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CoAStal and marine waters integrated monitoring systems for ecosystems proteCtion AnD managemEnt

CASCADE

Project ID: 10255941

Priority Axis: Environment and cultural heritage

Specific objective: Improve the environmental quality conditions of the sea and coastal area by use of sustainable and innovative technologies and approaches

D5.1.1

Report and datasets on assessment of hazards, impacts and vulnerability of endangered ecosystems for each Pilot

PP in charge: LP – Regione Puglia

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Contents

1 Introduction

The main objective of ***WP5 – Pilots for endangered species restoration and Integrated coastal/marine management system*** was to demonstrate the usefulness of monitoring (observing and modelling) and spatial planning techniques by implementing coastal integrated management, restoration of marine ecosystems and support for endangered species and related ecosystems. D5.1.1 reports the outcomes of the ***Activity 5.1*** **"Assessment of hazards, impacts and vulnerability of endangered ecosystems"**, which focused on the use of data collected in WP3 and WP4 to identify weaknesses in the different pilot areas and then define the resulting soft restoration and management activities more appropriate for each Pilot area.

On regard of P1 Intertidal communities settled on rocky coasts, like many other coastal communities, have always been exposed to human pressures. These biotopes provide valuable ecosystems services in term of biodiversity, contribution to primary productivity, fisheries and tourism (Seitz et al., 2013; Mearns et al., 2014; Vinagre et al., 2015) and therefore benthic macroalgae, macroinvertebrates and fish fauna are biological elements encompassed in the environmental quality assessments.

The Gulf of Trieste can boast a long tradition on benthic ecology, but the knowledge on macrozoobenthic communities is still far from being satisfactory (Pitacco et al., 2013). In that regard, hard bottom macrozoobenthos has received much less attention than soft bottom communities with a lower number of published works most of them focuseing on the circalittoral rocky outcrops in the off areas (e.g. Bettoso et al., 2023). The research on the ecological quality status on rocky shores communities mainly concerned the macroalgal community (e.g. Orlando-Bonaca et al., 2008), as well as interesting researches were performed on the characterization of the littoral fish fauna communities (e.g. Lipej et al., 2003; Orlando-Bonaca & Lipej, 2005; Lipej & Orlando-Bonaca, 2006). The last research on the intertidal rocky shore macrozoobenthos in the Italian side of the Gulf of Trieste goes back to over 50 years ago, when a first description of key species and benthic zonation was drawn in some areas such in Miramare (today a protected marine reserve) (Specchi, 1966), in a small littoral cave (Orel & Specchi, 1967) and in the Bay of Muggia, near Trieste (Specchi & Orel, 1969). On the other hand, a more recent study was conducted along the Slovenian shore in order to characterize the rocky macrozoobenthos community along a gradient of hydromorphological modifications (Pitacco et al., 2013) and thanks to this an ecological index was performed and tested in order to assess the environmental status using the WFD Directive criteria (Orlando-Bonaca et al., 2012).

In this context the CASCADE project represented a great opportunity to improve knowledge about the distribution of macrozoobenthic communities on mediolittoral rocky substrates, which are the most directly affected by the rapid widespread of human-induced coastal modifications.

On regard of P2 UNIBO identified the impacts of anoxic effects, climatic and anthropogenic stressors and invasive species on the ecosystem.
Activities of UNIBO in P2 consisted in:

- sampling activities in Goro Lagoon aimed at measuring water column biotic parameters and identifying phytoplankton abundance and composition especially in the presence of relevant phenomena (algal blooms, alien species presence)
- sampling in the Lake of Nazioni with measurement of biotic and abiotic water column parameters (on a monthly base)
- laboratory tests to understand the effect of nutrient conditions on the proliferation of blooming species
- field tests in the Lake of Nazioni to verify the capacity of controlled macroalgae cultivation to improve water column quality
- pilot laboratory and field tests in the Piallassa coastal lagoons to identify the impacts of anoxic effects.

On regard of P3 activities implemented included chemical analysis, biodincators and monitoring with multiparametric probe. Chemical analysis on water and sediments focused on heavy metals, chlorinated pesticides and microplastics.
Amphipods belonging to the *Gammarus insensibilis* species were used as bioindicators: samples were collected in spring from populations located in small basins. Animals sampled in a pristine area (Le Cesine) and/or stabulated in laboratory fish tanks were also used as controls.
Multiparametric probe allowed to collect a dataset in the Torre Guaceto Marine Protected Area (MPA) regarding depth, temperature, conductivity (salinity, density), dissolved oxygen, pH, chlorophyll a, turbidity. The dataset allowed to have the vertical distribution of the measured variables in all the 20 stations.

On regard of P4 data from WP3 and WP4 have been used to:

- assess threats, impacts and vulnerability of endangered ecosystems in pilot area (P) 4 - Neretva River by describing existing water and sediment quality in the Neretva River and comparing it with relevant guidelines for protection of aquatic life and human and animal health
- propose measures to prevent further degradation of the studied ecosystems
- discuss, where possible, seasonal variations in water quality
- analyze and harmonize existing data sets in the pilot area of interest to evaluate current strategies and plan optimal monitoring and modeling strategies

On regard of P5 several reef formations are found sparse in the area within few miles seaward from the Italian coast of the Northern Adriatic Sea. Each reef differs in extension and benthic assemblages, depending on a number of key factors: depth, abiotic features (e.g. mineralogical characteristics of the substrata (ARPAV 2011), distance from coast, geographic position and other outcrops proximity (Ingrosso et al. 2018). The calcareous bioconstructions of the Northern Adriatic

form the habitat that supports the life of several flora and fauna species. Among the species identified, several are prioritized for conservation. The specific habitat found on top of the Northern Adriatic rocky outcrops differ in composition and overall structure from most coralligenous outcrops found in the Mediterranean basin (Ponti et al. 2011; Curiel et al. 2012; Falace et al. 2015), and based on EUNIS classification, as reported in Annex I of the Habitat Directive, they are classified as Reef habitat (habitat code 1170). Concerning the focus of this report, the reef formation included in the SCI of Tegnùe di Chioggia, is the biogenic reef with the largest extension of the entire Northern Adriatic marine area (Casellato and Stefanon 2008). Calcareous bioconstructions of the Tegnùe di Chioggia site are composed mainly by calcareous algae, together with molluscs, Bryozoas, polychaetes and madrepores. These sessile organisms form an unique coralligenous-like habitat which provides trophic resources, reproduction niches and shelter for several benthic, demersal and pelagic invertebrate and vertebrate species through their different life stages. The great availability of nutrients in the water column typical of the northern Adriatic Sea, represents a main trophic source for several filter and suspension feeders' organisms living on the reef (Ingrosso et al. 2018).
Within the homogeneous sea bottom of the Northern Adriatic Sea, the Tegnùe di Chioggia ecosystem function as collectors of organic matter, which is then made available through the trophic chain to macrophage organisms that feed directly on the bioconstructor organisms of the reef as well as detrivorous species (Casellato et al. 2005).
In this context the CASCADE project represented a great opportunity to improve knowledge about benthic communities and fish assemblages on these hard substrates, which are the most directly affected by the cumulative effect of multiple human-induced pressures present in the area, and of climate changes. Temporal changes in ecosystem functioning indicators, estimated through food web modelling, were also investigated.

On regard of P6 the area of the city of Nin is located in the northwestern part of Zadar County. The city is surrounded on the eastern side by salt pans, on the northern side by sandy beaches, and on the western side by forests and natural areas. The Nin coast is characterized by shoals and a sandy coast, canals, lagoons and cliffs, and the fertile land of Ravni Kotar.
Due to the shallow water and high concentration of salt, the coastal zone and lagoons are a habitat for many endemic and rare plants, birds and various animals. Further offshore, the landscape changes from muddy and sandy shores to rocky grasslands (Breiner, 2017). The characteristic elements of this area: shallow water, low, sandy and rocky shores, grasslands and a high concentration of salt result in a unique environment, a habitat for several rare and endangered species. Therefore, the area of Nin and its surroundings is a unique landscape of rare plant and animal species with 8 NATURA 2000 habitats, 5 endemic, 4 critically endangered, 1 endangered and 5 sensitive plants. Specific rare habitats are formed in the Nin Lagoon: low muddy and sandy shores with wetlands with distinctive flora and fauna and sands with rare plants. The important ecological value of Nin's habitats has long been recognized, and plant communities associated with sandy and muddy shores are strictly protected and included as important habitats in the natural ecological network. Nature conservation is carried out according to the criteria of

8

the world's largest coordinated network of protected areas NATURA 2000 (https://www.nin.hr/hr/natural-heritage/natura-habitats).
A significant factor affecting these muddy and sandy coastal habitats and one of the main challenges in managing this area are the processes of coastal erosion, where wind and waves carry sandy material from one part of the coast to another or into the sea. The changes in the shape of the sea shores are especially visible on Ždrijac beach and Kraljičina beach. According to some previous researches, it is estimated that the coast line, due to erosive processes, has in some places moved up to 10-15 m during the last 10-15 years. As a result of these processes, eroded beaches are rehabilitated by filling in sand extracted from the sea in the nearby area, and also by deepening the waterway (Natura Jadera, 2021). Furthermore, the Nin area could be threatened by the potential consequences of climate change and sea level rise, given the aforementioned problems of coastal erosion (Barić et al., 2008).
As part of this task, an assessment of the danger, impact and vulnerability of endangered ecosystems and marine habitats, as well as the hitherto observed and most hidden species of flora and fauna in the area of the mouth of the Miljašić ravine, was made. Based on the data obtained in the implementation of task 4.1. and 4.2. and data collected from previous research during the implementation of the CASCADE project, primarily using the BRUV method and visual census, an assessment of the impact on regularly present species in this micro-site was presented, with special reference to sensitive and rare species observed in recent years. Namely, previous research carried out in this relatively small area over the past 15 or so years indicates that it is a location with extremely high biodiversity, especially represented by molluscs from the class of bivalves and gastropods (with species found that are extremely rare or have not yet been observed on other areas). Therefore, it is extremely important to assess which species from the ones observed so far, including threatened habitats such as seagrasses, will be affected by the newly planned interventions in this area. The research conducted in 2022, as well as those from before, indicate a good adaptation of the species present at the mouth of the Miljašić ravine and the existing embankment, considering the previous anthropogenic impacts caused during the construction of the embankment and concreting of the coast, as well as during previous intense flooding events (especially in 2017 year), which represents the basis for assessing the impact of the future planned intervention.

On regard of P7 UNIMOL conducted the assessment of the impacts of stressors on halophile meadows mosaic in P7 and developed bio-indicators (use of plant guilds as indicators of change, stress and ecosystem functioning).
In particular, after a brief exploration of threats and DIPSIR analysis for halophile meadows mosaic transition ecosystems the activity focused on the implementation of restoration actions on brackish formations. Data collected on areas in which restoration actions were implemented were analyzed and discussed ex post focusing on plant species as bioindicators of salt meadows mosaic integrity.
Coastal wetlands are complex and dynamic ecosystems widely distributed on the world’s shorelines. Occupying transitional waters between freshwater and marine realms they conform

intricate mosaics following steep environmental gradients (e.g. oxygen, pH, salinity) which encompass a particularly specialized flora and fauna. Wetland mosaics are shaped by seasonally changing abiotic (e.g. salinity, soil aeration, frequency and duration of inundations and elevation of the marsh surface and biotic factors (e.g. interspecific competition for light and nutrients).

In coastal salt marshes plant species are mainly stress tolerant and specialist, well adapted to highly variable and dynamic ecological conditions. Specifically, the Mediterranean coastal salt marshes vegetation consists of a mosaic of low-growing meadows with herbaceous plants able to dwell on wet and hydromorphic soils periodically flooded. Such meadows are composed by grasses, sedges, rushes and other herbaceous angiosperms distributed across an observable zonation, according to topographic and environmental variability as well as vegetation succession linked to the geo morphogenesis of salt marshes.

Coastal wetlands also play a key supporting role for animal biodiversity as they provide critical habitats for resident (e.g. arthropods) and migratory fauna (e.g. birds). Indeed, they ensure different stages of the life cycle to a great variety of species offering a suitable habitat for fish and invertebrate spawning as well as for the larval and juvenile stages. Many migratory birds use marshes as feeding.

Coastal wetlands are among the most imperiled ecosystems both, globally and in the Mediterranean basin. Approximately 50% of the world’s wetlands have been lost since 1900. In the Mediterranean, brackish marshes have undergone a drastic reduction due to land reclamation and conversion to croplands, changes in water regimes, urbanization and invasive alien species combined with climate change and coastal erosion which have caused a drastic reduction of ecosystem services and a loss of biodiversity. Specifically, the alterations on marshes hydrology (depth and hydroperiod) along with the increasing temperatures and the reduction of water supply registered during the last decades consistently threat more than 35% of wetland species.

For such outstanding threatened biodiversity, wetlands are protected by the intergovernmental Convention of Ramsar which provides the regulatory framework for defining national and international conservation sites (so called Ramsar sites; https://www.ramsar.org/) and dedicated actions for their conservation and management. Furthermore, in Europe, most of the salt marsh plant communities have been of conservation concern and listed in the Habitats Directive (HD: European Directive 92/43/EEC; European Commission (2013) for which conservation and restoration actions are claimed. According to HD, member states are committed to monitoring and preserving habitats extension into the Union and implementing the necessary management measures to keep them in a good “conservation status”.

Amongst the possible conservation measures, the restoration of salt marshes, aimed at bringing back the brackish mosaic to its original condition faster than nature does on its own and at establishing a self-sustaining ecosystem status, has rapidly accelerated over the last decades with the great support of government agencies and conservation organizations (Adams et al. 2021). There is evidence that salt marshes vegetation recovery time under natural conditions is quite fast (e.g. around 10 or more years, depending on the perturbation and the maturity of the marsh) (Broome et al. 1988), so after the necessary hydraulic reconstruction works, soft restoration schemes promoting spontaneous recovery of natural key species are advisable (Wolters et al.

2005, 2008). The assessment of the effectiveness of saltmarsh restoration actions in terms of plant species composition in some European wetlands (Wolters et al. 2005; Billah et al. 2022) have evidenced a good recovery of native plant diversity over time (Curado et al. 2014). Despite the importance of the restoration of salt marshes and its widely implementation in several coasts in the world (Billah et al. 2022), updated research and systematic monitoring activities aiming to assess biodiversity changes after restoration actions on Mediterranean salt marsh areas should be improved (Moreno-Mateos et al. 2015; Billah et al. 2022).

In this context, have been analyzed vegetation dynamics on a restored salt marsh mosaic, through a multi-temporal analysis of vegetation plots collected before and after the implementation of restoration actions in the Central Adriatic coast in Italy. Has been hypothesized a good response of vegetation that after restoration will evolve towards improved of ecosystems, with a gain of diagnostic native species and a reduction of alien and ruderal ones. Specifically, by a re-visitation study (data collection carried out in the years 2010 and 2021) have been explored plant species composition and ecology changes across the brackish habitats addressing the following questions: (i) Have the abundance and distribution of vascular plant species changed during the last decade?; (ii) which are the abundance trends in the main plant groups (diagnostic, ruderal and alien species) and in halophilous and thermophilous species over time in the brackish mosaic habitats?

Increasing the knowledge on vegetation dynamics and how it varies across the different habitats of the brackish mosaic after a restoration actions, contributed to improve the current scientific understanding on the effectiveness of implemented conservation strategies (Wolters et al. 2005; Billah et al. 2022) and gave new insights for the adaptive management and the prioritization of the conservation actions in such highly vulnerable environment.

<u>On regard of P8</u>, the area is characterized by shallow waters and steep ecological gradients influenced by freshwater inputs and the Adriatic Sea circulation. The study involved monthly sampling at two stations using CTD probes and Niskin bottles to collect oceanographic parameters, chlorophyll-a, and nutrients. Real-time data collection was supported by oceanographic buoys. Metabarcoding analysis was also performed to assess phytoplankton taxonomic richness and genetic diversity.

The results showed that the Northern-Eastern Adriatic Sea is a highly productive and dynamic marine ecosystem with diverse phytoplankton groups. The study highlighted the impacts of global warming and oligotrophication on phytoplankton composition and emphasized the importance of understanding their response to climate change. The total abundance of phytoplankton has decreased since 2003, but the abundance of diatoms remained unchanged or increased. The presence of new species has also been observed, with connections found between the origin of newly arrived species and the direction of currents entering the Adriatic Sea.

<u>On regard of P9</u> the objective of this deliverable for Sea and Karst, as managing authority for the Natura 2000 site Cetina estuary, was to define future actions and conservation measures addressing risks/factors impacting ecosystem biodiversity, especially habitat types and species protected by BD Directives. In this phase of assessing the state of the Cetina estuary as a complex

habitat, the focus was on one of its most representative, key habitat type: 1110 Sandbanks which are slightly covered by the sea water all the time, and key benthic habitat formers, i.e. little Neptune grass *Cymodocea nodosa*, Noble Pen Shell *Pinna nobilis* and cushion coral *Cladocora caespitosa*, which are strictly protected species according to Croatian Nature Protection Act.

On regard of P10 UNIMOL described the assessment of the impacts of stressors on dune-marine habitats transition in P10 and developed bioindicators (e.g. use plant guilds as indicators of change, stress and ecosystem functioning). In particular, after a brief exploration of threats and DIPSIR analysis for coastal dunes and psammopile transition ecosystems, the activity focused on the effects of trampling on dune integrity and on the implementation of boardwalks as conservation and management actions. Data collection and monitoring, as well as models were carried out on WP3 and WP4. Were analyzed and discussed ex post data collected on areas in which boardwalks were implemented focusing on plant species as bioindicators of dune integrity.
Coastal dune systems are characterized by strong environmental gradients which determine the coexistence of different plant communities in a relatively small area. One of the most outstanding features of coastal dunes is a high ecological diversity in terms of environmental heterogeneity and variability of species composition (Martinez et al. 2004). The unique dune plant diversity underpins essential benefits to society (MA, 2005). Coastal dunes are dynamic systems which supply ecosystem services (ES) that can have a clearly recognized market value, such as groundwater stored in dunes, or provide ES such as water purification and coastal defense, which have value in the form of replacement costs. They also provide less tangible cultural services such as opportunities for recreation and tourism. Some dune sites are highly visited for tourism, but dunes are also seen as wild spots and are valued as a place of escape and isolation and as a source of mental well-being. Another ES are the capacity to protect from wind and aerosols, and to regulate climate at local and global scales.
Coastal dunes also provide unique habitat assemblages due to a strong environmental sea-inland gradient, which supports a highly specialized flora and fauna sharing relatively few species with other terrestrial ecosystems (Acosta et al., 2009).
Despite their high biodiversity value and numerous benefits, coastal sand dunes are among the most threatened habitats both globally (Schlacher et al., 2007) and in the Mediterranean (Malavasi et al., 2016). Human activities in European littoral areas have been intensifying in the course of the 20th century; consequently, sand dunes across Europe had lost on average 25% of their extent by 1998, compared to 1900 (EUCC, 1998), with peaks of 80% area loss in some Mediterranean countries. In Italy, 86.7% of EU coastal habitats currently have an unsatisfactory (bad or inadequate) conservation status, having suffered a drastic reduction in both extent and ecological quality, mainly due to urban expansion (Genovesi et al., 2014; see Pilot10 - Figure 19. DIPSIR Model for Pilot 10).
In order to preserve the last intact coastal landscapes from human-driven threats, they were included by the Italian government in some protected areas (as is the case of Torre del Cerrano Marine Protected area, Pilot 10) and in ecological network of sites of European importance, called Natura 2000, as determined by the Council Directive 92/43/EEC (EEC, 1992; Habitats Directive).

During the last century, anthropogenic impact on coastal ecosystems has dramatically increased, especially in the Mediterranean area (Defeo et al. 2009). Unregulated urbanization, intensive farming and the increase of the road infrastructures coupled with massive beach tourism have led to pollution, biological invasions, over-exploitation of the natural resources and to a decline in quantity and quality of coastal habitats (Malavasi et al. 2014, Figure 19. DIPSIR Model for Pilot area 10). Tourists commonly see the beaches exclusively as vacation and leisure sports; thus, sand beaches and dunes are often cleaned mechanically to make room for sun beds, umbrellas or beach volleyball courts. However, a growing environmental awareness has highlighted the need to stop the loss of these habitats and, possibly, to reverse the negative trend ongoing during the last decades (Brown and McLachlan 2002). There is an increasing consensus that coastal dunes are not just a place for recreational activities and that it is necessary to integrate spatial planning, stakeholders' interests and the conservation of ecosystems processes for human well-being through a multidisciplinary approach (Drius et al 2019).

Conflicts frequently arise between the socio-economic interests in the beaches' exploitation for tourism and the conservation measures to protect these vulnerable ecosystems. Human trampling associated with recreational activities is the most common disturbance on sand dune habitats, especially during the summer period. Trampling often means an extremely negative effect, causing substrate erosion, decreasing of plant community's diversity and wildlife dismissal from no longer suitable habitats. To face trampling effects, regulatory and management tools are undertaken to balance conservation with tourism needs.

Several studies have focused on the negative impact of activities related to beach tourism on coastal dunes vegetation, coupled with the implementation of management plans and actions. Dune restoration usually foresee strategies to limit coastal erosion and trampling through fencing, building elevated boardwalks, vehicular access restrictions, revegetation of degraded areas and environmental education programs (Šilc et al. 2017). Previous studies found indicators of rehabilitation of coastal habitats, in terms of dune vegetation's density increase, coastal erosion reduction and revegetation with native plants leading to dune accretion (Johnston and Ellison 2014). Access control fencing, boardwalks and vegetation replanting are among the most effective methods largely used in many countries to promote successful dune restoration.

Itzkin et al. 2020) Although seriously threatened, a number of valuable stretches of sandy coast remain along the Italian shoreline.

Coastal dunes continuously change in response to sea level rise, natural erosion, climate change, fire, cattle and land transformations worldwide (Malavasi et al. 2016), however, the typical resilience of dune vegetation often offers a rapid short-term recovery as soon as the disturbance is interrupted or mitigated through simple management options, as the use of fence or boardwalks (Šilc et al. 2017).

In this deliverable for Pilot 10 have been assessed the changes in vegetation assembly after 2 years since the establishment of wooden boardwalks across different coastal dune habitats.

In particular:

– use of vascular plants as bioindicators and evaluation of changes of species richness and cover, floristic composition and ecological functional guilds among different dune habitats,

– exploration of which dune habitats showed steeper changes in vegetation assembly after the boardwalks set up,
– investigation of which species or group of species (species guilds as bio-indicators) are major responsible in vegetation assembly changes after the boardwalks set up
Have been measured changes in vegetation assembly after the establishment of wooden boardwalks (protecting dunes form human trampling) across different coastal dune habitats.
By increasing the knowledge on vegetation dynamics and how it varies across the different habitats on coastal dune zonation after a restoration actions, is possible to contribute to improve the current scientific understanding on the effectiveness of implemented conservation strategies and give new insights for the adaptive management and the prioritization of the conservation actions in such highly vulnerable environment.

On regard of P11, Marche Region implemented analysis of emerged and submerged biocenosis:

- monitoring of emerged biocenosis was carried out on transects of variable length between 100 and 200 meters;
- monitoring of submerged biocenosis was carried out through the use a ROV (remote Operated Vehicle) equipped with Full HD camera, lighting system and positioning system Built-in USBL applying the investigation protocol used for "Marine Strategy" projects.

The monitoring performed has shown a reduction in the quality of the coastal ecosystems, due to different reasons.
One of the main pressures affecting coastal ecosystems is coastal erosion consequent to climate change.
On the whole the activities allowed to take in to account the identified vulnerability in the management of coastal ecosystems.

14

2 Description of the pilot area and DPSIR model

The eleven pilot areas have been plotted into the cartography represented in Figure 1 and associated to the Project's Partner. A detailed description is reported in the following chapters.

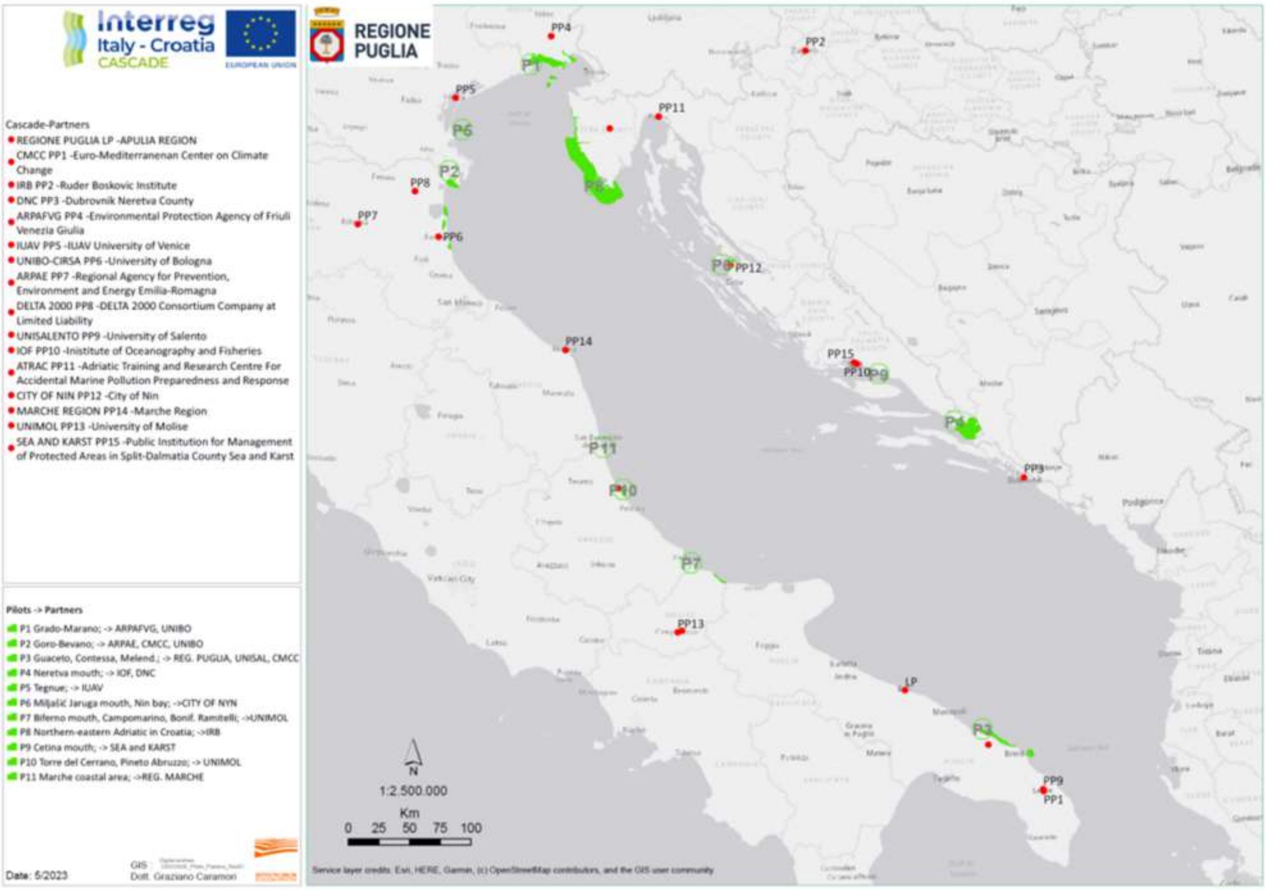


Figure 1: Distribution of the eleven CASCADE pilot areas.

P1. Grado and Marano Lagoon and Gulf of Trieste (IT)

The Gulf of Trieste is a shallow semi-enclosed gulf that represents the northernmost part of both Adriatic and Mediterranean Sea. It is shared by three countries, extending from Croatia to Italy and includes the entire Slovenian coast. With a maximum depth of 33 m, is characterised by the lowest winter temperatures (below 10 ◦C) in the whole Mediterranean Sea, as well as the widest tidal amplitude. The Gulf is subject to different anthropogenic pressures, such as fishery, mariculture, tourism and sewage disposal, but the major anthropogenic disturbances are related to the intensive maritime transport.

DPSIR model for Pilot 1_2 (Gulf of Trieste)

Driving forces

Urbanization;
Tourism;
Navigation;
Intense harbour activity;
Fishery and aquaculture;
Agricolture;
Industry

Pressures

Discharge of nutrients and contaminants;
Anthropic noise;
Fish cartches and fishing pressure

State

Alteration of geomorphology and sea bottoms;
Presence of alien species;
Presence of litter, microplastic 0,3-5 mm;
Ecological status: generally good;
Chemical status: presence of some contaminants detected

Impact

Alteration of trophic state;
Alteration of biological communities;
Bioaccumulation/biomagnification of contaminants, ingestion of microplastic and contaminants by fish fauna and invertebrates;
Impact on food quality

Responses

Area 'Carso triestino e goriziano' included in ZPS, MCS;
MPA Miramare included in ZSC, N2K;
Macroalgae restauration programs;
Monitoring (WFD, MS) and management programs for fishery safeguards (EMFAF, CFP);
Research and awareness programs;

Sources:

- Piano regionale di tutela delle acque (marzo 2018) Regione FVG
- Rapporto sullo stato dell'ambiente in FVG (2018) ArpaFVG, SNPA
- Definizione delle linee guida di classificazione e delle norme tecniche di gestione (2006) Project AdriBlu

The DPSIR model performed for the pilot area of the Gulf of Trieste can also be applied on the rocky shore habitat. The main driving forces who can act upon mesolittoral communities are related to urbanization, tourism and navigation/harbor activity. The urbanization caused the modification of the hydromorphological characteristics of the shoreline, whereas the tourism relates to bathing season. Finally, the navigation and the harbor activity can generate oil spill, potentially impacting the rocky shore habitats. In this context the driving forces such as fishery, aquaculture and agriculture can be neglected.
The pressures on this environment can be mainly related to contaminants and organic enrichment polluting the seawater and thus selecting those species more opportunistic in such conditions; another aspect to take into consideration is the anthropic noise which can disturb the ethology of many species, in particular during the breeding season.
On regard the state, the biological quality elements *sensu* WFD Directive are generally classified as good, although the chemical status shows some contaminants exceeding the environmental quality standard. In addition, the presence of alien species who can alter the ecosystem stability has to be considered, as well as that of microplastic, capable to alter the physiological adaptation of the organisms. On regard the alteration of geomorphology and sea bottoms, it is important to consider the hydromorphological status of rocky shores in order to evaluate the degree of modification from pristine condition.
Therefore, the main impacts are those related to alteration of trophic state and biological communities when environmental stress is particularly pronounced. Bioaccumulation of contaminants and ingestion of microplastics can act at organisms and community level.
The responses are connected to monitoring program following WFD and Marine Strategy directives, whereas the macroalgae restoration programs are specifically oriented to rocky habitats on upper infralittoral belt. Finally, the protected areas such as MPA Miramare and “Carso triestino e goriziano” have to be considered as pristine areas from an hydromorphological point of view.

P2. Transitional (e.g. Goro area and Bevano Mouth) and coastal areas in Emilia Romagna (IT)

Sacca di Goro (P2)

The Sacca di Goro is a shallow-water lagoon of the southern Po River Delta with a surface area of 26 km^2, an average depth of 1.5 m, connected to the sea by a large sea mouth. The main freshwater inputs are the Po di Volano River, the Canal Bianco and Giralda, freshwater inlets are also located along the Po di Goro. The fresh water or hydraulic residence time oscillates monthly between 2.5 and 122 days, whereas the water exchange time ranges from 2 to 4 days. The bottom of the lagoon is flat and the sediment is alluvial mud with high clay and silt content in the northern and central zones. Sand is more abundant near the southern shoreline, whilst sandy mud occurs in the eastern area. The climate of the region is Mediterranean with some continental influence (wet Mediterranean). In terms of hydrodynamics, Sacca di Goro is strongly influenced by the semi-diurnal tides, tidal amplitude is ca 80 cm., following what is observed in the Northern Adriatic. Wind also plays a major role altering the lagoon's circulation pattern: while Sirocco (southerly winds) tends to fill the lagoon up, Bora conditions (NE winds) normally increase the discharge.

Sacca di Goro is a Natura 2000 site both SPA and SAC (code IT4060005) furthermore is inside the Parco del Delta del Po of Emilia-Romagna Region; Valle di Gorino, to the east of Sacca di Goro is a Ramsar site of about 1,500 hectare and the outer bank is Natural State Reserve.

The ecological peculiarities of this environment allow the establishment and presence of important plant and animal communities. The mixture of freshwater and seawater are somehow balanced and the Sacca di Goro is a typical brackish lagoon of the category transitional water. This feature allows an uncommon biodiversity richness in terms of habitats and species.

To analyze hazards, impacts and vulnerability a **DPSIR Model** has been adopted by EEA (European Environmental Agency). It consists of a causal framework for describing the interactions between society and the environment: driving forces, pressures, states, impacts, responses (extension of the PSR model developed by OECD). Establishing a DPSIR framework for a particular setting is a complex task as are all the various cause-effect relationships that have to be carefully described in a context of environmental and global changes that can rarely be attributed to a single cause.

The Sacca di Goro is an over exploited environment where everything revolves around an intensive aquaculture farming pressure that drives all the secondary activities (figure 2).

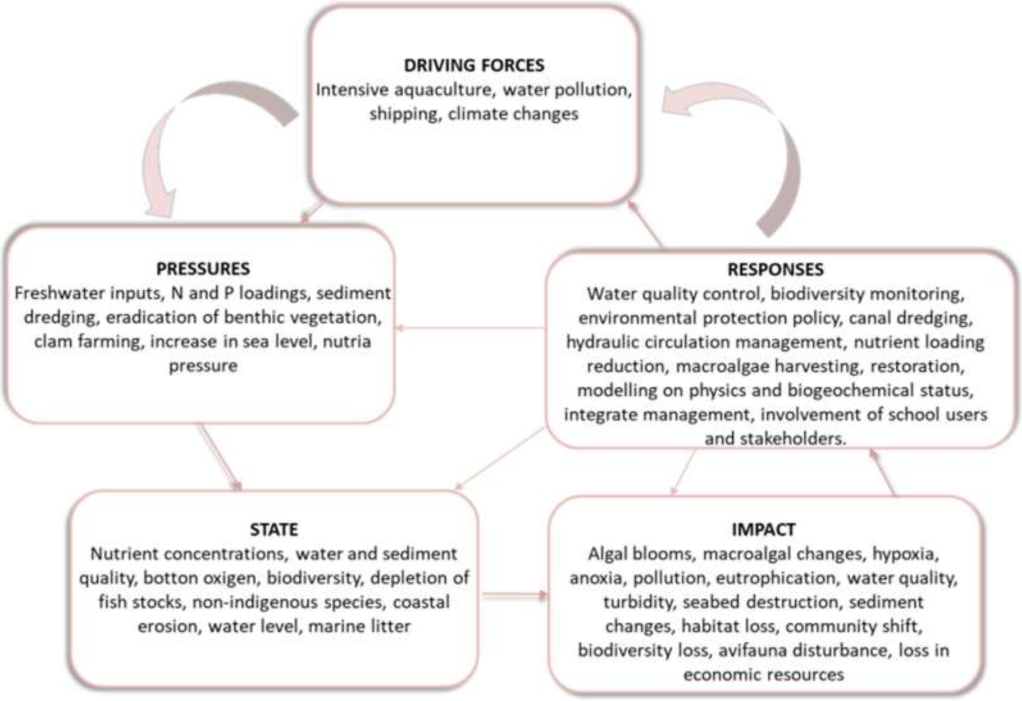


Figure 2. DPSIR model for the Sacca di Goro

Several pressures are related to aquaculture activities such as sediment dredging and the eradication of benthic vegetation. A great pressure derives from a constant river input that brings into the area a great volume of freshwater modifying and altering water column conditions often carrying a great load of nutrient inputs as nitrogen, urea, ammonium, phosphorous and others contributing to eutrophication processes. At present, the Sacca di Goro is featured by coastal erosion due to the increment of the water level, a decline of fish stocks and a continuous discharge of water pollutants derived from boats engaged on mussel farming. River runoff, mussel farming and climate changes constitute an important element of variability; given all, transitional water and coastal lagoons have a wide variety of hydrological, biological and sedimentological gradients that make those areas heavily affected by temporal variability and high spatial heterogeneity (Basset et al., 2006; Pérez-Ruzafa & Marcos, 2012; Newton et al., 2014).
These environments may create extreme conditions for many taxa, resulting in a biodiversity typified by a low number of highly specialized species (Crosetti & Massa, 2015); indeed, taxonomic diversity in transitional waters tends to be limited in comparison to fresh and sea water's diversity (Basset et al., 2013; Elliott & Quintino, 2007).
All those factors directly affect water quality, turbidity and contribute to biodiversity loss considering phytoplankton, benthos and avifauna and eventually impacting economic resources.
Some actions have been already taken to mitigate impacts; monitoring activities have been carried out yearly and monthly to establish water quality and assess biodiversity both in the water column

and in the sediment, the development of a physical and biogeochemical model to predict ecological and environmental conditions in the future is ongoing. Integrated and innovative monitoring techniques such as eDNA-metabarcoding has been applied to phytoplankton community to increase knowledge of microalgal community and dynamics in the Sacca.

Lago delle Nazioni (P2)

Lago delle Nazioni (FE, Emilia-Romagna), is a semi-artificial brackish lake located between Valle Nuova and the beaches of Volano and Lido delle Nazioni. Its area is about 100 hectares of which approximately 70 ha dedicated to wild breeding of bulls and Camargue-Delta horses. The valley originated from repeated inflow episodes of marine waters; its shape changed several times following the growth of the coast and was in direct contact with the sea until a few decades ago through “Bocca del Bianco”. Currently, the water exchange is ensured by a channel regulated by a siphon and a dewatering pump connected to the terminal section of the mouth of the Po di Volano. The external part of the lake is dominated by common reed, meanwhile the western site is surrounded by agricultural crops and mainly halophilic and halotolerant vegetation.

During summer, the lake is heavily influenced by human presence; Spiaggia Romea Village and Residence, situated in the northern part of the lake, has more than one direct access to the lake giving visitors both bathing and pedal boat renting possibilities. Moreover, the Southern area hosts the sailing and kayaking school Lega Navale Italiana; in the southern shore was also located an experimental breeding activity of clam carried out by the company Naturedulis s.r.l, whose headquarter is based in Goro, Ferrara.

DPSIR model for Lago delle Nazioni is reported in figure 3.

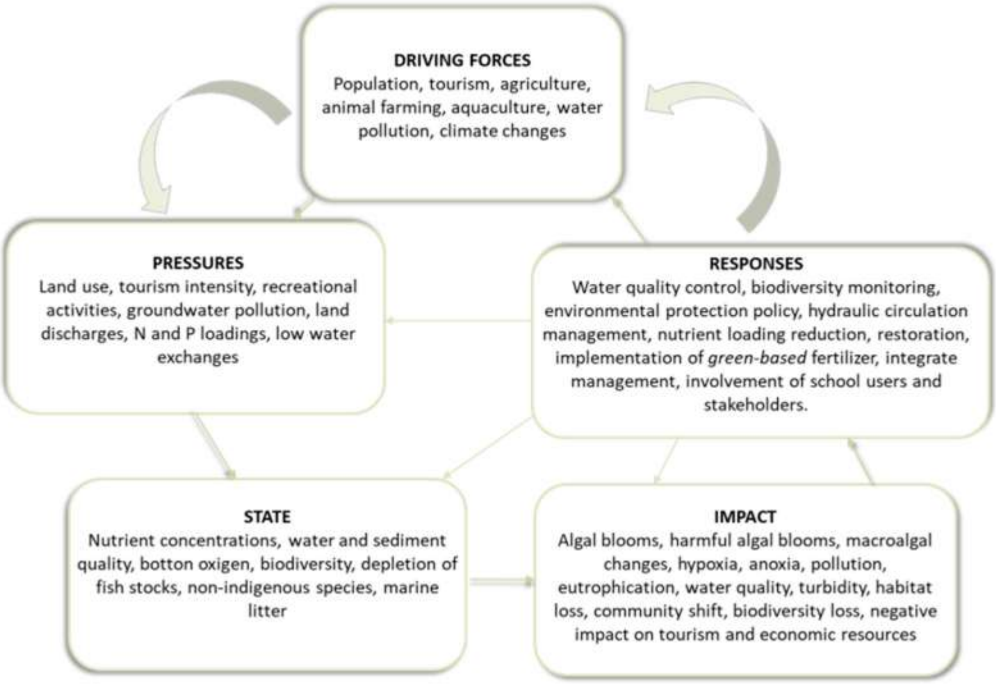


Figure 3. DPSIR model for Lago delle Nazioni

As indicated driving forces mainly consist of human related activities such as tourism and farming operation triggering groundwater pollution and nutrient loading processes which, in combination with low water exchange and climate changes, make the area extremely under pressure.
At the moment Lago delle Nazioni is characterized by a low water quality (ARPAE 2019), and a severe loss of biodiversity including phytoplankton, macroalgae and fish community. Main impacts on the area are algal blooms due to land discharges and tourism which enhance N and P loadings in the water. Algal blooms contribute to the formation of hypoxic or anoxic zones and alter turbidity of the water column preventing light to pass through. On several occasions algal blooms changed water color, an aspect that could lead to negative impact on tourism as well. Finally, in the last year a non-indigenous and invasive Ctenophora, ***Mnemiopsis leidyi,*** **has been detected in large numbers during water sampling.** As a response to such criticalities, water quality is under continuous control to assess biodiversity and nutrient composition. Some restoration activities have been tested using a macroalga *Gracilaria sp.* but no positive effects have been observed, specifically because the water column was not transparent and the algae could not grow. An integrated management could lead to a better evaluation of biodiversity and enhance knowledge of community; in fact, phytoplankton population has become increasingly smaller in dimension since water condition promote the expansion of nano- and pico-organisms which can be difficult to characterize. In this regard a novel monitoring approach using a remote sensing technique with hyperspectral camera has been tested already. One more action could be the implementation of green-based fertilizers promoting a nutrient loadings reduction.

Pialassa Baiona lagoon (P2)
North of the Ravenna canal port, there is a brackish coastal lagoon, the Piallassa Baiona, covering an area of about 10 km2. Discontinuous artificial embankments further divide the Piallassa Baiona into several shallow water ponds (average depth 1 m), connected to each other and to the sea by channels (depth ranging from 1 m, in the inner zones, to 8 m towards the sea). This arrangement of embankments, canals and ponds was initially created to promote the water circulation and the passage of small boats (Airoldi et al., 2016).
The Piallassa Baiona lagoon receives water inputs from five main channels that drain a watershed of 264 km^2, including urban (9%) and agricultural (87%) areas. The southern channel collects also the wastewater coming from urban and industrial sewage treatment plants and from two thermal power plants. Nutrient inputs are particularly high in the southern areas (Ponti et al., 2005). Complete water turnover in the lagoon has been estimated to take on average 3 days. The mean salinity value is about 28 and varies from 0, in areas close to the pinewood forests, to 37 in areas with low water exchanges during the summer. Salinity of the seawater in nearby coastal areas is low (mean value 30), due to the freshwater input form the Po River (Ponti et al., 2005).
In the Piallassa Baiona, bottoms are prevailingly sedimentary, ranging from mud to fine sand. The lagoon, ponds, and channels as well as a variety of man-made structures provide a variety of different habitats.

21

Ravenna harbour and the connected coastal lagoons (Piallassa Baiona and Piallassa Piomboni) receive several civil and industrial wastewaters carrying nutrients, different types of pollutants and cooling water from a power station and several industrial plants. Although nowadays discharges comply with the current laws, their accumulation still raises concerns, especially in confined areas of the lagoons. Further, legal constraints in the past were more permissive, or even absent, and during 1958 to 1976, Piallassa Baiona was heavily impacted by industrial pollution that has led to persistent accumulation in the sediment of numerous pollutants. The pollution gradient decreases from south to north, in relation to the distance from the southern industrial area (Trombini et al., 2003). Since 2000, part of the wastewaters has been diverted from the lagoon towards the harbour channel, reducing the pollution load, especially at low tide.

The Pialassa Baiona lagoon provides a wide range of habitats and conditions that over time have facilitated the establishment of populations of non-native species.

P3. Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT)

The coastline is characterized, along the western sector, by a series of small subrectangular coves with pocket beaches. At East, the coast is mainly sandy, with reduced rocky formations and low rocks emerging right, characterized by a regular, sinuous coastline. The eastern coastal sector is incised by ten little valleys, some continuing underwater. The marine bottoms are always characterized by the presence of two submarine cliffs, running parallel to the coastline, with a medium slope, whose physiognomy is articulated and presents a convexity. Important organisms are present and *Posidonia oceanica* meadows are one of the most characteristic habitats of the sandy infralittoral. Sampling of water column and sediments from the proposed sites are representative of the horizontal distribution.

Torre Guaceto

The sampling plan in Torre Guaceto marine protected area (MPA) was in collaboration between Unisalento (PP9) and CMCC (PP1), to have some stations in common and gather both chemical and physical/meteorological data.

In Torre Guaceto there is the outlet of Canale Reale. The Canale Reale, more than 60-km long, is perhaps the most extensive hydrographic system in Salento, naturally collecting the rainwater falling within its catchment area. The pollutants can enter the aquatic environment (surface and groundwater) through treated wastewater and industrial discharge, or as run-off from agricultural operations or simply as urban run-off.

Figure 4: Torre Guaceto map: the UniSalento sampling points are those found along the red transects. Geographical coordinates are in data tables (see below)

SALINA PUNTA DELLA CONTESSA

In Salina Punta della Contessa area, next to the Salina Vecchia, there is the outlet of a drain, Foggia di Rau, that is dry during most part of the year. The pollutants, mainly run-off from agricultural operations and industrial discharge, can enter the aquatic environment as surface and groundwater and could also reach the sea following leaching through the dunes, in front of the saltwork.

Figure 5: The UniSalento sampling stations in Salina Punta della Contessa. Geographical coordinates are in data tables (see below)

MELENDUGNO

In the area north of San Foca there is no important industrial activity, so run-off from agricultural operations represent the main source of pollutants. This area does not show any important water drain and it was not possible to select a station in the shoreline as significant pollutant input. In situ surveys showed the presence of different input of freshwater directly in the sea distributed along the shoreline. As a result, the sampling stations were placed parallel to the shoreline next to Torre Specchia Ruggeri which permit both to remain far from the sampling station of TransAdriaticPipeline and ARPA and next to naturalistic important coralline reef.

24

Figure 6: - The UniSalento sampling stations along the coast of Melendugno Municipality next to Torre Specchia Ruggeri. Geographical coordinates are in data tables (see below)

A DPSIR model for the management area as a whole is reported below and together with a DAPSI(W)R(M) model (Elliott et al., 2017), better highlighting the human need drivers requiring activities causing pressures on the ecological status changes which determine impacts on human welfare in the management area. DAPSI(W)R(M) model has also the advantage to clearly disentangle pressures which could be managed within the management area of interests and those pressures which are generated outside the area and, therefore, cannot be managed locally.

