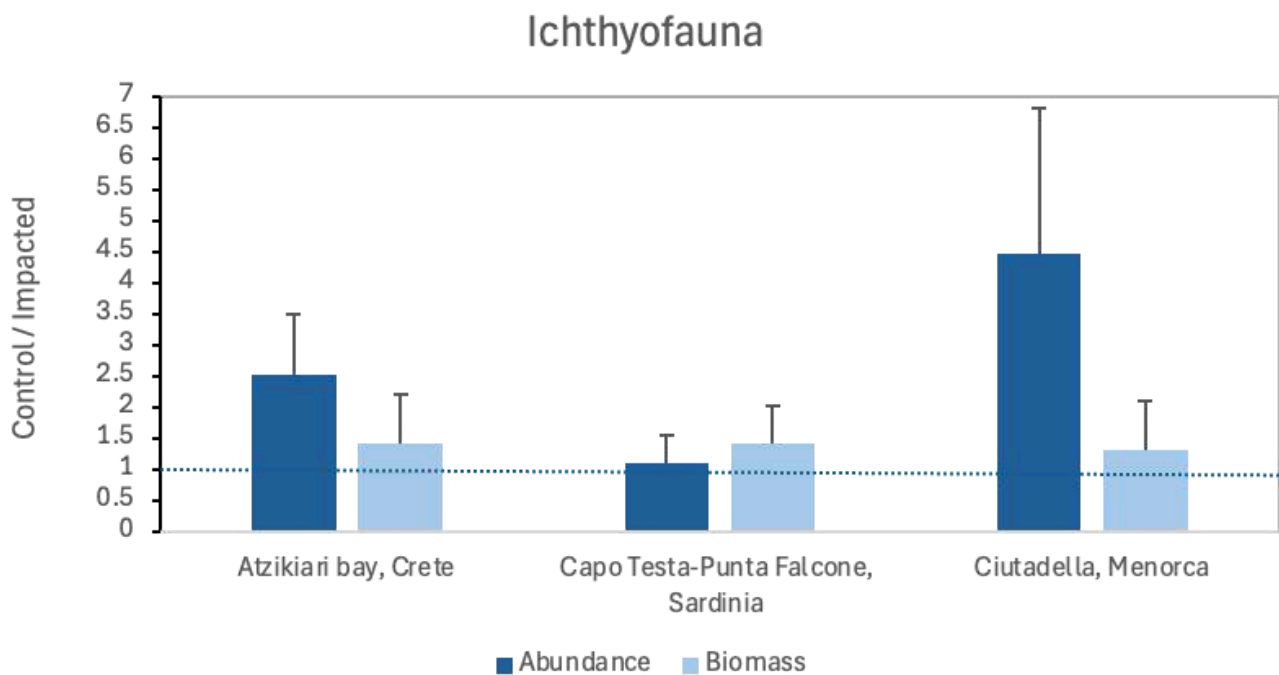


Figure 7.3. Relative abundance and biomass ($\pm$ SE) of ichthyofauna between the control and impacted stations at each pilot site.

Given the relatively different conditions across the pilot sites (i.e. different seagrass species, water depth, stations, sediment thickness, etc) it is difficult to provide a single estimate of the baseline conditions. Furthermore, the effect of disturbances on the seagrass ecosystems depends on the local conditions at small spatial scales like our case. Therefore, our estimates provide the baseline for biodiversity and carbon storage at each pilot site. But, in any case, the comparative analysis of results clearly shows the deterioration of the *P. oceanica* ecosystems studied here and the necessity of their restoration. Following the transplantation efforts carried out at each pilot site in the frame of ARTEMIS, the potential enhancement in biodiversity and increase in carbon accumulation should be followed. Available results from similar initiatives in other parts of the world show that this is a long process that may take several decades for the meadows to reach a good status (Orth et al., 2020) (Fig. 7.4).