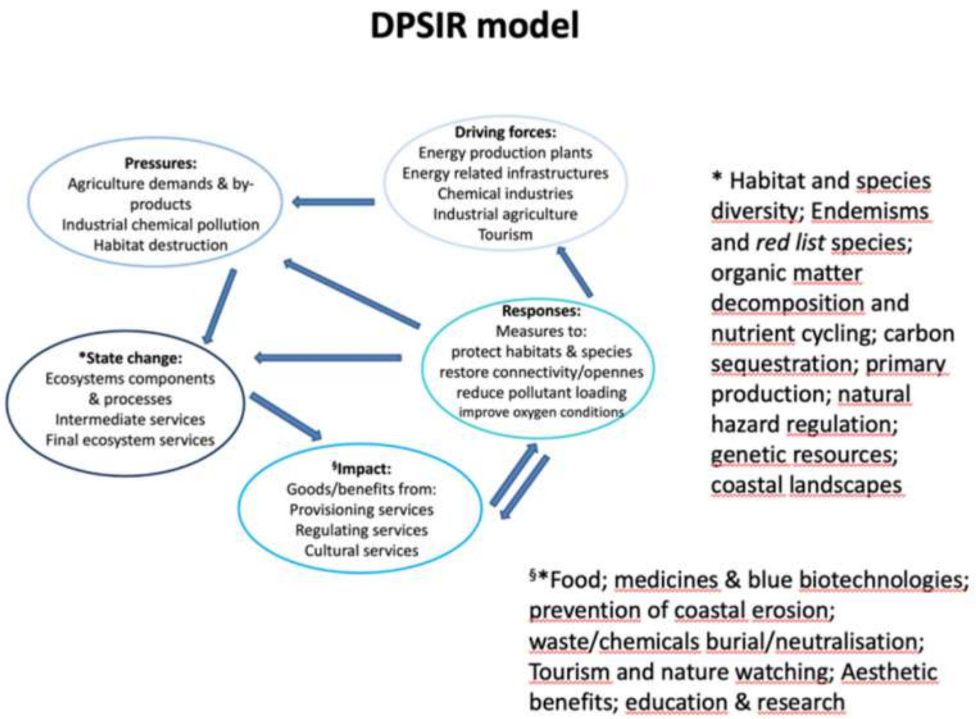


Figure 7. DPSIR model for the Pilot 3 management area

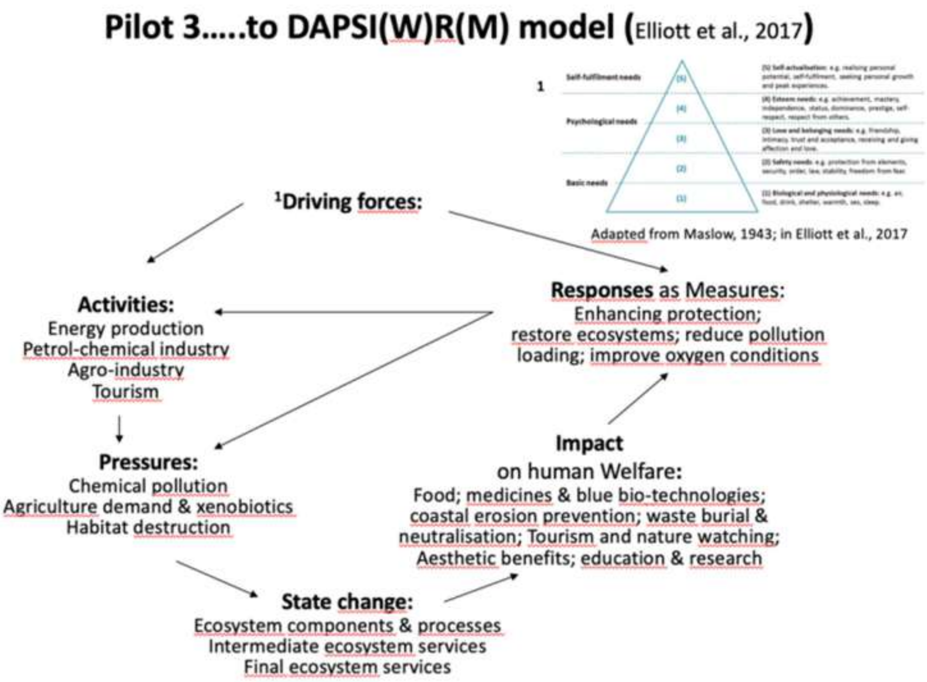


Figure 8. DASPI(W)R(M) model for the Pilot 3 management area

P4. Neretva river mouth (HR)

The study area is located in Croatia, more precisely on the Neretva River (P4). The Neretva River was included in Pilot Area 4 because it has a great diversity of habitats, especially aquatic and wetlands. Moreover, the delta area in Croatia is considered very important for the protection of nature and biodiversity. Therefore, in recent years, the government has taken measures to protect the Neretva Delta and its population from further destruction.
Naron is the Roman name for Neretva, the largest river in the eastern part of the Adriatic basin, which stretches for 225 km in Bosnia and Herzegovina and the last 20 km in Croatia. It occupies 20% of the total water catchment area of Bosnia and Herzegovina. This bioecological complex is divided between two countries: The delta-shaped river mouth with lakes Modro oko, Desne and Kuti belongs to Croatia, while the nature park Hutovo blato belongs to Bosnia and Herzegovina. In the Pleistocene, the region around today's Neretva estuary looked quite different. It was occupied by the then middle course of the Neretva River, whose bed stretched along today's Pelješac Peninsula. The river flowed into the sea near the present-day town of Vela Luka on the island of Korčula. With the end of the Ice Age, the sea level rose by about 100 m, which led to a shortening of the river and the formation of a new estuary, located approximately at the site of today's river mouth and near three triangular widenings. These widenings were not due to the erosive activity of the river, but to tectonic predisposition (Todorović, 2007). From then on, the material eroded from the upper reaches of the Neretva was deposited there and formed the present delta. In the past, the Neretva Delta was known for its abundance and diversity of plants, birds, and fish, as well as for hunting and fishing, which were practiced by almost all inhabitants of the region.
Water and wetland communities are the basic ecosystems in the Neretva. The most important factor influencing vegetation types is the water table. The Neretva River and its tributaries form the largest complex of wetlands in the Croatian coastal zone with well-developed coastal and other wetland vegetation (floating and submerged). The wetland communities are dominated by plant species common in Europe and the Holarctic. The *Scirpo-Phragmitetum* plant community represents the majority of wetland vegetation. *Myriophyllo-Nupharetum* and *Nymphoidetum-peltatae* are also common communities. The plant community *Fimbristylion dichotomae* is fragmentary, having disappeared primarily due to land reclamation. On the tidal flats in the Neretva estuary there are halophytic meadows with the species *Salicornietum herbaceae*. The grazed meadows near the sea are more or less saline and are covered by *Juncetum maritimo-acuti* and grassland of *Agropyro-Rumicion* type. Although the habitats have been significantly modified, the Neretva Delta still represents the largest and most valuable remnants of Mediterranean wetlands, which gives it significant value for the entire Mediterranean region (Mateljak et al., 2011.).

The Neretva Delta has many lagoons, shallow sandy bays, low sandy shores, sand flats, salt beaches, etc. Though a large area of the wetland habitat has been transformed into agricultural lands, due to the branching network of channels, these areas are still important habitats for aquatic birds and a very important ichthyological area. The delta, lagoons and brackish waters are an exceptionally important habitat which creates room for the intensive growth of fry, which later spend their life cycle in the sea or fresh water. Furthermore, these areas are important for the migration of anodromous and catadromous fish species. With a large number of endemic species and great diversity, the mouth of the Neretva River is one of the most interesting areas of Croatia.

There are three ornithological Special reserves in the area (Orepak, Pod gredom and Prud), ichthyological and ornithological Special reserve (Neretva Delta) and two Significant landscapes (Modro oko and Lake Desne, Predolac – Šibenica). Neretva River Delta is designated as internationally important wetland under the Convention on Wetlands (Ramsar, 1971). Also, it is a Natura 2000 site HR1000031 Delta Neretva (SPA), HR5000031 (SCI).

Figure 9. P4 Pilot Area

Today, Neretva is the only river in this region with a delta at its mouth. The karst system slows the flows of the Neretva River significantly. Moreover, entire project area, especially its sensitive ecosystems of karst and wetlands. Sensitive karst system is endangered by different activities such as negative human impact on freshwater ecosystems and global climate changes.

In many ways, the Neretva delta is different from other parts of coastal Croatia. Its unique landscape is a result of digging and depositing of the marsh soil. Furthermore, global climate changes have had and continue to have a strong influence on Neretva River and its flora and fauna. Constant oscillation of water levels, lack of water during summer, increased water temperature, etc. have led to disruption in the reproductive cycle of shellfish and fish. Unsuccessful spawning and increased fry mortality due to the impacts of climate change have reduced overall recruitment.

28

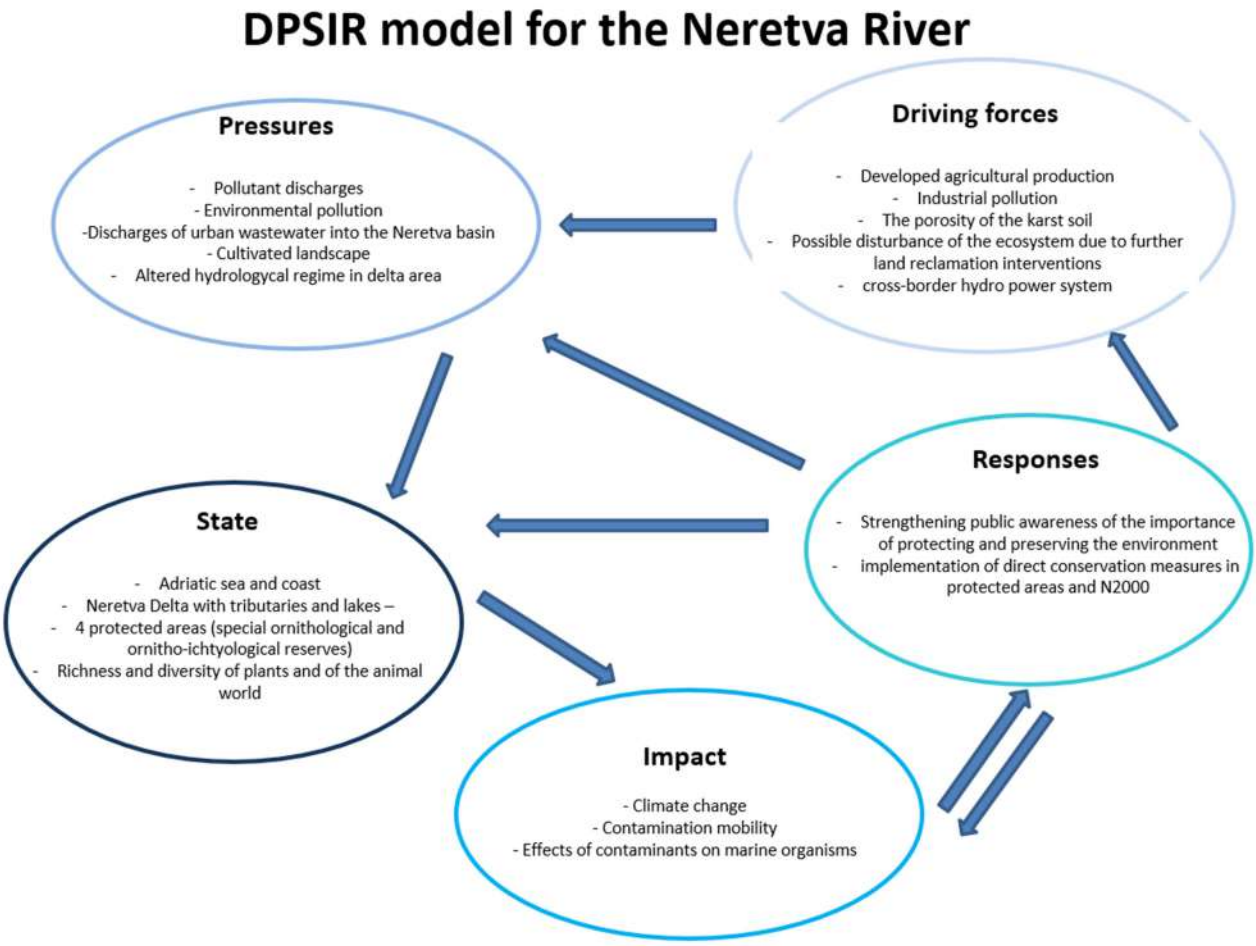
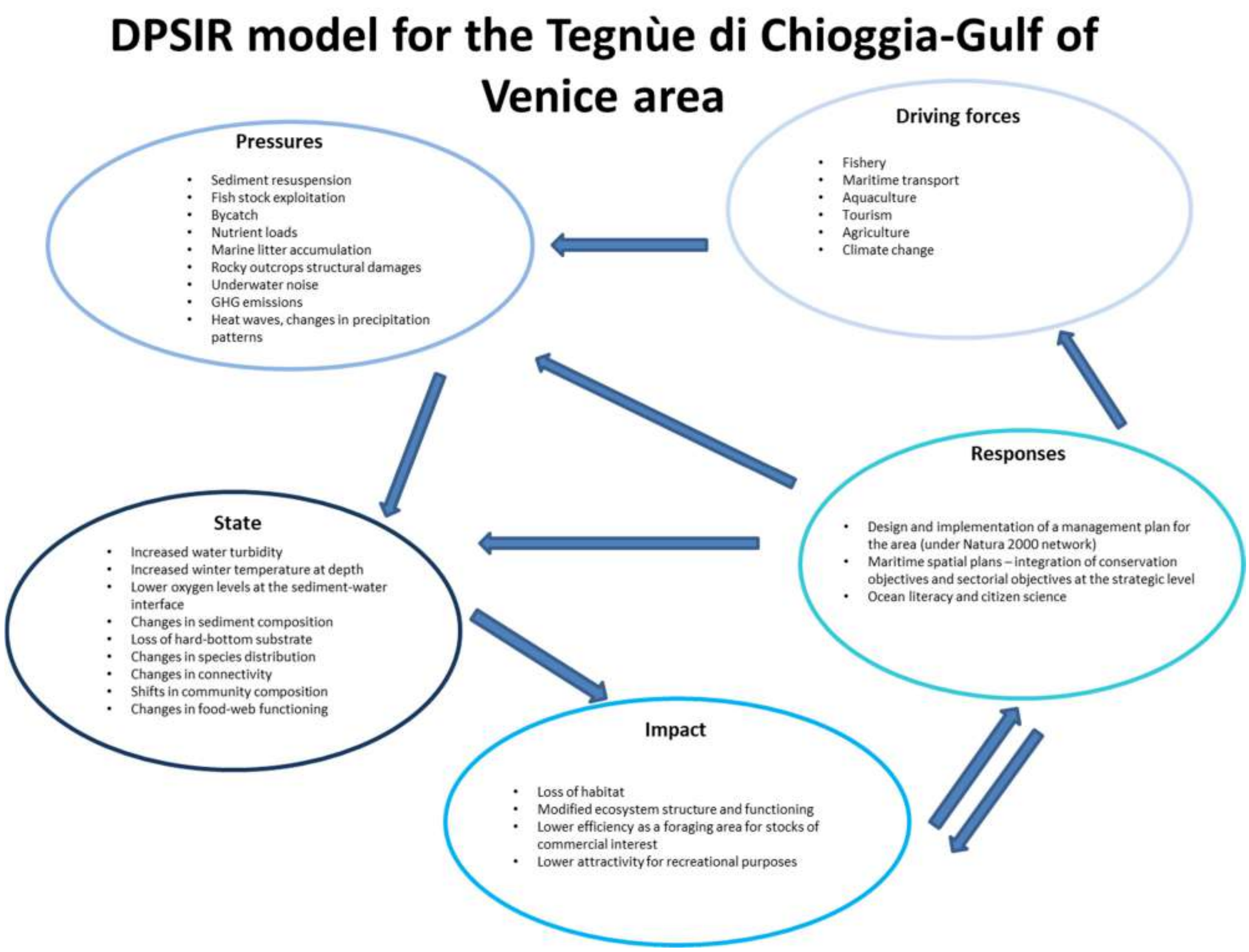


Figure 10. P4 Pilot Area DPSIR model

P5. Coastal area in Veneto (IT)

The marine area of "Tegnùe di Chioggia" IT3250047, was proposed as SCI under the European Habitat Directive in 2010 and consequently declared as a Special Area of Conservation (SAC) in 2018. Previously, from the year 2002, it has been declared a Biological Protection Zone (BPZ) under the Italian Ministry of Agricultural, Food and Forestry Policies (Ministero delle politiche agricole, alimentari e forestali), with the aim of prohibiting any type of fishing activity. The area is classified as Reef habitat (habitat code 1170).



The DPSIR model applied to pilot area 5 identifies maritime transport, fisheries, tourism, aquaculture and agriculture as main drivers of pressure. Some of these drivers act locally, while

30

others, such as agriculture and, to some extent, coastal tourism, exert a remote effect, which may be classified within the land-sea interactions category.

Pressures on this environment can be mainly related to sediment resuspension, marine litter accumulation, underwater noise, fish stock exploitation, and increased temperatures at the sea bottom. Contaminants and organic enrichment originated by maritime activities, and remotely by agriculture, are also included among the pressures. These pressures can cause changes on community composition in the area, both regarding benthic hard-bottom communities and demersal fish ones. Changes in food web functioning (e.g. degree of organic matter cycling, tot. prod./tot. resp., production of higher TLs) may also be an outcome of cumulative pressures effects. Impacts can be extended to the socio-economic dimension, through a change in the role of the Tegnùe as a foraging and aggregation ground for fish species of commercial interest, causing an impact on different fishery segments in the area (including artisanal fishery). These changes will eventually have an impact on recreational activities associated with the site, and primarily apnea and scuba-diving. Responses are primarily associated to the implementation of a management plan of the Natura 2000 site, which is still missing. An attention at the area at the strategic level is also possible through Maritime Spatial Planning, which can represent the strategic instrument fostering the integration between conservation objectives and the different sectorial objectives associated to maritime activities present in the area (such as artisanal fisheries, aquaculture, tourism).

P6. Miljašić Jaruga river mouth, Nin bay (HR)

The Miljašić jaruga together with its tributaries drains a part of Ravni Kotar with a total area of about 131 km^2. According to the study of the flood defense of the city of Nin and the protection against high waters of the Miljašić ravine, the protection against high water floods is provided for a 100-year return period. In its current state, the riverbed is partially covered with silt, unkempt, overgrown with grass and low vegetation, and the existing old stone lining is damaged and completely collapsed in parts. Collapsed buildings and a bed with accumulated silt reduce the flow profile of the watercourse, and parts of the stream slow down. An inspection of the marine habitat within the final part of the channel and in the immediate vicinity at the exit to the sea of the Miljašić jaruga, carried out in 2022, confirmed the presence of species typical for anthropogenic habitats of the sea coast and sea ports:

- **F. Sea coast**
  - ***F.5. Anthropogenic seashore habitats***
    - F.5.1. Anthropogenic seashore habitats
      - F.5.1.2. Seashore communities on firm ground influenced by man
        - F.5.1.2.1. Built and constructed banks
- **G. Sea**
  - ***G.1. Pelagial***
    - G.1.1. Pelagic communities of the neritic province
      - G.1.1.2. Pelagic communities of the neritic province under anthropogenic influence
      - G.1.1.2.1. Water areas of seaports
        - G.1.1.2.3. Water areas of inhabited places along the coast, and harbors
  - ***G.2. Mediolittoral***
    - G.2.5. Anthropogenic habitats in the mediolittoral
    - G.2.5.2. Medilittoral communities on firm ground influenced by man
      - G.2.5.2.1. The mediolittoral facies of concreted and built coasts (ports, marinas, shipyards) and other human constructions in the sea (e.g. gas platforms)

There is a visible anthropogenic influence on the biocenoses of the mediolittoral within the channel itself and the direct exit to the sea (G.2.5. Anthropogenic habitats in the mediolittoral; G.2.5.2.1. Facies of the mediolittoral of concreted and built coasts (ports, harbors, shipyards) and other human constructions in the sea (eg gas platforms)). The mediolittoral zone is dominated by the presence of snails *Patella rustica* and crabs *Chthamalus stellatus*. The concrete bottom at the final part of the channel descends vertically to a depth of about 1.5 m in the infralittoral zone. The mediolittoral zone on both sides of the canal consists of fine sand and is characteristic of the entire coastal area around the city of Nin (G.2.5.1. Mediolittoral communities on a moving substrate under the influence of man (silt, sand, gravel); G.2.5.1.1. Facies of tourist beaches and healing mud).

In the infralittoral zone, in the final part of the channel, the muddy bottom is covered with a thick layer of green algae of the genus Ulva spp (G.3.1. Infralittoral sandy muds, sands, gravels and rocks in euryhaline and eurythermal environments; G.3.1.1. Euryhaline and eurythermal biocenosis; G.3.1.1.8. Association with species of the genera Ulva and Enteromorpha). The depth is about 2 m in the middle of the channel. The above points to eutrophication and anthropogenic influence of the channel, given that these algae are characteristic of shallow polluted seas (Turk, 2011). Inside the channel, below the very edge of the concreted channel, there are communities typical of harbors and concreted shores, with the dominance of sea urchins of the genus Holothuria spp. and the wolf snail *Hexaplex trunculus* (G.3.8. Anthropogenic habitats in the infralittoral; G.3.8.2. Anthropogenic infralittoral communities on G.3.8.2.1 Infralittoral communities of concreted and constructed shores (ports, harbors, shipyards) and other human structures in the sea (eg gas platforms).

Green algae of the genera Codium and Ulothrix, brown algae *Zanardinia typus*, *Padina pavonica* and *Dictyota dichotoma* appear on the rocky substrate along the channel edges. On the outer part on the side of the channel (towards the north) on the muddy and sandy bottom, a sea grass meadow *Cymodocea nodosa* is developed. Among the benthic fauna, the muddy sediment is dominated by holothurians and the snail *Hexaplex trunculus*. Also, a population of the noble fan *Pinna nobilis* was observed, but a more detailed examination revealed no living individuals but only shells in the sediment. The aforemention is a consequence of the occurrence of mass mortality in the Mediterranean and the Adriatic during 2019 and 2020 (Saric et al. 2020). Moving away from the channel on both sides, the bottom changes from muddy to sandy (G.3.2. Infralittoral fine sands with more or less silt; G.3.2.2. Biocenosis of fine uniform sands), and is occasionally punctuated with smaller and larger stones overgrown with brown Algae of the genus Cystoseira spp. The dominance of sea urchins *Paracentrotus lividus* and sea urchins of the genus Holothuria was observed on the sand. The rocks and larger stones are dominated by the oyster oysters *Ostrea edulis* and the krill *Arca noae*. *Haxaplex trunculus* snails regularly appear on the sand. Of the sponges, the species *Crambe crambe* and *Aplysina aerophoba* were observed. Among the anemones, there is *Condylactis aurantiaca* and *Anemonia viridis*. The presence of the sea grass *Posidonia oceanica* fields was not observed in the vicinity.

33

P7. Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC) (IT)

Pilot area 7, in the Molise Coast, comprises two Special Areas of Conservation (SACs; Habitats Directive 92/43/EEC: Biferno river mouth-Campomarino Coast -IT7222216, and Saccione mouth Bonifica Ramitelli -IT7222217) included in the municipalities of Campomarino and Termoli, and is partially included in the ecological monitoring network called LTER (Long Term Ecological Research Netwotk - *https://deims.org/088fe3af-c5bb-4cc8-b479-fe1ea6d5be80*, Figure 11)

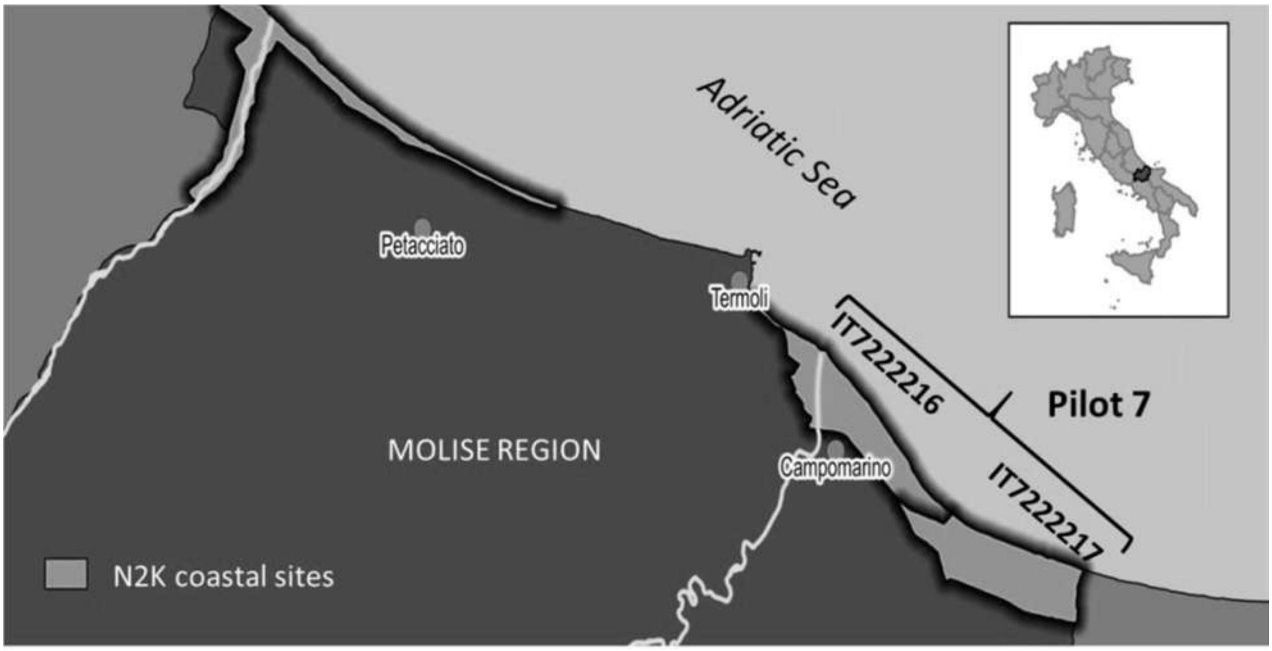


Figure 11. P7 Pilot Area is part of the Long Term Ecological Research network (LTER - https://deims.org/088fe3af-c5bb-4cc8-b479-fe1ea6d5be80) and includes two N2k sites: Biferno river mouth-Campomarino Coast (IT7222216), and Saccione mouth Bonifica Ramitelli (IT7222217).

The Pilot Area includes well-preserved coastal dune and halopsamophile ecosystems that are rapresentative of the Central Adriatic coast. In fact, coastal dunes are among the most valuable ecosystems due to the presence of a high specialized fauna and flora. The area is part of Nature 2000 (Biferno river mouth-Campomarino Coast (IT7222216), and Saccione mouth Bonifica Ramitelli (IT7222217)) and LTER network (https://deims.org/088fe3af-c5bb-4cc8-b479-fe1ea6d5be80) and hosts several species and habitats of European conservation concern (Habitats Directive 92/43/EEC). Major habitats are: Habitat 1210 (Annual vegetation of drift lines), Habitat 2110 (Embryonic shifting dunes), Habitat 2120 (Shifting dunes along the shorelines with Ammophila arenaria, white dunes), Habitat 2230 (Malcolmietalia dune grasslands), Habitat 2260 (Cisto-Lavanduletalia dune sclerophyllous scrubs) and the habitat of priority concern 2250 (Coastal dunes with Juniperus spp of priority concern). On the Biferno mouth, we also found the following

habitats: 1410 (Mediterranean salt meadows - Juncetalia maritimi); 1420 (Mediterranean and thermo-Atlantic halophilous scrubs -Sarcocornetiea fruticosi); the priority Habitats 1510* (Mediterranean salt steppes) and 3170* (Mediterranean temporary ponds). (Stanisci et al 2014)
Coastal areas in the Molise region hosting pilot area 7 hosts one of the most threatened landscapes at national and European level (see **Figure 12.** DIPSIR Model). These Mediterranean coastal ecosystems are extremely vulnerable and threatened by both erosion and increasing human pressure (e.g. urban expansion, trampling, beach litter accumulation, alien species invasion. These threats lead several dune habitats to reduce and/or even disappear, implying negative consequences on biodiversity and human well-being, as e.g. a loss of coastal defense ability, a low perception of environmental quality by the tourists and a reduction of the ability of mitigate extreme climate events.

This chapter is focused on Salt marshes occurring at Biferno river mouth area, that represent a residual wetland of larger one covering a century ago wide sectors of the Molise coast.
Salt marshes have not direct connections to the sea and are fed by salt water table and partially by artificial wetland drainages. Specifically, the saltmarshes of Campomarino and Biferno River mouth has been exposed to high erosion risk with strong coastal erosion processes (see **Figure 12.** DIPSIR Model). The period 1954–2014 registered an erosion rate of − 2.90 m/ year in the study area and such trend is expected to proceed over time.
The climate in the analyzed coastal tract, as in the whole Mediterranean region, is changing rapidly (IPCC 2022). The statistical analysis of climatic data recorded in the last fifty years (1970–2020) in the nearby weather station of Termoli evidenced a consistent rise of temperatures and a slight decrease of annual precipitations. The mean annual temperatures in the last half century have been of 16,74 °C with annual values that significantly increased from 15,5 °C to 18,6 °C ($R2=0,839$, $p\text{-value}<0,001$). Precipitations in summer (that is the period of greatest aridity stress for plants in the Mediterranean biome) registered a mean value of 21,50 mm and a slight decline from ≈30 mm to ≈19 mm.

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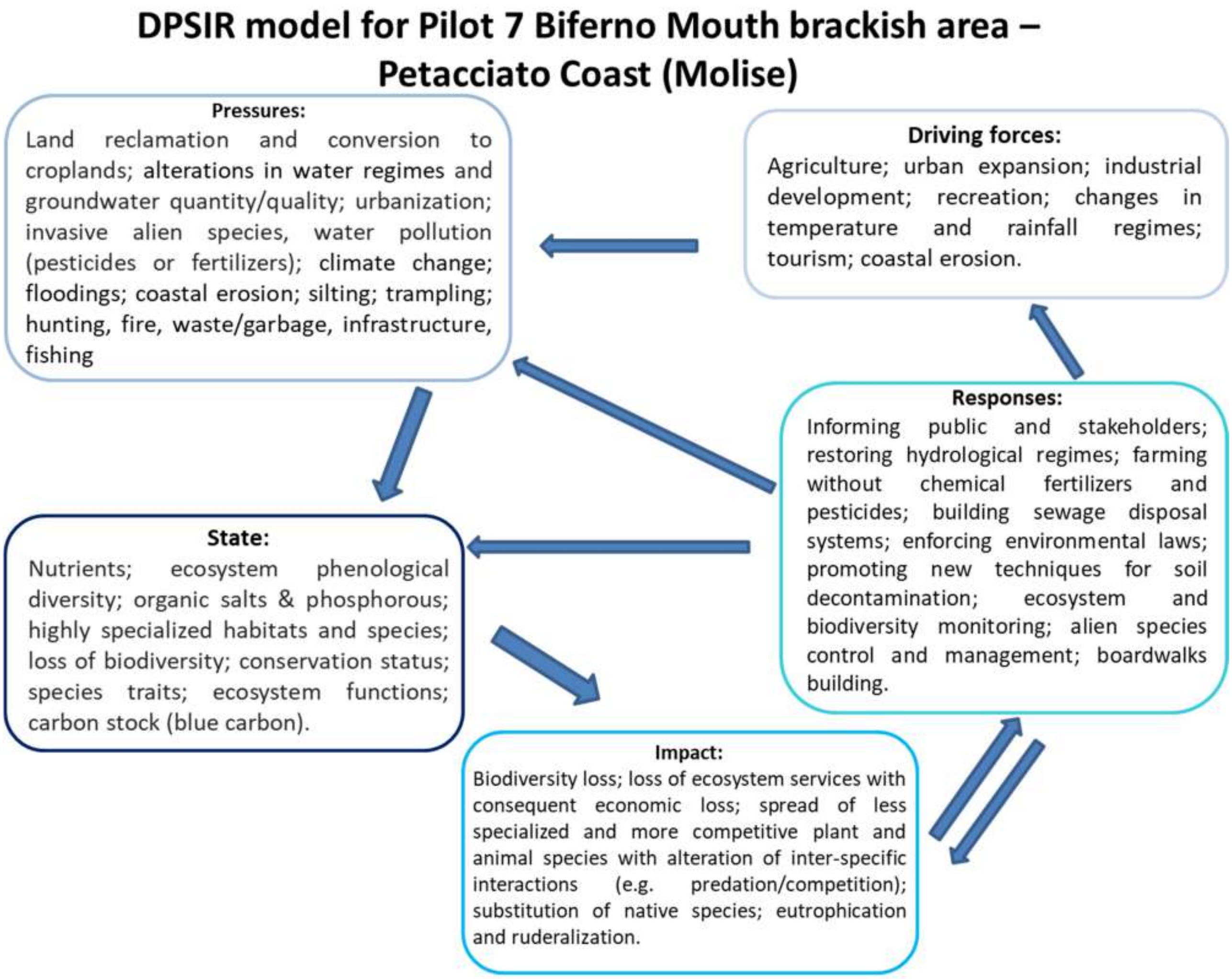


Figure 12. DIPSIR Model for *Pilot 7 – Molise Coast in the central Adriatic area*

P8. Northern-eastern Adriatic in Croatia (HR)

The North – Eastern Adriatic Sea is a shallow (up to 40 m depth), semi-enclosed coastal area with steep ecological gradients, which are mainly noticeable through changes in nutrient composition, salinity, temperature and oxygen saturation. The area is under significant influence of freshwater inputs (Po River as the largest freshwater input into the Adriatic) and the established Adriatic Sea circulation. In addition to river dilution and nutrient enrichment, the area is characterised with periodic inflows of high salinity waters transferred by the Eastern Adriatic Current (EAC) from the southern to the northern part of the Adriatic Sea. Given the ecological characteristics, the North – Eastern Adriatic Sea is a highly productive and dynamic marine ecosystem. It is an ideal basin to study the influence of freshwater inflows and fast oceanographic changes on marine ecosystem.
Observing campaigns in the Northern – Eastern Adriatic Sea were organised at two stations (Figure 13, Table 1) were included and monthly sampling conducted in the period from 2020 to December 2021. Variables that were measured include: temperature, conductivity (salinity), dissolved oxygen, Chlorophyll a and nutrients (N, P, Si). Samplings were conducted with the CMR – RBI research vessel Burin using CTD probe (temperature, conductivity and dissolved oxygen) from the surface to the bottom and collecting sea water with Niskin bottles (Chlorophyll a and nutrients) on depths of 5, 10 and 20 m (Figure 14). All the samples were immediately laboratory processed and analysed in the CMR – RBI. Observing system of the Nothern – Eastern Adriatic Sea was supported with the real-time data collecting of the CASCADE project installed 2 observing oceanographic buoys (Figure 14) on the Rovinj – Po transect, position 1 and 5 nm from the coast of Croatia during a period from January 2023 – June 2023. Oceanographic buoy enabled measuring of diverse set of meteorological (wind direction and speed, air temperature, relative humidity, atmospheric pressure, solar irradiation, precipitation and air visibility) and oceanographic variables (surface current, wave height, PCO2, temperature, conductivity (salinity), dissolved oxygen, light transmission, pH, phytoplankton pigments; phycocyanin, phycoerythrin and chlorophyll-a).

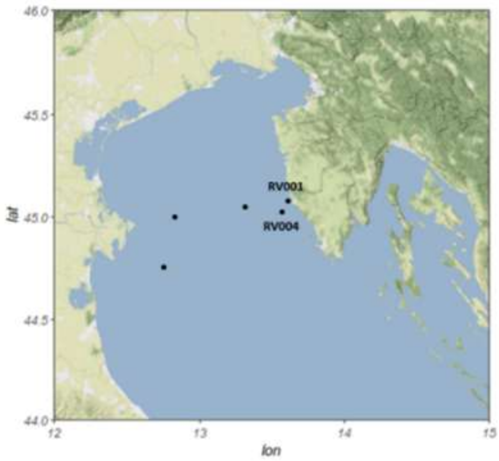


Figure 13. Selected sites within the Natura 2000 Map of the Rovinj – Po transect and sampling stations in the Northern – Eastern Adriatic Sea pilot area

Transect	Station ID	Lat (N)	Long (E)	Distance from the coast
Rovinj - Po	RV001	45.080000	13.610000	1 nm
Rovinj - Po	RV004	45.076450	13.568094	4 nm

Table 1. Description of the sampling stations included in the observing campaigns

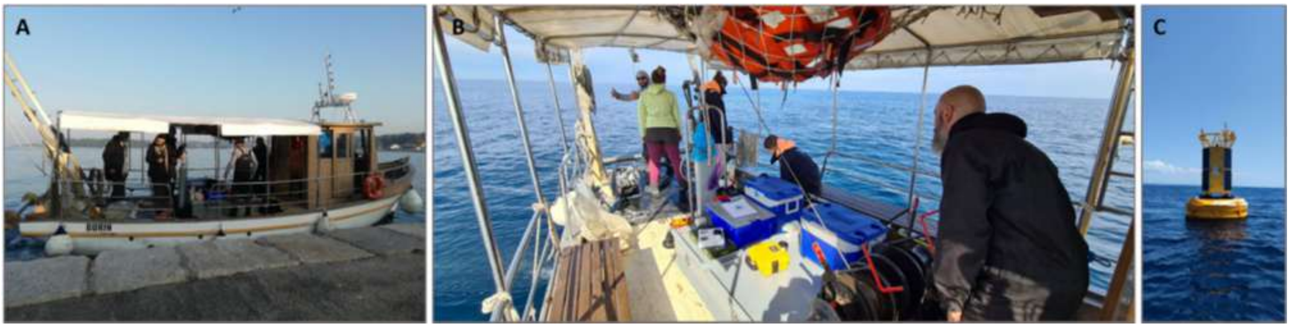


Figure 14. Research vessel (A), sampling procedure (B), and oceanographic buoy (C) in the Northern – Eastern Adriatic Sea pilot area

Figure 15. Research vessel Vila velebita, sampling with niskin samplers, phytoplankton nets and photos on board in the Northern –Adriatic Sea pilot area 8

38

P9. Cetina river mouth (HR)

CASCADE project pilot area no. 9 is Cetina estuary, a Natura 2000 site (code: HR3000126) proclaimed as such in 2013 managed by Public Institution Sea and Karst (Project Partner No. 15). The site is located in south of Croatia, in front of the town Omiš and includes the mouth of river Cetina (Figure 16). Cetina estuary is a 100% marine area of 6.67 km^2.

SITE DISPLAY

Figure 16. Map of Natura 2000 site Cetina estuary

Area is characterized by a brackish lagoon, coastal marine area with sandy and muddy bottoms. The coast is relatively flat with no pronounced bays. The seabed in the area under consideration can be described in the same way. Around the mouth and west of it, the seabed is shallow and relatively gently stretches from the coast towards the middle of the Brač Channel. Significant amounts of water are constantly flowing into this channel through the river Cetina, and with them organic and inorganic suspensions and various solutes. As such this is an important site for habitat types such as 1110 – sandbanks which are slightly covered by sea water all the time, 1130 – estuaries, 1140 - mudflats and sandflats not covered by seawater at low tide, as well as for *Petromyzon marinus* reproduction (Table 1). The description of the pilot area was accurately reported in CASCADE report D 3.2.1 Ecosystem Pilot characterization report.

ANNEX I HABITAT TYPES							SITE ASSESSMENT			
CODE	NAME	PF	NP	COVER (ha)	CAVES (number)	DATA QUALITY	A | B | C | D	A | B | C		
							Representativity	Relative surface	Conservation	Global
1110	Sandbanks which are slightly covered by sea water all the time	0	0	135		P	B	C	C	B
1130	Estuaries	0	0	677		G	B	B	C	B
1140	Mudflats and sandflats not covered by seawater at low tide	0	0	1	0	M	C	B	C	C

PF: Value "1" indicates the priority form for habitat types that can have a non-priority as well as a priority form (6210, 7130, 9430)
NP: Value "1" indicates a habitat type that no longer exists in the site
Caves: for habitat types 8310, 8330 (caves) the number of caves is entered if estimated surface is not available.
Data quality: G = 'Good' (e.g. based on surveys); M = 'Moderate' (e.g. based on partial data with some extrapolation); P = 'Poor' (e.g. rough estimation)
Representativity (Degree of representativity of the habitat type on the site): A= excellent, B=good, C=significant, D=non-significant
Relative surface (Area of the site covered by the natural habitat type in relation to the total area covered by that natural habitat type within the national territory): A = >15%, B = 2-15%, C = <2%
Conservation (Degree of conservation of the structure and functions of the natural habitat type): A = excellent conservation, B = good conservation, C = average or reduced conservation
Global assessment (Global assessment of the value of the site for conservation of the natural habitat concerned): A = excellent value, B = good value, C = significant value

Table 2. Habitat types present on the site and assessment for them (bioportal.hr)

39

The DPSIR model has been prepared for the Cetina Estuary (Figure 17). The entire considered coastal belt has undergone significant changes due to intensive use / settlement. Larger changes occurred in those areas that were more easily accessible and favourable for settlement, and these are flattened parts west of the mouth. By far the biggest changes have occurred in the area of the estuary where the town of Omiš developed. With the construction of the hydropower plants, the regime of the river flow changed significantly, so the banks of the river at the very mouth were arranged accordingly. Since the mouth of the river has been substantially altered, from a natural delta with shallow backwaters overgrown with sedges into a funnel-shaped regular bed, the conditions for the interaction between the river and the sea have completely changed. Today, town of Omiš is a touristic destination not only because of its large sandy and pebble beaches, but also because of tourist activities on the river Cetina (rafting, canyoning, etc.).

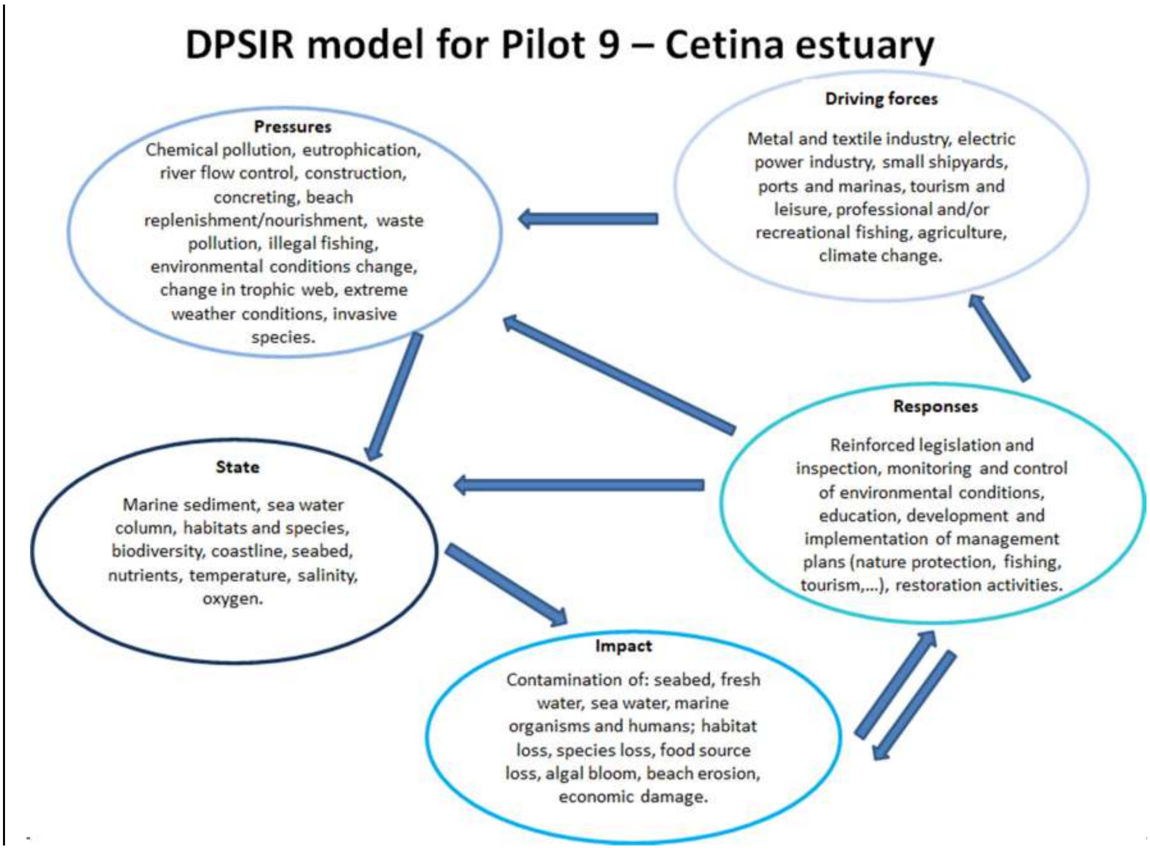


Figure 17. DPSIR model for Cetina estuary

On the coastline and in the Cetina estuary itself, a small shipyard and port open to public transport developed. While tourism is a growing industry, agriculture tends to decrease keeping its level to small family farms business that however have some impacts on Cetina River environmental conditions. Textile industry is still present there, that combined with still underdeveloped sewage network and old waste dumps degrade river environmental conditions. All these impacts are however significantly lower in scale compared to irreversible hydropower plants impacts. Like other marine ecosystems, the Cetina estuary is under the pressure of climate change, which introduces changes in the food chain, the emergency of invasive species, and occurrence of mass morality events.

Despite numerous pressures over this area, all parameters that are measured and monitored for Cetina estuary sea water, sediments and marine organisms show values that prove that the state of eutrophication of the Cetina estuary is very good, that biological quality of transitional waters is good, that sea bottom is oxygenised what provides very good conditions for development of organisms on the seabed. Also, ecological condition of the body of surface waters of the Cetina estuary is good. Sea beaches of Cetina estuary also have excellent sea quality for bathing. All hazardous substances, except TBT, are also below the legally prescribed values. Despite the influence of the river Cetina, the mouth of the Cetina belongs to oligotrophic areas.

However, it is necessary to continue all monitoring programs and to introduce additional monitoring of the state of the ecosystem, especially in relation to EU biodiversity directives. Probably there are changes in usual patterns of the water column salinity and temperature however there is no systematic monitoring that should be carried out. Management plan development shall be a priority to strategically plan nature conservation activities of Public Institution Sea and Karst for this Natura 2000 site that has a marine ecosystem under strong influence of freshwater unique to Split-Dalmatia County.

41

P10. Torre del Cerrano, Pineto Abruzzo (IT)

The Pilot area 10 is the Torre Cerrano Marine Protected area (AMP). Among the coastal biodiversity facets Torre Cerrano is known for (https://www.torredelcerrano.it/), the work focused on coastal dunes hosting valuable relicts of well-preserved natural ecosystems (Figure 18).
The Pilot area 10 includes well preserved coastal dune ecosystems representative of the Central Adriatic coast along a 6 km long seashore. It hosts several species and habitats of European conservation concern (Habitats Directive 92/43/EEC). Major dune habitats are: Habitat 1210: Annual vegetation of drift lines; 2110: Embryonic shifting dunes; 2120: Shifting dunes along the shorelines with Ammophila arenaria, white dunes; 2230 Malcolmietalia dune grasslands; 1410: Mediterranean salt meadows - Juncetalia maritimi; 2270: Wooded dunes with Pinus pinea and/or Pinus pinaster (Carranza et al 2008; Stanisci et al 2014).

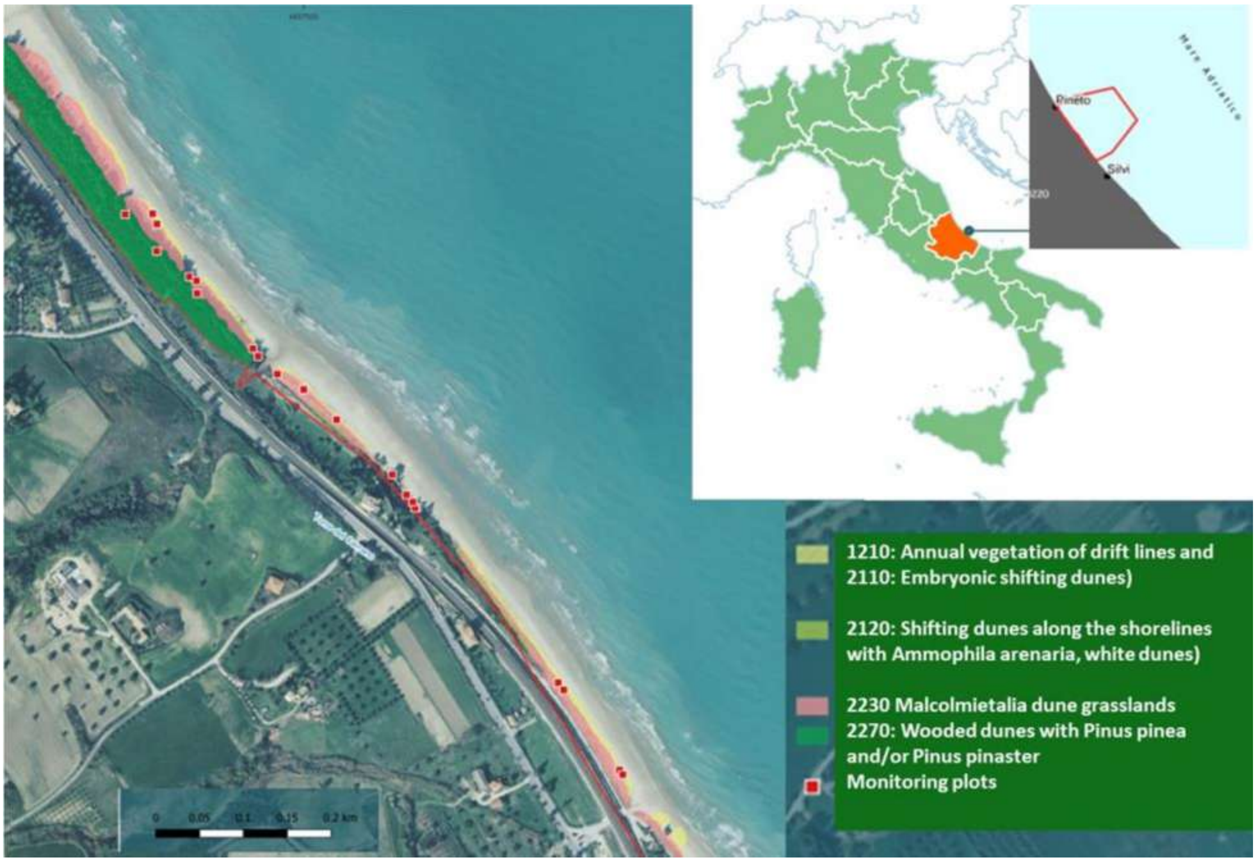


Figure 18. Pilot area is a Marine Protected area (https://www.torredelcerrano.it/area-marina-protetta.html) along with coastal dune habitats (Habitats Directive 92/43/EEC). Some monitoring plots are also reported.

Along the Adriatic coasts (as well as on most of the coastal areas in the world), unregulated urbanization, intensive farming and increasing road infrastructures coupled with massive beach tourism have led to pollution, biological invasions, over-exploitation of the natural resources and to a decline in quantity and quality of coastal habitats. Usually, tourists, tour operators and local administrators see the beaches exclusively as a place for vacation and leisure sports; thus, sand beaches are often cleaned mechanically and dunes severely trampled. In fact, human trampling associated with recreational activities is the most common disturbance on sand dune habitats, especially during summer, whose extremely negative effects include substrate erosion, decreasing of plant community's diversity and wildlife dismissal from no longer suitable habitats (Fig 19).
In this example, we measure changes in vegetation assembly after the establishment of wooden boardwalks (protecting dunes form human trampling) across different coastal dune habitats.

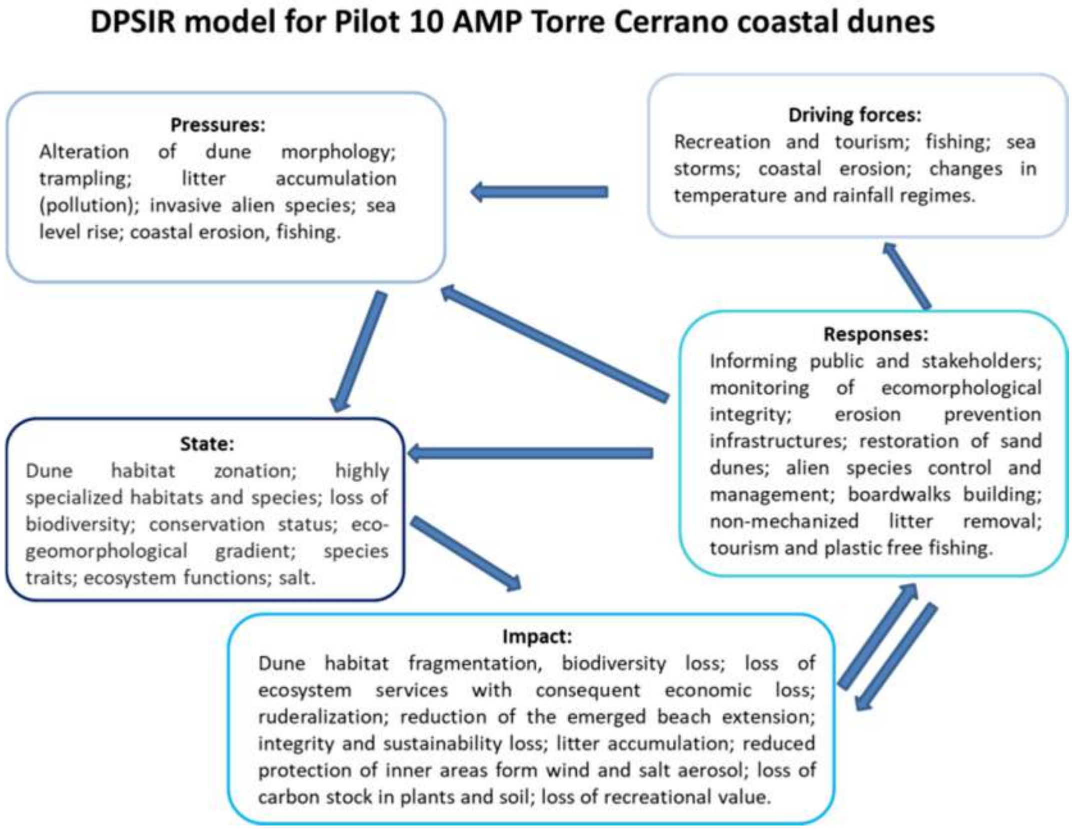


Figure 19. DIPSIR Model for Pilot 10 – Area Marina Protetta Torre del Cerrano

P11. Marche coastal area (IT)

Pilot area P11 includes the entire coastal marine area of the Marche region. The Marche coast is one of the most characteristic aspects of the Region. It extends for 180 km from the promontory of Gabicce Mare to the mouth of the Tronto river, awarded with numerous European Blue flags that sanction the excellent quality of bathing water and the care taken for the protection of the marine environment.
The coast alternates pebble, rocky and sandy beaches, on the northern coast, long and thin beaches alternate are interrupted by promontories, by small coves or by the mouth of a stream. From Ancona, capital of Marche Region, is visible the promontory of Monte Conero overlooking the Adriatic Sea. The Monte Conero is a Natura 2000 site and the coast hosts 7 Natura 2000 sites.
From here begins the most beautiful stretch of the Marche coast: the "riviera del Conero", full of white bays sometimes reachable only by boat or through paths, cut out in the green of the Mediterranean scrub.
To the south of the Conero, the coast offers wide and flat beaches up to an area full of pine forests in Porto Recanati, Porto Potenza Picena and Civitanova Marche.

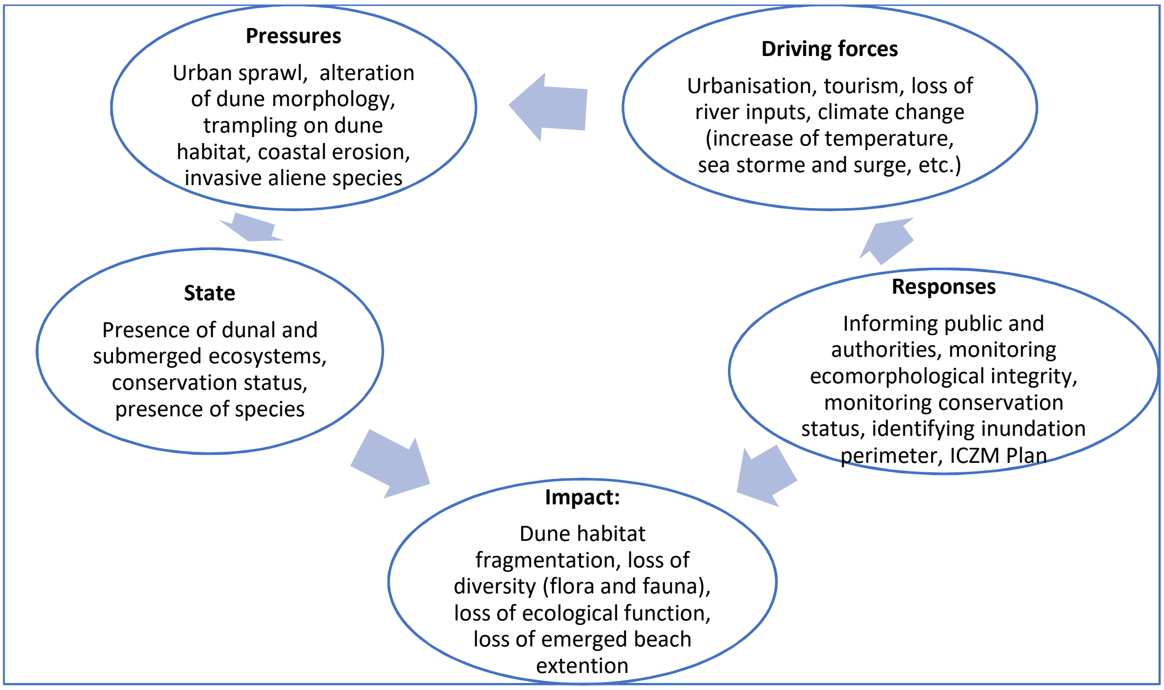


Figure 20 - DPSIR model for Pilot 11 Marche coastal area (IT)

3 Activities carried out in WP3 and WP4 and any effect of lockdown observed

P1. Grado and Marano Lagoon and Gulf of Trieste (IT)

Material and methods

The fieldwork was carried out *una tantum* within April and September 2021, between Villaggio del Pescatore and Lazzaretto (nearby the Slovenian border). The total number of sites was 15 (CAS1-CAS15) (Figure 21), resulting in 135 samples of macrobenthic invertebrates. A vessel and a small support boat with oars were used to transport all the equipment and approach the rocky coast.

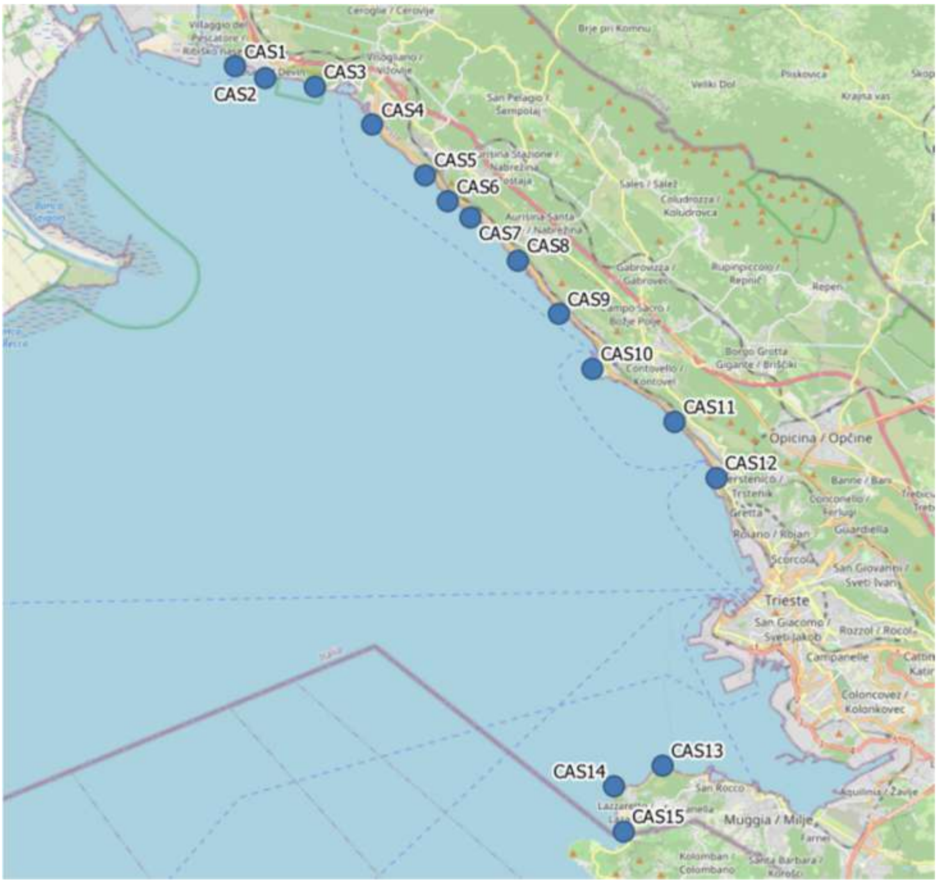


Figure 21. Map of the sampling stations in the Gulf of Trieste

The horizontal extent of upper mediolittoral (UML), lower mediolittoral (LML) and upper infralittoral (UIL) was determined through organisms' observation according to Orlando-Bonaca et al. (2012) and Pitacco et al. (2013). Sampling in the two mediolittoral belts was done by snorkeling,

45

whereas in the upper-infralittoral scuba tools were used at a depth of about 1.5 m. All the organisms were collected and scraped from the rocks by a rasp, within a metal square 20x20 cm that is the reference sampling area. The sampling in UIL was carried out by using a small sorbona. A total of 9 20x20 cm samples were collected in every sampling station (3 in UML, 3 in LML and 3 in UIL). Photos of every square were taken with a digital camera, before and after animals' collection, in order to obtain data on substrate characteristics and to monitor the effectiveness of the samplings. The material was sieved through a 0.5 mm mesh and preserved in ethanol 70%. All sampled organisms were counted and identified to the lower possible taxonomic level under a stereomicroscope and an optic microscope. The identified species of every belt were categorized in term of the frequency of occurrence in the 20x20 cm square, as follow: the species were marked as permanent (P) when found in all samples of the belt (100%), constant (C) (<100% but ≥ 50%), frequent (F) (<50% but ≥ 25%) or temporary (T) (< 25%).
Cluster, ANOSIM and SIMPER analysis were applied to evaluate the similarities and/or differences among belts and sampling sites (the software used was PRIMER 7).
AMBI index (AZTI software) was used to calculate the proportion sensitive/tolerant species (Borja et al., 2000), as suggested by Vinagre et al. (2016) for the rocky shore macrozoobenthos. AMBI is based on the classification of the benthic species in five (I–V) ecological groups (EG), according to their tolerance to pollution:

- EG-I = species which are very sensitive to organic enrichment, intolerant to pollution;
- EG-II = species which are indifferent to enrichment;
- EG-III = species which are tolerant to enrichment, slightly unbalanced environments;
- EG-IV = second-order opportunistic species, slight to pronounced unbalanced environments;
- EG-V = first-order opportunistic species, pronounced unbalanced environment.

  Then a formula was used to calculate the AMBI on a scale of increasing disturbance from undisturbed (0.0<AMBI<1.2) to extremely polluted (6.0<AMBI<7.0) as descrived with further details by Muxica et al., 2005. The hydromorphological stressor class (HM) was calculated for every sampling site by a series of stressor metrics (for details see Orlando-Bonaca et al., 2012). The total score range for every sampling station is between 0 and 14, as follow described (Orlando-Bonaca et al., 2012):

- class 1 (pristine) when HM range is 0-2.8;
- class 2 (slightly altered) HM 2.9-5.6;
- class 3 (moderately altered) HM 5.7-8.4;
- class 4 (substantially altered) HM 8.5-11.2;
- class 5 (heavily altered) HM 11.3-14.

During the main monitoring activities focused on benthic macroinvertebrates other two objectives were pursued on the same sampling stations:

1. A visual census on the presence/absence of the brown algae *Fucus virsoides*;

2. Identification of the main fish species of medio/infra littoral belt by the means of non-invasive methods, locating video cameras on the sea bottom and by visual census along 120 m horizontal transects. Every transect line (60 m) was parallel to the coast and laid at a constant depth. Again, fish were counted within 1m on each side of the transect line. With a constant swimming speed, a sampling generally took 25 minutes (Lipej & Orlando-Bonaca, 2006). In particular, it will be investigated the presence/absence of adults and/or juvenile of the giant goby *Gobius cobitis.*

Results

A total of 275 taxa from 54,717 individuals were identified from 9 phyla. 197 taxa were just identified to species level (Table S1), because of some specimens were partially damaged and the identification was not possible. In term of species richness, phylum Mollusca was the most represented (108 taxa), followed by Annelida (70) and Arthropoda (67), together representing 89% of the macrozoobenthos richness. Other phyla were Porifera (11), Echinodermata (8), Tunicata as phylum Chordata (4), Bryozoa (3), Cnidaria (2) and Platyhelminthes (2). On regard the abundance, The phylum Arthropoda, represented by subphylum Crustacea, reached 50% of the abundance in the samples, followed by Mollusca represented by Gastropoda and Bivalvia (47%) and Annelida represented by Polychaeta (2.7%). Taking into consideration the belt zonation, 19 taxa were recorded on the upper mediolittoral (UML), 121 on lower mediolittoral (LML) and 252 on upper infralittoral (UIL). UML belt was populated by Mollusca (11 taxa), Arthropoda (6) and Annelida (2). In term of abundance, comparing with the other belts, 44% of individuals were recorded on UML, of which 94% were represented by Arthropoda and 5.6% by Mollusca. The cirriped *Chthamalus montagui* (Southward, 1976) constituted alone 93% of the total abundance, followed by the gastropod *Melarhaphe neritoides* (Linnaeus, 1758) (3.5%), the blue mussel *Mytilus galloprovincialis* (Lamarck, 1819) (0.9%), the cirriped *Chthamalus stellatus* (Poli, 1791) (0.9%) and the limpet *Patella caerulea* (Linnaeus, 1758) (0.6%).
LML belt was mostly populated by Mollusca (49 taxa), Annelida (41), Arthropoda (25), who represented 95% of the richness. Some species belonging to phyla Cnidaria, Platyhelminthes, Bryozoa and Echinodermata were found too; it is also interesting to mention the presence of the typical intertidal species *Actinia equina* (Linnaeus, 1758). In term of abundance 23% of individuals were recorded on this belt, mostly represented by Mollusca (91%), followed by Arthropoda (7%) and Annelida (1.8%). On regard the species, *M. gallo provincialis* dominated with 75% of the total abundance on this belt, *P. caerulea* represented 2.6% of the community and among cirripeds, *Perforatus perforatus* (Bruguière, 1789) was the most abundant (2.4%).
Finally, UIL was prevalently populated by phyla Mollusca (104 taxa), Arthropoda (61) and Annelida (57), followed by Porifera (11), Echinodermata (8), Chordata (4), Bryozoa (3), Cnidaria (2) and Platyhelminthes (2). In term of abundance UIL represented 33% of collected specimens, where Mollusca constituted 69% of individuals, Arthropoda 23% and Annelida 7%. Among species, *M. galloprovincialis* was always dominant (46%), followed by amphipods of the family Corophiidae Leach, 1814 (7.5%).

47

The mean number of taxa per sampling station was 3±2 in UML belt, 8±6 in LML and 33±12 in UIL; the minimum was recorded in CAS6 UML (1±1) and the maximum in CAS8 UIL (48±7) (Table 3). The mean abundance per station was 536±446 in UML, 284±389 in LML and 403±609 in UIL; the minimum was recorded in CAS10 LML (18±13) and the maximum in CAS5 UIL (1,910±1,682) (Table 3).
The frequency of occurrence on the three belts for every species in the study area is reported in Table S1: on UML *C. montagui* was constant (C) in the square samples, *P. caerulea* and *M. neritoides* were frequent (F) and 76.9% of the species in UML were temporary (T). On LML *P. caerulea* and *M. galloprovincialis* were constant, *P. perforatus* was frequent and 96.6% of the species were temporary. On UIL belt 10 species were constant (7 Mollusca, 1 Annelida, 2 Arthropoda) as *M. galloprovincialis* and *Paranthura japonica* (the latter is a non-indigenous species); 20 species were frequent and 83% of species were temporary (for further details see Table S1).

n. taxa	UML		LML		UIL	
	Average	S.D.	Average	S.D.	Average	S.D.
CAS1	3	0	11	4	30	6
CAS2	3	2	10	2	29	12
CAS3	3	2	5	2	47	3
CAS4	3	1	6	2	30	8
CAS5	2	0	14	9	32	7
CAS6	1	1	4	2	35	13
CAS7	2	2	8	6	31	20
CAS8	3	1	4	2	48	7
CAS9	3	1	7	1	36	9
CAS10	2	1	2	1	16	4
CAS11	6	2	11	2	28	11
CAS12	2	1	13	10	45	9
CAS13	2	1	6	3	34	10
CAS14	2	0	5	1	27	10
CAS15	3	1	17	10	25	11

abundance	UML		LML		UIL	
	Average	S.D.	Average	S.D.	Average	S.D.
CAS1	179	63	540	210	185	123
CAS2	306	51	225	87	249	176
CAS3	427	353	478	488	359	282
CAS4	749	699	292	49	297	102
CAS5	774	342	431	337	1910	1682
CAS6	398	235	29	13	169	26
CAS7	816	413	84	64	282	163
CAS8	237	30	112	134	204	22
CAS9	424	16	230	110	274	117
CAS10	407	373	18	13	68	21
CAS11	605	799	386	196	264	138
CAS12	919	284	96	130	427	93
CAS13	737	630	176	266	468	342
CAS14	787	850	35	43	774	860
CAS15	279	435	1124	1008	110	57

Table 3. Number of taxa and abundance per sampling station and belt (average±S.D.)

The multivariate analysis discriminated the community in the three belts, as represented by the dendrogram, where all samplings stations where grouped in UML, LML or UIL clusters (Fig. 22). UML community showed a null degree of similarity with other two belts, whereas LML and UIL showed a degree of similarity of about 5%.

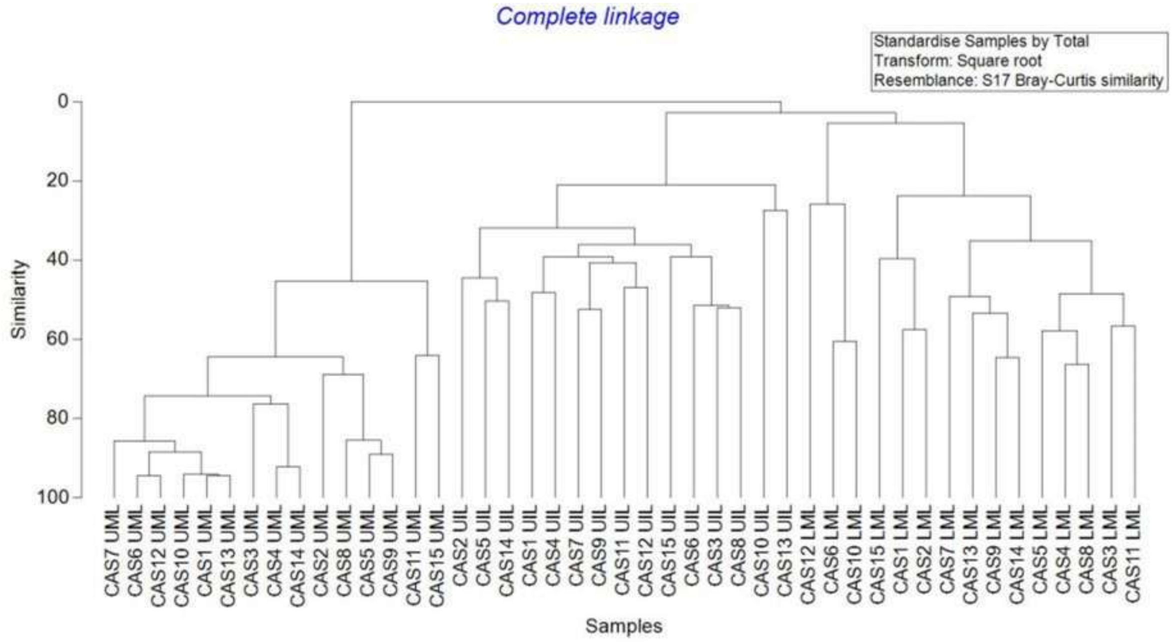


Figure 22. Dendrogram with belt clusters

The AMBI index has been calculated for every sampling station considering the benthic intertidal community as a whole (UML+LML+UIL). The index shows the prevalence of species belonging to EG-I (species very sensitive to organic enrichment, intolerant to pollution) and those belonging to EG-III (species which are tolerant to enrichment, slightly unbalanced environments) (Fig. 23).

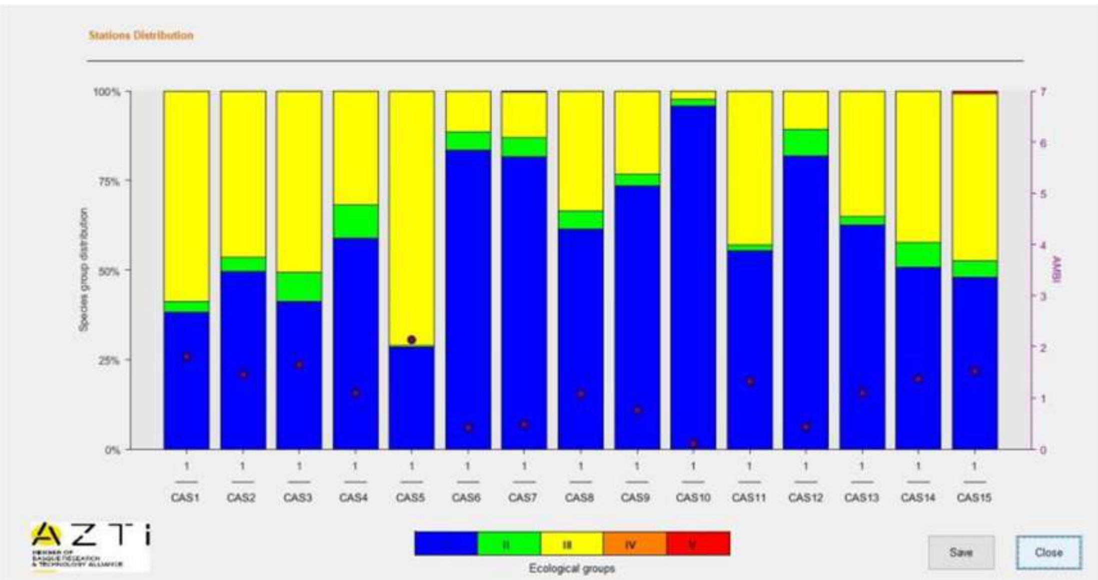


Figure 23. Percentage of ecological groups (EG) and AMBI values for every sampling station

Stations	I(%)	II(%)	III(%)	IV(%)	V(%)	Mean AMBI	Disturbance Classification
CAS1	38,2	2,9	58,7	0	0,2	1,816	Slightly disturbed
CAS2	49,6	3,8	46,5	0,1	0,1	1,458	Slightly disturbed
CAS3	41,2	8,2	50,6	0	0	1,642	Slightly disturbed
CAS4	58,9	9,3	31,8	0	0	1,094	Undisturbed
CAS5	28,5	0,5	71	0	0	2,137	Slightly disturbed
CAS6	83,5	5,1	11,4	0	0	0,418	Undisturbed
CAS7	81,5	5,3	12,9	0,3	0	0,479	Undisturbed
CAS8	61,3	5,2	33,3	0,2	0	1,085	Undisturbed
CAS9	73,4	3,4	23	0,2	0	0,75	Undisturbed
CAS10	95,7	1,9	2,3	0	0,1	0,103	Undisturbed
CAS11	55,3	1,6	42,9	0	0,2	1,323	Slightly disturbed
CAS12	81,7	7,6	10,7	0	0	0,436	Undisturbed
CAS13	62,6	2,2	35,2	0	0	1,09	Undisturbed
CAS14	50,7	6,9	42,3	0	0,1	1,376	Slightly disturbed
CAS15	47,8	4,6	46,7	0	0,8	1,519	Slightly disturbed

Table 4. Ecological groups (EG-I to V %), AMBI index and disturbance classification for every sampling station

The highest percentage of EG-I was recorded in CAS10 (95.7%), whereas 58.7% was recorded in CAS1 for EG-III (Table 4); on the opposite the species belonged to EG-IV and EG-V, indicating a pronounce unbalanced environment, were almost absent (Fig. 23, Table 4).
On the basis of AMBI values, 8 stations were classified as undisturbed cause EG-I species exceeded 48% of the total species, while 7 stations resulted slightly undisturbed due to EG-III species exceeding 42% of the total species (Table 4).
The hydromorphological stress class (HM) estimation, applied on all sampling sites, revealed 6 sites in pristine status (class 1), 5 in slightly altered status (class 2) and 4 in moderately altered status (class 3) (Table 5). The lowest value of total HM pressure was recorded in CAS10 (Miramare marine reserve), whereas the highest was detected in CAS12 (Saturnia e Nettunia), characterized by an artificial breakwater enclosing a bathing area.

HM pressure	CAS1	CAS2	CAS3	CAS4	CAS5	CAS6	CAS7	CAS8	CAS9	CAS10	CAS11	CAS12	CAS13	CAS14	CAS15
Material	0,00	0,00	0,00	0,00	0,00	1,00	1,00	1,00	1,00	0,00	1,00	1,00	1,00	1,00	1,00
Texture	1,00	0,67	0,33	1,00	0,67	1,67	1,67	1,67	2,00	0,00	1,00	1,67	2,00	1,00	1,00
Structure	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	1,00	0,00	1,00	1,00	1,00	1,00	1,00
Sedimentation (visibility)	0,00	1,00	0,33	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,33	0,00	0,00	0,00
Water retention	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00
Artificially induced mobility (walking/bathing)	1,00	0,00	0,00	1,00	1,00	1,00	1,00	1,00	1,00	0,00	1,00	2,00	1,00	1,00	1,00
Belt length	0,00	1,00	0,67	0,00	0,00	0,00	0,00	0,67	1,00	0,00	1,00	1,00	1,00	0,33	0,33
Total HM pressure	2,00	2,67	1,33	2,00	1,67	3,67	3,67	5,33	6,00	0,00	6,00	8,00	6,00	4,33	4,33
Class	1	1	1	1	1	2	2	2	3	1	3	3	3	2	2

Table 5. Hydromorphological (HM) pressures and total HM pressure class

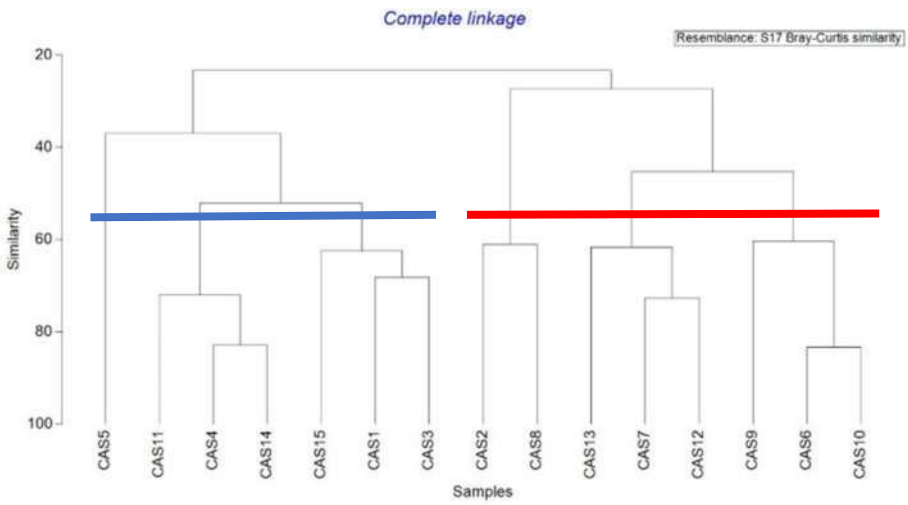


Figure 24. Dendrogram with clusters of sampling stations considering the whole intertidal community (UML+LML+UIL); blue line (group A), red line (group B)

The dendrogram obtained by considering the whole community in every station showed two groups, where stations were mostly clustered into undisturbed (group B) and slightly disturbed (group A) on the basis of AMBI, excepting for CAS2 and CAS4 (Fig. 24). ANOSIM applied to AMBI classes revealed in fact significant differences between undisturbed and slightly undisturbed groups (R=0.435, 0.05%), whereas no differences were detected among HM classes (R=-0.004, 47%).
Simper analysis performed on group A and B, identified by the dendrogram of Fig. 24, showed that Group Ais mainly characterized by higher average abundance of *M. galloprovincialis*, whereas abundance of *C. montagui* was comparable between groups (Table 6). *M. galloprovincialis* is considered as EG-III species, while *C. montagui* as EG-I and this could partially explain the correspondence between AMBI index and clusters of the dendrogram. In this term a positive Pearson linear correlation (r=0.77; p≤0.001) was found between AMBI values and abundance of *M. galloprovincialis*.

Groups A & B
Average dissimilarity = 55,09

Species	Group A Av.Abund	Group B Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
Mytilus galloprovincialis	738	108,08	23,39	1,87	42,46	42,46
Chthamalus montagui	483,24	507,54	11,26	1,47	20,44	62,91
Corophiidae indet.	24,95	39,5	2,11	0,69	3,83	66,73
Mytilaster minimus	43,62	2,13	1,9	0,69	3,45	70,19

Table 6. SIMPER analysis between group A and B

51

Table S1. List of species and their occurrence on every belt: constant (C), frequent (F) and temporary (T)

Phylum	species	UML	LML	UIL
POR	Aplysina aerophoba (Nardo, 1833)			T
POR	Chondrosia reniformis Nardo, 1847			T
POR	Cliona viridis (Schmidt, 1862)			T
POR	Geodia conchilega Schmidt, 1862			T
POR	Ircinia variabilis (Schmidt, 1862)			T
POR	Polymastia mamillaris (Müller, 1806)			T
POR	Sarcotragus spinosulus Schmidt, 1862			T
POR	Spongia (Spongia) officinalis Linnaeus, 1759			T
POR	Sycon raphanus Schmidt, 1862			T
CNI	Actinia equina (Linnaeus, 1758)		T	T
CNI	Anemonia viridis (Forsskål, 1775)			T
PLA	Discocelis tigrina (Blanchard, 1847)		T	T
MOL	Acanthochitona fascicularis (Linnaeus, 1767)		T	F
MOL	Rhyssoplax olivacea (Spengler, 1797)			T
MOL	Alvania discors (T. Brown, 1818)		T	F
MOL	Alvania dorbignyi (Audouin, 1826)			T
MOL	Alvania lanciae (Calcara, 1845)			T
MOL	Alvania lineata Risso, 1826		T	F
MOL	Alvania mamillata Risso, 1826			T
MOL	Alvania strangei (Brazier in Henn, 1894)			T
MOL	Bittium latreillii (Payraudeau, 1826)		T	C
MOL	Bittium reticulatum (da Costa, 1778)		T	T
MOL	Cerithiopsis petanii Prkic & Mariottini, 2010			T
MOL	Cerithium renovatum Monterosato, 1884			T
MOL	Cerithium vulgatum Bruguière, 1792		T	T
MOL	Columbella rustica (Linnaeus, 1758)		T	T
MOL	Dendropoma cristatum (Biondi, 1859)		T	T
MOL	Felimare villafranca (Risso, 1818)			T
MOL	Gibbula ardens (Salis Marschlins, 1793)		T	T
MOL	Gracilipurpura craticulata B & D, 1882			T
MOL	Hexaplex trunculus (Linnaeus, 1758)		T	C
MOL	Jujubinus exasperatus (Pennant, 1777)	T	T	F
MOL	Mangelia brusinae van Aartsen & Fehr-de Wal, 1978		T	T
MOL	Mangelia unifasciata (Deshayes, 1835)			T
MOL	Melarhaphe neritoides (Linnaeus, 1758)	F	T	
MOL	Mitrella scripta (Linnaeus, 1758)			T

Phylum	species	UML	LML	UIL
MOL	Ocenebra edwardsii (Payraudeau, 1826)		T	T
MOL	Patella caerulea Linnaeus, 1758	F	C	T
MOL	Phorcus mutabilis (Philippi, 1851)		T	
MOL	Phorcus turbinatus (Born, 1778)	T	T	
MOL	Pisania striata (Gmelin, 1791)		T	T
MOL	Pusia ebenus (Lamarck, 1811)			T
MOL	Rissoa guerinii Récluz, 1843			T
MOL	Rissoa membranacea (J. Adams, 1800)			T
MOL	Rissoa monodonta Philippi, 1836		T	T
MOL	Rissoa similis Scacchi, 1836			T
MOL	Rissoa splendida Eichwald, 1830			T
MOL	Rissoa variabilis (Megerle von Mühlfeld, 1824) cf.			T
MOL	Steromphala adansonii (Payraudeau, 1826)			T
MOL	Steromphala albida (Gmelin, 1791)			T
MOL	Steromphala divaricata (Linnaeus, 1758)			T
MOL	Steromphala racketti (Payraudeau, 1826)			T
MOL	Steromphala rarilineata (Michaud, 1829)		T	T
MOL	Steromphala umbilicalis (da Costa, 1778) cf.	T	T	T
MOL	Steromphala varia (Linnaeus, 1758) cf.		T	T
MOL	Tricolia pullus (Linnaeus, 1758)			T
MOL	Tritia corniculum (Olivi, 1792)		T	F
MOL	Tritia cuvierii (Payraudeau, 1826)			T
MOL	Tritia incrassata (Strøm, 1768)		T	F
MOL	Tritia reticulata (Linnaeus, 1758)			T
MOL	Abra alba (W. Wood, 1802)		T	T
MOL	Acanthocardia aculeata (Linnaeus, 1758)			T
MOL	Acanthocardia tuberculata (Linnaeus, 1758)			F
MOL	Anomia ephippium Linnaeus, 1758		T	T
MOL	Arca noae Linnaeus, 1758			T
MOL	Arcopagia crassa (Pennant, 1777)			T
MOL	Astarte sulcata (da Costa, 1778)			T
MOL	Chama gryphoides Linnaeus, 1758			T
MOL	Hiatella arctica (Linnaeus, 1767)		T	T
MOL	Hiatella rugosa (Linnaeus, 1767)		T	C
MOL	Irus irus (Linnaeus, 1758)		T	F
MOL	Kurtiella bidentata (Montagu, 1803)			T
MOL	Lasaea rubra (Montagu, 1803)		T	
MOL	Limaria hians (Gmelin, 1791)			T
MOL	Lithophaga lithophaga (Linnaeus, 1758)			T
MOL	Mimachlamys varia (Linnaeus, 1758)			T

Phylum	species	UML	LML	UIL
MOL	Modiolus barbatus (Linnaeus, 1758)		T	C
MOL	Musculus costulatus (Risso, 1826)			T
MOL	Musculus subpictus (Cantraine, 1835)		T	C
MOL	Mytilaster marioni (Locard, 1889)		T	T
MOL	Mytilaster minimus (Poli, 1795)	T	T	T
MOL	Mytilus galloprovincialis Lamarck, 1819	T	C	C
MOL	Neopycnodonte cochlear (Poli, 1795)			T
MOL	Ostrea edulis Linnaeus, 1758		T	T
MOL	Ostrea stentina Payraudeau, 1826		T	T
MOL	Petricola lithophaga (Retzius, 1788)			F
MOL	Placamen lamellosum (G. B. Sowerby I, 1825)			T
MOL	Polititapes aureus (Gmelin, 1791)			T
MOL	Pseudochama gryphina (Lamarck, 1819)			T
MOL	Rocellaria dubia (Pennant, 1777)		T	C
MOL	Ruditapes decussatus (Linnaeus, 1758)			T
MOL	Spondylus gaederopus Linnaeus, 1758			T
MOL	Talochlamys multistriata (Poli, 1795)			T
MOL	Varicorbula gibba (Olivi, 1792)			T
MOL	Venus verrucosa Linnaeus, 1758			T
ANN	Amphiglena mediterranea (Leydig, 1851)			T
ANN	Amphitritides gracilis (Grube, 1860)		T	T
ANN	Arabella iricolor (Montagu, 1804)			T
ANN	Capitella capitata (Fabricius, 1780)		T	T
ANN	Ceratonereis (Composetia) costae (Grube, 1840)		T	T
ANN	Cirriformia tentaculata (Montagu, 1808)		T	T
ANN	Desdemona cf. ornata Banse, 1957			T
ANN	Eumida punctifera (Grube, 1860)		T	
ANN	Eunice pennata (Müller, 1776)			T
ANN	Eunice vittata (Delle Chiaje, 1828)			T
ANN	Hydroides elegans (Haswell, 1883)			T
ANN	Hydroides pseudouncinata Zibrowius, 1968			T
ANN	Hypsicomus cf. stichophthalmos (Grube, 1863)			T
ANN	Lumbrineris coccinea (Renier, 1804)			T
ANN	Lumbrineris latreilli Audouin & Milne Edwards, 1833		T	T
ANN	Lysidice ninetta Audouin & H Milne Edwards, 1833			F
ANN	Lysidice unicornis (Grube, 1840)		T	T
ANN	Marphysa sanguinea (Montagu, 1813)			T
ANN	Nereis pelagica Linnaeus, 1758		T	T
ANN	Nereis rava Ehlers, 1868		T	T
ANN	Nereis zonata Malmgren, 1867		T	C

Phylum	species	UML	LML	UIL
ANN	Palola siciliensis (Grube, 1840)			T
ANN	Paucibranchia bellii (Audouin & Milne Edwards, 1833)			T
ANN	Perinereis cultrifera (Grube, 1840)		T	F
ANN	Platynereis dumerilii (Audouin & Milne Edwards, 1833)			F
ANN	Polyophthalmus pictus (Dujardin, 1839)			T
ANN	Sabellaria spinulosa (Leuckart, 1849)			T
ANN	Serpula vermicularis Linnaeus, 1767			T
ANN	Spirobranchus polytrema (Philippi, 1844)			T
ANN	Spirobranchus triqueter (Linnaeus, 1758)			F
ANN	Syllis alternata Moore, 1908			T
ANN	Syllis amica Quatrefages, 1866		T	T
ANN	Syllis armillaris (O.F. Müller, 1776)		T	T
ANN	Syllis compacta Gravier, 1900		T	T
ANN	Syllis gerlachi (Hartmann-Schröder, 1960)		T	T
ANN	Syllis gracilis Grube, 1840			T
ANN	Syllis hyalina Grube, 1863		T	
ANN	Syllis krohnii Ehlers, 1864		T	
ANN	Syllis pectinans Haswell, 1920		T	
ANN	Syllis pulvinata (Langerhans, 1881) cf.		T	
ANN	Syllis rosea (Langerhans, 1879)		T	T
ANN	Syllis tyrrhena (Licher & Kuper, 1998)		T	
ANN	Syllis variegata Grube, 1860		T	
ANN	Syllis vittata Grube, 1840		T	
ANN	Terebellides stroemii Sars, 1835		T	
ANN	Trypanosyllis aeolis Langerhans, 1879		T	
ANN	Vermiliopsis striaticeps (Grube, 1862)			T
ART	Achelia echinata Hodge, 1864			T
ART	Ampithoe ramondi Audouin, 1826		T	T
ART	Apocorophium acutum (Chevreux, 1908)		T	F
ART	Apohyale crassipes (Heller, 1866)		T	T
ART	Apohyale perieri (Lucas, 1846)		T	
ART	Apseudopsis latreillii (Milne Edwards, 1828)			T
ART	Athanas nitescens (Leach, 1814 [in Leach, 1813-1815])			T
ART	Balanus balanus (Linnaeus, 1758)		T	T
ART	Caprella acanthifera Leach, 1814			T
ART	Caprella scaura Templeton, 1836			T
ART	Chondrochelia savignyi (Kroyer, 1842)		T	F
ART	Chthamalus montagui Southward, 1976	C	T	T
ART	Chthamalus stellatus (Poli, 1791)	T	T	
ART	Cyathura carinata (Krøyer, 1847)			T

Phylum	species	UML	LML	UIL
ART	Cymodoce truncata Leach, 1814			T
ART	Dexamine spiniventris (Costa, 1853)			C
ART	Dexamine spinosa (Montagu, 1813)			T
ART	Dynamene bidentata (Adams, 1800)		T	F
ART	Elasmopus brasiliensis (Dana, 1853)		T	T
ART	Eriphia verrucosa (Forskål, 1775)	T		
ART	Eualus cranchii (Leach, 1817)			T
ART	Gammaropsis maculata (Johnston, 1828)			T
ART	Ianiropsis serricaudis Gurjanova, 1936			T
ART	Janira maculosa Leach, 1814			T
ART	Jassa marmorata Holmes, 1905			T
ART	Lekanesphaera rugicauda (Leach, 1814)		T	
ART	Leptocheirus pilosus Zaddach, 1844			T
ART	Ligia italica Fabricius, 1798	T		
ART	Lysianassa costae H. Milne Edwards, 1830			F
ART	Melita palmata (Montagu, 1804)			T
ART	Microdeutopus chelifer (Bate, 1872)			T
ART	Microeuraphia depressa (Poli, 1791)	T		
ART	Monocorophium sextonae (Crawford, 1937)		T	F
ART	Nymphon brevirostre Hodge, 1863			T
ART	Pachygrapsus marmoratus (J.C. Fabricius, 1787)		T	T
ART	Palaemon elegans Rathke, 1836			T
ART	Paragnathia formica (Hesse, 1864)			T
ART	Paranthura japonica Richardson, 1909		T	C
ART	Parapseudes latifrons (Grube, 1864)		T	T
ART	Parhyale plumicornis (Heller, 1866)		T	T
ART	Perforatus perforatus (Bruguière, 1789)	T	F	T
ART	Phtisica marina Slabber, 1769			T
ART	Pilumnus hirtellus (Linnaeus, 1761)			T
ART	Processa macrophthalma Nouvel & Holthuis, 1957			T
ART	Protohyale (Boreohyale) camptonyx (Heller, 1866)		T	T
ART	Stenothoe cf. monoculoides (Montagu, 1813)			T
ART	Tanais dulongii (Audouin, 1826)		T	T
ART	Uromunna petiti (Amar, 1948)			T
BRY	Schizobrachiella sanguinea (Norman, 1868)		T	T
ECH	Amphipholis squamata (Delle Chiaje, 1828)			T
ECH	Amphiura chiajei Forbes, 1843		T	F
ECH	Holothuria (Holothuria) helleri Marenzeller von, 1877			T
ECH	Ophioderma longicaudum (Bruzelius, 1805)		T	F
ECH	Paracentrotus lividus (Lamarck, 1816)			T

Phylum	species	UML	LML	UIL
CHO	Pyura microcosmus (Savigny, 1816)			T

On regard the visual census activity, no specimens of the brown algae *Fucus virsoides* were recorded in the whole study area.
33 species of fish fauna were recorded, belonging to 9 families (Table 7). Blennidae was the richest in term of recorded species (11 sp.), followed by Sparidae (7 sp.), Gobiidae (5 sp.) and Labridae (4 sp.), together accounting 82% of the total species in the sampling stations. *Atherina boyeri* was recorded in all sampling sites, whereas 10 species were constantly recorded in the whole study area; among them, it was interesting the presence of the giant goby (Gobius cobitis). Overall, 13 species were frequent and 9 were temporary.