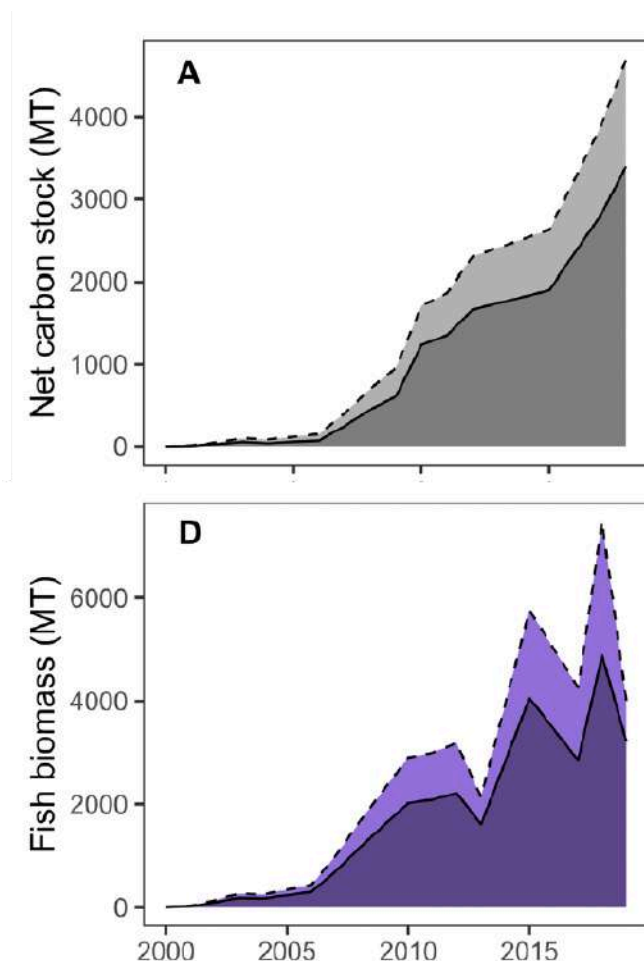


Figure 7.4. Net sediment carbon stock and fish biomass associated with the restoration of eelgrass over time. Mean (solid lines) and 95% confidence intervals (dotted lines) over time (mT = metric tons). Modified from Orth et al. 2020.

To this end, regular monitoring should be scheduled by applying appropriate methods (e.g. deployment of permanent plots to assess shoot recruitment, permanent transects to identify biodiversity trends, collection of new cores and use of surface elevation tables to follow carbon accumulation in the sediment, *in situ* measurements of dissolved inorganic and organic carbon to estimate their lateral fluxes, and CO₂ and other greenhouse gases fluxes to determine air-sea exchanges). In order to do so, future funding should be secured to allow multiannual monitoring of the restored sites. Most importantly, effective protection measures should be implemented to each site to prevent future seagrass losses and protect the meadows under restoration.



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9 Annexes

9.1 Annex 1 – WePOSI (Weighted POSidonia Index)

The *P. oceanica* metrics examined for the assessment of ecological status are incorporated into the multimetric WePOSI index using the following equation:

$$EQR'_{WePOSI} = (EQR'_{MEADOW} * 0.5 + EQR'_{PLANT} * 0.3 + EQR'_{COMMUNITY} * 0.2) / 3$$

where:

$$EQR'_{MEADOW} = EQR'_{typeLL} + EQR'_{depthLL} + EQR'_{cover} + EQR'_{density} + EQR'_{plagiotropic}$$

$$EQR'_{PLANT} = EQR'_{shootlength}$$

$$EQR'_{COMMUNITY} = EQR'_{epiphbiomass}$$

with:

$$EQR'_{typeLL} \text{ value of LL type (progressive/erosive}=1; \text{sharp } 0.75; \text{sparse}=0.50; \text{regressive}=0.25)$$

$$EQR'_{depthLL} \text{ value measured - worst value/reference value- worst value}$$

$$EQR'_{cover} \text{ value measured /reference value,}$$

the ratio of the two supporting metrics, Meadow Cover and Dead meadow Cover, expressed as Meadow Cover / Meadow Cover + Dead meadow Cover (i.e., Conservation Index, Moreno et al. 2001).

$$EQR'_{density} \text{ value measured - worst value/reference value- worst value}$$

$$EQR'_{plagiotropic} \text{ value measured - worst value/reference value- worst value}$$

$$EQR'_{shootlength} \text{ value measured - worst value/reference value- worst value}$$

$$EQR'_{epiphbiomas} \text{ value measured - worst value/reference value- worst value}$$

The multimetric index is calculated as follows:

$$EQR = (EQR'_{WePOSI} + 0.11) / (1 + 0.1)$$



9.2 Annex 2 – Ecological indices

Table 9.1 Calculation of synthetic ecological indices CI, SI, PSI and conservation status classification scales.