Family	species	occurrence		Family	species	occurrence
Atherinidae	*Atherina boyeri*	P		Sparidae	*Diplodus puntazzo*	F
				Gobiidae	*Gobius fallax crf*	F
Serranidae	*Serranus scriba*	C		Labridae	*Symphodus tinca*	F
Sparidae	*Diplodus vulgaris*	C		Sparidae	*Diplodus sargus*	F
Blennidae	*Microlipophrys dalmatinus*	C		Blennidae	*Salaria pavo*	F
Blennidae	*Parablennius sanguinolentus*	C		Sparidae	*Diplodus annularis*	F
Sparidae	*Sarpa salpa*	C		Blennidae	*Lipophrys trigloides*	F
Blennidae	*Aidablennius sphynx*	C		Blennidae	*Microlipophrys adriaticus*	F
Gobiidae	*Gobius cobitis*	C		Blennidae	*Microlipophrys canevae*	F
Blennidae	*Parablennius tentacularis*	C		Blennidae	*Parablennius incognitus*	F
Atherinidae	*Atherina hepsetus*	C		Moronidae	*Dicentrarchus labrax*	F
Tripterygiidae	*Tripterygion tripteronotum*	C		Sparidae	*Oblada melanura*	F
				Sparidae	*Sparus aurata*	F
				Belonidae	*Belone belone*	T
				Gobiidae	*Pomatoschistus sp.*	T
				Blennidae	*Coryphoblennius galerita*	T
				Labridae	*Labrus merula*	T
				Labridae	*Symphodus cinereus*	T
				Gobiidae	*Gobius cruentatus*	T
				Gobiidae	*Gobius niger*	T
				Labridae	*Labrus viridis*	T
				Blennidae	*Parablennius gattorugine*	T

Table 7. Fish fauna species and their occurrence in the study area: permanent (P), constant (C), frequent (F) and temporary (T)

Any effects due to lockdown were not detectable on intertidal community during the sampling period within April and September 2021.

P2. Transitional (e.g. Goro area and Bevano Mouth) and coastal areas in Emilia Romagna (IT)

Sacca di Goro and Lago delle Nazioni

A monthly monitoring activity has been carried out to examine phytoplankton communities and investigate their dynamics. Both in-situ physical parameters and biological samples have been collected in four sites for Sacca di Goro: Porto Gorino (PG), Gorino (G), Bocca Mare (BM) and Foce Volano (FV), and in the southern part of Lago delle Nazioni (figure 25).

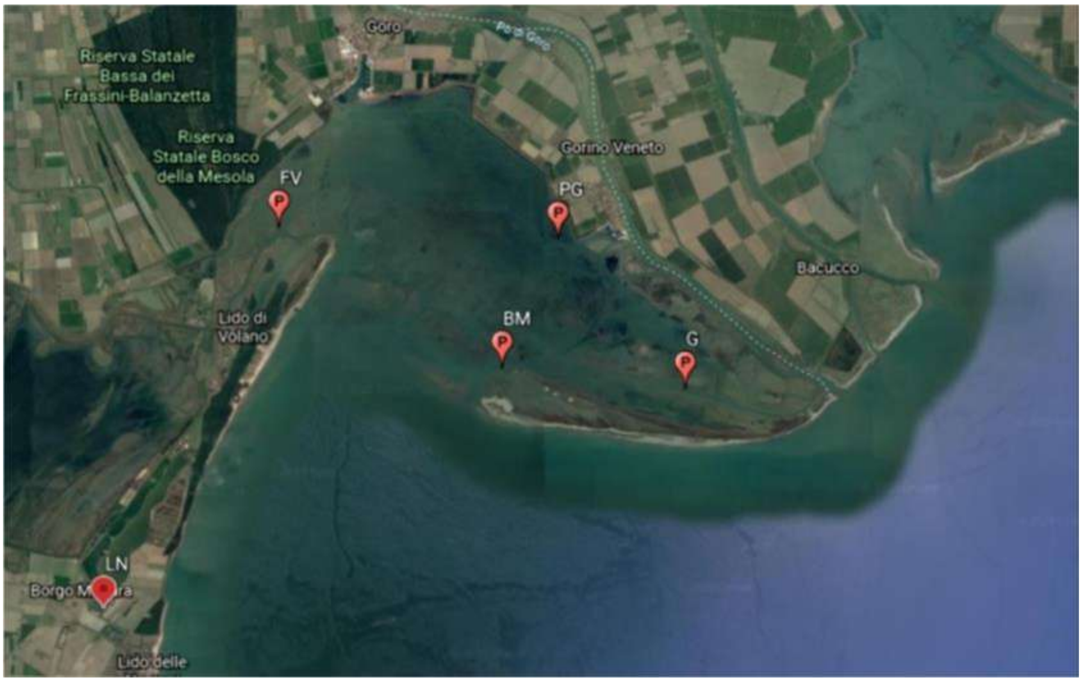


Figure 25. Monitoring areas in Sacca di Goro and Lago delle Nazioni

Macrobenthic assemblages were sampled 4 times to cover the different seasonal conditions (i.e.: October 2020, March 2021, June 2021, February 2022) in the same sites, except Porto Gorino that was replaced with a more central site (Centro Sacca, CS) for consistency with past monitoring.

During WP4 monitoring activities in the Sacca di Goro have become less frequent, since September 2021 the area was monitored every 3 months. Lago delle Nazioni did not undergo a reduction of monitoring frequency instead. In-situ measurements were made for temperature, salinity, dissolved oxygen (DO) and depth.

Within the Sacca di Goro temperature ranged from a minimal average value of 7.6 °C in December to a maximal value of 29°C during summer (July 2020 and June 2022); temperature did not change among different sites during the monitoring program. Salinity reflected the strong influence of the freshwater inputs from the rivers in the external parts of the lagoon. Foce Volano (FV) and Gorino (G) have the lowest values, with an average of 11 and a minimum peak of 0 in FV in May 2021. On the other side, Porto Gorino (PG) and Bocca Mare (BM), due to their central position and connection to the sea, showed average values of 24 with maximum peak of 35 during April 2022. Ecologically critical oxygen concentrations (2-3 mg L^{-1}) were found at the station FV in the late spring period (May-June 2021) and at the station PG in June 2022, with a minimum peak of 2,25 mg L^{-1}, while an intermediate condition (DO 3-5 mg L^{-1}) was found during 2020 and 2021 autumn season and during summer 2021 and 2022 in station FV and during summer 2021 in station PG. Oxygen depletion is more commonly observed as a consequence of the sinking and decay of blooms. Microbial respiration (e.g., nitrification) may deplete the water column and sediments of dissolved oxygen in the presence of organic matter (Best et al., 2007). Oxygen usually ranged from 6 to 14 mg L^{-1} in the water, a shortage of DO – hypoxia (below 2-3 mg L^{-1}) or anoxia (<0.5 mg L^{-1}) - can have dramatic impacts on marine life conditions and affect the ecosystems, including direct loss of habitat or habitat compression, altered trophic relationships, changes in migration patterns, and changes in biodiversity (NOAA). Overall, FV represents the most sensitive site strongly influenced by the freshwater input of the Po di Volano, which could negatively affect the life of benthic organisms.

On the other side Lago delle Nazioni showed across the first year a steady-state situation of salinity due to its limited water exchange (22); however, during the last year of monitoring, salinity values increased reaching 25 during July 2022. Temperature ranged from a minimum of 5.5 °C (January 2022) to a maximum of 29°C (July 2022). Dissolved oxygen normally ranged from 7 to 14 mg L^{-1} with an average concentration of 10 mg L^{-1}. A critical oxygen depletion has been attained during May 2022 with a value of 3.6 mg L^{-1}.

Surface water samples have also been collected along with in-situ measurements of physical parameters. These have enabled several analyses to explore water quality such as dissolved inorganic nitrogen (DIN) and reactive phosphorous (RP) and characterize microalgal community trough chlorophyll-a, phaeopigments, particulate organic carbon (POC), total particulate nitrogen (TPN) and intracellular phosphorous. Water samples fixed with Lugol's iodine solution have been analysed with the Utermhöl method (Utermöhl, 1931) to determine phytoplankton abundances.

59

Sacca di Goro showed high variability in the phytoplankton composition. Any anomalous bloom events was registered during the sampling period, except for the proliferation of *Heterosigma akashiwo* (Y.Hada) in October 2020 (data not personally recorded).

Foce Volano (FV) has been one of the most variable sites, reflecting the strong freshwater influence. Chlorophyceae reached a maximum relative abundance of 72% (December 2020) with an average of 32% through the year. Diatoms were always accounted with an average abundance of 25% and a peak during winter/spring 48% (March 2021). Total average abundances were 2 x 10^6 cells L^{-1}, with peaks in summer and spring. Bocca Mare (BM), due to its position alongside the Adriatic Sea, showed typically marine species, with a dominance of diatoms (42%) followed by cryptophytes (27%). Diatoms were represented mainly by *Chaetoceros* spp. and *Skeletonema* spp., the latter is a species common to proliferate during the winter. Total abundances ranged from a min value of 2.4x 10^5 cells L^{-1} in (December 2020) to 2.7 x 10^6 cells L^{-1} in spring. Porto Gorino (PG), along the year, showed a dominance of diatoms (45%) followed by cryptophytes (30%). Along the sampling time, it has been recorded a peak of phytoplankton abundance in April 2021 of 1.1 x 10^7 cells L^{-1} due to the seasonal *Skeletonema* bloom. Gorino (G) showed a predominance of diatoms (39%) followed by cryptophytes (28%). The diatom group was widely represented by benthic Naviculales (5-80%). The average abundances were 1.2 x 10^6 cell L^{-1}, with a peak of 2.5 x 10^6 cell L^{-1} in June 2020.

Macrobenthic assemblages were mainly composed by polychaetas (e.g.: *Streblospio shrubsolii*, *Hediste diversicolor*, *Alitta succinea*), amphipods (e.g., *Corophium orientale*, *Microdeutopus anomalus*), hydrobiids snails and clams (e.g., *Cerastoderma glaucum*, *Rutitapes phylippinarum*). Compared to historical data (1984-1985), species richness has undergone a dramatic reduction (30-50%), especially in Gorino (G) and Centro Sacca (CS). This reduction in diversity has been accompanied by a profound change in the community structures. Basically, 30 species have resulted locally extinct, 16 have significantly decreased in abundance, 10 new species have colonized the lagoon and of these 4 are non-indigenous species. The average ratio of non-indigenous to native species increased from 3.8% to 12.5%.

Lago delle Nazioni is characterized by high chlorophyll-*a* concentration with the dominance of picoplanktonic species that were not quantified during the monitoring; among the micro-phytoplankton, the dominant group in the lake was represented by Dinophyceae (about 60%) followed by cryptophytes and diatoms. Total average abundances were 1.3 x 10^6 cell L^{-1}. The presence of algal cells with large dimensions was also recorder, like *Nitzschia* spp., *Dinophysis* sp., *Ceratium fusus* and dinocysts. In addition, it was observed a shift to zooplankton communities with the dominance of small species (ciliates and rotifers), which feed on other resources, like bacteria or picoplankton. Algal blooms have been observed in Lago delle Nazioni; the first bloom occurred before the monitoring program began, between December 2019 and January 2020 caused by the dinoflagellate *Prorocentrum cordatum* that reached 4.7 x 10^7 cells L^{-1}. Another algal bloom occurred between autumn 2020 and spring 2021 and lasted for several months. This bloom was caused by an unidentified species (<2μm) probably belonging to the green-algae group. These events completely overwhelmed other phytoplanktonic species and resulted into noticeable water discoloration events (figure 26).

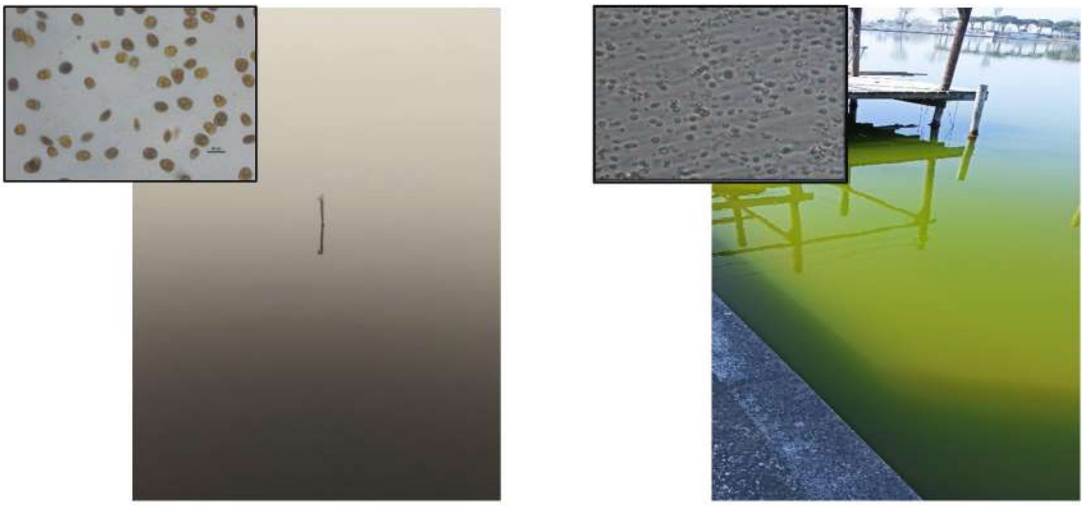


Figure 9. Water discoloration caused by Prorocentrum minutum (left) and an unidentified green algae (right).

Concerning laboratory analyses, chl-*a* concentration, station FV was the most variable, ranging from 0.4 ± 0.2 µg L^{-1} during winter 2020 to 21.3 ± 4.5 µg L^{-1} in late summer. The other stations (PG – G – BM) except for high values in summer (July 2020), did not exceed 8 µg L^{-1} along the first year, while values between 17-20 µg L^{-1} occurred during late spring-summer 2022 in station PG and G. Overall, based on the OECD criteria (1982), the mean chl-*a* concentration determined in the Sacca di Goro ranged from a mesotrophic (means 2.5 – 8 µg L^{-1}) to eutrophic (means 8 – 25 µg L^{-1}) condition.

Lago delle Nazioni showed high chlorophyll concentrations along the two years period, ranging from a minimum value of 7.9 ± 0.8 µg L^{-1} in July 2020 to a maximum of 67.3 ± 10.5 µg L^{-1} during the spring season (May 2021). Based on OECD criteria, Lago delle Nazioni showed constant hypereutrophic conditions where massive proliferation of phytoplankton can cause dystrophic events.

Regarding inorganic nutrients in the Sacca di Goro, total inorganic nitrogen along the year ranged from a minimum average value of 0.03 ± 0.02 mg L^{-1} in July 2022 in the station Gorino (G) to a maximum of 3.6 ± 0.1 mg L^{-1} in May 2021 in the station Foce Volano (FV), corresponding to critic level of oxygen concentration and low salinity; presumably a high volume of freshwater inputs arriving from rivers have led to this increase. On the same ground Gorino (G) station often showed the highest N concentration, due to its enclosed position and freshwater inputs from the Po di Goro River. The other stations showed comparable patterns with fluctuation about 0.5 to 1.0 mg L^{-1}.

Soluble reactive phosphorous (RP) showed different concentrations among the stations and times. The maximum value was recorded in December 2020 in the Gorino station (G) reporting 53.9 ± 5.7 µg L^{-1}. Overall, mean values of RP ranged from <10 to 40 µg L^{-1}.

Lago delle Nazioni showed very low concentrations of both N and P: from 0 to 25.3 µg L^{-1} and 0.2 to 10.5 µg L^{-1}, respectively; as a result of algae uptake, there was a depletion of inorganic nutrients in the upper water layer, thus the concentrations were very low (<15 µg L^{-1}).

Particulate organic carbon, total particulate nitrogen and intracellular phosphorous have been extracted from algal cells. The concentration of organic C followed a temporal distribution with the highest values during the autumn and spring season and the lowest during winter; similar pattern is spatially distributed in the four sampling sites. FV showed the highest and variable values than other stations, ranging from 3.3 ± 0.3 mg C L^{-1} (Sept. 2020) to 0.5 ± 0.2 mg C L^{-1} (Dec. 2020). Likewise, concentration of organic N followed the same trends, resulting in a good removal and transformation of matter and subsequent incorporation trough the food web.

Lago delle Nazioni particulate matter showed a higher C content than those measured in the lagoon, ranging from 2.0 ± 0.01 mg C L^{-1} (Dec. 2020) to 11.6 ± 0.5 mg C L^{-1} (May 2021). A similar pattern is showed for the N content that ranged from 0.2 ± 0.01 (Dec. 2020) to 3.8 ± 0.8 mg N L^{-1} (Apr. 2021).

Neither positive nor negative effects due to lockdown conditions have been observed in the areas.

P3. Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT)

Chemical parameters

UniSalento collected already available data regarding the chemical parameters of Pilot Area 3, organizing them with metadata. Data were collected from Final Reports and/or dataset of:

- Surface Water body monitoring service of Arpa Puglia (from 2010 to 2018);
- ECODO-NET INTERREG IIIA/ Greece – Italy project (September 2007 – April 2008).

Other data were collected from 2 papers:

Dell'anno A., Mei M. L., Ianni C. and Danovaro R. "Impact of bioavailable heavy metals on bacterial activities in coastal marine sediments". *World Journal of Microbiology & Biotechnology.* 2003, 19, p. 93-100;

Matteucci G., Magagnini M., Armeni M., Giaccaglia L., Fiesoletti F., Ciotti C., Pari P., Riccio S., Rossini P., Ambrosini P., Buongarzone E., Patata L., Trovarelli L. and Tentoni P. "Ecological assessment of a Marine Coastal Area Affected by a Power Plant Water Discharge (Brindisi, Adriatic Sea). *The Open Environmental & Biological Monitoring Journal.* 2011, 4, p. 45-56.

The water and sediment samples collected in Pilot 3 were analysed for:

- heavy metals,
- chlorinated pesticides
- microplastics.

Chlorinated pesticides were always below the limit of quantification, so in the present document only the list of the determined pesticides is reported. In the following tables the main results are collected.

eventDate	Matrix	latitude	longitude	station name	unit	As	Cd	Cr Tot	Cu	Hg	Ni	Pb	Zn
28/07/21	sediment	40,7050	17,8071	TG1	mg/kg d.w.	1,5	<LOQ	5,4	1,0	<LOQ	1,4	1,8	15,1
28/07/21	sediment	40,7099	17,8065	TG2	mg/kg d.w.	6,3	<LOQ	5,7	0,8	<LOQ	1,5	9,5	9,5
28/07/21	sediment	40,7166	17,8049	TG2B	mg/kg d.w.	9,4	<LOQ	14,8	2,0	<LOQ	3,0	3,4	25,9
28/07/21	sediment	40,7153	17,8032	TG3	mg/kg d.w.	13,2	<LOQ	7,9	0,9	<LOQ	2,1	1,9	6,0
28/07/21	sediment	40,7075	17,8029	BAY2	mg/kg d.w.	12,3	0,29	24,6	3,1	<LOQ	5,9	3,7	16,6
22/12/21	sediment	40,3223	18,3824	SF1	mg/kg d.w.	1,8	<LOQ	4,9	0,5	<LOQ	1,2	1,2	5,7
22/12/21	sediment	40,3207	18,3835	SF2	mg/kg d.w.	1,9	<LOQ	5,0	0,4	<LOQ	1,0	1,3	7,7
22/12/21	sediment	40,3191	18,3847	SF3	mg/kg d.w.	1,7	<LOQ	5,0	0,5	<LOQ	1,1	1,5	5,9
14/02/22	sediment	40,6050	18,0358	PC1	mg/kg d.w.	4,2	<LOQ	3,4	0,8	0,2	1,3	1,6	4,6
14/02/22	sediment	40,6081	18,0377	PC2	mg/kg d.w.	6,1	<LOQ	7,1	1,6	0,4	2,7	2,9	28,7
14/02/22	sediment	40,6138	18,0376	PC2B	mg/kg d.w.	6,2	<LOQ	4,4	1,1	0,1	1,4	2,3	9,0
14/02/22	sediment	40,6051	18,0417	PC3	mg/kg d.w.	14,1	<LOQ	6,0	1,0	0,3	1,8	2,6	7,4
14/02/22	sediment	40,6045	18,0479	PC3B	mg/kg d.w.	41,8	<LOQ	22,0	3,1	0,6	5,3	5,7	99,2
28/07/21	water	40,7050	17,8071	TG1	µg/l	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
28/07/21	water	40,7099	17,8065	TG2	µg/l	0,3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
28/07/21	water	40,7166	17,8049	TG2B	µg/l	0,5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
28/07/21	water	40,7153	17,8032	TG3	µg/l	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
28/07/21	water	40,7075	17,8029	BAY2	µg/l	0,4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
22/12/21	water	40,3223	18,3824	SF1	µg/l	1,2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

22/12/21	water	40,3207	18,3835	SF2	µg/l	0,7	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
22/12/21	water	40,3191	18,3847	SF3	µg/l	0,6	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14/02/22	water	40,6050	18,0358	PC1	µg/l	0,4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14/02/22	water	40,6081	18,0377	PC2	µg/l	0,4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14/02/22	water	40,6138	18,0376	PC2B	µg/l	0,4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14/02/22	water	40,6051	18,0417	PC3	µg/l	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
14/02/22	water	40,6045	18,0479	PC3B	µg/l	<LOQ	<LOQ	1,3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Table 8. Results of heavy metals searched in seawater samples from Pilot 3 area.

Aldrin
Atrazina
Alpha-BHC
Beta-BHC
Delta-BHC
Gamma-BHC
4,4'-DDD
4,4'-DDE
4,4'-DDT
Dieldrin
Endosulfan I
Endosulfan II
Endosulfan Sulfate
Endrin
Endrin Aldehyde
Heptachlor
Heptachlor Epoxide isomero B
Methoxychlor

Table 9. List of pesticides searched in seawater samples from Pilot 3 area.

Event Date	**Matrix**	**Decimal latitude**	**Decimal longitude**	**Station name**	**unit**	**<50**	**50 - 100**	**100 - 150**	**150 - 300**	**300 - 500**	**>500**
28/07/21	sediment	40,7050	17,8071	TG1	item/30 g dry sediment	2	3	3	3	10	18
28/07/21	sediment	40,7099	17,8065	TG2	item/30 g d.s.	0	8	1	7	8	12
28/07/21	sediment	40,7166	17,8049	TG2B	item/30 g d.s.	2	10	16	8	15	20
28/07/21	sediment	40,7153	17,8032	TG3	item/30 g d.s.	0	2	4	9	8	18
28/07/21	sediment	40,7075	17,8029	BAY2	item/30 g d.s.	0	3	8	17	12	16
22/12/21	sediment	40,3223	18,3824	SF1	item/30 g d.s.	0	10	5	12	12	17
22/12/21	sediment	40,3207	18,3835	SF2	item/30 g d.s.	1	11	4	14	14	20
22/12/21	sediment	40,3191	18,3847	SF3	item/30 g d.s.	0	8	6	9	14	24
14/02/22	sediment	40,6050	18,0358	PC1	item/30 g d.s.	3	13	13	26	24	20
14/02/22	sediment	40,6081	18,0377	PC2	item/30 g d.s.	0	4	5	6	5	15

14/02/22	sediment	40,6138	18,0376	PC2B	item/30 g d.s.	0	2	4	5	6	21
14/02/22	sediment	40,6051	18,0417	PC3	item/30 g d.s.	1	2	5	18	15	31
14/02/22	sediment	40,6045	18,0479	PC3B	item/30 g d.s.	0	1	1	2	2	8
28/07/21	water	40,7050	17,8071	TG1	item/L seawater	5	8	5	3	5	6
28/07/21	water	40,7099	17,8065	TG2	item/L seawater	0	2	6	11	16	20
28/07/21	water	40,7166	17,8049	TG2B	item/L seawater	0	5	2	7	8	14
28/07/21	water	40,7153	17,8032	TG3	item/L seawater	3	5	4	10	21	20
28/07/21	water	40,7075	17,8029	BAY2	item/L seawater	4	11	5	8	6	19
22/12/21	water	40,3223	18,3824	SF1	item/L seawater	1	1	2	2	4	10
22/12/21	water	40,3207	18,3835	SF2	item/L seawater	1	6	9	14	20	15
22/12/21	water	40,3191	18,3847	SF3	item/L seawater	0	9	6	7	9	14
14/02/22	water	40,6050	18,0358	PC1	item/L seawater	0	6	12	17	22	50
14/02/22	water	40,6081	18,0377	PC2	item/L seawater	1	3	4	4	2	10
14/02/22	water	40,6138	18,0376	PC2B	item/L seawater	1	6	5	12	7	11
14/02/22	water	40,6051	18,0417	PC3	item/L seawater	1	1	2	0	2	8
14/02/22	water	40,6045	18,0479	PC3B	item/L seawater	1	8	9	30	6	33

Table 10. Results of microplastics determination in samples collected in Pilot 3

The chemical measurements did not evidence any measurable effect clearly attributable to the pandemic lockdown.

Bioindicators

Unisalento monitored Pilot 3 transitional water ecosystem status by using amphipods as bioindicators: samples belonging to the *Gammarus insensibilis* species were collected in spring from populations located in small basins. Animals sampled in a pristine area (Le Cesine) and/or stabulated in laboratory fish tanks were also used as controls. Measurements of the enzymatic activity of enzymes involved in both metabolism and antioxidant response systems were carried out. Antioxidant defence enzymatic activities, i.e. catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and metabolic enzymes ones, like cytochrome c oxidase (COX) and lactate dehydrogenase (LDH), were measured using lysates of the whole body of amphipods.
The results are collected in table 11 and on histograms **Figure** , **Figure** .

Enzymatic activity (EA) = nmol/min/mg prot
Specific activity (SA) = nmol/min x mg protein in 1 mg Gam
Specific activity (SA) = nmol/min x µg protein in 1 mg Gam

Sampling Date	Origin	Name	Gammarus (size)	SA Total xA-Prot/Gam	CAT H2O2 µmol/mi	µmol/min A-Prot/Gai	% SA	SOD Cyto-c red U/mg	U/mg -Prot/Ga	% SA	GPX NADPH nmol/min/	nmol/m -Prot/Ga	% SA	COX Cyto-c ox nmol/min/m	nmol/m -Prot/Ga	% SA	LDH-pyr mol/min/m equaz	NADH ox nmol/min/ A-Prot/Ga	% SA
15/03/22	*Sal Contessa*	G1	large	4,281	12,70	0,19	4,50	99,70	1,51	35,34	122,20	1,85	43,32	26,50	0,40	9,39	20,99	0,32	7,44
15/03/22	*Sal Contessa*	G2	large	4,809	13,80	0,39	8,10	47,20	1,33	27,70	77,30	2,18	45,37	15,20	0,43	8,92	16,89	0,48	9,91
15/03/22	*Sal Contessa*	M1	medium	6,412	15,00	0,48	7,46	53,50	1,71	26,60	89,40	2,85	44,45	13,00	0,41	6,46	30,21	0,96	15,02
15/03/22	*Sal Contessa*	M2	medium	6,013	7,10	0,28	4,63	41,30	1,62	26,92	75,90	2,98	49,48	14,60	0,57	9,52	14,50	0,57	9,45
15/03/22	*Sal Contessa*	P	small					51,10	4,34		114,10	9,70		11,30	0,96				
26/04/22	*Torre Guaceto*	G1 L	large	3,224	2,75	0,07	2,24	47,91	1,26	39,13	44,88	1,18	36,65	14,98	0,39	12,23	11,92	0,31	9,74
26/04/22	*Torre Guaceto*	G2 T	large	3,572	3,98	0,13	3,64	35,65	1,17	32,62	59,61	1,95	54,55				10,05	0,33	9,20
26/04/22	*Torre Guaceto*	M1 L	medium	3,867	5,08	0,13	3,46	43,10	1,14	29,36	58,62	1,54	39,94	33,20	0,87	22,62	6,77	0,18	4,61
26/04/22	*Torre Guaceto*	M2 T	medium	2,971	6,73	0,20	6,82	29,65	0,89	30,04	54,60	1,64	55,32				7,72	0,23	7,82
26/04/22	*Torre Guaceto*	P L	small	9,796	2,52	0,13	1,29	44,53	2,24	22,87	94,28	4,74	48,41	53,43	2,69	27,43			
04/04/22	*Cesine*	C1	large	6,971	37,11	1,07	15,40	65,60	1,90	27,22	29,30	0,85	12,16	106,50	3,08	44,19	2,51	0,07	1,04
04/04/22	*Cesine*	C2	nedium/sma	8,086	21,77	0,63	7,73	49,70	1,43	17,66	32,60	0,94	11,58	173,00	4,97	61,48	4,34	0,12	1,54
04/04/22	*Cesine*																		
17/05/22	*Cesine-Lab-ctr1*	L	medium	6,901	3,20	0,14	1,98	42,10	1,79	25,99	25,10	1,07	15,49	87,00	3,71	53,70	4,60	0,20	2,84
17/05/22	*Cesine-Lab-ctr1*	M	medium	5,517	5,80	0,16	2,87	50,60	1,25	22,66	23,30	0,64	11,53	113,90	3,11	56,36	13,30	0,36	6,58
17/05/22	*Cesine-Lab-ctr1*	H	medium	6,006	3,20	0,13	2,12	37,00	1,47	24,52	29,00	1,15	19,22	74,00	2,95	49,04	7,70	0,31	5,10
26/05/22	*Cesine-Lab ctr2*	G	large	3,929	61,90	0,88	22,40	85,00	1,21	30,76	128,30	1,82	46,43	64,70	1,93		13,00	0,39	
26/05/22	*Cesine-Lab ctr2*	M	medium	4,888	92,40	1,74	35,66	66,40	1,25	25,63	100,30	1,89	38,71	65,80	2,11		14,00	0,45	
26/05/22	*Cesine-Lab ctr2*	P	small	3,416	30,30	0,55	16,03	74,60	1,35	39,46	84,17	1,52	44,52	73,50	1,31		3,60	0,06	

Table 11. Data of enzymatic activities in PILOT 3 samples.

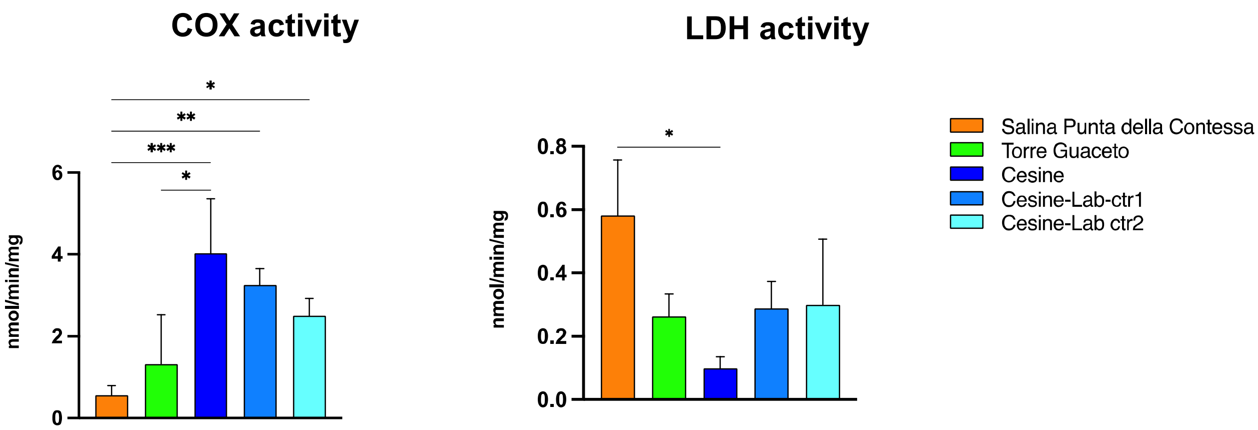
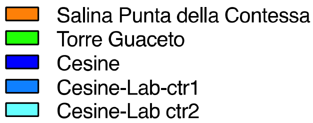


Figure 27. Quantification of metabolism enzymatic activity (COX and LDH).



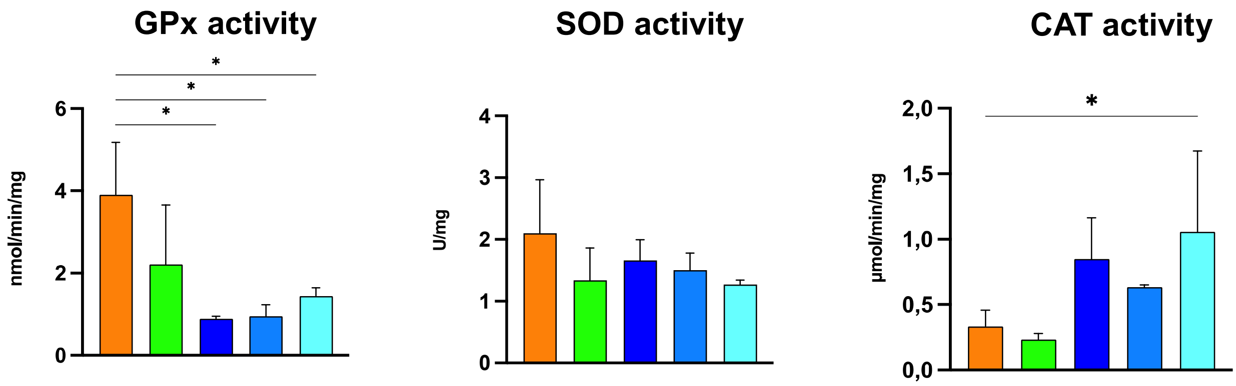


Figure 28. Quantification of antioxidant enzymatic activity (CAT, SOD and GPx).

The biological measurements did not evidence any measurable effect clearly attributable to the pandemic lockdown.

Monitoring system

For what concern the Torre Guaceto pilot site (Puglia, Italy), CMCC installed a fixed monitorng system; indeed, one of the delimitation buoys moored in the Torre Guaceto Marine Protected Area was made available for the integration of a low-cost measurement instrumentations. The station is based on low-cost and open access technologies and allows to acquire different types of parameters.

67

Figure 10. Monitoring System installed on the Torre Guaceto MPA buoy.

The system is based on low-cost electronics and allows to manage analog and digital sensors and acquires data with a frequency of 10 minutes. The data are transmitted to a server in .csv format. In July 2021 was installed and the first data were collected. Below examples of data acquired during the first period of installation.

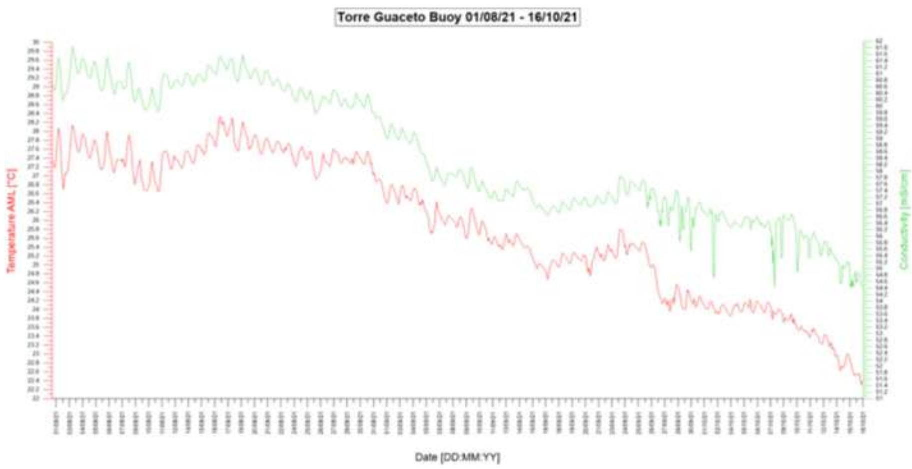


Figure 30. An example of Conductivity and Temperature data collected during the first period of installation

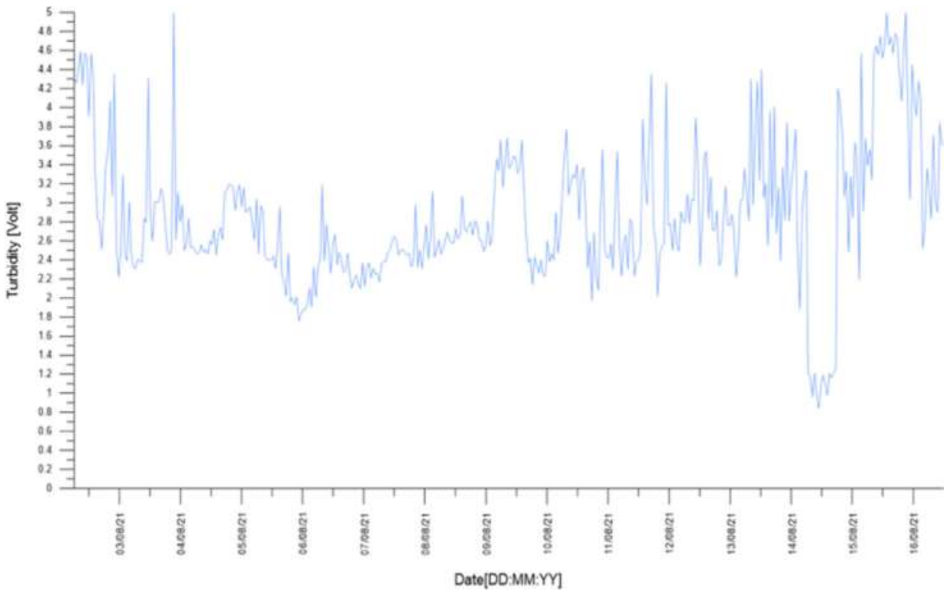


Figure 31. An example of Turbidity data collected during the first period of installation

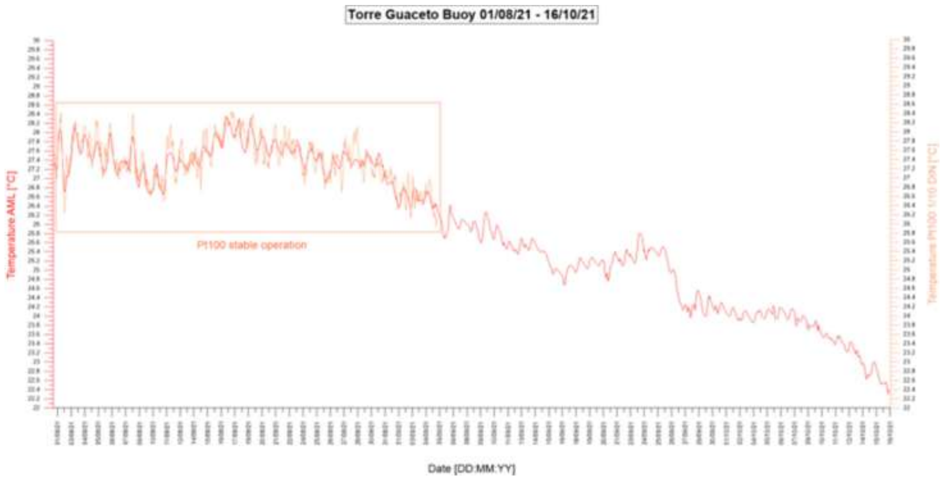


Figure 32. An example of comparison between different low-cost Temperature sensors data

Torre Guaceto MPA monitoring survey data

CMCC performed in July 2021 an extended survey in the Torre Guaceto MPA marine waters.

The campaign consists of n.20 stations, in each station a multiparametric probe was used allowing to perform a series of vertical profiles and to acquire along the water column the following parameters: depth, temperature, conductivity (salinity, density), dissolved oxygen, pH, chlorophyll a, turbidity.

69

Figure 33. Survey stations

The data acquired by the multiparametric probe were processed in order to have the vertical distribution of the measured variables in all the stations. All the data will be used for numerical model validation.

Below some results processed by Ocean Data View software.

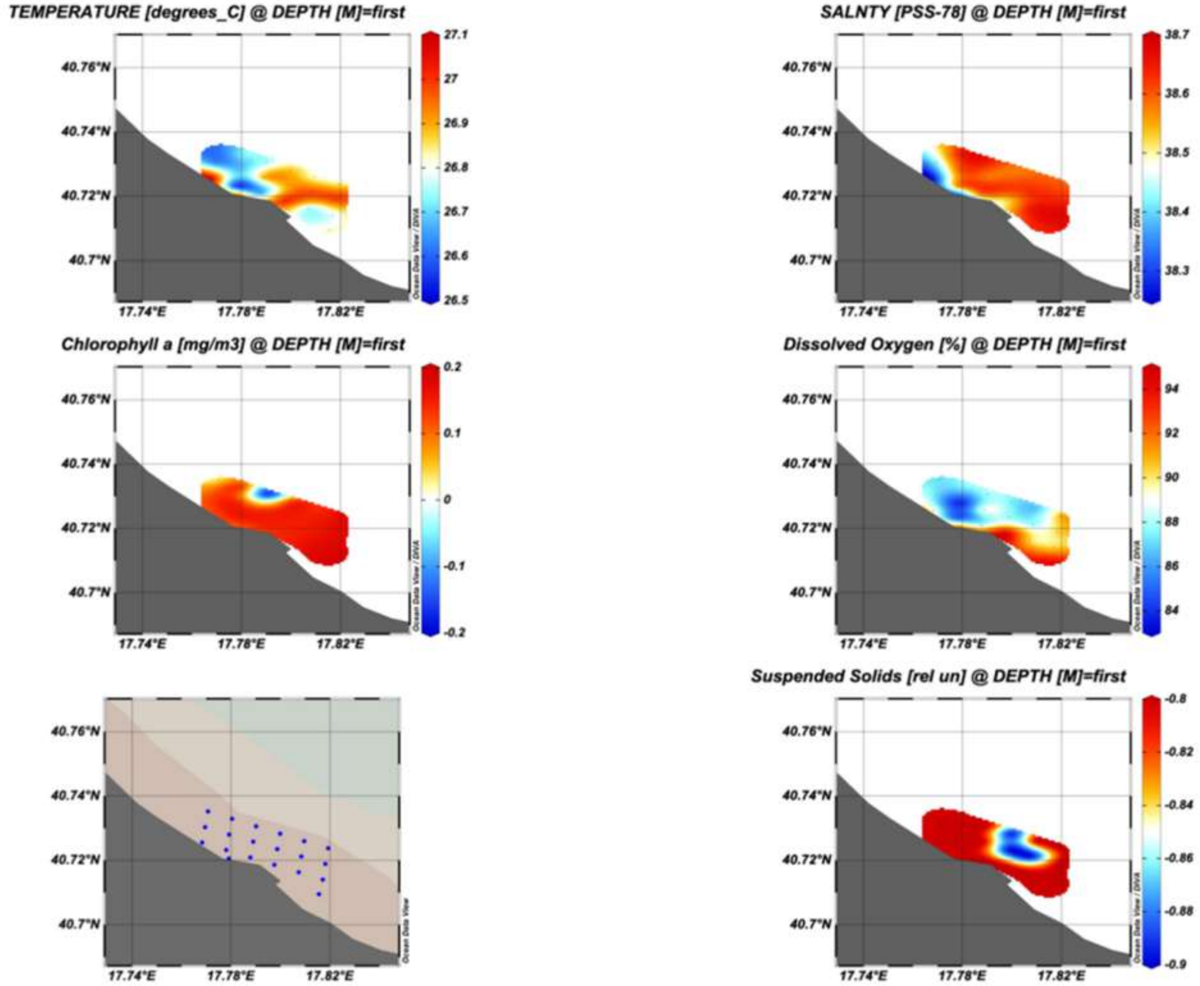


Figure 34. Isosurfaces at 5m of temperature, salinity, dissolved oxygen, chlorophyll and suspended solids.