Conservation Index: $CI = P / (P + D)$

where P is the percent cover of living *Posidonia oceanica* and D is the percent cover of dead matte

- 1 - $CI < 0.3$: bad conservation status
 - 2 - CI between 0.3 and 0.5 excluded: poor conservation status
 - 3 - CI between 0.5 and 0.7 excluded: moderate conservation status
 - 4 - CI between 0.7 and 0.9 excluded: good conservation status
 - 5 - $CI \geq 0.9$: high conservation status
-

Substitution Index: $SI = S / (S + P)$

where S is the percent cover of substitutes and P is the percent cover of living *P. oceanica*

- 1 - $SI < 0.1$: no ($SI = 0$) or little substitution; high conservation status
 - 2 - SI between 0.1 and 0.25 excluded: low substitution; good conservation status
 - 3 - SI between 0.25 and 0.4 excluded: moderate substitution; moderate conservation status
 - 4 - SI between 0.4 and 0.7 excluded: significant substitution; poor conservation status
 - 5 - $SI \geq 0.7$: strong substitution; bad conservation status
-

Phase Shift Index: $PSI = \{[D / (P + D) \times 1] + [Cn / (P + Cn) \times 2] + [Cp / (P + Cp) \times 3] + [Ct / (P + Ct) \times 4] + [Cr / (P + Cr) \times 5]\} / 6$

where D is the percent cover of dead matte, P that of living *P. oceanica*, Cn of *Cymodocea nodosa*, Cp of *Caulerpa prolifera*, Ct of *C. taxifolia*, and Cr of *C. racemosa*

- 1 - $PSI < 0.08$: no ($PSI = 0$) or early stage of phase shift; high conservation status
 - 2 - PSI between 0.08 and 0.16 excluded: low phase shift; good conservation status
 - 3 - PSI between 0.16 and 0.25 excluded: moderate phase shift; moderate conservation status
 - 4 - PSI between 0.25 and 0.5 excluded: significant phase shift; poor conservation status
 - 5 - $PSI \geq 0.5$: strong phase shift, irreversible; bad conservation status
-

Regional scale from Montefalcone et al. (2009)



9.3 Annex 3 – EBQI results in Atzikiari bay

Table 9.2 Calculation of the EBQI for assessing the status of the different Posidonia meadows ecosystem's functional compartments.

Box n°	Functional compartment	Weighting (W)	Parameter	Estimates	Status	CI	Status grade (WxS)	CI grade (W x CI)
1	Roots and rhizomes ('rhizomes')	3	Growth of orthotropic rhizomes (mm y ⁻¹)	8.78	4	4	12	12
2	Posidonia oceanica leaves ('leaves')	5	Density (shoots m ⁻²)	679.17	4	4	20	20
			Cover (%)	61.42	3	4	15	20
3-4	MPOs, filter- and suspension-feeder s leaf epibiota ('leaf epibiota')	4	Biomass (g DM shoot ⁻¹)	0.008	1	4	4	16
5	Benthic filter-feeder: Pinna nobilis (bivalve) ('Pinna')	2	Density (ind. 100 m ⁻²)	0	0	4	0	8
6	HOM	2	Density (ind. m ⁻²)	0	4	4	8	8
	LOM		Density (ind. m ⁻²)	27.64	4	4	8	8
7	Litter detritus: dead leaves and broken rhizomes ('litter')	2	Biomass (g DM m ⁻²)	46.57	0	4	0	8
8	Detritus-feeders 2 and 3 (Holothuria spp.) ('Holothuria')	2	Density (ind. 10 m ⁻²)	0	1	4	2	8
9	Herbivores 1 ('herbivores')	5	Density of Paracentrotus lividus (ind. m ⁻²)	0	1	4	5	20
			Grazing index (% leaves)	0	1	4	5	20
10	Predatory teleosts, cephalopods and seastars ('predators')	5	Biomass (kg 100 m ⁻²)	0.005	0	4	0	20
11	Piscivorous teleosts ('piscivores')	5	Biomass (kg 100 m ⁻²)	0.005	0	4	0	20
12	Planktivorous teleosts ('planktivores')	3						