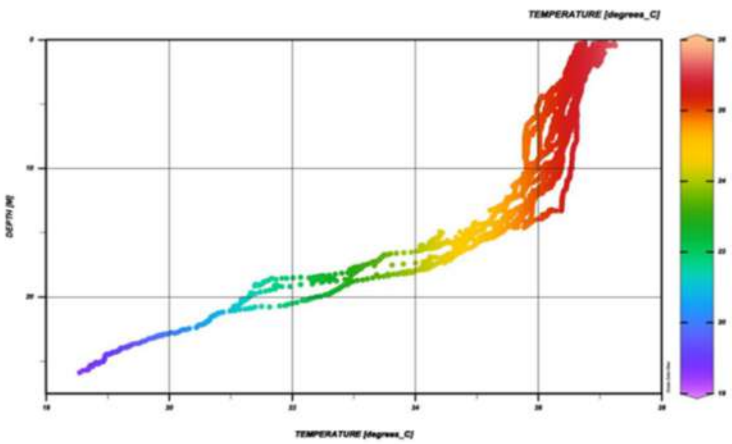


Figure 35. Temperature vertical profiles.

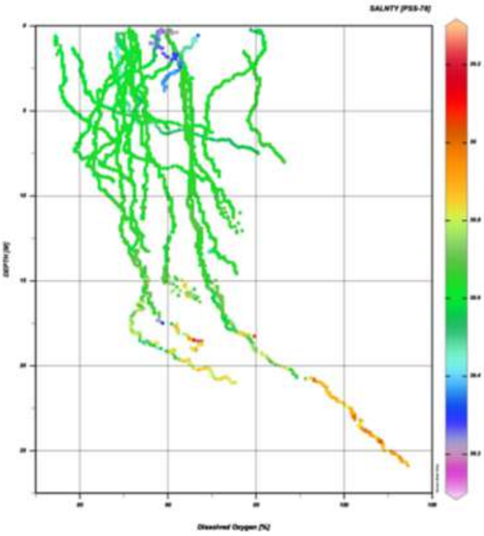


Figure 36. Salinity vertical profiles.

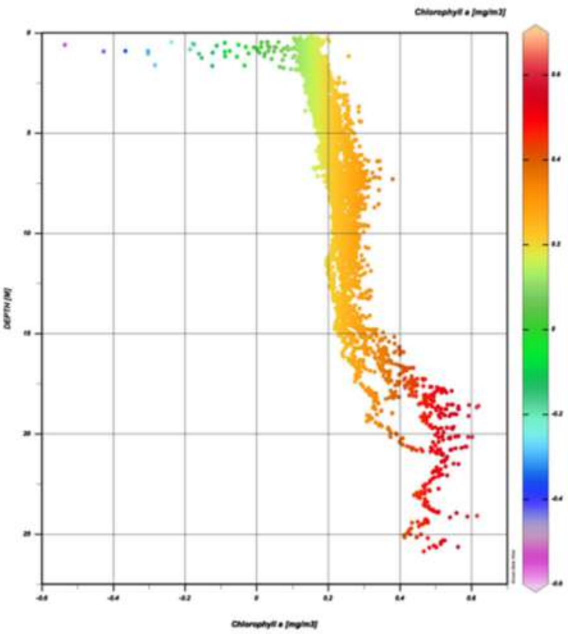


Figure 37. Chlorophyll a vertical profiles

P4. Neretva river mouth (HR)

The main objective of the activities carried out in WP3 and WP4 is to assess potential pollution and ecological status of the study area (Neretva estuary) by characterizing general water and sediment quality as an important component of river ecosystems as they integrate pollution in time and space and they are persistent and environmentally toxic.

The data obtained will contribute to the conservation of the ecosystem of the Adriatic Sea and especially the estuaries. Anthropogenic impacts of agriculture, industrial and other economic activities in coastal areas lead to significant input of pollutants such as metals (Cd, Cu, Cr, Pb, Zn, Hg) and polycyclic aromatic hydrocarbons (PAHs) into the marine environment, which may lead to conditions conducive to the production of biotoxins in shellfish. Poisoning of humans and animals is a possible consequence of consuming contaminated seafood. Through filter-feeding process shellfish accumulate nutrients and other components which are suspended in the water column. In this way, they can accumulate metals, PAHs, and toxic phytoplankton species that produce toxic organic compounds (marine biotoxins or phycotoxins). The accumulated toxins are not harmful to shellfish, but after consumption they are the cause of toxicity in humans, marine mammals, and birds. Depending on the amount of toxins ingested during consumption of contaminated shellfish, the consequences of poisoning can range from mild digestive and neurological disorders to fatality. Increased levels of metals and PAHs in shellfish also affect the health of the shellfish, as well as providing additional information about the ecological status of the area in which they live.

In addition, sediments serve as both a sink and source for heavy metals and PAHs. Most heavy metals are rapidly deposited in sediment after entering rivers and are much more concentrated there than in the water body of river systems. Analysis of PAHs in biota is particularly important because they are considered as pollution indicators, since they give a view of the spatial distribution of the pollutants. Therefore, seafood is an interesting example of the various routes by which human food sources may become contaminated by PAHs. PAHs enter the marine environment from a variety of sources, including petroleum pollution, fallout from air pollution, effluents from industries and sewage treatment plants and creosoted wharves. The analyze of PAHs, metals and toxins in samples includes on-site sampling, extraction, purification and instrumental analysis.

The granulometric composition of sediment samples is also determined and is important because it depends on the physicochemical properties that affect the accumulation of contaminants. To determine the grain size, the dried sediments are separated into two fractions and determined by sieving (> 0.063 mm) and hydrometry (< 0.063 mm) after laser granulometry.

In the Neretva area, sandy sediments are deposited close to the land, while silty sediments (mainly silt and clay particles) are deposited farther from the coast or in protected areas such as the Parila lagoon. The Ploče station is located near the entrance to the Ploče harbour, and the high proportion of sandy particles is a result of the Neretva River, currents, and anthropogenic influence (passage of ships and occasional dredging in the harbor). At the Blaca station, in comparison with the station under direct influence of the Mala Neretva, the proportion of sand predominates, while the proportions of organic matter and carbonates are equal. At the Škoj station, the proportion of organic matter is the highest in this study, which is a consequence of the highest proportion of silt particles and is due to the lower influence of the Neretva. The determined values are in the range determined for the Neretva area (Parila Bay, station before the mouth of Neretva, station near the mouth of Mala Neretva).

Water samples were collected at three relevant depths (0, 5, and 7 m). Dissolved oxygen content in the seawater samples was determined titrimetrically using thiosulfate. Temperature, salinity, and depth of seawater were measured with a CTD probe, while concentrations of nutrient salts were determined photometrically. Oxygen saturation varied from 97% to 131%, and no station was found to have an ecologically critical oxygen concentration (2-3 mg/L) that could negatively impact marine life. Temperature varied between 13.3 and 24.1 °C depending on the depth and season, while salinity varied between 34.1 and 37.9.

Heavy metal contamination of surface sediment and biota (mussels) samples collected from three stations of the Neretva River was studied to assess water quality conditions, possible ecological risks and heavy metal distribution. Heavy metal concentrations in sediment were statistically analyzed at all sampling sites and did not exceed the proposed ERLs (cadmium 1.2 mg/kg body weight; copper 34 mg/kg body weight; mercury 0.15 mg/kg body weight; lead 47 mg/kg body weight; zinc 150 mg/kg body weight; concentrations above the ERL may have adverse effects on marine organisms). Effects Range Low (ERL) is an evaluation criterion for assessing the ecological significance of sediment concentrations and was developed by the United States Environmental Protection Agency. For biota, the moisture content of mussels was calculated by recording the difference between fresh weight and dry weight. The average moisture content in soft tissue of mussels is 80-85%. The data sets were statistically evaluated and the concentrations of all analyzed metals in mussels were below the permitted levels (according to the EU Regulation 1881/2006, the maximum permitted level for Cd is 1.0 mg/kg and for Pb 1.5 mg/kg wet weight); moreover, no concentrations unsuitable for human consumption were detected at any station.

Also, the maximum level of lipophilic and hydrophilic toxins established in Regulation (European Community, 2013) No. 786/2013 was not exceeded in any of the samples. The low toxin levels in the bivalve molluscs analyzed in this report demonstrate the good status of the areas studied and the safety of the bivalve molluscs for public health.

Overall, it can be concluded that the lockdown did not have a measurable impact on the P4 in terms of contaminants such as metals or PAHs.

74

P5. Coastal area in Veneto (IT)

Monitoring activities programmed within CASCADE were designed to achieve two main goals:

- characterizing hard-substrata communities and fish assemblages located in the different Tegnùe sub-areas, ensuring a continuity with monitoring efforts carried out in the area in the framework of previous research initiatives;
- combining four different data acquisition methodologies for hard-substrata communities and fish fauna, and assessing their complementarity, applicability and cost-effectiveness.

In order to achieve these goals, a comprehensive experimental plan was designed. This included the following 4 actions:

A. A video recording survey, carried out by means of a ROV in June 2021;

B. A non-destructive photographic sampling survey, carried out by scuba diving in July 2021;

C. Three campaigns with gillnets targeting fish fauna (November and March 2021, December 2022);

D. Three campaigns implementing an active bioacoustics survey, targeting fish community present in the area, and carried out from fall 2021 until March 2022.

Experimental activities at points A-D were integrated with a desk-based one, focused on the development and application of a dynamic and spatially explicit food web model (EwE - Christensen e Walters 2004) in the Tegnùe area. Model application was carried out by starting from a similar application performed in the lagoon of Venice (Anelli Monti et al., 2021), and integrating the field data observed in-situ with existing literature information, and with data from earth observation (Copernicus, EMODNET).

This allowed to obtain a representation of food web functioning in the area, and to explore scenarios of change in food-web functioning under expected climate trends. The latter ones were defined by changes in water temperature and primary production over a 50 years time-window, predicted by the Polcoms-Ersem model on combined European GCOMS domains (CLIMEFISH-CERES H2020 projects). A comprehensive description of sampling and modelling activities carried out within CASCADE can be accessed on WP4 reports. In the following we will focus on specific aspects related to the DPSIR framework defined within the previous paragraph, and defining the potential vulnerability of the system.

ROV surveys (June 23, 2021) were performed at three weeks of distance from the scuba diving sampling (July 14-15, 2021). ROV acquisitions were carried out over 4 transects within the A SCI area, each one of 30’ duration and > 100m distance. Scuba-diving photographic acquisitions were carried out at 6 selected sites, located within 3 of the 4 zones of the SCI. From the films and HD photographs obtained through ROV surveys and relating to the 4 sampled areas, it was possible to build the list of species present in the area, distinguishing a total of 76 different taxa, 60 of which recognized at species level (Table 1). Overall, the most represented taxon was that of porifera (18%), followed by crustaceans and fish (12%), molluscs and tunicates (11%), polychaetes and algae (9%) and echinoderms (8%). Bryozoans, cnidarians and flatworms were the least represented taxa, with values of 5%, 4% and 1%, respectively.

Phylum	Specie	Ecologia
Algae (Rhodophyta)	*Botryocladia botryoides* (Wulfen) (Feldmann, 1941)	sessile
	Ceramiales nd (Oltmanns, 1904)	sessile
	Colpomenia sinuosa (Mertens ex Roth) (Derbès & Solier, 1851)	sessile
	Cryptonemia lomation (Bertoloni) (J. Agardh, 1851)	sessile
	Halymenia floresii (Clemente) (C. Agardh, 1817)	sessile
	Lithophyllum stictiforme (J.E. Areschoug) (Hauck, 1877)	sessile
	Lithothamnion sp. (Heydrich, 1897)	sessile
Porifera	*Antho (Antho) inconstans* (Topsent, 1925)	sessile
	Aplysina aerophoba (Nardo, 1833)	sessile
	Chondrilla nucula (Schmidt, 1862)	sessile
	Cliona viridis (Schmidt, 1862)	sessile
	Dictyonella incisa (Schmidt, 1880)	sessile
	Dysidea avara (Schmidt, 1862)	sessile
	Dysidea fragilis (Montagu, 1814)	sessile
	Geodia cydonium (Linnaeus, 1767)	sessile
	Haliclona (Soestella) mamillata (Griessinger, 1971)	sessile
	Irciniidae nd (Gray, 1867)	sessile
	Petrosia (Petrosia) ficiformis (Poiret, 1789)	sessile
	Phorbas tenacior (Topsent, 1925)	sessile
	Pleraplysilla spinifera (Schulze, 1879)	sessile
	Terpios fugax (Duchassaing & Michelotti, 1864)	sessile
Cnidaria	*Caryophyllia (Caryophyllia) smithii* (Stokes & Broderip, 1828)	sessile
	Cornularia cornucopiae (Pallas, 1766)	sessile
	Epizoanthus arenaceus (Delle Chiaje, 1836)	sessile
Anellida (Polychaeta)	*Chaetopterus variopedatus* (Renier, 1804)	sessile
	Lanice conchilega (Pallas, 1766)	sessile
	Protula sp. (Risso, 1826)	sessile
	Sabellidae nd (Latreille, 1825)	sessile
	Serpula vermicularis (Linnaeus, 1767)	sessile
	Serpulidae nd (Rafinesque, 1815)	sessile
	Terebellidae nd (Johnston, 1846)	sessile
Mollusca	*Chama gryphoides* (Linnaeus, 1758)	sessile
	Ostrea sp. (Linnaeus, 1758)	sessile
	Rocellaria dubia (Pennant, 1777)	sessile
Bryozoa	*Pentapora fascialis* (Pallas, 1766)	sessile
	Schizomavella (Schizomavella) mamillata (Hincks, 1880)	sessile
	Schizoporella errata (Waters, 1878)	sessile
	Smittina cervicornis (Pallas, 1766)	sessile
Chordata (Ascidiacea)	*Aplidium cf conicum* (Olivi, 1792)	sessile
	Aplidium sp. (Savigny, 1816)	sessile
	Aplidium tabarquensis (Ramos-Espla, 1991)	sessile
	Botryllus sp. (Gaertner, 1774)	sessile
	Microcosmus vulgaris (Heller, 1877)	sessile
	Phallusia fumigata (Grube, 1864)	sessile
	Polycitor adriaticus (Drasche, 1883)	sessile
	Pyura microcosmus (Savigny, 1816)	sessile
Plathyelminthes	*Pseudobiceros splendidus* (Lang, 1884)	vagile
Mollusca	*Bolinus brandaris* (Linnaeus, 1758)	vagile
	Calliostoma sp. (Swainson, 1840)	vagile
	Flabellina sp. (McMurtrie, 1831)	vagile
	Mimachlamys varia (Linnaeus, 1758)	vagile
	Pecten jacobaeus (Linnaeus, 1758)	vagile
Arthropoda (Crustacea)	*Dromia personata* (Linnaeus, 1758)	vagile
	Galathea sp. (JC Fabricius, 1793)	vagile
	Galathea strigosa (Linnaeus, 1761)	vagile
	Maja crispata (Risso, 1827)	vagile
	Munida cf rugosa (JC Fabricius, 1775)	vagile
	Paguristes eremita (Linnaeus, 1767)	vagile
	Paguroidea nd (Latreille, 1802)	vagile
	Pagurus anachoretus (Risso, 1827)	vagile
	Pilumnus sp. (Leach, 1816)	vagile
Echinodermata	*Amphipholis squamata* (Delle Chiaje, 1828)	vagile
	Holothuria (Holothuria) tubulosa (Gmelin, 1791)	vagile
	Ocnus planci (Brandt, 1835)	vagile
	Ophiothrix fragilis (Abildgaard, in O.F. Müller, 1789)	vagile
	Ophiuroidea nd (Gray, 1840)	vagile
	Paracentrotus lividus (Lamarck, 1816)	vagile
Chordata (Pisces)	*Monochirus hispidus* (Rafinesque, 1814)	bentonica (B)
	Pegusa lascaris (Risso, 1810)	bentonica (B)
	Parablennius tentacularis (Brünnich, 1768)	bentonica (B)
	Scorpaena porcus (Linnaeus, 1758)	bentonica (B)
	Scorpaena scrofa (Linnaeus, 1758)	bentonica (B)
	Serranus hepatus (Linnaeus, 1758)	bentonica (B)
	Chromis chromis (Linnaeus, 1758)	bentonectonica (BN)
	Spicara maena (Linnaeus, 1758)	bentonectonica (BN)
	Trisopterus minutus (Linnaeus, 1758)	bentonectonica (BN)
Totale	76	

Table 12. Comprehensive list of observed species.

76

Photographic survey through scuba-diving allowed to consider a wider area, focusing on 6 stations located within 3 of the 4 sub-areas of the SCI (Figure 38). Epibenthic assemblages found on the investigated northern Adriatic coralligenous reefs were very heterogeneous in terms of percent cover of the most abundant taxa. The epibenthic assemblages were characterized by algal turf (percent cover between 1.46 and 36.63%), crustose coralline algae (17.15–91.17%), *Peyssonnelia* spp. (0.73-27.32%), encrusting sponges (2.64–53.32%), massive sponges (9.59–60.76%), boring sponges (6.14–25.11%), large massive colonial ascidians (1.27–9.63%), erect sponges (0–11.88%) and boring molluscs (3.83–11.6%). The percent cover of "detritus + sediment" ranges from 43.68 to 89.16%.

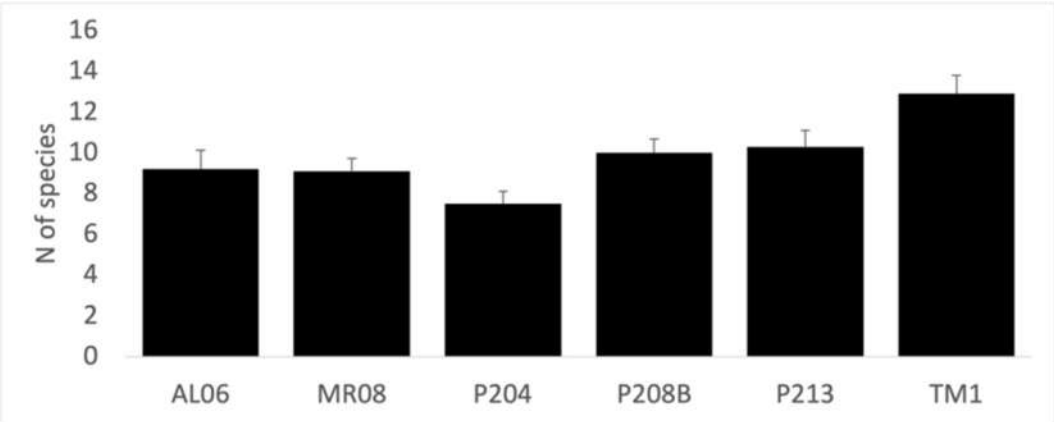


Figure 38. Mean (+SE, n = 10) species richness (S) in photographic samples (21 × 28 cm).

The scientific fishing activity with gillnets, conducted in three successive campaigns (18-19 December 2021, 26-27 March 2022, 14-15 December 2022), overall highlighted that the difference in the composition of the seabed has a greater influence on the diversity of the fish community living near the Tegnùe (Table 13), which are confirmed to be a fundamental area for species of commercial interest that live associated with coral-like structures, such as the *sparidae*, but they are also important areas for pelagic species such as *S. scombrus* and *T. mediterraneus*.

	Df	SumOfSqs	R2	F	Pr(>F)
Fondale	1	1.6976	0.24355	18.198	**0.001** ***
Area	1	1.0839	0.1555	11.6189	**0.001** ***
Maglia	1	0.4264	0.06117	4.5706	**0.017** *
ZTB	1	0.1031	0.01479	1.1048	0.388
Mese	1	0.3543	0.05083	3.7981	**0.026** *
Fondale:Area	1	0.756	0.10846	8.1041	**0.003** **
Fondale:Maglia	1	0.2681	0.03846	2.8736	0.059

Area:Maglia	1	0.1526	0.02189	1.6358	0.195
Area:ZTB	1	0.1084	0.01556	1.1626	0.364
Maglia:ZTB	1	0.3346	0.04801	3.5873	**0.032 ***
Fondale:Mese	1	0.1762	0.02528	1.8887	0.146
Area:Mese	1	0.2293	0.03289	2.4579	0.075
Maglia:Mese	1	0.1664	0.02387	1.7834	0.155
ZTB:Mese	1	0.1725	0.02475	1.8496	0.156
Area:Maglia:ZTB	1	0.2045	0.02934	2.1919	0.109
Fondale:Area:Mese	1	0.2403	0.03447	2.5758	0.068
Maglia:ZTB:Mese	1	0.1486	0.02132	1.5934	0.191
Residual	3	0.0677	0.00971	0.7257	0.614
Total	21	0.2798	0.04015		
		6.9702	1		

Table 13. PERMANOVA test results. Substrate type, along with the location (area) results to be the most significant factor.

Overall, 2237 targets were identified in the December 2021 acoustic surveys, 2217 in January and 2048 in February. The analysis of the signals showed that, regardless of the period, the average TS of the targets was located between -77 and -31 dB, with the average TS at about -67 dB, around which most of the observations were located. A second frequency peak located around -77 dB was associated with macroplankton organisms; this peak was absent only in the January 2022 campaign. From a temporal point of view, targets presenting higher TS were detected less frequently and in a decreasing manner starting from December and continuing in subsequent samplings (Figure 39). This data suggests a change in the distribution of the nektonic component in the winter period, and agrees with what is reported anecdotally by local fishermen, therefore it is attributable to natural seasonal cycles.

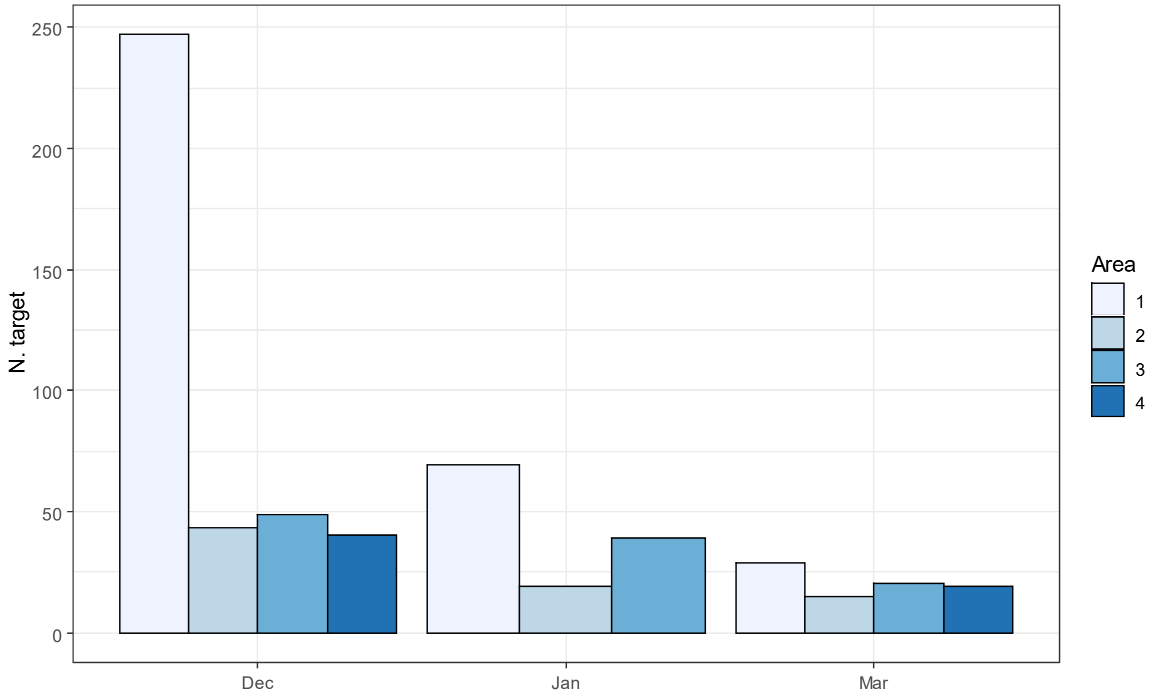


Figure 39. Number of targets detected with TS > -65 dB (ascribable to large macroplankton or small nektonic organisms) within the 4 areas of the SCI, during the three campaigns.

P6. Miljašić Jaruga river mouth, Nin bay (HR)

The use of the BRUV (Baited remote underwater video) method proved to be very successful for use in monitoring the ichthyo populations of the mouth of the Miljašić jaruga, especially in combination with the visual inspection that was used earlier. Although the same number of species was determined as with the visual examination by diving, a very large diversity of ichthyopopulations was observed in a relatively small research area. This especially applies to the second half of May and June 2022, when the temperature is above 22°C, and the number of species, their abundance as well as the average size of individuals increases. Longer recording lasting a few hours a day and the invisibility of the BRUV system definitely enables the detection of a larger number of individuals compared to recording with the use of diving equipment. In this way, adults of commercially important species such as sea bream and other species of the Sparidae family, as well as mullets were observed. The research using this method should continue in other periods of the year in order to get a more complete insight into the composition and condition of ichthyopopulations near the Miljašić ravine.
All activities performed were not affected anyhow by the lockdown during the pandemia.

80

P7. Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC) (IT)

During the project have been analyzed the vegetation dynamics on a restored salt marsh mosaic, through a multi-temporal analysis of vegetation plots collected before and after the implementation of restoration actions in the Central Adriatic coast in Italy. Have been specifically analyzed plant communities of the residual brackish wetlands occurring in the inter-dunal humid depressions of the Biferno river mouth area before and after a restoration action. This area includes a rich mosaic of ecosystems of Conservation Concern in Europe (included in Annex I of the Habitats Directive – HD- EEC 1992; European Commission 2013).

During the years 2020-21, have been re-visited (hereafter T2), 33 vegetation plots collected in 2010 (hereafter T1; Figure 40 - field data collection). Vegetation plots, collected within the Biferno brackish wetlands before and after the restoration of wetlands carried out in the year 2016 are representative of the brackish mosaic dominated by the following habitats of conservation concern (HD: 92/43/EEC): 1410: Mediterranean salt meadows - Juncetalia maritime; 1420: Mediterranean and thermo-Atlantic halophilous scrubs - Sarcocornietea fruticose − 1510*: Mediterranean salt steppes – Limonietalia). Phytosociological relevés of 16 m2 (4 × 4 m) were carried out following a stratified random protocol that used a detailed land cover map (1: 5000 scale; A.VV. 2008) and high-resolution color digital orthophotos (flight 2007, granted by the Civil Protection) for identifying the strata.

For re-visitation, have been sampled the same T1 plots following the description of the location reported in the reference study. Have been carried out phytosociological relevés following the same sampling protocol and in the same season (April-October) to remove the effects of phenological differences.

In addition, in order to limit the pseudo-turnover caused by observer bias, one of the researchers who had conducted the T1 sampling campaign was also involved in T2 field work activity. For each georeferenced vegetation plot we registered the complete list of vascular plants and their cover values in compliance with Braun-Blanquet scale using the classical phytosociological approach. Species nomenclature follows the updated checklist of “Flora d’Italia”.

Figure 40. Picture of the field data collection campaign implemented by UNIMOL CASCADE working group aimed at detecting vascular plant changes after wetland restoration actions and to identify plant species (species guilds) as bioindicators of change and ecosystem functioning

P8. Northern-eastern Adriatic in Croatia (HR)

Report on observing campaigns

Sampling took place in 2020-2021 at two stations monthly (RV001 and RV004) in the Northern – Eastern Adriatic Sea. CTD probe (Sea Bird, USA) measurements of bottom – surface water column oceanographic parameters (temperature, conductivity and dissolved oxygen) were collected for each campaign on two sampling stations (RV001 and RV004). In each campaign, Niskin bottles water column collections were conducted and 6 samples (2 stations x 3 depths) of 500 mL per parameter (chlorophyll-a and nutrients) were prepared for laboratory measurements. After prefiltration (200 µm mesh size), chlorophyll-a Niskin samples were filtered on Whatman GF/C filters and immediately frozen at -20°C until analysis (within a week). Total chlorophyll a concentrations were determined on a Turner TD-700 fluorometer after three hours extraction in 90% acetone (in the dark, with grinding). Nutrients analyses were performed using methods described in Strickland and Parsons (1972). All measuring instruments are calibrated by the manufacturer once per year, and CMR scientific team always checks the quality of the data collected during monitoring to avoid any issue.

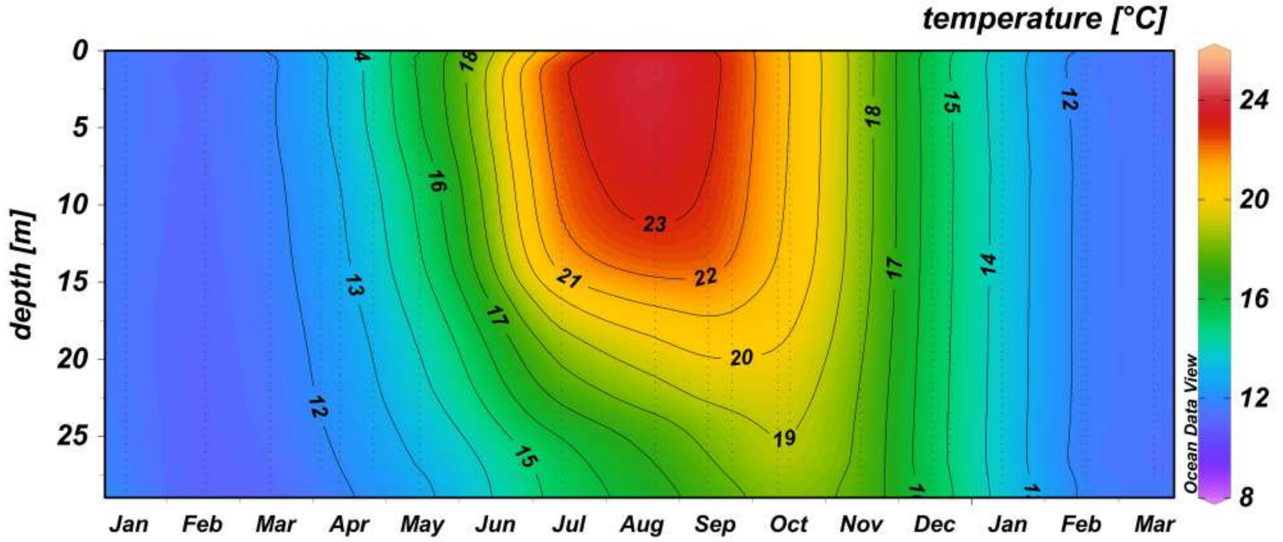


Figure 41. Yearly temprerature distribution in water column on station RV001

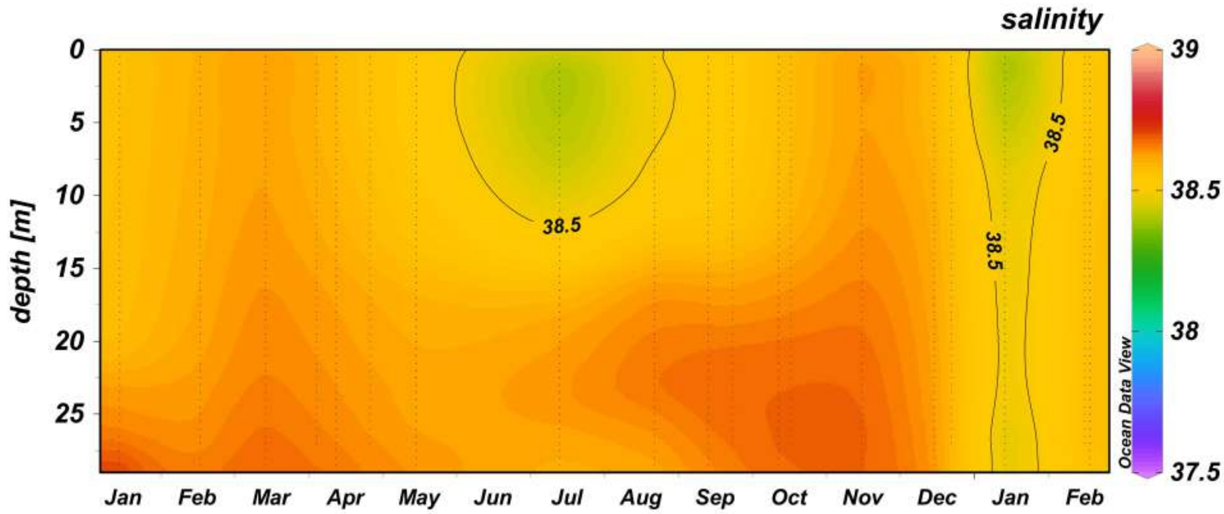


Figure 42. Yearlly salinity distribution in water column on station RV001

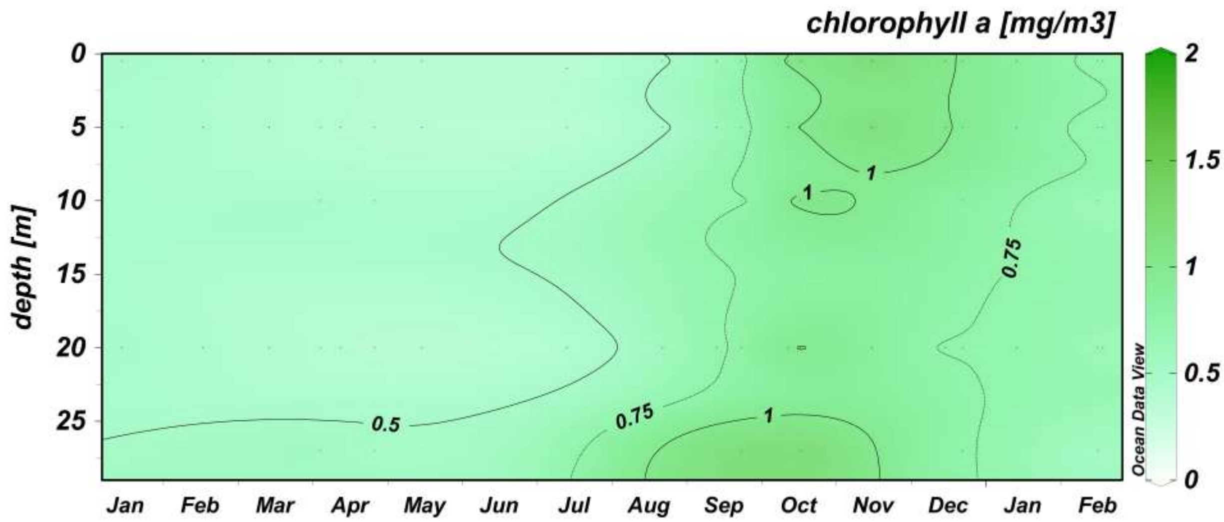


Figure 43. Yearlly chlorophyll a distribution in water column on station RV001

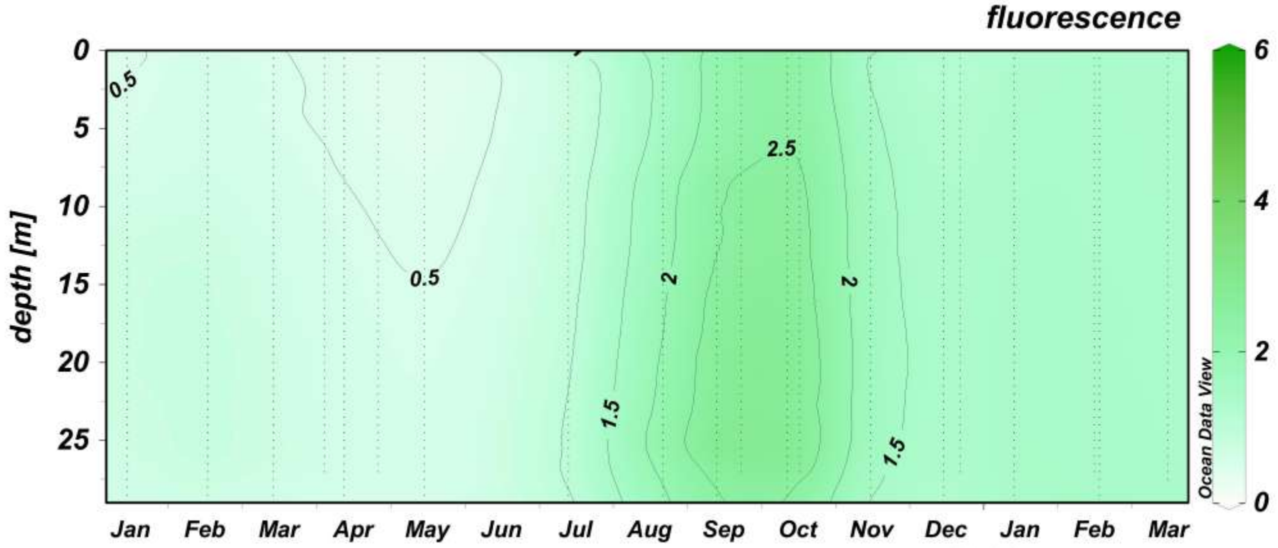


Figure 44. Yearlly fluorescence distribution in water column on station RV001

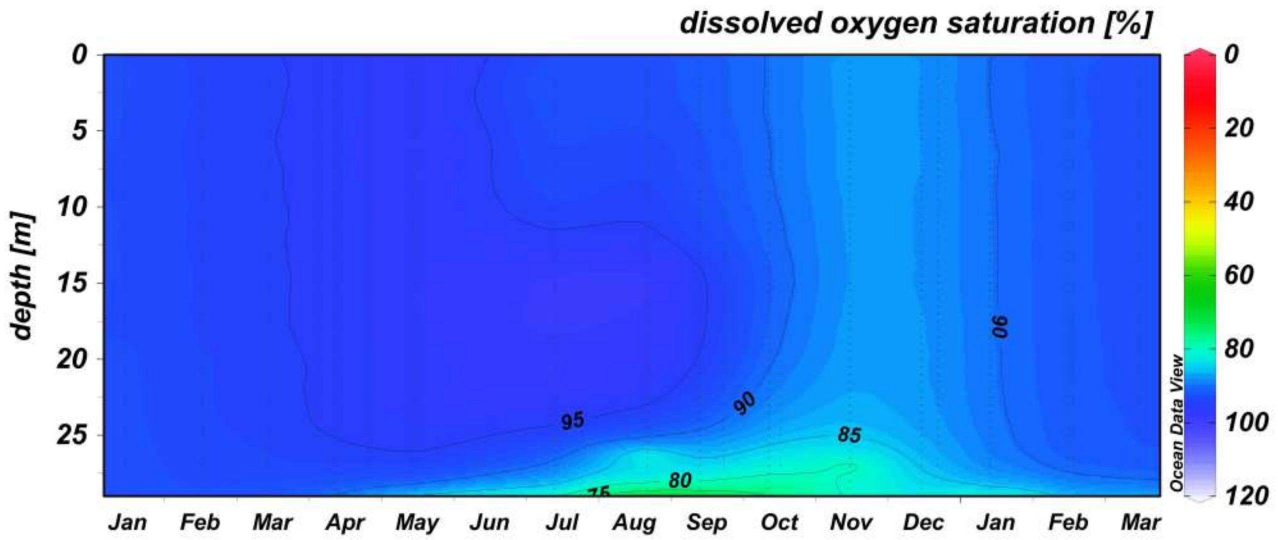


Figure 45. Disolved oxigen saturation in water column on station RV001

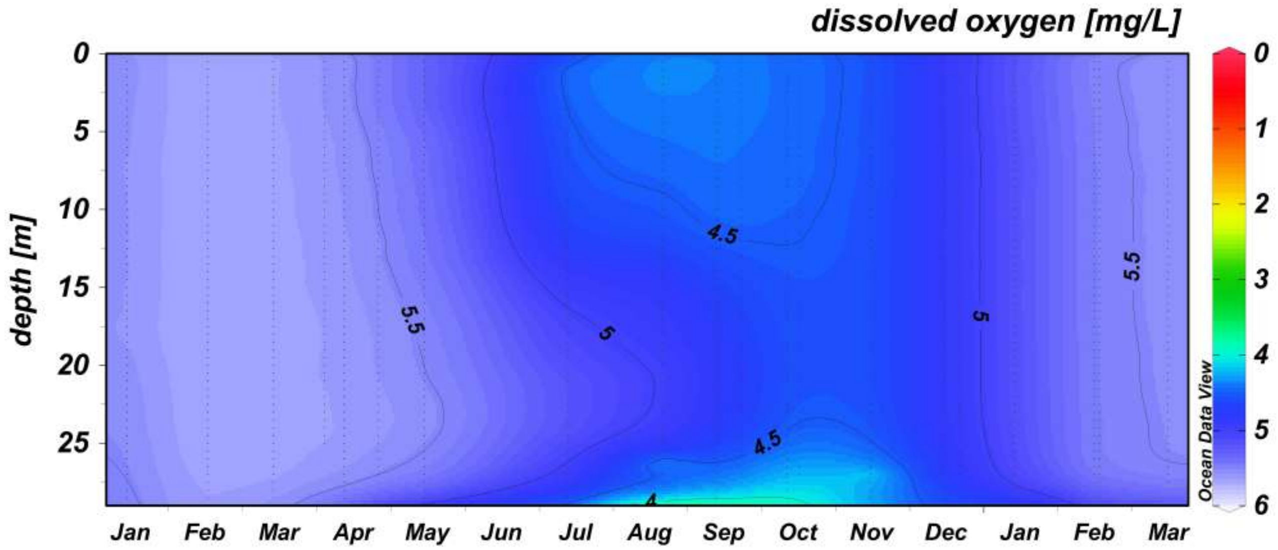


Figure 46. Disolved oxigen concentration in water column on station RV001

Materials and methods for phytoplankton analisys:

For phytoplankton community composition and abundance subsamples were taken form niskin bottles. Niskin bottles were used to sample the seawater from 0, 5, and 20 m depth (from January 2020 to May 2020 only 5 and 20 m depths were sampled). Samples for phytoplankton analysis (200 ml) were prefiltered through 300 µm mesh net and fixed with neutralized formaldehyde (2% final concentration). Subsamples of 50 ml were settled for 40 hours in sedimentation chambers and analysed following the Utermöhl's method. In total 130 samples were analysed on inverted zeiss axiovert microscope. All data analysis was made using R software (package vegan for ecological analysis and ggplot2 for graphical representation of results).

Nanophytoplankton dynamics

Nanoplankon taxa were divided into main groups: Chlorophyceae, Chrysophyceae, Coccolithophoridae, Cryptophyceae, Diatomeae, Dinophyceae. Pachysphaera, *Phaeocystis* (counted in one sample (22.2.2021. RV001, 0m) and *Emiliania* were identified as separate taxa. The most abundant nanophytoplankton taxon is *Emiliania* (peak abundance is 1,7x 10^{6}cells per liter, recorded on RV001, 11.6.2021). Peaks in *Emiliania* abundance corresponded to late spring and early summer months when other groups like cryptophytes also increased their abundance and contribution. Increase in abundance of *Emiliania* also occurs in late autumn and early winter months, when contribution to total nanoplankton is also high. During rest of the winter and summer abundances of *Emiliania* are low. Other nano coccolithophores did not reach high abundances and contribution. *Emiliania* and cryptophytes increased their mean abundance in the year 2021. while nano diatoms decreased their mean annual abundance.

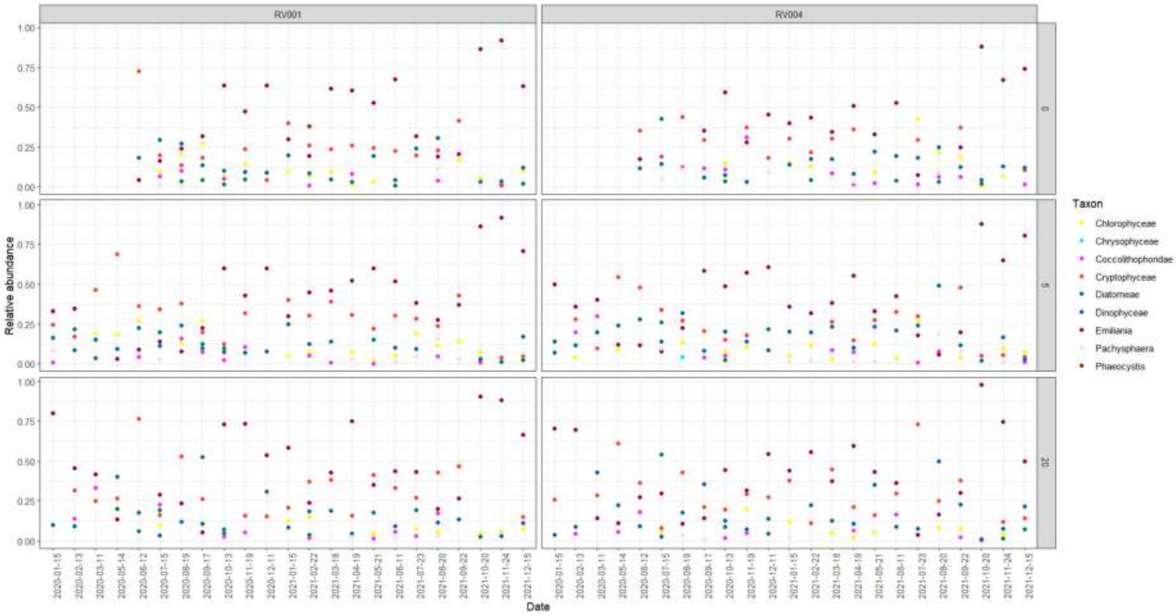


Figure 47. Relative abundances of nanophytoplankton taxa

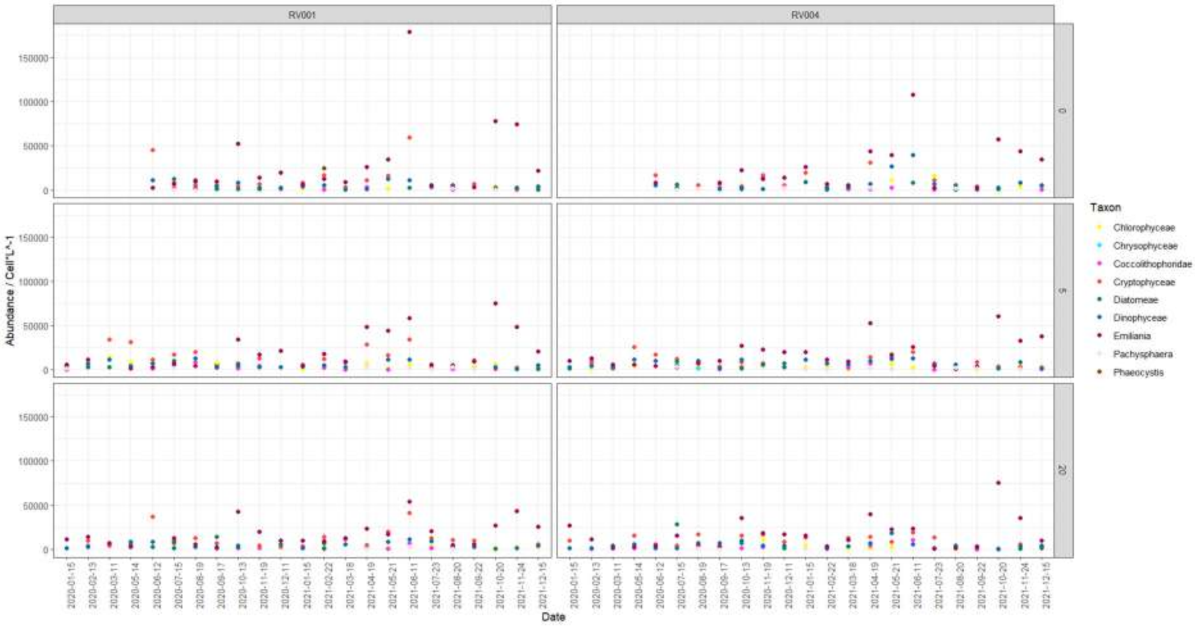


Figure 48. Total abundances of nanophytoplankton taxa

Highest relative abundance of nanophytoplankton (0.9539805) was recorded in RV004, 18.3.2021. while lowest (0.05549364) was recorded in RV004, 13.10.2020. Nanophytoplankton contribution tends to be low in autumn months (September, November and October) and in June and July as well. This periods of low nanophytoplankton contribution corresponds to periods of high microphytoplankton abundance where microphytoplankton, especially diatoms outnumbered the nanophytoplankton. In year 2020. nanophytoplankton dominated the community from January to March. Then microphytoplankton dominated community, but with low total abundances until late summer when nanophytoplankton shortly dominated before increasing of microphytoplankton abundances in early autumn. Domination of mikrophytoplankton lasted until the end of the year. The end of 2020. and first half of 2021. (until June) was characterized by very low abundances of microphytoplankton, especially diatoms and domination of nanophytoplankton size fraction. This domination lasted until September when abundances of microphytoplankton increased during autumn months.

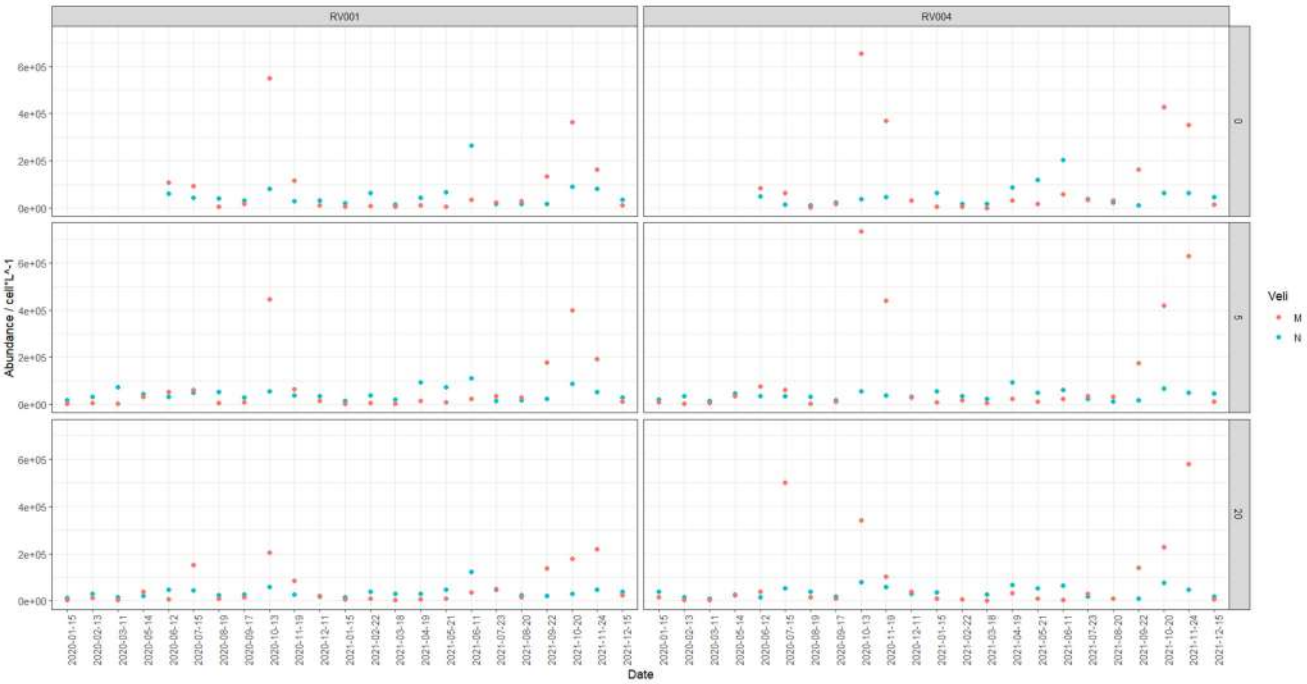


Figure 49. Total abundances of n anophytoplankotn (N, pink) and microphytoplankton (M, blue).

Microphytoplankton dynamics

During the observed period on both stations (RV001 and RV004) all main taxa of microphytoplankton (total number of taxa: 202): Bacillariophyceae (B, 83 taxa), Dinophyceae (D, 101 taxa), Haptophyceae (H, 12 taxa), Chrysophyceae (C, 4 taxa) and Euglenophyceae (E, 3 taxa) were identified. Abundances of microphytoplankton ranged from 1220 to 732 790 cells per liter. Bacillariophyceae mean abundances showed more differences between sites (RV001 and RV004) than between years. Abundances of diatoms were higher in RV004 station. Dynamics of diatoms on both stations showed unimodal pattern with one strong peak in autumn months. Maximum abundance of diatoms (710850 cells per liter) was recorded on RV004, 0 m in October 2020 while minimum (80 cells per liter) was in April 2021, 0 m. Although diatom abundance peaked in 2021, in year 2021 abundance of diatoms peaked also in autum, with maximum abundance of 620 910 cells per liter, station RV004, 5m. Diatoms slightly increased abundances in early summer months but with lower intensity compared to autumn. In terms of relative abundance, diatoms were main group that contributed to microphytoplankton community in 2020. At the beginning of the 2021. contribution of diatoms decreased while contribution of coccolithophores and dinoflagellates begun to increase. In spring and early summer dinoflagellates contributed most to the total microphytoplankton, athough maximum abundances of dinoflagellates was only 33650 cell per liter on RV004 in April, 0 m depth. On this station on all three sampled depths in April none of diatoms were found. Therefore, these results may suggest that diatom community shifted to dinoflagellate community in period from March to May or June. Coccolithophores which are part of microhytoplankton community showed no strong trend in change of mean abundances. Contribution changed and, as said before, coccolithophorids contributed mostly during the winter months but contribution increased in late summer/early autumn as well. Chrysophyceans showed higher mean abundances on RV001, and their mean abundances slightly increased in 2021. Eugelnophyceans showed strong decrease in mean abundance in 2021.

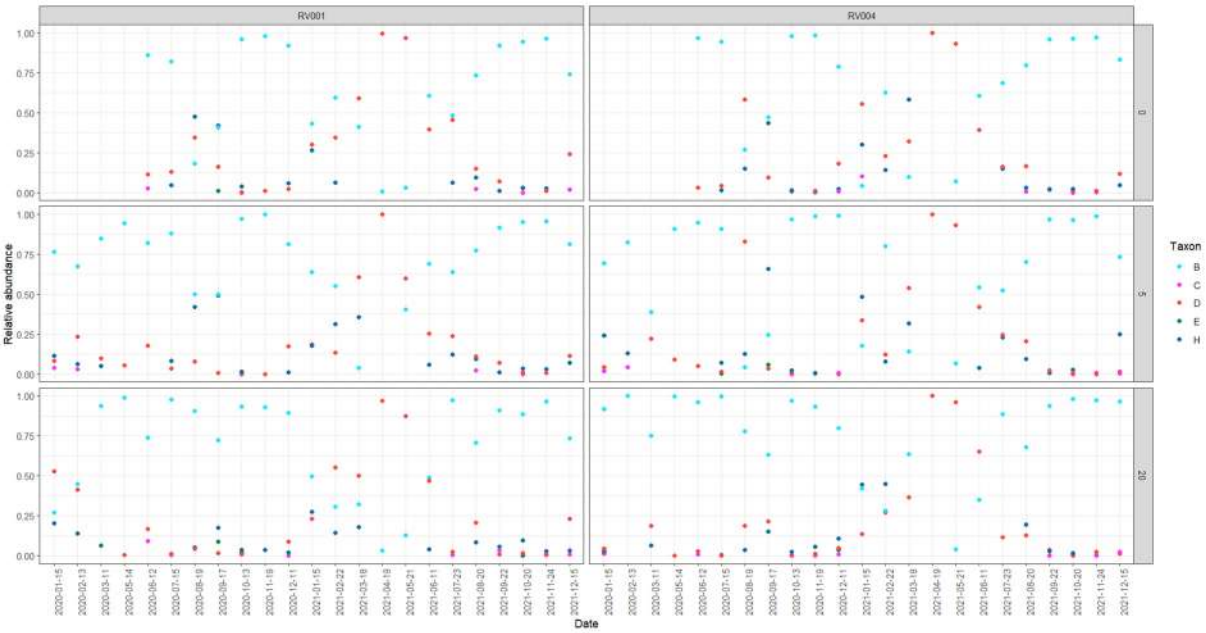


Figure 50. Relative abundances of microphytoplankton taxa: B - Bacillariophceae, C-Chrysophyceae, D- Dinophyceae, E - Euglenophyceae, H - Haptophyta

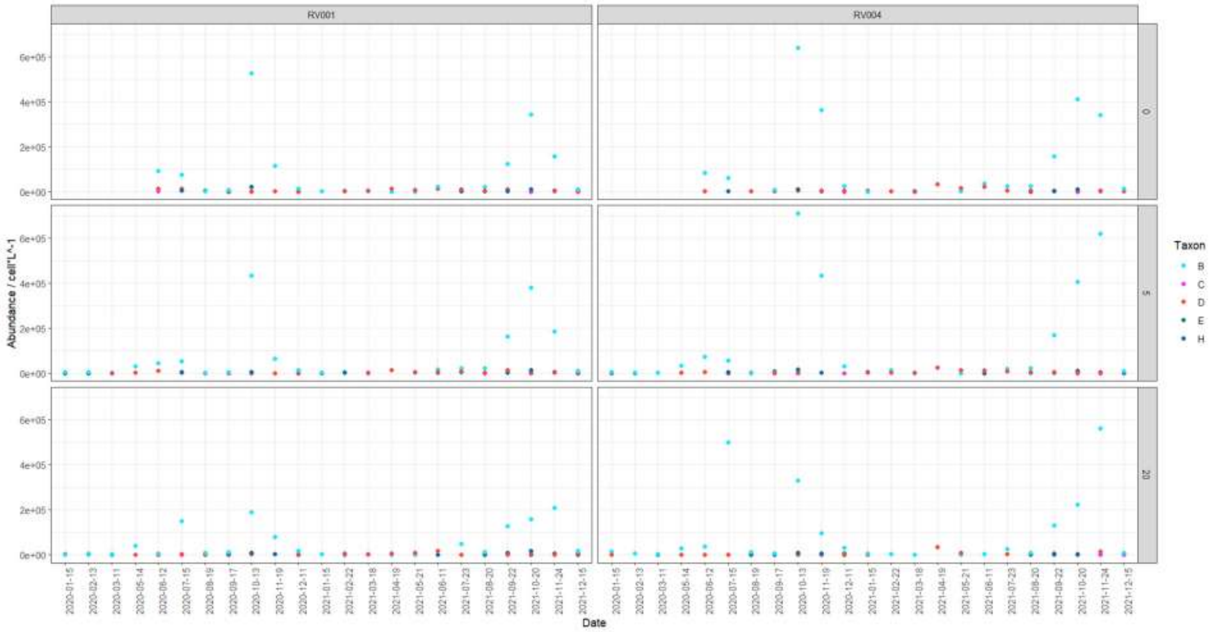


Figure 51. Total abundances of microphytoplankton taxa: B - Bacillariophceae, C- Chrysophyceae, D- Dinophyceae, E - Euglenophyceae, H - Haptophyta

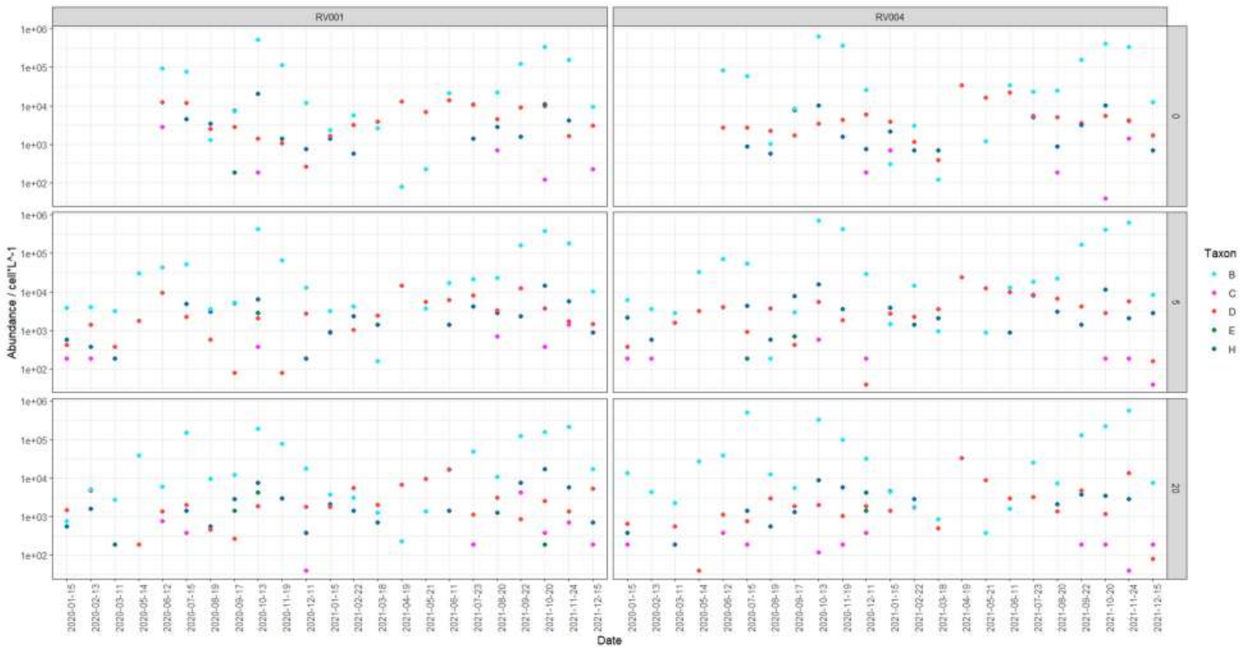


Figure 52. Total abundances of microphytoplankton taxa: B - Bacillariophceae, C- Chrysophyceae, D- Dinophyceae, E - Euglenophyceae, H - Haptophyta (log scale)

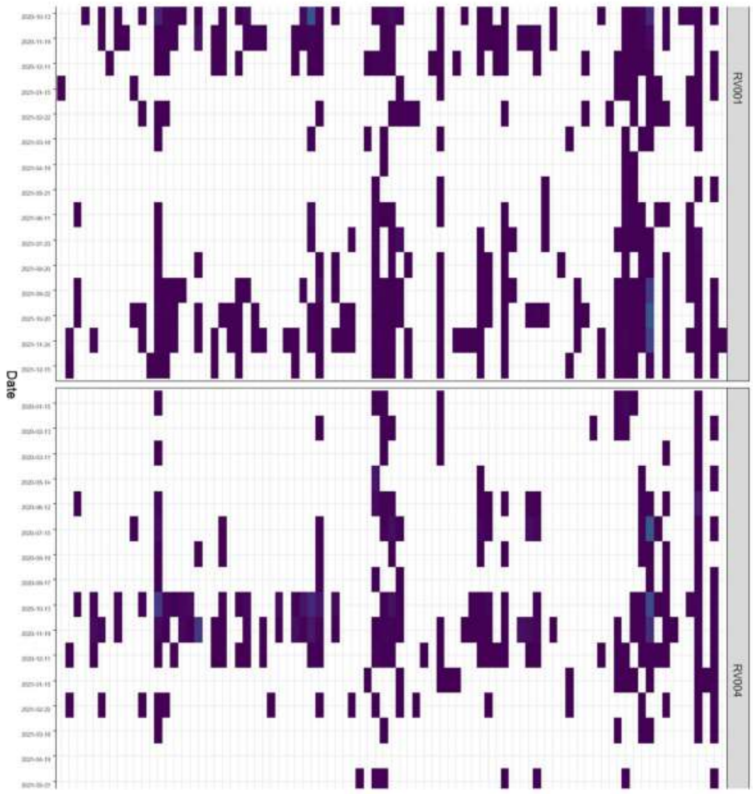


Figure 53. Monthly mean abundance dynamics of diatom species

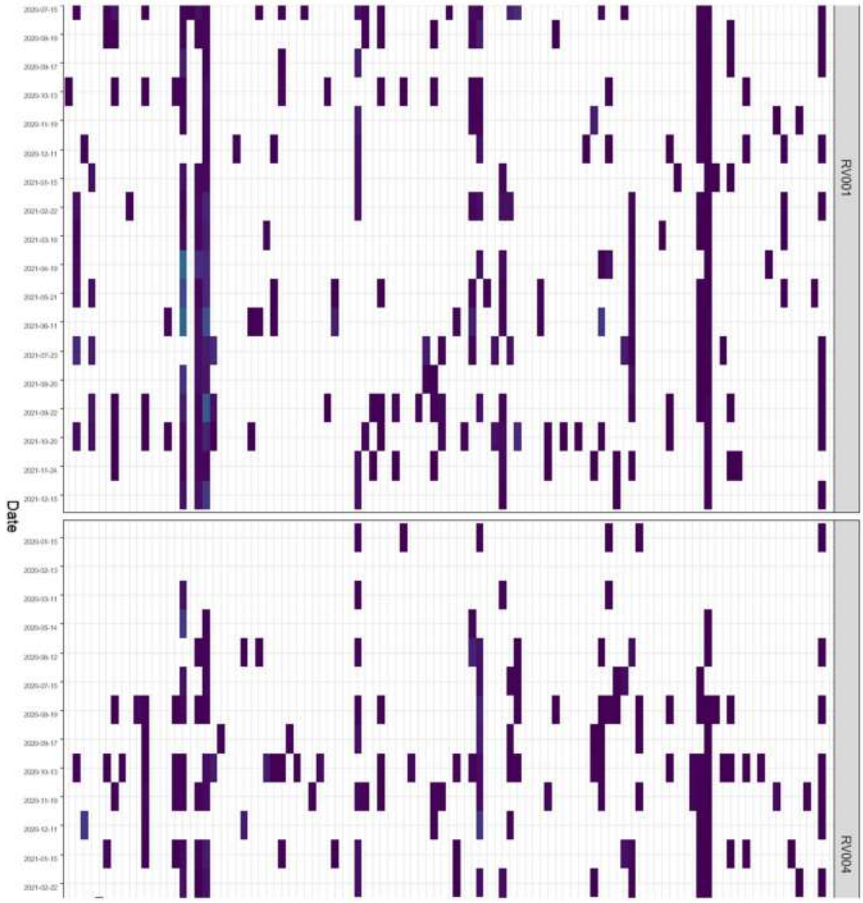


Figure 54. Monthly mean abundance dynamics of dinoflagellate species

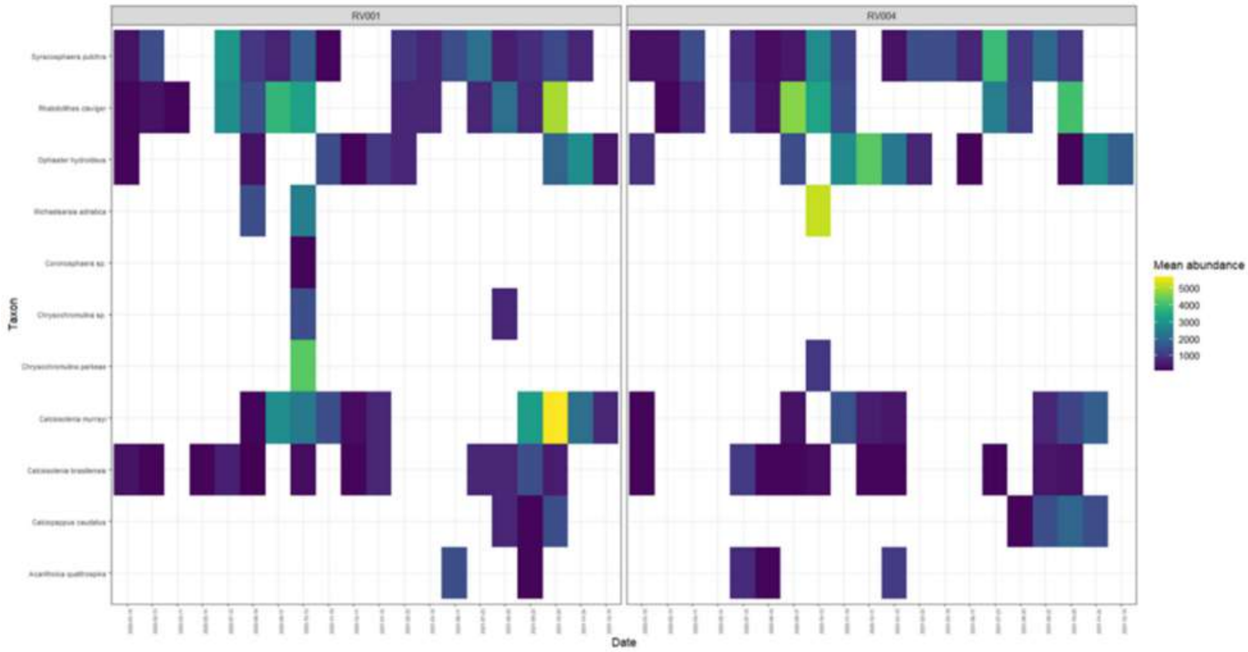


Figure 55. species Monthly mean abundance dynamics of coccolithophorid species

Metabarcoding analysis

This study aimed to report an up-to-date list of the phytoplankton taxonomic richness and phylogenetic relationship in eastern northern Adriatic, based on occurrence of their genetic material resolved with advanced molecular tools, namely metabarcoding. Monthly two-years net sampling aimed to target 6 phytoplankton groups including Dinophyceae (dinoflagellates), Bacillariophyceae (diatoms) and Chrysophyceae (golden algae) belonging to Ochrophyta,

Cryptophyceae (cryptophytes), Haptophyta (mostly coccolithophorids) and Chlorophyta with Prasinophyceae (prasinophytes) and Chlorophyceae (protist green algae). Generated sequence data were taxonomic assigned and redistributed in two kingdoms, five phyla, 46 orders, 67 families and 110 genera. Most diverse group were dinoflagellates, comprising of 45 found genera (41,3%), following by diatoms with 33 (30,3%) and coccolithophorids with 10 genera (11,0%). In the terms of genetic diversity, results were a bit different: more than half sequences with one nucleotide tolerance (ASVs) assigned to species or genus level were dinoflagellates (51,3%), 16,6% diatoms and 14,7% crytophytes. Although many taxa have not been detected that have been considered as a common in this area, metabarcoding revealed three diatoms and nine dinoflagellate genera that not reported in previous checklist, along with numerous species from other targeted groups that have been poorly reported previously.
In this metabarcoding study, a total of 7 785 ASVs, 3 229 99%-OTUs and 420 97%-OTUs were detected on genus or species level, of which 364, 453 and 172 were present with five of more reads per cluster (ASVs*, 97%-OTUs* or 99%-OTUs*). Most present phytoplankton groups were *Dinophyceae* and *Bacillariophyceae* no matter of clustering method used (Table 14).

Phytoplankton group	ASVs (%)	99%-OTUs (%)	97%-OTUs (%)	ASVs* (%)	99%-OTUs* (%)	97%-OTUs* (%)
Bacillariophyceae	16,57	18,33	25,89	14,72	19,33	27,65
Dinophyceae	51,28	49,54	55,82	55,56	46,22	46,47
Cryptophyceae	14,66	14,29	6,41	10,83	8,44	5,88
Chlorophyta	11,49	11,00	5,94	9,44	18,67	8,82
Prymnesiophyceae	5,48	6,10	4,99	8,06	5,78	8,24
Chrysophyceae	0,52	0,74	0,95	1,39	1,56	2,94

Table 14. Proportions of differences for each phytoplankton group and clustering method used for sequences assigned to genus or species level

As expected, different clustering methods, tend to show different taxonomic coverage. As a result, 148 taxa were assigned at a species level based on ASVs clustering, while with OTU clustering were retrieved 144 species (99%-OTUs) and 123 species (97%-OTUs). Using an additional restriction step which relates to number of reads generated for each ASV or OTU and keeping only those clustering units represented in five or more reads reduced significantly number of assigned species. Total number of species generated were 84 assigning ASVs*, 98 99%-OTU and 85 regarding 97%* which corresponds to 56,8%, 68,1% and 69,1% of ASVs, 97%-OTUs and 99%-OTUs retained after removing low abundant sequences/clustering units.

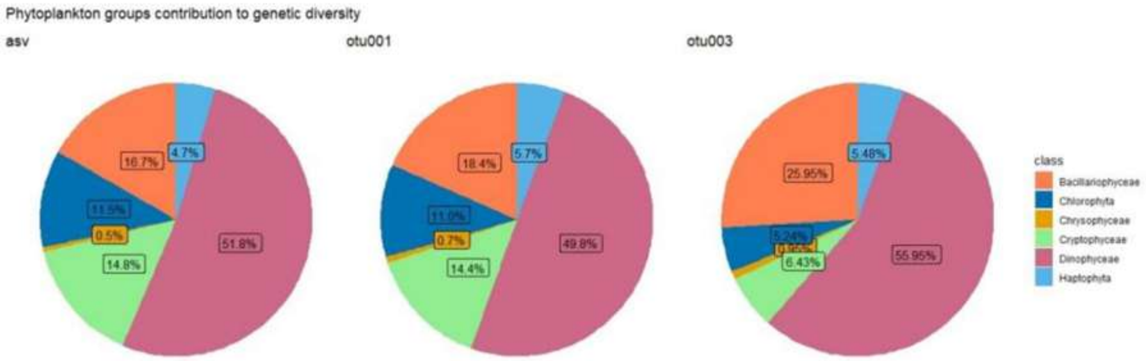


Figure 56. Relative contribution of phytoplankton groups. Changes in relative contribution in genetic diversity of different phytoplankton groups depending on clustering method used.

Five diatoms genera were revealed with molecular methods that have not been reported in phytoplankton checklists before. Four of them were assigned to species level containing one or more species per genus (*Cymbella*, *Meuniera*, *Mediolabrus*, *Minidiscus*), while *Fragilariopsis* was assigned up to genus level. Dinoflagellates group showed higher number of non-reported genera (9), and higher number assigned to species not mentioned in earlier complete checklist of this area of interest.

Class	Order	Genus	Species
Bacillariophyceae	*Chaetocerotales*	*Bacteriastrum*	*Bacteriastrum jadranum*
Bacillariophyceae	*Chaetocerotales*	*Bacteriastrum*	*Bacteriastrum parallelum*
Bacillariophyceae	*Cymbellales*	*Cymbella*	*Cymbella prostrata*
Bacillariophyceae	*Chaetocerotales*	*Chaetoceros*	*Chaetoceros eibenii*
Bacillariophyceae	*Leptocylindrales*	*Leptocylindrus*	*Leptocylindrus aporus*
Bacillariophyceae	*Thalassiosirales*	*Mediolabrus*	*Mediolabrus comicus*
Bacillariophyceae	*Naviculales*	*Meuniera*	*Meuniera membranacea*
Bacillariophyceae	*Thalassiosirales*	*Minidiscus*	*Minidiscus trioculatus*
Bacillariophyceae	*Thalassiosirales*	*Minidiscus*	*Minidiscus variabilis*
Bacillariophyceae	*Bacillariales*	*Pseudo-nitzschia*	*Pseudo-nitzschia galaxiae*
Bacillariophyceae	*Bacillariales*	*Pseudo-nitzschia*	*Pseudo-nitzschia pseudodelicatissima*
Bacillariophyceae	*Rhizosoleniales*	*Rhizosolenia*	*Rhizosolenia formosa*
Bacillariophyceae	*Thalassiosirales*	*Skeletonema*	*Skeletonema pseudocostatum*
Bacillariophyceae	*Thalassiosirales*	*Thalassiosira*	*Thalassiosira mala*
Bacillariophyceae	*Thalassiosirales*	*Thalassiosira*	*Thalassiosira profunda*
Dinophyceae	*Noctilucales*	*Abedinium*	*Abedinium dasypus*

Dinophyceae	*Gonyaulacales*	*Alexandrium*	*Alexandrium affine*
Dinophyceae	*Gonyaulacales*	*Alexandrium*	*Alexandrium andersonii*
Dinophyceae	*Gonyaulacales*	*Alexandrium*	*Alexandrium margalefii*
Dinophyceae	*Gonyaulacales*	*Alexandrium*	*Alexandrium ostenfeldii*
Dinophyceae	*Gonyaulacales*	*Alexandrium*	*Alexandrium tamarense*
Dinophyceae	*Amphidiniales*	*Amphidinium*	*Amphidinium massartii*
Dinophyceae	*Gonyaulacales*	*Amylax*	*Amylax triacantha*
Dinophyceae	*Blastodiniales*	*Blastodinium*	*Blastodinium contortum*
Dinophyceae	*Gymnodiniales*	*Balechina*	*Balechina pachidermata*
Dinophyceae	*Blastodiniales*	*Blastodinium*	*Blastodinium mangini*
Dinophyceae	*Blastodiniales*	*Blastodinium*	*Blastodinium navicula*
Dinophyceae	*Blastodiniales*	*Blastodinium*	*Blastodinium spinulosum*
Dinophyceae	*Gonyaulacales*	*Cucumeridinium*	*Cucumeridinium coeruleum*
Dinophyceae	*Pyrocystales*	*Dissodinium*	*Dissodinium pseudolunula*
Dinophyceae	*Gymnodiniales*	*Erythropsidinium*	*Erythropsidinium agile*
Dinophyceae	*Gonyaulacales*	*Fragilidium*	*Fragilidium mexicanum*
Dinophyceae	*Gonyaulacales*	*Fragilidium*	*Fragilidium subglobosum*
Dinophyceae	*Gonyaulacales*	*Gonyaulax*	*Gonyaulax cochlea*
Dinophyceae	*Gonyaulacales*	*Gonyaulax*	*Gonyaulax ellegaardiae*
Dinophyceae	*Gymnodiniales*	*Karenia*	*Karenia brevis*
Dinophyceae	*Gymnodiniales*	*Karlodinium*	*Karlodinium veneficum*
Dinophyceae	*Noctilucales*	*Kofoidinium*	*Kofoidinium pavillardii*
Dinophyceae	*Gymnodiniales*	*Lepidodinium*	*Lepidodinium chlorophorum*
Dinophyceae	*Dinophysiales*	*Ornithocercus*	*Ornithocercus heteroporus*
Dinophyceae	*Gymnodiniales*	*Paragymnodinium*	*Paragymnodinium shiwhaense*
Dinophyceae	*Gymnodiniales*	*Pelagodinium*	*Pelagodinium bei*
Dinophyceae	*Dinophysiales*	*Phalacroma*	*Phalacroma porodictyum*
Dinophyceae	*Prorocentrales*	*Prorocentrum*	*Prorocentrum cordatum*
Dinophyceae	*Gonyaulacales*	*Protoceratium*	*Protoceratium reticulatum*
Dinophyceae	*Gymnodiniales*	*Torodinium*	*Torodinium robustum*

Table 15. *Diatoms and dinoflagellates species revealed by metabarcoding, assigning taxonomy based on ASVs as a clustering method with no reads cutoff, not present in checklist*

Phylum	Class	Order	Species
Cryptophyta	*Cryptophyceae*	*Pyrenomonadales*	*Hemiselmis andersenii*
Cryptophyta	*Cryptophyceae*	*Pyrenomonadales*	*Hemiselmis cryptochromatica*
Cryptophyta	*Cryptophyceae*	*Pyrenomonadales*	*Hemiselmis rufescens*
Cryptophyta	*Cryptophyceae*	*Pyrenomonadales*	*Proteomonas sulcata*
Cryptophyta	*Cryptophyceae*	*Pyrenomonadales*	*Rhodomonas salina*
Cryptophyta	*Cryptophyceae*	*Pyrenomonadales*	*Teleaulax amphioxeia*
Cryptophyta	*Cryptophyceae*	*Pyrenomonadales*	*Teleaulax gracilis*
Ochrophyta	*Chrysophyceae*	*Hibberdiales*	*Helicopedinella tricostata*
Ochrophyta	*Chrysophyceae*	*Chromulinales*	*Paraphysomonas bandaiensis*
Ochrophyta	*Chrysophyceae*	*Chromulinales*	*Paraphysomonas imperforata*
Ochrophyta	*Chrysophyceae*	*Parmales*	*Triparma pacifica*
Ochrophyta	*Chrysophyceae*	*Parmales*	*Triparma strigata*
Haptophyta	*Pavlovophyceae*	*Pavlovales*	*Pavlova pinguis*
Haptophyta	*Pavlovophyceae*	*Pavlovales*	*Rebecca salina*
Chlorophyta	*Prasinophyceae*	*Mamiellales*	*Bathycoccus prasinos*
Chlorophyta	*Prasinophyceae*	*Mamiellales*	*Micromonas bravo*
Chlorophyta	*Prasinophyceae*	*Mamiellales*	*Micromonas commoda*
Chlorophyta	*Prasinophyceae*	*Mamiellales*	*Micromonas pusilla*
Chlorophyta	*Prasinophyceae*	*Nephroselmidales*	*Nephroselmis pyriformis*
Chlorophyta	*Prasinophyceae*	*Mamiellales*	*Ostreococcus tauri*
Chlorophyta	*Prasinophyceae*	*Halosphaerales*	*Pterosperma cristatum*
Haptophyta	*Prymnesiophyceae*	*Prymnesiales*	*Chrysochromulina leadbeateri*
Haptophyta	*Prymnesiophyceae*	*Prymnesiales*	*Chrysochromulina simplex*
Haptophyta	*Prymnesiophyceae*	*Isochrysidales*	*Dicrateria rotunda*
Haptophyta	*Prymnesiophyceae*	*Isochrysidales*	*Gephyrocapsa oceanica*
Haptophyta	*Prymnesiophyceae*	*Prymnesiales*	*Imantonia rotunda*
Haptophyta	*Prymnesiophyceae*	*Phaeocystales*	*Phaeocystis globosa*
Haptophyta	*Prymnesiophyceae*	*Phaeocystales*	*Phaeocystis jahnii*
Haptophyta	*Prymnesiophyceae*	*Prymnesiales*	*Prymnesium pienaarii*
Chlorophyta	*Chlorophyceae*	*Chlamydomonadales*	*Chlamydomonas parkeae*

Table 16. Species belonging to phytoplankton groups Cryptophyceae, Chrysophyceae, Haptophyta and Chlorophyta, revealed by metabarcoding, assigning taxonomy based on ASVs as a clustering method with no reads cutoff, not present in checklist

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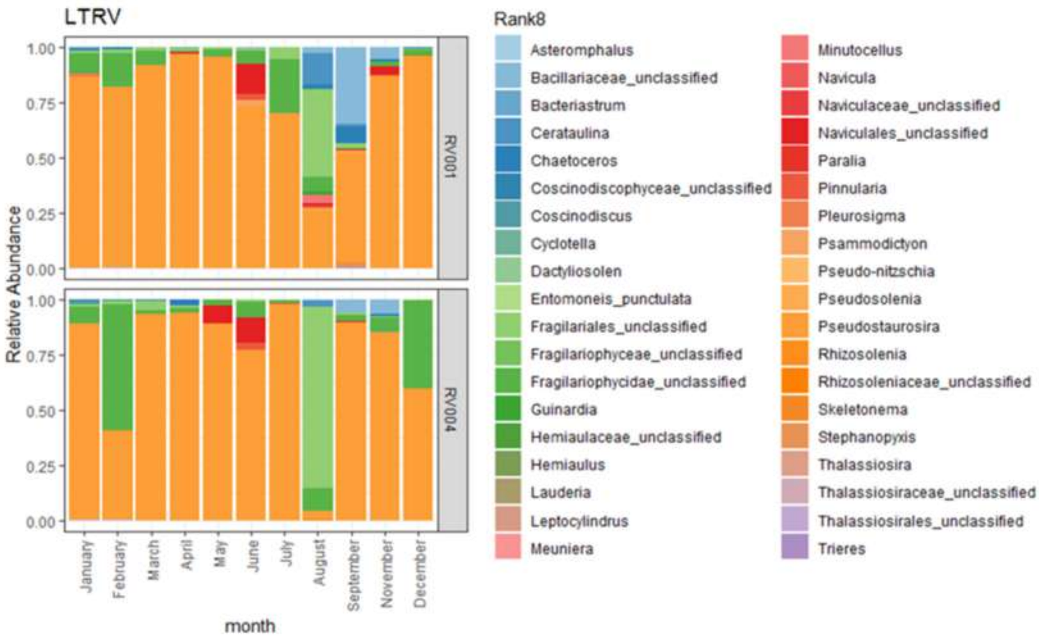


Figure 57. Comparison of the 2 sampling stations (RV001 and RV004) diatom community composition at the genus level

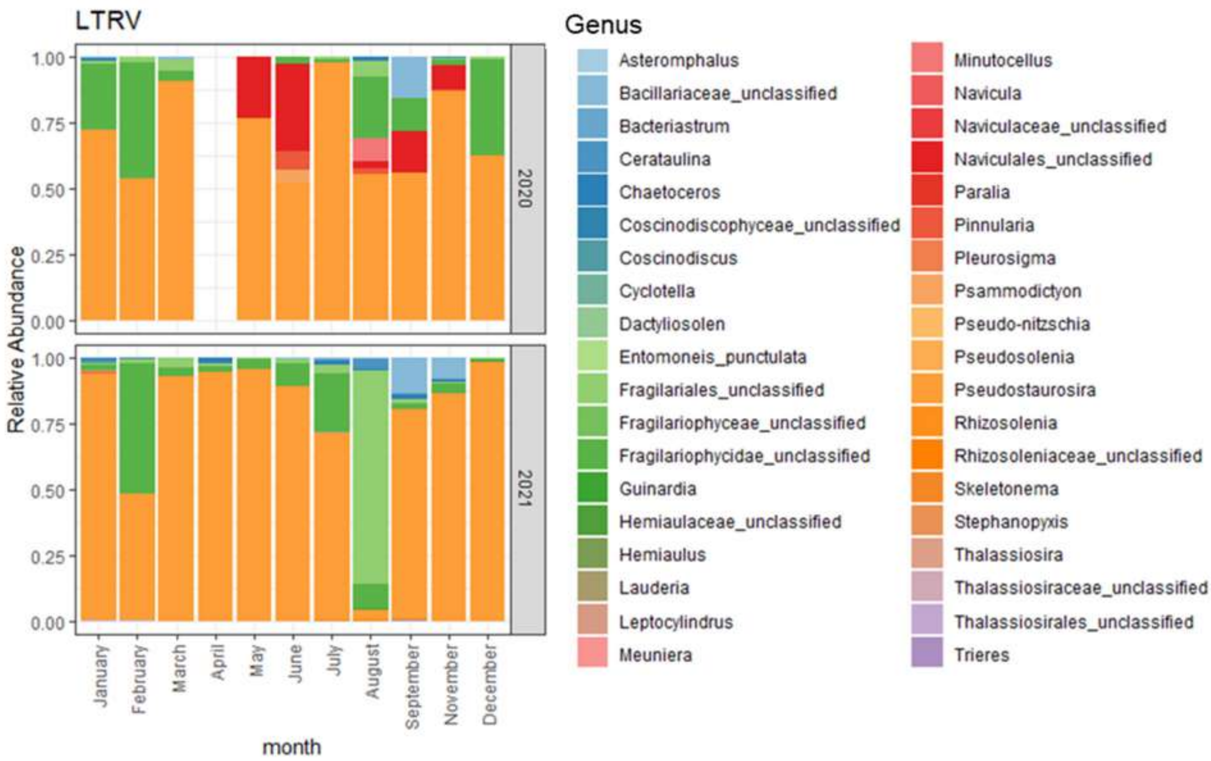


Figure 58. Comparison of the 2 sampling stations (RV001 and RV004) diatom community composition at the genus level Bacillariophyta unclassified removed.

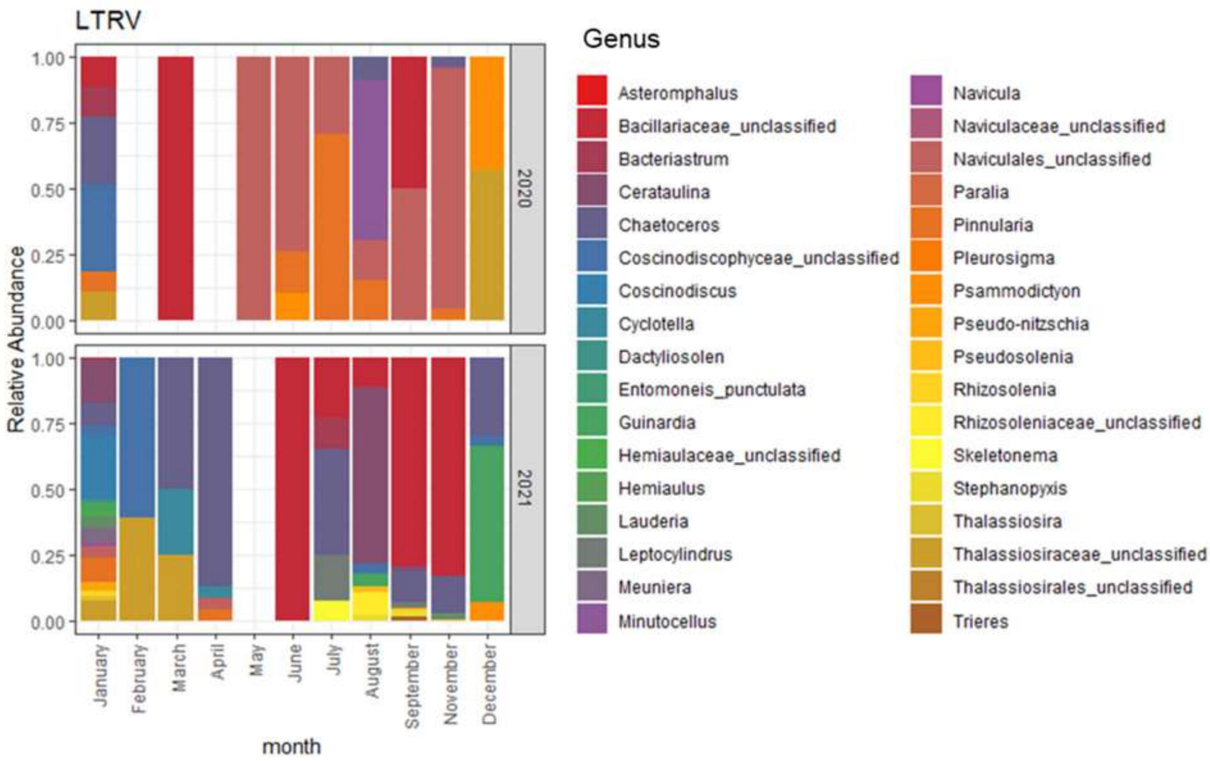


Figure 59. Fragilariophyceae removed because it dominated the samples in abundance (genus Pseudostaurosira)

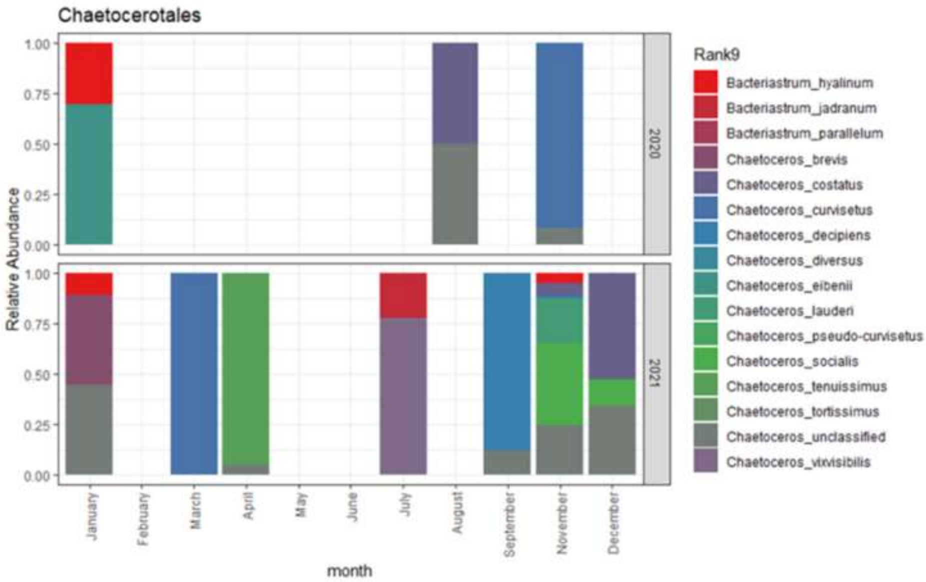


Figure 60. Comparison of the 2 sampling stations (RV001 and RV004) Chatoceros community composition at the species level revealing 16 different species in the genus.