	Zooplankton feeders		Biomass (kg 100 m ⁻²)	0.06	0	4	0	12
	Omnivores		Biomass (kg 100 m ⁻²)	0	0	4	0	12
9-1 2	All teleosts ('SRDI')	3	Specific Relative Diversity Index (SRDI)	3.67	1	4	0	12
13	Sea birds	1						
	Phalacrocorax spp.		Distance to the nearest nesting site (km)	10	2	4	2	4
				Sums of the weighted status and weighted CI grades of all boxes				
						5	52.	16
							8	
				EBQI and CI		3,1		4,0



9.4 Annex 3 – Standarized records Atzikiari bay

Table 9.3 Ichthyofauna standardized records (n/m^2) in Atzikiari bay

Sample	15M-TR1	15M-TR2	15M-TR3	15M-TR4	15M-TR5	15M-TR6	12M-TR1	12M-TR2	12M-TR3	12M-TR4	12M-TR5	12M-TR6	05M-TR1	05M-TR2	05M-TR3	05M-TR4	05M-TR5	05M-TR6	05M-TR7
Species																			
<i>Boops boops</i>	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bothus podas</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
<i>Chromis chromis</i>	75	26	30	12	6	40	0	0	0	0	15	0	5	0	0	0	0	0	1
<i>Coris julis</i>	2	0	0	0	1	3	2	2	1	0	0	0	0	0	0	0	0	0	0
<i>Dasyatis pastinaca</i>	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
<i>Diplodus annularis</i>	1	7	3	4	1	0	2	1	1	0	1	0	1	0	0	0	0	0	0
<i>Epinephelus costae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
<i>Gobius</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
<i>Hippocampus guttulatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mullus surmuletus</i>	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oblada melanura</i>	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
<i>Parupeneus forsskali</i> †	0	0	4	0	0	0	6	8	5	0	0	4	4	10	3	15	0	0	0
<i>Serranus scriba</i>	1	0	2	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Siganus luridus</i> †	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
<i>Siganus rivulatus</i> †	0	4	0	0	1	0	0	0	0	1	0	2	2	14	3	4	0	0	0
<i>Sparisoma cretense</i>	0	1	0	0	1	1	0	0	0	0	0	0	0	11	2	20	3	0	0
<i>Spicara smaris</i>	30	0	0	0	0	0	0	100	0	0	30	0	0	0	0	0	0	0	0
<i>Spondiosoma cantharus</i>	0	0	0	0	0	0	0	7	5	0	0	0	0	0	0	0	0	0	0
<i>Stethophis diasprios</i> †	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
<i>Symphodus mediterraneus</i>	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Thalassoma pavo</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1
<i>Torquigaster flavimaculosus</i> †	3	0	1	1	0	3	0	0	0	4	0	0	0	0	0	2	0	3	1
<i>Trochitus ocellatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Xyrichtys novacula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	0

Table 9.4 Macroalgae and invertebrates standardized records (n/m^2) in Atzikiari bay

Sample	12M-Q1	12M-Q2	12M-Q3	12M-Q4	12M-Q5	12M-Q6	12M-Q7	12M-Q1	12M-Q2	12M-Q3	12M-Q4	12M-Q5	12M-Q6	12M-Q7	05M-TR1	05M-TR2	05M-TR3	05M-TR4	05M-TR5	05M-TR6	05M-TR7	05M-Q1	05M-Q2	05M-Q3
Species																								
<i>Aplysina aerophoba</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Arborea thule</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0
<i>Astropecten platyacanthus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Balanus balanoides</i>	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	10	10	10	0	0	0	0
<i>Balanus rugosa</i>	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cerastium mediterraneum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chondria nucula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Codium bursa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conomuricea constricta</i> †	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hermotice constricta</i>	2	0	0	1	0	0	0	0	6	0	0	0	6	0	0	0	0	0	0	0	6	0	6	6
<i>Hexaplex trunculus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Halosira polii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Liquidula enasperatus</i>	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mafia squamula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
<i>Muticaria</i> sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Octopus vulgaris</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Orange unidentified sponge</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paguridea</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	56	6	25
<i>Penicillaria oblongulata</i>	83	76	87	76	78	70	56	113	125	38	75	106	163	88	0	0	0	0	0	0	0	0	0	0
<i>Physomella squamata</i>	25	19	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Procladius rufatus</i> †	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0
<i>Red unidentified sponge</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Reptadeonella violacea</i>	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sabella</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
<i>Schizoporella errata</i>	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Septa officinalis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenagella varda</i>	13	0	1	2	4	1	1	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
<i>Spongia</i> sp.	2	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trochalia pulvis</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Valoniopsis ventricosa</i>	4	0	0	0	0	1	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0



9.5 Annex 4 – Counts and relative abundance in Atzikiari bay

Table 9.5 Species of ichthyofauna recorded their counts and relative abundance over the high seagrass cover meadow (ST5) at 15m depth. The symbol † indicates non-indigenous species.

Species	N	Relative abundance %
<i>Boops boops</i>	50	15.8
<i>Bothus podas</i>	0	0
<i>Chromis chromis</i>	189	59.8
<i>Coris julis</i>	6	1.9
<i>Dasyatis pastinaca</i>	0	0
<i>Diplodus annularis</i>	16	5.1
<i>Epinephelus costae</i>	0	0
<i>Mullus surmuletus</i>	0	0
<i>Oblada melanura</i>	0	0
<i>Parupeneus forsskali</i> †	4	1.3
<i>Serranus scriba</i>	4	1.3
<i>Siganus luridus</i> †	0	0
<i>Siganus rivulatus</i> †	5	1.6
<i>Sparisoma cretense</i>	3	0.9
<i>Spicara smaris</i>	30	9.5
<i>Spondyllosoma cantharus</i>	0	0
<i>Stephanolepis diaspros</i> †	0	0
<i>Symphodus mediterraneus</i>	0	0
<i>Thalassoma pavo</i>	1	0.3
<i>Torquigener flavimaculosus</i> †	8	2.5
<i>Xyrichtys novacula</i>	0	0
Total	316	



Table 9.6 Species of ichthyofauna recorded, their counts and relative abundance over the low seagrass cover meadow (ST4) at 12m depth. The symbol † indicates non-indigenous species.

Species	N	Relative abundance %
<i>Boops boops</i>	0	0
<i>Bothus podas</i>	0	0
<i>Chromis chromis</i>	15	7.6
<i>Coris julis</i>	5	2.5
<i>Dasyatis pastinaca</i>	0	0
<i>Diplodus annularis</i>	5	2.5
<i>Epinephelus costae</i>	0	0
<i>Mullus surmuletus</i>	2	1.0
<i>Oblada melanura</i>	0	0
<i>Parupeneus forsskali</i> †	15	7.6
<i>Serranus scriba</i>	2	1.0
<i>Siganus luridus</i> †	2	1.0
<i>Siganus rivulatus</i> †	3	1.5
<i>Sparisoma cretense</i>	0	0
<i>Spicara smaris</i>	130	65.7
<i>Spondyllosoma cantharus</i>	12	6.1
<i>Stephanolepis diaspros</i> †	0	0
<i>Symphodus mediterraneus</i>	2	1.0
<i>Thalassoma pavo</i>	0	0
<i>Torquigener flavimaculosus</i> †	4	2.0
<i>Xyrichtys novacula</i>	0	0
Total	198	



Table 9.7 Species of ichthyofauna recorded, their counts and relative abundance over the degraded area (ST2) at 5 m depth. The symbol † indicates non-indigenous species.

Species	N	Relative abundance %
<i>Boops boops</i>	0	0.0
<i>Bothus podas</i>	2	1.6
<i>Chromis chromis</i>	6	4.9
<i>Coris julis</i>	0	0.0
<i>Dasyatis pastinaca</i>	2	1.6
<i>Diplodus annularis</i>	1	0.8
<i>Epinephelus costae</i>	3	2.5
<i>Mullus surmuletus</i>	0	0.0
<i>Oblada melanura</i>	4	3.3
<i>Parupeneus forsskali</i> †	32	26.2
<i>Serranus scriba</i>	0	0.0
<i>Siganus luridus</i> †	0	0.0
<i>Siganus rivulatus</i> †	23	18.9
<i>Sparisoma cretense</i>	36	29.5
<i>Spicara smaris</i>	0	0.0
<i>Spondyllosoma cantharus</i>	0	0.0
<i>Stephanolepis diaspros</i> †	1	0.8
<i>Symphodus mediterraneus</i>	0	0.0
<i>Thalassoma pavo</i>	2	1.6
<i>Torquigener flavimaculosus</i> †	6	4.9
<i>Xyrichtys novacula</i>	4	3.3
Total	122	

Table 9.8 Taxa recorded in the high cover meadow (ST5) at 12 m depth, and their abundances standardized as counts per square meter. The symbol † indicates non-indigenous species.