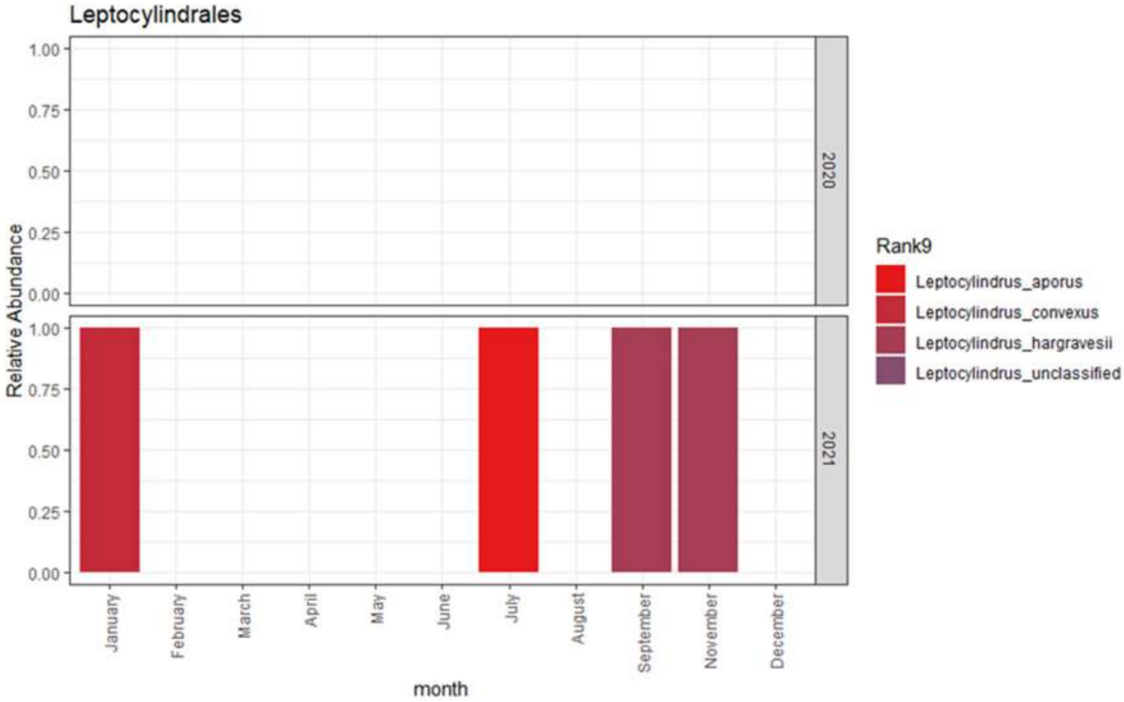


Figure 61. Comparison of the 2 sampling stations (RV001 and RV004) *Leptocilindrus* community composition at the species level. Discerning three species that cant be identified in light microscopy by traditional methods.

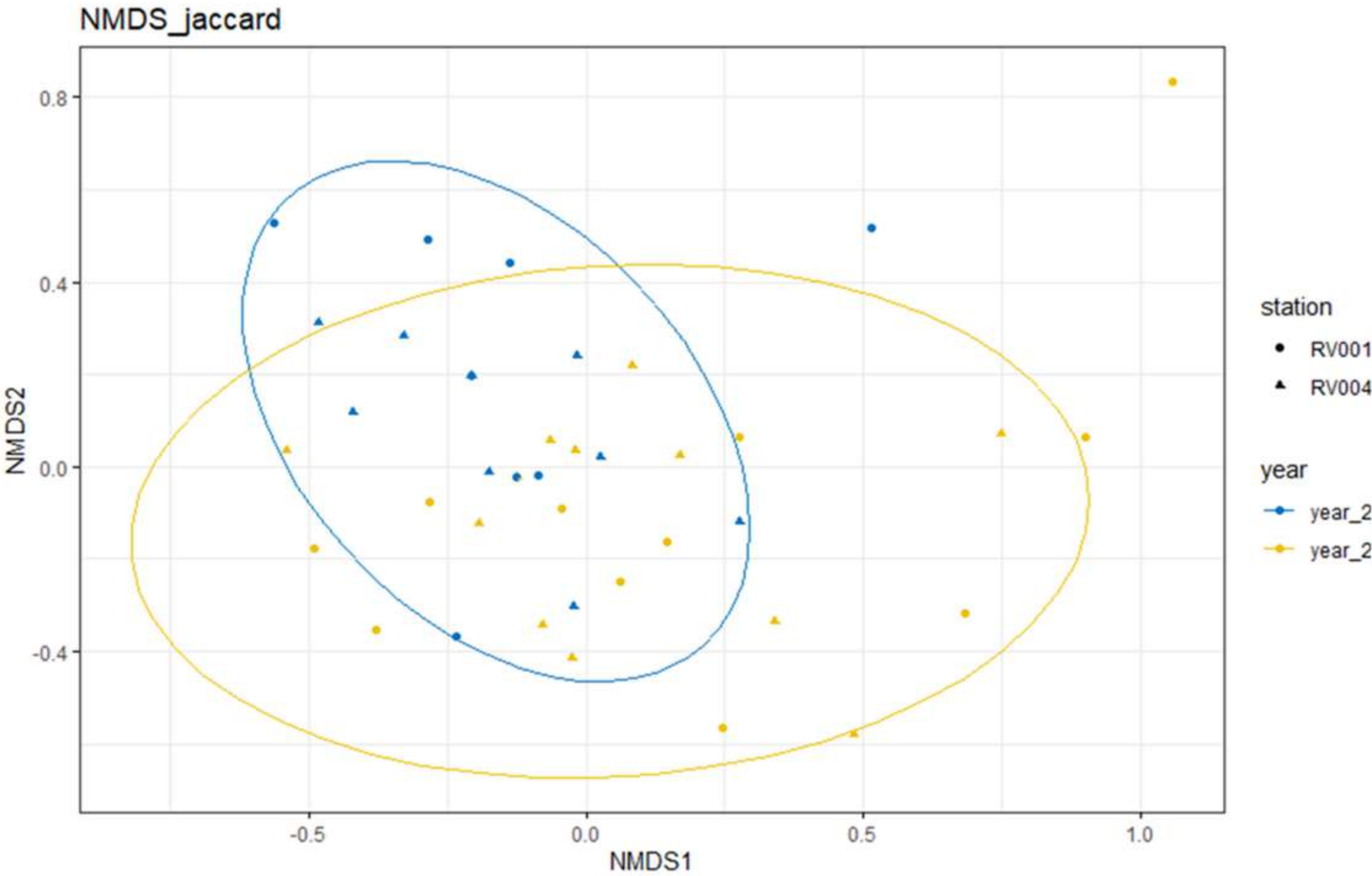


Figure 62. No significant diference between the stations and years based on the taxa abundance (Bray-Curtis distance matrix: station p=0.561, year p=0.639) but there is a significant difference between years based on taxa composition, presence absence (Jaccard distance matix: station p=0.962, year p=0.003 **)

P9. Cetina river mouth (HR)

Through WP 3 Sea and Karst firstly did a Review of existing observing and modelling systems (act 3.1) and then an Ecosystem characterization report (act 3.2) for the pilot area. Based on these outputs Sea and Karst engaged external experts to help the management authority in Design of the optimal observing systems for marine coastal environment characterization (act 3.3) and to Set-up and testing of the observing system (act 4.1). As planned in the Application Form, Public Institution Sea and Karst did not carry out any modelling activities as some other CASCADE partners did for their pilot areas.

As pointed out earlier, the state of the ecosystem in relation to EU biodiversity directives is not carried out. Accordingly, Sea and Karst developed and tested monitoring (observing) system for pilot area. The purpose of the observing system is to improve knowledge on the state of Natura 2000 site key habitat types for the purpose of carrying out conservation activities. In this initial phase of assessing the state of the Cetina estuary as a complex habitat, Sea and Karst focused on one of its most representative, key habitat types: 1110 Sandbanks which are slightly covered by the sea water all the time. The envisaged activities included: (1) establishment of high-resolution monitoring of environmental conditions in the sea (temperature, salinity, oxygen, etc.); (2) assessment of a dominant benthic habitat i.e. *Cymodocea nodosa* meadows; (3) testing of a monitoring system focusing on key benthic habitat formers (e.g. *Cymodocea nodosa, Pinna nobilis*); (4) testing of a monitoring system for *Cladocora caespitosa* bioconstruction that was discovered adjacent to the current southern border of the pilot Natura 2000 site Cetina estuary; (5) formulation of a proposal for the revision of the existing Natura 2000 site to include *Cladocora caespitosa* bioconstruction, belonging to the priority habitat type 1170 Reefs; (6) capacity building of managing authority for carrying out monitoring and conservation activities; (7) identification of risks and threats to the Pilot Area; (8) identification of conservation measures for the Pilot Area. Furthermore, in WP5 Sea and Karst tested soft pilot restoration actions supporting critically endangered species and coastal and transitional ecosystems in relation to *Pinna nobilis*.

The results of testing activities 3 and 4 are briefly outlined here while details of setting-up and testing of monitoring (observing) system are available in CASCADE project reports, namely, D.3.3.1 Report on design the optimal observing system for marine coastal environment characterization and D.4.1.2 In situ observing campaigns, as well as in external experts' reports, prepared by Kipson (2023) and OIKON (2023).

In summary, a constant increase in the area covered by *Cymodocea nodosa* of approximately 6-7% every two to three years over the entire area of the Cetina Estuary Natura 2000 site is observed. The only exception was a period between 2017 and 2019 when such trend was not present. This however coincides with the putative increase in sand excavation activities in the period from 2016 to 2017. Overall, in a decadal period (2011-2021) *C. nodosa* extent has increased from 43.65 to 54.04 ha within the Cetina Estuary Natura 2000 site (Figure 63.), ultimately comprising 40% of the

Annex I 1110 Habitat type present within the site (in total 135 ha reported in Natura 2000 Standard Data Form).

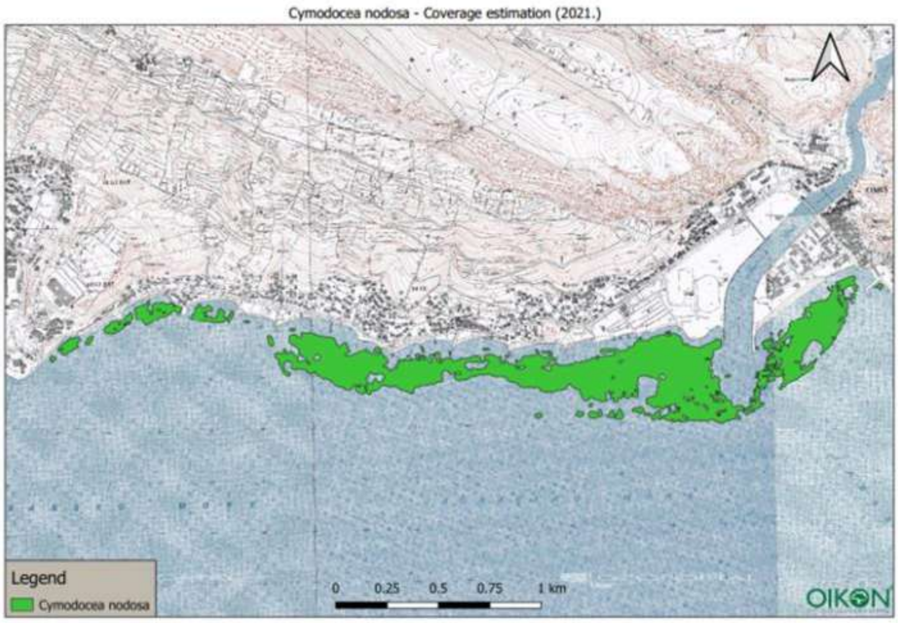


Figure 63. Meadows cover estimation map - 2021

Overall, as already mentioned, the surface of the *Cymodocea nodosa* bed has increased over a decadal period (2011-2021) within the Cetina Estuary Natura 2000 site by 10.4 ha (OIKON 2023).

It has increased even after the occurrence of the most recent event of sand extraction in 2016. However, it seems this surface increment mainly results from lateral expansion of the outer meadow edge and not so much due to recolonization of the areas suspected to be previously extracted. It is clear that seagrass has disappeared from some of the areas where it was formerly present (over 2.36 ha) and furthermore, such areas seem to “heal“slowly and have not fully recovered 5-yr after the adverse event. To reemphasize, although initiation of regeneration can be identified over 53% of meadow area formerly affected by human interventions, in most cases *Cymodocea nodosa* density and habitat structure are still far from the ones we would expect for mature, well preserved meadows in the area. Based on the results of Kipson (2023) study, habitat extent assessed by OIKON (2023) and previous results of periodic monitoring in the scope of the WFD (summarized in Kurtović-Mrčelić 2021), we can conclude that *C. nodosa* and its meadows, as a representative habitat of 1110 *Sandbanks which are slightly covered by the sea water all the time* Habitat type remained in favourable status within the Cetina Estuary during the past 10 years, despite of sporadic mechanical damage caused by sand extraction and human activities along the coastline. This would further confirm *Cymodocea nodosa* being quite a resilient species (Kipson 2023).

Starting from 2020, i.e. from the beginning of the CASCADE project, collectors of Noble Pen shell (*Pinna nobilis*) larvae are set up every year, a total of 24 of them at 3 locations with different characteristics in terms of the former population density inside the Cetina Estuary. The results of the analysis of the collectors showed that in 2020, there was a recruitment of the species, because a juvenile individual of Noble Pen shell with a size of 1.2 cm was found in the collector at a depth of 6 m at the Mala luka location. During 2021 and 2022, we can conclude that there was no recruitment, because no juveniles of *Pinna nobilis* were found in the installed collectors. The individual found in 2020. died in in-vitro breeding conditions in the Pula Aquarium, and analyses by the Spanish LIMIA (Laboratory of Marine Research and Aquaculture - Government of the Balearic Islands) laboratory showed that the individual was positive for the parasite *Haplosporidium pinnae*, considered as the main cause of the Mass Mortality Event (MME) of the Noble Pen shell. In February 2022, thanks to citizens science approach, a live in-situ individual of Noble Pen shell with total length (TL) of 22 cm was recorded. It has been protected by a cage, and its development is being monitored for the purpose of restoring the population (Figure 64.). After the occurrence of the MME, this is for now the only recorded living individual of the Noble Pen shell south of Zadar.

Figure 64. Living specimen of the Noble Pen shell

The implemented observing system show that cushion coral *Cladocora caespitosa* bioconstruction that was discovered adjacent to the current southern border of the pilot Natura 2000 site Cetina stretches from 24.6 m to 28 m depth. It occupies an area of 36.1 m^2 with the maximum length of 15.8 m and the maximum width of 3.8 m. The bioconstruction is thicker in its lower part (from 27-

28 m depth; with mean thickness of 50.17 ± 4.67 cm) than in the upper one (at approx. 25 m depth; 42.88 ± 6.24 cm). Moreover, the reef appeared to be the healthiest in its lower part (from 27-28 m depth), with mean live coral cover of 86.78 ± 19.63%, followed by considerably lower mean live coral cover in its central (29.52 ± 33.85%) and upper part (24.6-25 m depth) which hosted mere 6.96 ± 16.90% (mean ± SD) of live coral cover. When assessing the condition on the scale of the whole reef, the mean percentage of live coral cover amounted to 45.03 ± 42.09% (n = 52). Besides the main bioconstruction in form of colonies "fused" into a bank, several non-fused colonies are present next to the reef. Their total surface area amounts to additional 4.77 m^2, ranging in size from 0.01 to 2.1 m^2 (Figure 65.).

Figure 65. The orthophoto mosaic of the *Cladocora caespitosa* reef and nearby colonies at the Mala Luka site.

Moreover, there are numerous smaller colonies present nearby, scattered over an area that stretches for at least 100 m. Overall, mean number of all colonies per m^2 was 4.1 ± 1.9, whereas the mean number of alive colonies per m^2 was approximately half that value, 2.2 ± 0.9. In total, size of 387 colonies could be assessed from video frames, and mean values together with minimum and maximum measured sizes (in terms of colony surface area) per each individual transect. While most of alive colonies were attached to the substrate, also unattached “spherical” colonies were observed, referred to as “coral nodules”. Moreover, small individual colonies were observed also in the shallows of the same site, around 10-15 m depth (Kipson, 2023).

In terms of establishment of high-resolution monitoring of environmental conditions in the sea (temperature, salinity, oxygen, etc.) Sea and Karst performed two activities. External expert installed in-situ HOBO® Pendant temp/light dataloggers which will record seawater temperature every hour, starting from July 16th 2022 at 1 am along depth gradient at Mala luka in the vicinity of the *Cladocora caespitosa* reef (Kipson 2023). Sea and Karst also installed in-situ Aqua TROLL 600 probe measuring temperature, salinity and dissolved oxygen at the Shallow mark at 5 m depth starting from March 7th 2023. Measurements are taken in vicinity of resistant Noble Pen shell individual, at location that once had moderate population density of critically endangered shell and that is under strong influence of the freshwater. Results of such monitoring will be visible at longer term and cannot be discussed during CASCADE project implementation period.

Sea and Karst did not have data before the lockdown event, so estimation of its effects is not discussed.

P10. Torre del Cerrano, Pineto Abruzzo (IT)

UNIMOL analyzed the vegetation dynamics after the establishment of wooden boardwalks (protecting dunes form human trampling) across different coastal dune habitats, through a multi-temporal analysis of vegetation plots collected before and after the implementation of conservation actions in the Central Adriatic coast in Italy. Sampling strategy and ecosystem models were prepared on WP3 and WP4 and then were discussed the observed changes and focused on the effects of conservation actions on ecosystems using plants as bioindicators of change and eventual improvement of ecosystem integrity and functioning.

During the years 2019-20, have been performed vegetation monitoring plots (hereafter T2), on areas in which broad-walks to protect dunes form trampling were implemented in and compared with plots collected before the implementation of conservation actions (e.g. control data before the boardwalks installation; Fig 66, 67). Specifically, have been generated random plots in a GIS environment, placed within a 100-m radius of each planned boardwalk. The dune vegetation has been sampled in 53 random plots before conservation action implementation and re-sampled after the boardwalks' installation. Have been then re-sampled the dune vegetation in the same plots in the year 2019-20across the vegetation zonation, following a typical sea-inland gradient, ranging from pioneer annual communities on the beach to Mediterranean scrubs on the landward fixed dunes: upper beach (EU Habitat 1210), embryo dune (EU Habitat 2110), mobile dune (EU Habitat 2120), dune grasslands (EU Habitat 2230), Mediterranean maquis (EU Habitat 2260) and pine forest
(EU Habitat 2270) (Stanisci et al. 2014). For each georeferenced vegetation plot has been registered the complete list of vascular plants and their cover values. Species nomenclature follows the updated checklist of "Flora d'Italia". For each dune-habitat has been compared species richness in control and testing plots through rarefaction curves for overall species and for specific species guilds indicating conservation status (e.g. focal species) as well as disturbance (e.g. ruderals) Figure 66.


Figure 66. UNIMOL-CASCADE working group collecting data on coastal dunes monitoring areas

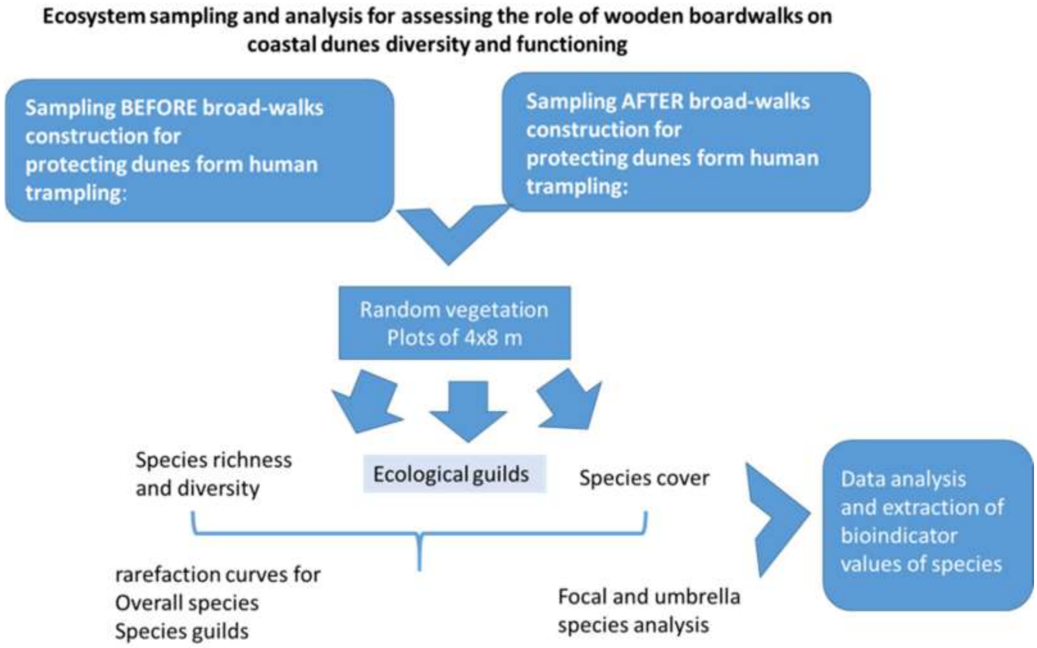


Figure 67. Flowchart depicting methodical approach implemented for the assessment and monitoring of conservation actions effects on coastal dune ecosystems

P11. Marche coastal area (IT)

In situ observing campaigns are made up of two different types of analyses which concern the entire coastal area of the Marche region:

1. Analysis of emerged biocenosis
2. Analysis of submerged biocenosis

1. Analysis of biocenosis emerged

The Analysis of emerged biocenosis is a visual campaign, which concerns the updating of the dune vegetation data and the definition of emerged biocenoses. It has 3 main objectives:

- Identification and updating of coastal habitats;
- The identification of specimens of *Charadrius alexandrinus*;
- The eventual identification of the *Charadrius alexandrinus* nesting sites.

The investigated transects are listed below in the table, with reference to the time schedule that was followed in the field.

20/03/2023	21/03/2023	22/03/2023	23/03/2023	24/03/2023	25/03/2023	26/03/2023	27/03/2023	28/03/2023
01bis_fr_220	06_fr_255	03_he_039	13_fr_535	17_he_410	20_he_587	10_he_116	20_fr_772	12bis_fr_534
02_fr_223	07_fr_260	02_he_036	14_fr_537	15_he_404	21_he_622	11_he_117	21_fr_774	13bis_he_324
03_fr_224	08_fr_261	01_he_034	15_fr_539	14_he_401	17_fr_626	12_he_118	22_fr_775	01_fr_168
04_fr_226	09_fr_262	09_he_105	16_fr_550	16_he_399	17bis_fr_635	13_he_120	23_fr_777	
05_fr_227	10_fr_266	08_he_104	15bis_fr_544	19_he_448	18_fr_651		24_fr_780	
	11_fr_272	07_he_102			19_fr_652		24_he_706	
	12_fr_274						23_he_701	
							22_he_700	

The monitoring of emerged biocenosis was carried out on transects of variable length between 100 and 200 metres, with a lateral buffer that included any dune and back-dune belt, for the identification of habitats. For what concerns the identification of the plover, the sighting buffer was greater, extended from the vegetated belt to the shoreline (in many cases individuals were sighted intent on feeding).

An inspection report was drawn up for each transect, and it contains the data relating to the present detectors, the weather conditions, the start of the transect coordinates and the time.

Furthermore, each monitoring has provided for the drafting of field cards regarding the sighting of the plover and the habitats identification.

Three different habitats have been surveyed on the coast:

Habitat 1210: Annual vegetation of marine deposit lines

Annual herbaceous formations (therophytic-halonitrophilous vegetation) that colonize the sandy beaches and with thin pebbles, near the shoreline where the organic material carried by the

waves accumulates and decomposes, creating a substrate rich in sea salts and decomposing organic matter. It is an exclusively coastal habitat and therefore linked to the dynamism of the shoreline but which, given the ecological characteristics of the present organisms, is able to maintain itself even in conditions of high anthropogenic pressure.
The indicator species of the habitat are represented by *Cakile maritima, Salsola kali, Euphorbia peplis, Polygonum maritimum, Matthiola sinuata, Xanthium orientale subsp. italicum*. The presence of young individuals of *Elymus farctus* and/or *Sporobolus virginicus* is frequent, due to the chain contact with the vegetation of the embryonic dunes.
The newly discovered phytocoenosis connected to this habitat is the Salsolo-Cakiletum one.

Habitat 2110: Mobile embryonic dunes

It refers to sandy micro-dunes, which are formed due to some specialized plants' soil retaining action. These plants, with their underground apparatus formed by long and intricate rhizomes, create a network which locks the sand in place thus forming small accumulations which form a dune cordon, with the capability of opposing the wind action from the sea.
The habitat indicator species are Elymus with: *Sporobolus pungens* (= *S. arenarius*; more recently referred to as *S. virginicus*), *Euphorbia peplis, Otanthus maritimus, Medicago marina, Anthemis maritima, A. tomentosa, Eryngium maritimum, Echinophora spinosa, Calystegia soldanella, Cyperus capitatus, Polygonum maritimum, Silene corsica, Rouya polygama, Lotus creticus, Lotus cytisoides ssp. conradiae, Solidago litoralis, Centaurea subciliata, Spartina juncea*.
The found phytocoenosis connected to this habitat is the *Echinophoro-Agropyretum*.

Habitat 2120: Shifting dunes of the coast with the presence of *Ammophila arenaria*

It refers to the sand dunes of the line behind the embryonic dunes which are higher and more structured than the latter. Also in this case, the dune construction architect is a grass, called *Ammophila arenaria*, equipped with a robust system of underground stems and roots capable of growing vertically and horizontally, retaining large quantities of sand.

Habitat 2230: Dunes with meadows of the Malcolmietalia

Predominantly annual vegetation, with prevalent late-winter-spring phenology of sandy substrates, weakly to strongly nitrophilous, located in clearings of perennial vegetation belonging to the *Ammophiletea* and *Helichryso-Crucianelletea* classes; it is affected by the evolution of the dune system, in relation to the winds action and to the animals and people passage. The habitat is distributed on sandy coasts with both Mediterranean and temperate macrobioclimates. The phytocoenosis connected to this habitat is the *Sileno-Vulpietum* one.
In some cases, these habitats are found as mosaic associations since the structure does not allow a clear differentiation of the habitats (1210-2110 and 2110-2120).

The described habitats are in some cases strongly altered, and due to anthropization and the massive presence of tourism, combined with the beaches narrow width and the presence of cycle paths, roads and railways that are located immediately behind the beach, they cannot find themselves in optimal conditions.

Often, the presence of plant associations and vegetation could be indicators of habitat, however, the strong pressures and threats to the area meant that these could not be registered as such.

Below, there are 3 examples and photos of the monitoring carried out.

3 types of habitats have been identified on the north transects of Senigallia, found in a good state of conservation in the transects 01bis_fr_220, 02_fr_223 and 03_fr_224:

- 1210: Annual vegetation of marine deposit lines
- 2110: Mobile embryonic dunes
- 2230: Dunes with meadows of the Malcolmietalia

Figure 68. Transept 01bis_fr_220 - The signs of mechanical means on the sand are visible

In the south transept of Senigallia 07_fr_260, 3 specimens of *Charadrius alexandrinus*, 2 males and one female, have been sighted.

Figure 69. Transetto 07_fr_260 - Esemplare (*Charadrius alexandrinus*)

The **beaches in the Conero area** are mainly sandy, with gravel deposits in the area back to the sea, between 15 and 30 meters wide.

Figure 70. Transetto 14_he_401 - Type of beach with gravel filling

No habitats of interest have been surveyed in these areas, as the conformation of the mountain slope overlooking the beach means that no dune and back-dune vegetation could be created.
There are no sea cliffs and the action of the waves significantly erodes the mountain clayey side, causing frequent landslides.

Figure 71. Transept 15_he_404 - Landslides and landslides of the upstream slope

The vegetation that settles in these areas is limited to some rocky herbaceous species, such as *Matthiola sinuata* and *Helichrysum*, some tree and shrub species typical of the thermo-Mediterranean shrub communities, such as *Cytisus spp* and *Genista spp*, and other species such as *Smilax aspera, Pittosporum tobira*, *Pistacia lentiscus* and *Phyllirea angustifolia*.

Figure 72. Transept 19_he_448 - Vegetation present

The avifauna consists of some specimens of herring gull, cormorants intent on fishing and crows on the vegetation of the slope. Some specimens of unidentified prey birds have been spotted in the distance.

The area investigated in **the Sentina Nature Reserve (Porto d'Ascoli coast**) has common characteristics for all transepts, except 20_fr_772 which falls further north and is much poorer in vegetation. There are no sea cliffs and the beach is characterized by reduced width, between 15 and 25 meters, with very fine sand. Throughout the area you can see deposits of organic material and woody material, even of large dimensions, transported and deposited by the sea.
The area corresponding to the transept 21_fr_774 is heavily eroded in the northern part, while the other transects show no evident signs of erosion.
The present phytocenoses are those of *Salsolo-Cakiletum* for the annual deposit vegetation (habitat 1210) and *Echinophoro-Agropyretum* for the mobile embryonic dunes (habitat 2110).
The species that characterize the strip of vegetation in front of the dune, when present and not eroded by the sea, are those that are able to settle among the timber deposits of the beach, mainly *Salsola kali* and *Cakile maritima*; there are also some specimens of *Raphanus raphanistrum* on the transept 20_fr_772.

Figure 73. Transept 23_fr_777 - Vegetation in front of the dune

The species that instead colonize the dune belt are mainly *Xanthium italicum*, *Limbarsa crithmoides* and *Atriplex portulacoides*, the latter more typical of the salty environments behind the dunes.

The rear part is characterized by the humid environments vegetation, with the *Arundo donax* and *Phragmites australis*.


Figure 74. Transept 21_fr_774 - Vegetation behind the dune belt

In an area of the transept 22_fr_775, close to the present building, we find a settlement of *Glycyrrhiza glabra*.

The present avifauna is mainly connected to the presence of the humid environments behind it: in fact, some specimens of egret, a gray heron, a flock of ducks in flight and some common gulls have been identified.

The main threats and pressures are related to the marine erosion, which plays a fundamental role in the erosion of the embryonic dune and in the annual renewal of the deposit lines.

Figure 75. Transept 20_fr_772 - Presence of strong erosion

Furthermore, trampling and human presence also cause disturbance, even if it is not decisive for conservation purposes, to the present vegetation.

2. Analysis of biocenosis submerged

The third activities phase was represented by the investigations with inspections for the identification and delimitation of submerged biocenoses and habitats.
The analysis of submerged biocenosis were carried out in the month of June (19-25 June 2023), since bad weather in May did not allow boat trips.
Instrumental detection surveys were carried out through the use of a state-of-the-art ROV generation equipped with Full HD camera, lighting system and positioning system Built-in USBL.
The investigation protocol normally used for "Marine Strategy" projects were applied.
On board of the vessel that has been used for this activity, in addition to the ROV Video Ray Pro4 and yours integrated positioning system USBL Micronav, the following instrumentation was present:
- Multi Beam Echosounder R2 Sonic for morphobathymetric data acquisition;
- IMU model Ixea Octans III for data acquisition of: Heading, Pitch, Roll, Heave and relative compensation of the dynamic effects induced with a frequency of 80Hz refresh;

- SVP On line Mini SVS Valeport for determining the speed of sound in real time;
- DGPS Trimble NetR9S for determining:
    - absolute position (NMEA CGA string),
    - synchronization parameters (NMEA ZDA string and PPS Output pulse),
    - real-time range of the tide.

All sensors have been pre-installed on the vessel in order to reduce installation and calibration time and possible delays. In any case, at the beginning of each survey, we acted in order to calibrate the system through the procedure "Patch Test", as required in the Technical Regulations for the Standardization of Hydrographic Surveys drawn up by the Hydrographic Institute of the Navy, 2021 edition.

For navigation, control and data recording operations, we used the Software Teledyne PDS 2000 release 4.3.8.0 2020.

In relation to submerged biocenosis, nine different transepts (between 100 and 150 metres of length) have been analysed, and two different habitats have been surveyed. The analysis of transepts allowed the seabed and the marine ecosystem surveys for the identification and delimitation of biocenoses and submerged habitats.

Figure 76. Transepts position (in black) 01_hs_420, 02_hs_423, 03_hs_428, 04_hs_432, 05_hs_434, 06_hs_438, 07_hs_444

Figure 77. Position of transepts (in red) 08_hs_772 and 09_hs_779 (it falls within the SCI area IT5340001 - Porto d'Ascoli coast)

The species found on the area are: *Liocarcinus vernalis*, *Cystoseira* spp., *Peyssonnelia*, *Aliclona* spp., *Mesophyllum lichenoides*, *Ircinia* spp., *Sabella spallanzanii*, *Axinella* spp.
The habitats identified within the analysed area could be described as follow:

Habitat 1170 : Reef

This environment is characterized by a substrate composed by rock with *Sabella spallanzanii* species polychaete and yellow sponge, which, probably, could be identified as the *Axinella* spp. In some specific transepts, this mixed environment is composed by sandy and rocky substrate. The *Cystoseira* spp., sponges and calcareous red algae, which suggests the probable presence of *Peyssonnelia*, were found on rocks. However, the fine sediment deposited on all structures did not

allow the exact species identification.

Figure 78. Transept 03_hs_428: habitat 1170 in Sirolo (1)

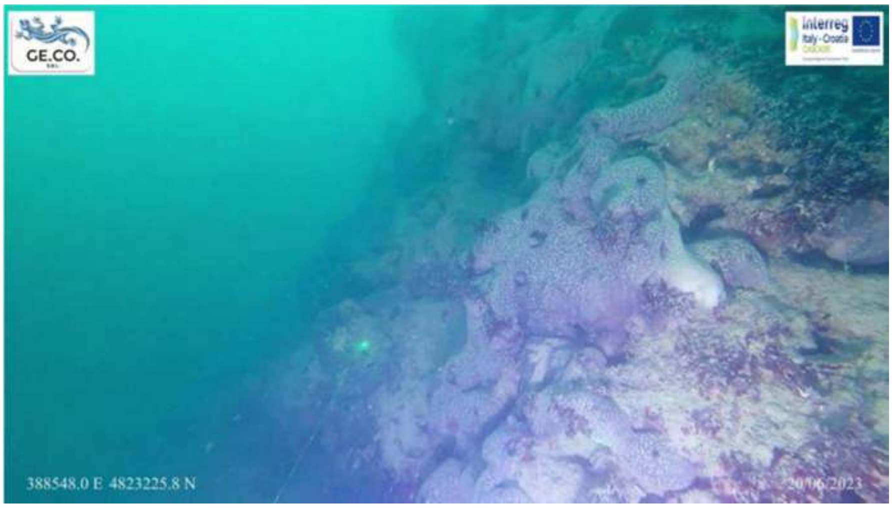


Figure 79. Transept 03_hs_428: habitat 1170 in Sirolo (2)

Habitat 1110 : Sandbanks with a weak permanent sea water cover

This habitat is characterized by sandy substrates with the presence of crustacean of the species *Liocarcinus vernalis* as well as by sandy substrates with rocky outcrops, bioconstructive polychaete of the genus *Sabellaria* and algal cover of *Ulva lactuca* with *Cystoseira* spp.

Figure 80. Transept 06_hs_438: habitat 1110 in Sirolo (1)

Figure 81. Transept 07_hs_444: habitat 1110 in Sirolo

4 Assessment of hazards, impacts and vulnerabilities of identified endangered ecosystems

P1. Grado and Marano Lagoon and Gulf of Trieste (IT)

Intertidal rocky shore represents a naturally stressful environment for benthic organisms. It is more or less regularly exposed and submerged according to sea level variations due to tides, winds, waves, atmospheric pressure and wider temperature range. Consequently, upper infralittoral community (UIL) shows higher values of species richness compared to lower mediolittoral (LML) and upper one (UML). These communities are naturally adapted to live in this environment, but the human disturbances could produce heavy impacts on their, due to hydromorphological alterations (including walking and bathing) and/or water pollution. In addition, the climate change represents an additionally threat for the intertidal rocky shore community through the arising sea level and increasing heatwaves. Nevertheless, the monitoring of ecological status of the macrozoobenthic intertidal community revealed a substantially good quality status. The characteristics key species of this environment were recorded within a quite diversified community; about 75% of the sampling sites were classified as pristine or slightly altered and 25% as moderately altered on the hydromorphological point of view. The disturbance classification on the basis of the proportion sensitive/tolerant species to organic enrichment (AMBI index) ranged between undisturbed to slightly disturbed condition. The relative dominance of *Mytilus galloprovincialis* appeared to be among the key factors of such classification, being considered a species tolerant to enrichment in slightly unbalanced environment (EG-III *sensu* AMBI).

Pérès & Picard (1964), in fact, identified the notable abundance of *M. galloprovincialis* as a facies of the biocenosis of the lower mediolittoral rock, when the sea water is particularly enriched with organic matter. The northern Adriatic Sea, and in particular the Gulf of Trieste, has been always characterized by the presence of a typical belt represented by the association *Mytilus*/*Fucus*/*Patella* which is bordering the intertidal rocky shore. A survey conducted in May 2009 on the assessment of *Fucus virsoides* distribution in the Gulf of Trieste identified the presence of 22 discontinuous populations (Orlando-Bonaca et al., 2013). On the contrary the survey on its presence/absence conducted in 2021 revealed the actual total disappearance of this species. The causes of this disappearance should be investigated, although Orlando-Bonaca et al. (2013) concluded that *F. virsoides* cannot be considered an ecological indicator for seawater pollution nor for climate change, since it is subjected naturally to high pollution levels and environmental variability. At present particular investigations are carrying on this species as well as experimental restoration in protected areas. Finally, on regard the fish fauna, the constant presence on the giant goby *Gobius cobitis* recorded in the study area represents an encouraging result. Any way a specific research devoted to the distribution and the status of the populations should be conducted and extended to the northern Adriatic, being this species vulnerable to coastal morphology modifications.

P2. Transitional (e.g. Goro area and Bevano Mouth) and coastal areas in Emilia Romagna (IT)

Transitional waters (TW) unfold across the freshwater to marine continuum, those areas rank among the most vulnerable coastal systems due to both environmental and anthropogenic stressors (Newton et al., 2014; Sgarzi et al., 2019)
These environments may create extreme conditions for many taxa, resulting in a biodiversity typified by a low number of highly specialized species (Crosetti & Massa, 2015); indeed, taxonomic diversity in transitional waters tends to be limited in comparison to fresh and sea water's diversity (Basset et al., 2013; Elliott & Quintino, 2007). Figuring out the mechanisms underlying phytoplankton dynamics is crucial to predict the impact of environmental changes, particularly in such a threatened ecosystem.
As we discussed earlier, P2 areas are either impacted by freshwaters inputs, or excessive nutrient loadings from agricultural and farming activities that trigger changes in nutrient stoichiometry and so in phytoplankton composition. This may also impact benthic communities, leading to the observed ecological shift over time. By now, some actions have been tested to evaluate, reduce or mitigate impacts and hazards in such ecosystems.
Regarding phytoplankton community in the Sacca di Goro, a novel and integrated monitoring approach has been explored to add further information to such a complex and wide group of organisms. The eDNA-metabarcoding technique has been applied to several samples in each station besides traditional monitoring activities. During the procedure a universal primer for eukaryotic organisms (18S rDNA gene) was used. Some relevant results have been achieved mainly identifying small and cryptic species that was not possible to identify using classical microscopy. However, the incompleteness of the available algae DNA database and the use of non-specific primers have probably limited the final dataset and represent a common constraint when dealing with environmental DNA. Broadly speaking, conventional microscopic identification is useful to assess the abundance and the richness of phytoplankton but may be limited in taxonomic identification of rare, endangered or cryptic species and can be ineffective in assessing the actual biodiversity (Kim et al., 2019; van der Heyde et al., 2020). Coupling innovative and traditional tools could lead to an in-depth assessment of biodiversity in marine habitat. As to Lago delle Nazioni, different tests were carried out to define vulnerabilities and hazards to which the lake is subjected. Firstly, considering the two major algal bloom events which occurred between 2020 and 2022, laboratory trials have been set up to characterize nutritional preferences and growth capacities. *Prorocentrum cordatum* and the green algae responsible for the aforementioned blooms were isolated and growth response to different N substrates (NO_3^-, NH_4^+, Urea) was tested.
Results showed that N forms which most stimulated algal growth were NH_4^+ and Urea (figure 82).

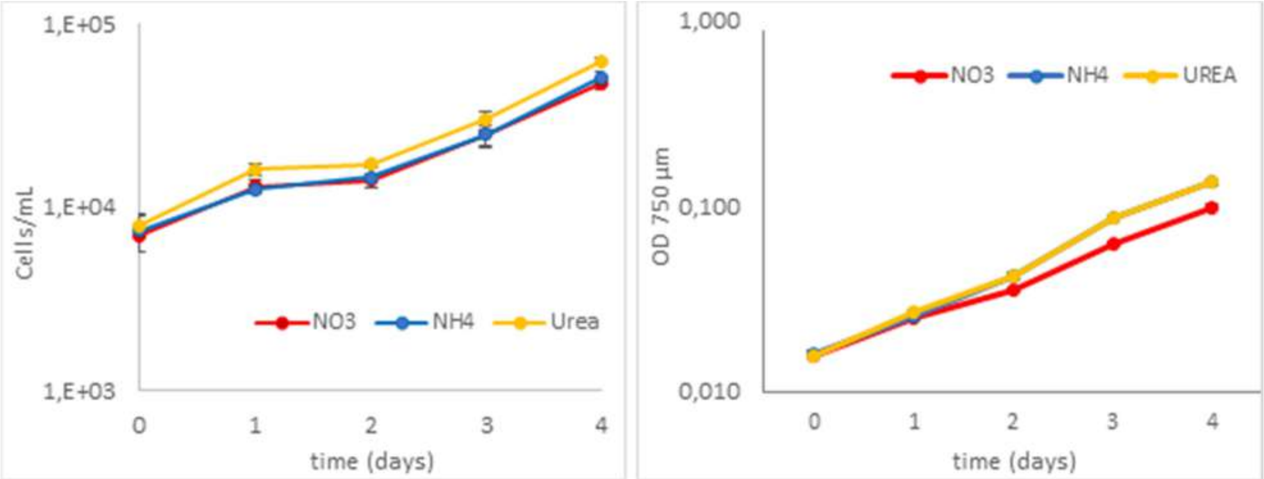


Figure 82. Growth response to different N substrates. Different algae were tested: Prorocentrum cordatum (left), green algae (right)

Giving the findings of the laboratory tests and considering that CASCADE water monitoring activities in this area has been focused on nitrate [NO^3 + NO^2] only exhibiting extremely low values, it must be taken into account that the lake is surrounded by agricultural lands and farms and to assume that other N forms, in particular urea, can lead to unusual algal blooms events.
Prorocentrum cordatum, isolated and cultured, has been tested for toxicity since several strains across Europe exhibited toxic compound, however, this strain was not found to produce any toxin and also the application of different nutrient conditions did not stimulate toxin production. On the other hand, the green algae has not yet been identified at species level and, presumably, a DNA analysis will be required.
In Lago delle Nazioni a restoration trial using macroalgae has also been tested: *Gracilaria* sp. has been placed on several ropes in an attempt to raise oxygen concentration and promote organisms' colonization. Sadly, each attempt ended in failure probably due to shortage of light caused by both microalgal biomass and suspended organic matter. In Lago delle Nazioni we could develop another innovative and integrated technique based on remote sensing. An UAV equipped with an hyperspectral camera was used to extract reflectance spectral signatures of the water surface. As one of the very first application of such tecnique, a laboratory calibration of the hyperspectral sensor was carried out by using both monospecific microalgal culture at different cellular concentrations and different mixes to simulate environmental microalgal community condition. Reflectance of tested algae remains consistent for each cellular concentration exhibiting a constant pattern (figure 83).

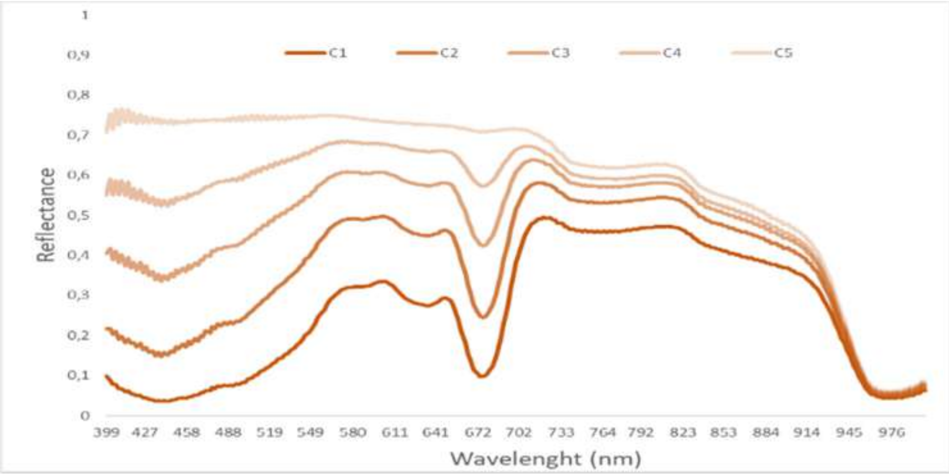


Figure 83. Spectral signature of a tested algae (Phaeodactylum tricornutum) at five different cellular concentrations.

Remote sensing data could offer immediate, accurate and not operator dependent results, in addition algae are interesting target for spectral imaging due to their bioptical properties since every microalgal group owns discriminant pigmentary composition that interact with light in different ways. Results from laboratory and field have been processed to identify absorbance and reflectance peaks, furthermore, an algal index has been developed to correlate reflectance measured by the sensor and the phytoplankton biomass; algal index showed a strong correlation with increasing phytoplankton biomass for each tested species (figure 84).

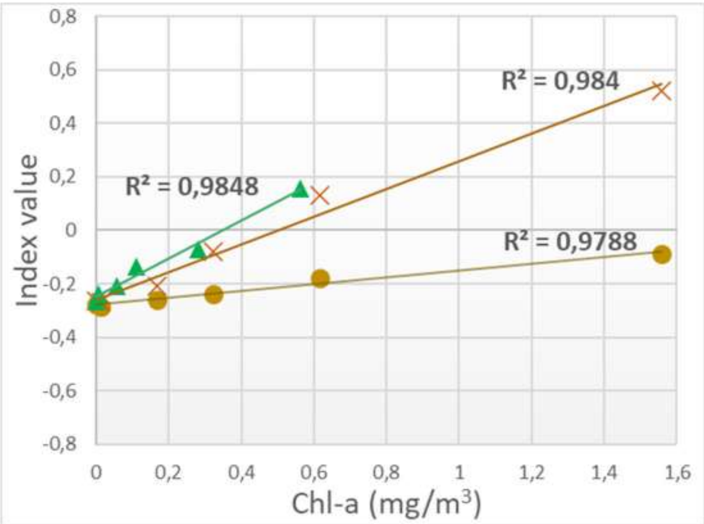


Figure 84. Correlation analysis result between phytoplankton biomass and Algal Bloom Index Value for Prorocentrum cordatum (●), Phaeodactylum tricornutum (×) and Chlorophycea (▲).

Furthermore, during UAV field trials, biological samplings have been collected in the north area of the lake as well. Microscopic analysis identified different algal species compared to those that are usually observed in the south area during the same period. This could state the need of implement monitoring station in the lake to fully describe algae community.

These integrated techniques tested in the Sacca di Goro and Lago delle Nazioni could represent the starting point to set innovative algal bloom monitoring applications for coastal zones and brackish waters.

With regards to Piallassa Baiona a field experiment has been performed to assess the effects of different timing regimes of hypoxic stress on the macrobenthic communities. The idea that sequential hypoxic events could be responsible of major changes in the benthic community has only been rarely studied (Benedetti-Cecchi al., 2006), even though the general consensus is that, after an impactful hypoxic event the resilience of the ecosystems against subsequent hypoxic periods might be eroded (Conley et al., 2009).