Taxon	n/m²
<u>Algae</u>	
<i>Peyssonnelia squamaria</i>	69
<i>Valonia ventricosa</i>	5
<i>Codium bursa</i>	0
<u>Porifera</u>	
<i>Sycon</i> sp.	10
<i>Chondrilla nucula</i>	0
<i>Aplysina aerophoba</i>	0
<u>Cnidaria</u>	
<i>Cerianthus membranaceus</i>	0
<u>Annelida</u>	
<i>Hermodice carunculata</i>	3
<i>Sabella</i> sp.	0
<u>Mollusca</u>	
<i>Bittium latreillii</i>	3
<i>Bolma rugosa</i>	0
<i>Conomurex persicus</i> †	0
<i>Hexaplex trunculus</i>	0
<i>Jujubinus exasperatus</i>	6
<i>Octopus vulgaris</i>	0
<i>Pinctada radiata</i> †	0
<i>Sepia officinalis</i>	0
<i>Smaragdia viridis</i>	20
<i>Tricolia pullus</i>	1
<u>Arthropoda</u>	
<i>Maja squinado</i>	0
Paguroidea	0
<u>Bryozoa</u>	
<i>Pentapora ottomulleriana</i>	535
<i>Schizoporella errata</i>	2
<u>Echinodermata</u>	
<i>Arbacia lixula</i>	0
<i>Astropecten platyacanthus</i>	0
<i>Holothuria poli</i>	0

Table 9.9 Taxa recorded in the low cover meadow (ST4) at 12 m depth, and their abundances standardized as counts per square meter. The symbol † indicates non-indigenous species.



Taxon	n/m ²
<u>Algae</u>	
<i>Peyssonnelia squamaria</i>	0
<i>Valonia ventricosa</i>	6
<i>Codium bursa</i>	0
<u>Porifera</u>	
<i>Sycon</i> sp.	0
<i>Chondrilla nucula</i>	0
<i>Aplysina aerophoba</i>	0
<u>Cnidaria</u>	
<i>Cerianthus membranaceus</i>	0
<u>Annelida</u>	
<i>Hermodice carunculata</i>	13
<i>Sabella</i> sp.	6
<u>Mollusca</u>	
<i>Bittium latreillii</i>	0
<i>Bolma rugosa</i>	6
<i>Conomurex persicus</i> †	0
<i>Hexaplex trunculus</i>	0
<i>Jujubinus exasperatus</i>	0
<i>Octopus vulgaris</i>	0
<i>Pinctada radiata</i> †	6
<i>Sepia officinalis</i>	0
<i>Smaragdia viridis</i>	6
<i>Tricolia pullus</i>	0
<u>Arthropoda</u>	
<i>Maja squinado</i>	0
Paguroidea	0
<u>Bryozoa</u>	
<i>Pentapora ottomulleriana</i>	706
<i>Schizoporella errata</i>	0
<u>Echinodermata</u>	
<i>Arbacia lixula</i>	6
<i>Astropecten platyacanthus</i>	0
<i>Holothuria poli</i>	0



Table 9.10 Species of macroalgae and invertebrates recorded with their abundances standardized as counts per square meter over the dead matte (ST2) at 5 m depth. The symbol † indicates non-indigenous species.

Taxon	n/m ²
<u>Algae</u>	
<i>Peyssonnelia squamaria</i>	0
<i>Valonia ventricosa</i>	0
<i>Codium bursa</i>	0.18
<u>Porifera</u>	
<i>Sycon</i> sp.	0.02
<i>Chondrilla nucula</i>	0.01
<i>Aplysina aerophoba</i>	0.01
<u>Cnidaria</u>	
<i>Cerianthus membranaceus</i>	0.01
<u>Annelida</u>	
<i>Hermodice carunculata</i>	0.05
<i>Sabella</i> sp.	0
<u>Mollusca</u>	
<i>Bittium latreillii</i>	33
<i>Bolma rugosa</i>	0
<i>Conomurex persicus</i> †	0.01
<i>Hexaplex trunculus</i>	0.04
<i>Jujubinus exasperatus</i>	0
<i>Octopus vulgaris</i>	0.01
<i>Pinctada radiata</i> †	0.01
<i>Sepia officinalis</i>	0.01
<i>Smaragdia viridis</i>	0
<i>Tricolia pullus</i>	0
<u>Arthropoda</u>	
<i>Maja squinado</i>	0.01
Paguroidea	0.34
<u>Bryozoa</u>	
<i>Pentapora ottomulleriana</i>	0
<i>Schizoporella errata</i>	0
<u>Echinodermata</u>	
<i>Arbacia lixula</i>	0
<i>Astropecten platyacanthus</i>	0.01
<i>Holothuria poli</i>	0.01

Table 9.11 Families of Annelids and Molluscs found on the canopy (ST5) with their average number standardized to 1 m² area and their abundances.

Taxon	Average n/m²	Abundance % (Within group)
<u>Annelida</u>		
Amphinomidae	57	0.5
Aphroditidae	28	0.3
Capitellidae	455	4.1
Chaetopteridae	38	0.3
Cirratulidae	549	5.0
Dorvilleidae	152	1.4
Eunicidae	170	1.6
Fabriciidae	2121	19.3
Glyceridae	57	0.5
Hesionidae	246	2.2
Maldanidae	19	0.2
Nereididae	492	4.5
Opheliidae	38	0.3
Phyllodocidae	170	1.6
Poecilochaetidae	19	0.2
Sabellidae	1212	11.0
Serpulidae	1136	10.3
Spionidae	199	1.8
Syllidae	3807	34.7
Terebellidae	19	0.2
<u>Mollusca</u>		
Arcidae	152	4.3
Caecidae	133	3.8
Cerithiidae	909	26.1
Eulimidae	19	0.5
Margaritidae	57	1.6
Mytilidae	1534	44.0
Neritidae	625	17.9
Rissoidae	57	1.6



Table 9.12 Families of Cnidarians and Bryozoans found on the canopy (ST5) with their average occurrences and average abundances (within their group).

Family	Phylum	Av. Occurrences	Av. Abundance %
Plumulariidae	Cnidaria	24	54
Sertulariidae	Cnidaria	21	46
Aeteidae	Bryozoa	7	6
Alcyonidiidae	Bryozoa	41	31
Crisiidae	Bryozoa	37	28
Electridae	Bryozoa	23	17
Lichenoporidae	Bryozoa	24	16
Mimosellidae	Bryozoa	4	4
Schizoporellidae	Bryozoa	1	0

Table 9.13 Average counts of Annelids in living *P. oceanica* matte and dead matte.

Annelida	Average living matte (n/m ²)	Average dead matte (n/m ²)
Amphinomidae	0.3	1.3
Aphroditidae	0.0	1.0
Capitellidae	5.7	22.7
Chaetopteridae	0.0	0.7
Cirratulidae	0.3	9.3
Dorvilleidae	0.3	9.3
Eunicidae	0.3	34.7
Fabriciidae	0.7	59.0
Glyceridae	1.7	0.3
Hesionidae	0.3	4.3
Lumbrineridae	0.0	10.3
Maldanidae	1.0	2.7
Nephtyidae	0.0	1.7
Nereididae	0.7	38.0
Oeonidae	0.0	0.7
Opheliidae	0.7	1.3
Orbiniidae	0.0	0.3
Paraonidae	0.3	2.0
Phyllodocidae	0.0	2.0
Sabellidae	4.3	41.3
Spionidae	1.7	8.7
Syllidae	10.3	69.7
Terebellidae	0.0	1.7
Family richness	15	23

Table 9.14 Average counts of Molluscs in living *P. oceanica* matte and dead matte.

Mollusca	Average living matte (n/m^2)	Average dead matte (n/m^2)
Arcidae	0.7	0.3
Caecidae	3.0	0.3
Cerithiidae	38.0	262.3
Cerithiopsidae	0.7	0.0
Costellariidae	0.3	0.0
Eulimidae	0.3	0.0
Lucinidae	0.0	1.7
Mytilidae	4.0	0.0
Neritidae	0.3	0.0
Rissoidae	0.3	0.0
Rissoinidae	0.3	0.3
Trochidae	0.3	0.3
Family richness	11	6



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