In transitional environments and lagoons hypoxia is influenced by climate, nutrients discharge and freshwater inputs (sometimes anthropically controlled) that make the appearance of hypoxia often stochastic and unpredictable. Hence, understanding the effects of different temporal regimes of hypoxia on the benthic communities can be a key factor for planning and management in the future. Experimental units consisted of 15,5 cm diameter pots covered with a 0,5 mm mesh plastic net. In the lagoon, pots were positioned at about five meters from the shore at low tide, completely submerged on two parallel rows. Hypoxic conditions were imposed by covering the treated pots with a black plastic bag. The controls were left uncovered to see how the structure of the benthic community change during the time without any change caused by treatment. Treatments consisted of two hypoxic periods (2 or 4 days) separated by a normoxic pause of different durations (Absent, Long, Short, figure 85).

Communities were strongly reduced in all the treatments. However, further analysis of species composition and abundances revealed that a normoxic pause between two hypoxic events can be beneficial for the community, provided that *i)* the pause is long enough and that *ii)* the hypoxic period is short enough.

Figure 85

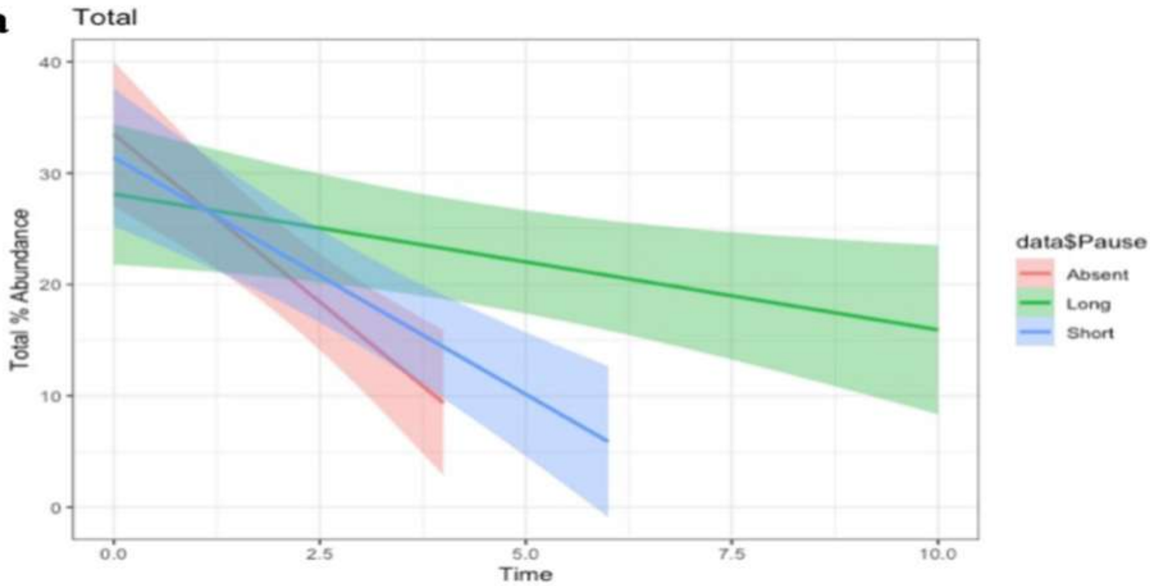
If the hypoxic event is short the presence of a longer normoxic pause appeared to be beneficial to the community. That is, at the end of the experiment, the community after two consecutive 2 days hypoxic periods resulted similar to that where the 2 days hypoxic events were separated by 2 days of normoxia (Fig. 86a). Organisms abundance, on the other hand, where significantly higher and the community resulted richer and more diverse if the hypoxic periods were separated by a 6 days normoxic pause. The situation reversed with hypoxic periods of 4 days (Fig. 86b): in terms of abundance the community decreased slightly faster in the treatment with no Pause while with a Short or Long Pause the normoxic periods appeared to be beneficial for the community.

From a management point of view our results suggest that multiple short repeated hypoxic events separated by periods with no stress would allow to maintain a richer and more diverse community compared to a scenario with a single long hypoxic period.

The field experiments give a more complete view on the whole community and focuses on how to minimize the effects of repeated hypoxic events by manipulating the timing of their occurrence, their duration and their distance in time. Short repeated hypoxic events, lasting not more than 48 hours, are expected to have a relatively smaller effect on the benthic community than a single long event of the same total duration. The effects of these short hypoxic events may be further limited by allowing for longer periods with no stress between one and the next.

In general, in an environment where the water flow can be partially controlled, three main aspects need to be taken into consideration to limit hypoxia: temperature, nutrient supply and mixing. Having a more open system will typically create a basin with a more mixed water with limited stagnation and stratification. However, a higher input of water may also cause an increased supply of nutrients, potentially causing eutrophication and subsequent hypoxia. Taking into account the complexity of factors contributing to the development of an hypoxic environment remains the key to put in place an effective management.

a



b

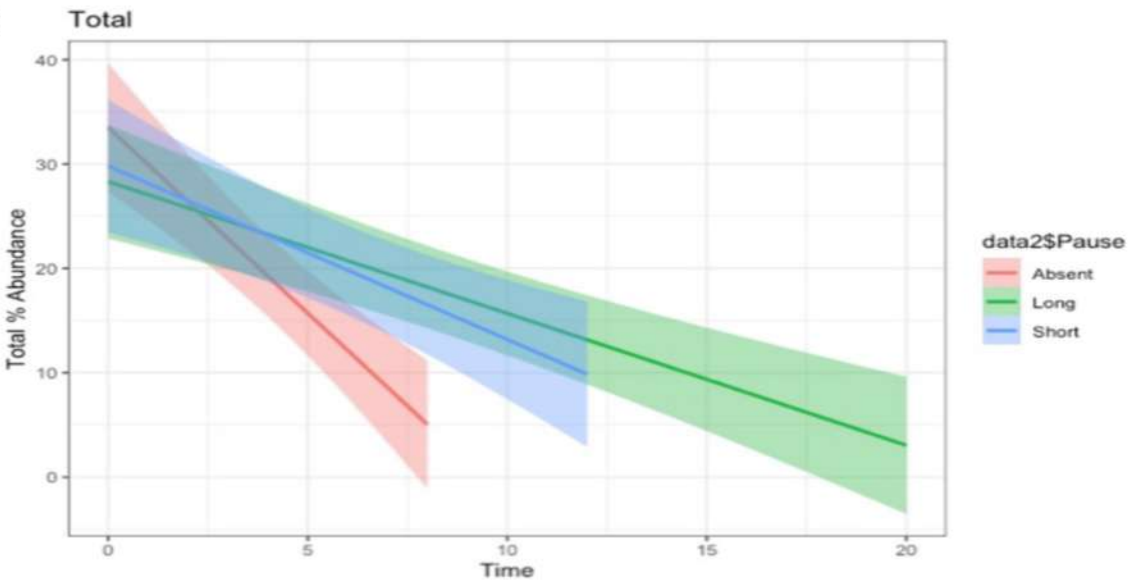


Figure 86. Organisms abundance, relative percentage-transformed.
a) 2 days hypoxic periods. In red 'Absent Pause', in green 'Short Pause, in blue 'Long Pause'.
b) 2 days hypoxic periods. In red 'Absent Pause', in green 'Short Pause, in blue 'Long Pause'.

P3. Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT)

Chemical parameters

Among the available data those collected by ARPA are the most regular. Data collected in the Surveillance monitorings in 2010-2011 and in 2016 and during the Operational monitoring in 2012-2013, 2013-2014, 2014-2015, and 2017 are available for the river Canale Reale (which output is in MPA Torre Guaceto), the transitional waters Torre Guaceto, Punta della Contessa, Cesine (which is north of the coasts of the municipality of Melendugno), Alimini Grande (which is south of the coasts of the municipality of Melendugno), and for the coastal waters T.Canne-Limite Nord MPA T.Guaceto, MPA Torre Guaceto, Limite Sud MPA T.Guaceto-Brindisi, Brindisi-Cerano, Cerano-Cesine, Cesine-Alimini.
In the following tables there are the chemicals which were not below the maximum allowable concentrations.

	Annual average	**Single measurement**
2010-2011		Hg = 0.07 µg/L
2012-2013	Benzo(a)pyrene = 0.6 µg/L	Benzo(a)pyrene = 1.8 µg/L
2013-2014	Hg = 0.10 µg/L	Hg = 1.60 µg/L
2014-2015	Cd = 0.37 µg/L	Cd = 3.3 µg/L; Hg = 0.14 µg/L
2016	Trichloromethane = 3,9 µg/L	
2017		Hg = 0,46 µg/L

Table 17. Canale Reale river. Chemicals whose concentrations (reported as annual averages or single measurements) exceeded the Italian regulatory thresholds (data from ARPA Puglia)

	Water- Annual Average					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
Torre Guaceto	PBDE=0.0027 µg/L			Cd= 1.0 µg/L		
Punta della Contessa		As= 19.5 µg/L	As= 5.61 µg/L	As= 9 µg/L	As= 7.6 µg/L	As= 8.6 µg/L
Cesine			Cd= 0.3 µg/L			
Alimini Grande						
	Sediment - Annual Average (µg/kg)					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
Torre Guaceto	Fluorantene= 202 µg/kg d.w.		DDT= 5 µg/kg d.w.; DDE= 5 µg/kg d.w.			

Punta della Contessa			Benzo(a)pyrene = 88 µg/kg d.w.; Benzo(b)fluoranthene= 50 µg /kg d.w.; Benzo(k)fluoranthene= 31 µg/kg d.w.; Anthracene= 724 µg/kg d.w.; Hexachlorobenzene= 7,8 µg/kg d.w.			Pb= 190.5 mg/kg d.w., As = 14.8 mg/kg d.w.
Cesine					Pb= 45 mg/kg d.w.	
Alimini Grande						

Table 18. Chemical status of transitional waters. Chemicals whose concentrations (reported as annual averages) exceeded the Italian regulatory thresholds (data from ARPA Puglia); d.w.= dry weight.

	Water- Annual Average					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
MPA Torre Guaceto 500						
Lim. sud MPA T. Guaceto-Brindisi						
Brindisi-Cerano						Tributyltin = 0.001 µg/L
Cerano-Cesine						
Cesine-Alimini						
	Sediment - Annual Average					
	2010-2011	**2012-2013**	**2013-2014**	**2014-2015**	**2016**	**2017**
MPA Torre Guaceto 500		Cd= 0.4 mg/kg d.w.				As = 12.9 mg/kg d.w.
Lim. sud MPA T.Guaceto-Brindisi						
Brindisi-Cerano		As= 23 mg/kg d.w.; Tributyltin =20 µg/kg d.w.	As= 20 mg/kg d.w.;		As= 43 mg/kg d.w.	
Cerano-Cesine		As= 38 mg/kg d.w.; Cd= 0.4 mg/kg d.w.; Tributyltin = 20 µg/kg d.w.	As= 28 mg/kg d.w.;			
Cesine-Alimini						

Table 19. Chemical status of coastal waters. Chemicals whose concentrations (reported as annual averages) exceeded the Italian regulatory thresholds (data from ARPA Puglia); d.w.= dry weight.

Data collected by ARPA showed high levels of arsenic in Salina Punta della Contessa water samples. This is a long-lasting contaminant as also in 2003 Dell'Anno et al. underlined the presence of arsenic in Salina Punta della Contessa sediment sample.
ECODO-NET results for inner and outer areas of Torre Guaceto MPA showed for priority substances a value lower than the threshold envisaged by the Italian and European legislation.
Among the other priority substances that were detected at concentration higher than threshold there are cadmium, lead and organotin. Attention must be paid to Canale Reale river whose water also contains mercury above threshold in different surveys. As to the organic priority substances there are not continuous records of exceedings the threshold in water and sediments collected in the reference period in the area in which pilot P3 is found. In particular, trichloromethane, benzo(a)pyrene and polybrominateddiphenylethers have been found in water samples whereas DDT, DDE, benzo(a)pirene, benzo(b)fluorantene, benzo(k)fluorantene, antracene, esaclorobenzene are the substances exceeding the thresholds in sediments.
The sampling point within 2 km from the coastal marine area of Melendugno Municipality were reviewed. Water samples had total petroleum hydrocarbons (TPH) concentrations lower than the Limit of Detection (<0.1 µg/L). Dissolved metals in water matrix, Cd and Hg values were also lower or close to the limit of detection (LOD) (0,1 µg/g) at all sampling points.
For all stations, polycyclic aromatic hydrocarbons (PAH), PCBs and organochloride compounds concentrations detected in sediment samples were lower than the LOD. The concentrations of hexachlorobenzene in sediment samples were in the range 0,01 ng/g-0,86 ng/g.

On considering data in the survey campaign carried out during Cascade project metal and organic pollutant in all Pilot 3 area sea water samples did not exceed the maximum admissible concentration. Arsenic and mercury in some sediment samples showed to be higher than legal thresholds. In fact sediments from the sampling points TG3 and Bay2 in Torre Guaceto and PC3 and PC3B in Punta della Contessa had arsenic concentration higher than 12 mg/kg d.w. whereas PC2, PC3 and PC3B in Punta della Contessa had mercury levels equal or higher than 0,3 mg/kg d.w..

Overall arsenic and mercury threat remain still real in the sediments of the north area of pilot 3 (Torre Guaceto and Punta della Contessa), whereas both cadmium and chlorinated pesticides deserve less attention as thresholds were not exceeded in any sample.
There are no legal thresholds for microplastics. There are also no time series to compare. However, the data suggest that if we consider the points on the coastline, Punta della Contessa has a higher amount of MPs than Torre Guaceto, as would be expected considering that the latter is an MPA, while there is no significant difference in all the samples at sea.

Bioindicators

Analysis of metabolism enzymatic activities in gammarids showed a decrease in aereobic metabolism (COX reduction) and an increase in anaerobic one in specimens sampled in P3 sites compared to controls (LDH increase). The kinetic activation of LDH indicates an acclimatory response of *G. Insensibilis* to systemic hypoxia and may serve as a short-term ATP-generating system under critical conditions.
GPx and SOD activities increased in amphipods. Besides protecting against ROS, these enzymes have multiple functions, including xenobiotic detoxification. Thus, their higher activity may be related to the enhanced tolerance level to various environmental factors. The activity of these enzymes is reduced by negative feedback resulting from the excess of the substrate or the damage caused by the oxidative modification.
Furthermore, CAT activity was reduced. This data seems to be in contrast with the other assessments, but CAT activity may increase or decrease in contaminated environments depending on the substances present. Decreases in CAT activity has been reported in aquatic organisms exposed to pollutants able to interfere with the activity of this enzyme.
Therefore, alterations in metabolism and antioxidant enzymes may potentially be used as sensitive biomarkers for risk assessment of pollutants in the environment.

Hazards
There are no legal thresholds for microplastics. There are also no time series to compare. However, the data suggest that if we consider the points on the coastline, Punta della Contessa has a higher amount of MPs than Torre Guaceto, as would be expected considering that the latter is an MPA, while there is no significant difference in all the samples at sea.

Impacts
On considering data in the survey campaign carried out during Cascade project metal and organic pollutant in all Pilot 3 area sea water samples did not exceed the maximum admissible concentration. Arsenic and mercury in some sediment samples showed to be higher than legal thresholds. In fact sediments from the sampling points TG3 and Bay2 in Torre Guaceto and PC3 and PC3B in Punta della Contessa had arsenic concentration higher than 12 mg/kg d.w. whereas PC2, PC3 and PC3B in Punta della Contessa had mercury levels equal or higher than 0,3 mg/kg d.w..
Overall arsenic and mercury threat remain still real in the sediments of the north area of pilot 3 (Torre Guaceto and Punta della Contessa), whereas both cadmium and chlorinated pesticides deserve less attention as thresholds were not exceeded in any sample.

P4. Neretva river mouth (HR)

Hazards

As mentioned above, the Neretva Delta has numerous lagoons, shallow sandy bays, low sandy beaches, sandy plains, salt beaches, etc. Today, the Neretva is the only river in this region that has a delta at its mouth. The karst system considerably slows down the flow of the Neretva. The dense marshes covered with hydrophilic vegetation provide excellent conditions for fish spawning, bird nesting, migration and wintering of almost 200 regularly occurring bird species, as well as habitats for several endemic species recognized by official nature conservation organizations at national and international level. In addition, the entire project area, especially its fragile karst and wetland ecosystems, is threatened by several activities, such as:

Anthropogenic impacts on freshwater ecosystems	• Ilegal waste disposal on the banks and in the surrounding area is not only an esthetic problem, but also poses a threat to water quality. The Neretva River flows through a karst landscape characterized by its porosity and numerous underground channels that carry all the water from the watershed to the river. This means that the rain washes all the toxic substances from the illegal waste dumps into the Neretva. • Depletion of natural resources has led to significant changes in the water balance, such as changes in the rate and variability of water flow, sediment quantity, temperature, oxygen availability, and nutrient concentrations in water, as well as groundwater levels and subsurface flow, making sustainable development in the region particularly difficult.
Neretva River freshwater influx	• Affects the thermohaline properties of water and the sediment quantity in the coastal area of the mouth, directly affecting marine biodiversity of the area. The Neretva estuary is permanently low salinity area with significant temperature changes. The Adriatic Sea as a whole is classified as low-productive sea, whilst the Neretva mouth is one of the highest productivity area due to the strong continental influence (freshwater and forests) (Jardas et al., 2008). It is protected from the strong currents, which favours the sedimentation of organic and inorganic materials, mostly from the land. Mouth bottom sediments are silty sand and fine sand, and the surrounding sea is up to 20 m deep. Sea quality in 2016 for all stations was characterized as excellent and satisfactory, while in 2017 it was assessed as good and satisfactory. The sewerage vent exists are located in front of Cape Višnjica near Ploče, and in front of Cape Dračevo in Neretva Channel, with 4 more locations in the lower reaches of the Neretva River from Metković to the mouth.
Global climate change:	

River flood	• Due to extreme precipitation in the Neretva River basin and overgrowth of watercourses in neighboring Bosnia and Herzegovina, as well as the inability to accept sewage, the Neretva River in the area of the town of Metković, municipality of Kule, leads to overflowing. The town of Norinska and the town of Ploče, endangering a certain number of inhabitants and legal entities.
Fires	• In the P4 the high temperatures in the spring and the dry vegetation favor a large number of wildfires with great environmental damage.

Impacts

Neretva River has undergone numerous environmental changes due to the generally low environmental awareness of the local population and various anthropogenic activities, such as:

River's water balance	• The river's water balance is disrupted by hydropower infrastructure. Seven dams, six artificial reservoirs and six tunnels with a total length of 74 km provide multifunctional use of water resources, from an altitude of 900 m to sea level. They reduce sedimentation and productivity of the Neretva River and increase erosion of the riverbed. Due to the operation of the dam, the water level changes frequently and rapidly, which has a serious impact on native fish, especially during the spawning season. Although there is no complete list of flora (ferns and spermatophytes) in the Neretva delta, according to available literature, field research conducted between 1995-2005 and in April 2011, approximately 820 plant species were found in the Neretva delta which represents about 15% of Croatian flora. According to the floristic research conducted from 1950 to the present days The Flora Croatica Database has established 81 plant varieties protected by the Nature Protection Act (OG 80/13, 15/18) and the Ordinance on Strictly Protected Species (OG 144/13, 73/16). Of these, 72 species are endangered at the national level and are listed in the Croatian Red Book of vascular flora (Nikolić and Topić, 2005).
Exploitation of sand and gravel mining	• Uncontrolled intensive exploitation of sand and gravel mining along the Neretva River. This has led to deepening of the Neretva riverbed and, together with the construction of dams upstream, has significantly disrupted the transport of heavy sediments (mainly gravel).
Expansion of human settlements	• The most significant transformation of the delta took place in the last decades with the expansion of human settlements. Intensive and long-term melioration of the terrain to obtain agricultural land and to protect the region from flooding, as well as the development of extensive transportation infrastructure, have changed land use and led to habitat loss. A significant attack on the marshes was the draining of Lake Modrič and the entire lagoon. This resulted in the loss of a large portion of wetland habitat for migratory

	birds and spawning fish.
Specific geographic location of the Neretva delta	• Created precondition for forming important traffic intersections of main roads, rail and maritime transport. The important part of local economy in area is cargo seaport in Ploče, second in Croatia by the amount of transshipment, a multi-purpose port for transshipment of almost all kinds of commodities represented in international maritime transport. An integral part of the port of Ploče is Metković port which is located 20 km upstream on the river Neretva.
Saltwater intrusion	• Due to the mixing of fresh and seawater, the Neretva River mouth area is mostly brackish water habitat and it's not easy to make clear difference between fresh and seawater fish. Along the course of the Neretva, certain species are differently distributed. Some are present along the entire length of the course, while others are located only in the lower course of the river. The lower course of the Neretva is populated by 22 fish species. More recent research of the lower course of the Neretva River and its tributaries, at the border of sweet and brackish water biotope, found 49 fish species. Due to number of endemic species and diversity of the Neretva mouth, it is one of the most interesting areas in Croatia. Eight species of fish – *Alburnus neretvae, Lampetra soljani, Squalius microlepis, Cobitis narentana, Chondrostroma nasus, Rutilus basak, Knipowitschia croatica, Knipowitschia radovici* live in Neretva River catchment. They represent a rare endemic ichthyofauna and a biologically remarkable natural heritage. There also live 18 endemic species in the Adriatic basin, three of which are endemic species of Croatia 69 different species in the form of juveniles were recorded in the mouth of the Neretva River, Mala Neretva River and Parila lake indicating the importance of this area as a hatchery site. Among other, flathead grey mullet (*Mugil cephalus*), vulnerable species according to IUCN category of threatened species whose number has been significantly reduced in recent decades, mainly due to loss of favorable habitats. Furthermore, this area is important for the migration of anadromous and catadromous fish. Lagoon Parila is one of the rare remaining brackish habitats on the eastern Adriatic coast of European eel (*Anguilla Anguilla*) which is today one of the most endangered fish species in Europe (PI DNC, 2018). The area is significant for seasonal migrations of eels and many different species of fish and cephalopods. Daily migrations are also significant, so that fish of Sparidae family come from deeper sea at night (gilt-head breams, sand steenbras, annular sea breams, white sea breams, common two-banded sea breams etc.) and surmullets (DZZP, 2007). The impact of saltwater intrusion as a result of natural processes or human activities on local biodiversity in brackish and freshwater ecosystems is an issue of increasing international concern because of the negative environmental impacts (Nicholls & Cazenave, 2010.). It reduces inflow of fresh water and reduce sediment. This concern stems primarily from the fact that saltwater intrusion has negative impacts on coastal environments, including the loss of habitat for freshwater

	biota such as vegetation, phyto and zooplankton, and fish, as well as the associated invasion of brackish and saltwater organisms (Love et al., 2008.). All of this directly affects the biodiversity of the area, target species and the habitat Natura 2000. Furthermore, as a consequence of human activity in the area, there is the distribution of contaminants in water and soil. All previously listed has a direct impact on reducing the quality of life of local communities, and loss of extremely valuable areas of biodiversity. Neretva River Delta is an area with a lot of different influences, so there are numerous activities that have or could have negative impact on the natural values site Delta Neretve, such as: expansion and intensification of agriculture; planned tourist zone in Natura 2000; excessive use of pesticides and fertilizers; fragmentation of wetland habitats; spreading of urban zones on account of wetland; water pollution with non-purified urban and industrial waters; unsolved land property rights; illegal taking of state owned agricultural land, including marshes; non-regulated recreational and touristic activities, especially on the river mouth, illegal hunting and fishing; frequent fires in reedbeds. The future of this area should be based on balancing the need for further development and the need to protect natural resources. Within the project it’s important to define guidelines for preservation of wetland area in Neretva River Delta whilst respecting the needs of the development of the local communities.

Vulnerabilities

Based on the values listed above, have been discussed problems and pressures that threaten various aspects of the area, namely human health, tourism and the economy, and marine ecosystems and biodiversity.

- **Vulnerability of the coast**

The coast is at risk primarily due to the expected rise in sea level, which would lead to a lowering of the coastline and erosion, which may lead to ecosystem degradation after prolonged exposure to waves. It is important to plan adequate protection of beaches, that is, to minimize the loss of sand from the beaches, in accordance with the rules of the profession, which are common in such interventions in the world.

- **Vulnerability of human health**

Extreme precipitation events in the coastal region have negative impacts on human health as runoff spills onto outdoor surfaces, therefore it is important to plan and implement appropriate solutions.

- **Vulnerability of tourism**

The impact on tourism will be twofold. Because of the increase in air and sea temperatures, it will be possible to extend the tourist season, both in the early and late seasons. In addition, the warmer weather will certainly be more attractive to tourists who are not bound to the bathing season, who are already attracted by the climate and who spend an active holiday in terms of non-maritime activities (hiking, inland cycling, etc.). The negative effects will be concentrated in the summer, especially in July and August, when the heat waves will be many times more intense and long-lasting than in the current climate.

- **Vulnerability of agriculture**

Due to the increase in air temperature and heat waves, as well as the decrease in precipitation during the warm season in the future climate and the associated increase in evapotranspiration (evaporation), certain agricultural crops that require more water for their growth and development will be at risk. The local population could suffer major economic losses as a result; crop failures and production declines will affect not only the agricultural sector, but also economic sectors in the city center.

- **Vulnerability of marine ecosystems and biodiversity**

In the sea, rising temperatures and salinity will affect biodiversity, especially in the coastal areas. here will be a spread of thermophilic species toward coastal areas, and the total number of marine plant and animal species will likely decrease as a result of changes in primary production, ocean acidity, dissolved oxygen, and amounts of nutrient salts.

P5. Coastal area in Veneto (IT)

During ROV sampling activities it was possible to observe numerous remains of nets and ropes from the videos. The high degree of colonization of the remains of fishing gear observed, such as their incorporation by large porifera, lead us to assume that, at least most of them, may derive from fishing activities prior to the establishment of the ZTB, occurred in 2002.

RETI	min. video A4
Pezzo di sacco di rete da strascico	16.01
Geodia cynodium inglobata in pezzo di sacco di rete da strascico	16.31
Pezzo di sacco di rete da strascico	17.22-27
Pezzo di sacco di rete da strascico	18.16
Groviglio di pezzi di rete da strascico e cime	21.25-49
Pezzo di sacco di rete da strascico inglobato in Geodia cynodium	22.09
Pezzo di sacco di rete da strascico su Geodia cynodium	26.53
Pezzo di sacco di rete da strascico	28.17

Table 20. Example: list of networks found during the monitoring of tegnùa A4, with the corresponding minutes within the video.

The scuba-diving photographic survey reported differences in assemblages inhabiting different sites. In particular (Figure 87) the MR08 site is characterized by the cover of CCA, *Peyssonnelia* spp. and large massive colonial ascidians, while the P213 site by massive and erect sponges. The other four sites (i.e. AL06, P204, P208B and TM1) show high variability of the assemblages and are mainly described by algal turf, encrusting sponges and "detritus + sediment". This is in line with previous knowledge (Falace et al., 2015), suggesting the role of water turbidity and sediment resuspension in defining the assemblages in the area.

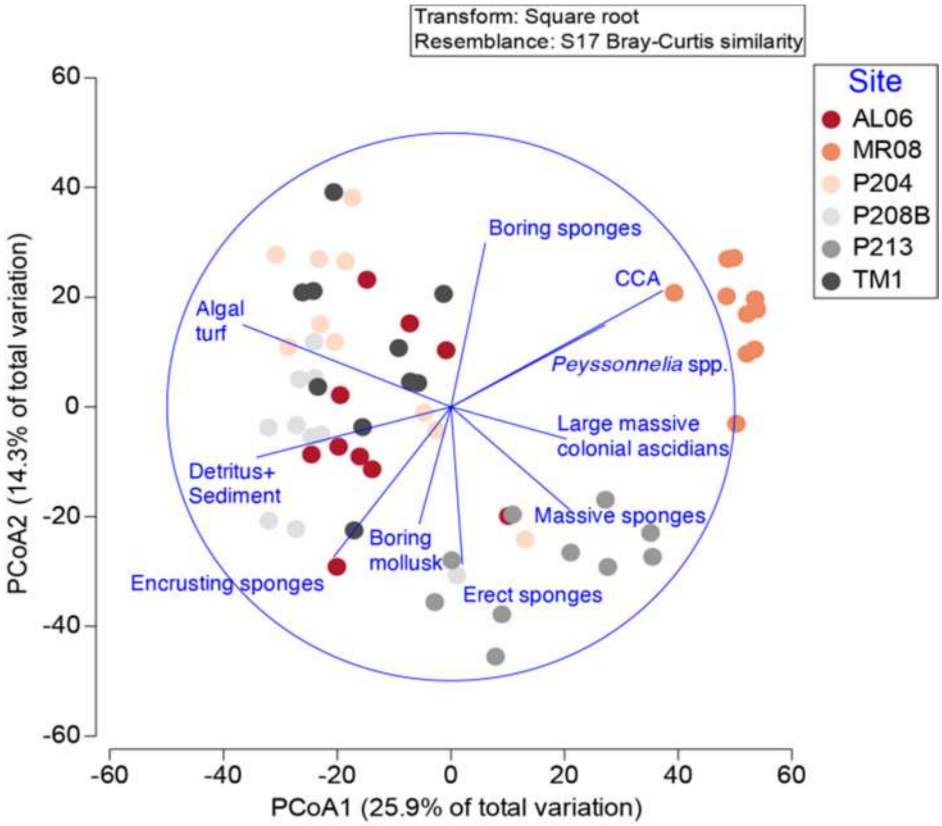


Figure 87. Unconstrained principal coordinate analysis (PCoA) ordination plots of benthic assemblage data in 2021at the 6 study sites, illustrated in different colors. Vectors superimposed to plot represent the correlations with the PCoA axes.

As concerns the fish community, comparing the data collected in December 2021 and March 2022 with previous studies (Sabatini, 2008), a decrease in the quantity of fish caught compared to past years is evident. Among the possible factors to be investigated, which could have influenced this negative trend, there are:

- the increase in water temperature, linked to climate change, which may not favor the transit of some species associated with the Tegnùe at certain times of the year;
- the increase in fishing pressure in the area, which despite being a biologically protected area, could be subject to illegal fishing (professional and/or recreational).

These seasonal changes in the occupancy of the area by the nektonic community were also corroborated by the results of the acoustic surveys.

Within the food web modelling activity, two time series of forcings associated to climate modifications (water temperature, and phytoplankton concentration) were considered under RCP4.5 and RCP8.5 scenarios. Both climate scenarios were combined with two contrasting scenarios of evolution of the fishing effort:

1. Increase the fishing effort of professional fleets (trawlers and clam dredgers) by 30% in 10 years.

2. Decrease in the fishing effort of professional fleets (trawlers and clam dredgers) by 30% in 10 years.

All the simulations lasted 40 years (January 2014 - December 2053). Results obtained (Figure 88) from the model suggest that, overall, fishery pressure have a more confined impact with respect to climate induced pressure on food web functioning indicators. Nonetheless, a sensitivity to a reduced fishery pressure is visible in specific indicators (total cach, System Omnivory Index).

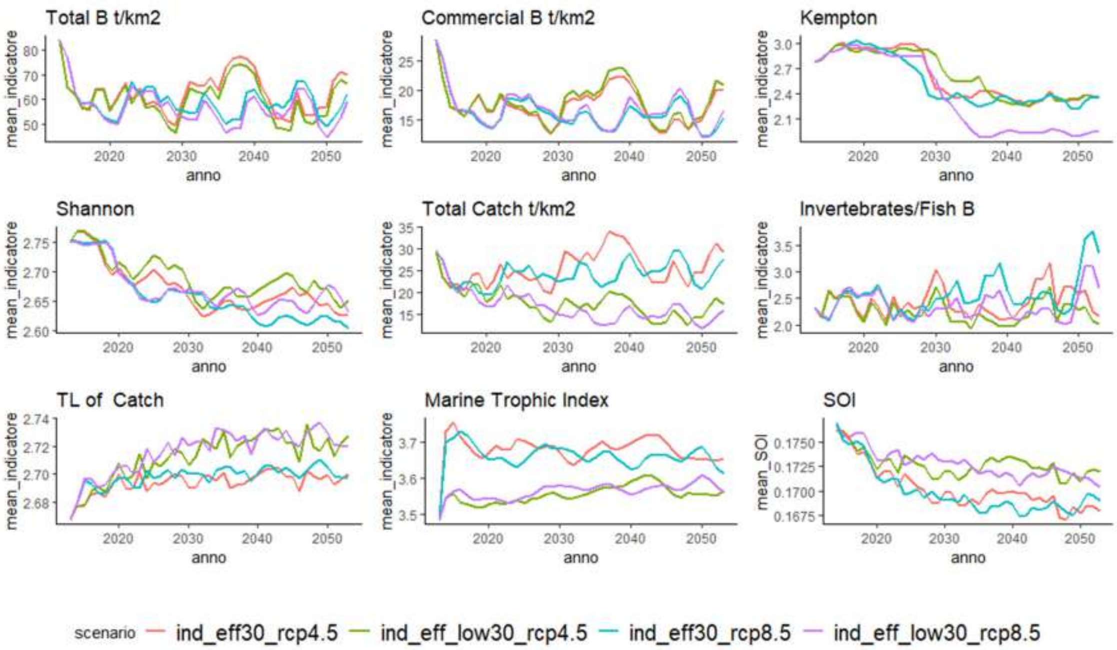


Figure 88. Food web model at Tegnùe di Chioggia area. Forecasted indicators of food web functioning under climate change and fishery pressure scenarios (ind_eff: increase in fishery pressure in the surroundings of the SCI; ind_eff_low: decrease in fishery pressure in the surroundings of the SCI).

P6. Miljašić Jaruga river mouth, Nin bay (HR)

In the Nin Bay area, erosion processes have been going on for several decades as a result of natural (wind, waves, currents) and anthropogenic influences (use of sand for construction purposes).
The Miljašić ravine together with its tributaries drains a part of Ravni Kotar with a total area of about 131 km2. In addition to the waters of the natural catchment area, through the "Bokanjac" hydraulic tunnel, the watercourse of the Miljašić ravine is also connected to the drainage of the Bokanjački mud area. The water regime of the watercourse in question is torrential in nature and forms occasionally only during heavy rainfall, when large waters occur that pose a danger to the surrounding lands and buildings due to possible flooding and erosion. According to the study of the flood defense of the city of Nin and the protection against high waters of the Miljašić ravine, the protection against high water floods is provided for a 100-year return period. The picture below shows a flood defense solution that envisages the construction of a dam that forms the Rašinovac retention. It is planned to construct an earth embankment dam from material secured from a loan site in its immediate vicinity. The elevation of the crown of the dam is 17.0 m.a.s.l. while the maximum height is 7.50 m. The length of the partition building in the crown is 750.0 m.

A number of regulatory works are planned along the entire bed of the Miljašić ravine, and the largest intervention is planned for the last 430 m with the aim of increasing the flow of the bed and securing private, agricultural and commercial facilities. In its current state, the riverbed is partially covered with silt, unkempt, overgrown with grass and low vegetation, and the existing old stone lining is damaged and completely collapsed in parts. Collapsed buildings and a bed with accumulated silt reduce the flow profile of the watercourse, and parts of the stream slow down.
With regard to the data of geotechnical investigations, hydrological-hydraulic calculations, and with regard to the surrounding terrain, the possibility of placing the building and fitting into the space, a technical design solution was chosen. For the arrangement of the mouth of the Miljašić ravine, this project envisages the construction of coastal walls on the left and right banks of the bed of the Miljašić ravine, along with accompanying walkways with supporting walls.

Vertical coastal walls maximize the flow profile of the bed and at the same time adequately protect the banks from the erosive action of the waters. The development of both banks would thus include the construction of a primary wall with an elevation of the crown at 1.50 m above sea level, except for shorter sections where the elevation rises to 2.50 m above sea level. Next to the primary wall, there is a walkway of variable width and a secondary wall with a constant height of the crown at 2.50 m above sea level, which ensures additional height of the bed. In this way, the area of the promenade, during extreme rainfall and high water levels, becomes a kind of inundation (flooding area) in such a way that the trough is expanded by the width of the promenade and an additional meter of trough height is obtained from the secondary wall. With this promenade solution, the possibility of building a pier for vessels with direct access from the promenade is ensured, regardless of the water level in the trough. The promenade is located at the same height as the primary wall (1.50 m above sea level), and even at lower water levels in the trough, there is no insurmountable height difference for getting down to the boats. In the future, moorings for barges and other vessels can be foreseen along the primary walls on both shores.

Therefore, a variant was chosen where both feathers are made as a combination of a vertical wall on the inside and a mound type on the outside. Part of the construction is made of larger stone blocks (scullery), which will be used for the dissipation of wave energy, and part of the construction is made with flat surfaces for access to piers, bathing and mooring of recreational vessels.

The conceptual solution for the reconstruction of the mouth of the Miljašić ravine envisages the following works:
1. Removal of the stone lining from the eastern and western wings
2. Removal of concrete parts of the eastern wing
3. Excavation of the bottom (deepening of the bed)
4. Construction of the eastern wing
5. Construction of the western wing
6. Connection to pedestrian infrastructure

The conceptual solution envisages the arrangement of pedestrian areas with stone, and the use of part of the existing stone on the location. In order to ensure a uniform flow in the area of the mouth, it is necessary to deepen the bottom of the bed to an elevation of -2.9 m.a.s.l. Therefore, it is planned to excavate 4,800 m3 (silt, sand, gravel) that needs to be disposed of at an underwater location or at a landfill on land.

In view of the planned interventions, it is to be expected that there will be increased sedimentation in this micro-locality during the execution of the works and the impact on the organisms present. The ichthyopopulation in this locality consists mostly of pelagic species that only occasionally visit the channel in search of food (e.g. mullet, sea bream, sea bream and sheepshead), while there are a small number of species that permanently inhabit this channel (e.g. some species of bream) . Therefore, it is to be expected that these populations will adapt to the conditions during and for a certain period of time after the implementation of the planned measures and construction works.

Among the more endangered species, benthic organisms such as bivalves and mantled clams can be considered, but given their low abundance (e.g. mussels) in this micro-locality due to large fluctuations in environmental conditions, and their greater presence in the surrounding areas, this impact of the construction of the project should not have significant influence.

The special feature of the Miljašić ravine microlocality is the large number of constantly present species of sea snails, such as Alvania rudis, Turbonilla sp. and Onda's modiola. Due to their lower mobility, these species can be considered more endangered in the processes of sedimentation and burial during construction. Especially in the case of extracting the existing stone elements of the Miljašić ravine from the sea for the purpose of building new ones.

P7. Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC) (IT)

The analysis of vegetation dynamics on restored salt marshes in the Adriatic coast in Central Italy (Biferno brackish area) revealed consistent changes on floristic composition and an improved conservation status.

The significant increment of diagnostic species along with the significant decrease of ruderal and alien plants, are likely related to the improvement of the environmental conditions after the restoration actions carried out in 2016 by the project LIFE + MAESTRALE (NAT/IT/000262; Prisco et al 2017).

As observed in other wetland ecosystems after naturalization interventions in America and Europe, also in the Adriatic coast the native plant diversity tends to recover. The observed recolonization can suggest the incipient establishment of a self-sustaining ecosystem status.

Besides the floristic and ecological changes depicting an ongoing successional recover of the brackish communities following the restoration actions (reconstruction of water ponds and wetlands and boardwalk construction), the observed vegetation dynamics could be also linked to a variety of environmental processes affecting the Central Adriatic coast (e.g. coastal erosion, climate change; Pilot 7 Fig 12: DIPSIR Model).

As, monitoring studies after restoration actions, based on ecological groups and key species abundance pattern, in salt marshes and coastal wetlands are very few so a comparative analysis between different geographical regions is not possible and the recovery of wetlands following restoration as currently practiced is often slow, incomplete and in the long term does not restore all ecosystem functions.

P8. Northern-eastern Adriatic in Croatia (HR)

The assessment was done of risks/factors impacting ecosystem biodiversity in Northern Adriatic Sea with the focus on biodiversity in phytoplankton.
Global warming significantly changes the seas and life in the sea through numerous indirect changes that include reduced solubility of carbon dioxide (which is needed by phytoplankton for photosynthesis), acidification, deoxygenation, increased stratification that reduces the mixing of the water column and contributes to oligotrophication, i.e. the reduction of nutrients inupper layer of the water column and reducing the thermohaline circulation. (Vrana et al 2023).
Understanding the effects of climate change on marine phytoplankton is of great importance. Since phytoplankton is the base of the food chain, higher trophic levels in the oceans and seas in the future will depend on phytoplankton species that will successfully acclimatize and adapt to global change. The impact of global change is also visible in the northern Adriatic. Summer temperatures approach 30 °C, and occasionally exceed it, and oligotrophication has progressed.

Global changes cause changes in the composition of phytoplankton that can have a significant impact on the functioning of the entire ecosystem. Different species of phytoplankton react differently to climate change, and some species will adapt better to the changes. In this research we focused on comparison of the biodiversity today with the check list published 2002.
From other research papers Vrana et al. 2023 Long-term field research has shown that *C. curvisetus* and *C. pseudocurvisetus* adapt more successfully to climate changes compared to other diatom species. From previous research we know that the total abundance of phytoplankton was significantly reduced after 2003 (Maric et al 2011) , when a significant increase in temperature was recorded in the northern Adriatic, while the abundance of *C. curvisetus* and *C. pseudocurvisetus* remained unchanged, and their contribution to the total community increased significantly (Vrana et al. 2023).
Grižančić et al (under review) aimed to report an up-to-date list of the phytoplankton taxonomic richness and phylogenetic relationship in eastern northern Adriatic, based on occurrence of their genetic material resolved with advanced molecular tools, namely metabarcoding on samples collected on CASCADE. Monthly two-years net sampling aimed to target 6 phytoplankton groups including Dinophyceae (dinoflagellates), Bacillariophyceae (diatoms) and Chrysophyceae (golden algae) belonging to Ochrophyta, Cryptophyceae (cryptophytes), Haptophyta (mostly coccolithophorids) and Chlorophyta with Prasinophyceae (prasinophytes) and Chlorophyceae (protist green algae). Generated sequence data were taxonomic assigned and redistributed in two kingdoms, five phyla, 46 orders, 67 families and 110 genera. Most diverse group were dinoflagellates, comprising of 45 found genera (41,3%), following by diatoms with 33 (30,3%) and coccolithophorids with 10 genera (11,0%). Although many taxa have not been detected that have been considered as a common in this area, metabarcoding revealed three diatoms and nine dinoflagellate genera that not reported in previous checklist, along with numerous species from

other targeted groups that have been poorly reported previously. Hence we have increased biodiversity in the area.

In the Adriatic we were recording more new species. Garić and Bastić (2022, 2022, 2016) are constantly recording new species in Plankton (Meduse, Tunicata, Apendicularia) samples in southern Adriatic as well. In past years they recorded 20 new species. They discovered a connection between the origin of newly arrived species and the type of currents entering the Adriatic, which depends on the direction of the eddy in the Ionian Sea (clockwise - anticyclonic and counter-clockwise - cyclonic eddy). During the anticyclonic flow, the colder and less salty Atlantic current enters the Adriatic, bringing species of western Mediterranean/Atlantic origin, and during the cyclonic eddy, the warmer and saltier East Mediterranean current enters the Adriatic, bringing Indo-Pacific species, which enter that part of the Mediterranean across the Suez Canal.

P9. Cetina river mouth (HR)

The assessment was done of risks/factors impacting ecosystem biodiversity in Cetina estuary with the focus on main habitat type protected by Habitat Directive, i.e. 1110 Sandbanks which are slightly covered by the sea water all the time, and key benthic habitat formers, i.e. little Neptune grass *Cymodocea nodosa,* Noble Pen Shell *Pinna nobilis* and cushion coral *Cladocora caespitosa,* which are strictly protected species according to Croatian Nature Protection Act.

The analysis of human and natural impacts on the *Cymodocea nodosa* meadows revealed negative effects on the meadows. Sand extraction activities and coastal construction projects, as well as natural coastal degradation led to disturbances in the meadow structure and coverage. However, in majority of cases, the meadows started to regenerate after the disturbance events, but the coverage and the density of the meadows are far from the original condition before the human interference (OIKON, 2023). It seems this surface increment mainly results from lateral expansion of the outer meadow edge and not so much due to recolonization of the areas suspected to be previously extracted. It is clear that seagrass has disappeared from some of the areas where it was formerly present (over 2.36 ha) and furthermore, such areas seem to "heal" slowly and have not fully recovered 5-yr after the adverse event. To reemphasize, although initiation of regeneration can be identified over 53% of meadow area formerly affected by human interventions, in most cases *Cymodocea nodosa* density and habitat structure are still far from the ones we would expect for mature, well preserved meadows in the area (Kipson, 2023).

The MME recorded in easter Adriatic in 2019 caused devasted and almost irreversible impact on Noble Pen shell population that used to thrive in Cetina Estuary area with population density being at some locations of 57 species/100m^2. In half a year period all individuals in monitored plots died, and till recording of a resistant individual in 2022, population of the Noble Pen shell was considered extinct in the area. As causes of the MME are still to be understood, currently the only hope for the recovery of the species is discover and protection of resistant individuals from predation and physical damage.

Several pressures are threatening *Cladocora caespitosa* reef and colonies at the Mala luka site. Whereas some physical damage due to elevated hydrodynamism may be naturally expected at the site, human-induced one must be eliminated through a priority conservation measure. It is absolutely pivotal to prevent, as soon as possible, any further mechanical damage, caused either by anchoring or destructive fishing. Somewhat paradoxically, it is among the most adverse threats and at the same time it is the easiest to manage since it completely depends on a human factor.
However other stressors are noted at the Mala luka site, causing physical effect of covering, such as mucilaginous algal aggregates and cyanobacterial mats which may be particularly harmful to sessile suspension feeders such as corals. Elevated seawater temperature may, alongside eutrophication, contribute to development of cyanobacterial mats and to the development and persistence of mucilaginous algal aggregate). These disturbances, alongside already mentioned

effect of covering and "suffocating" sessile organisms, may contribute to the spread of microbial pathogens and/or production of biotoxins and thus assist in development and spread of different diseases and appearance of necrosis (Kipson, 2023).

P10. Torre del Cerrano, Pineto Abruzzo (IT)

Observations collected few years after the boardwalks' installation across the dune system, evidenced encouraging results with species richness increase across all dune habitats, especially in the first vegetation communities near to the shoreline. It is well known that the species of pioneer communities near to the sea are adapted to the natural disturbance to which they are usually exposed and recovery after such disturbance is usually very fast.

However, effects of trampling, as well as changes in species cover or richness, strongly depend on the plant community analysed. Previous studies reported that the number of individuals and the resulting total species' cover of dune grasslands depend on the quantity of seeds produced the previous year, making these habitats highly subjected to random fluctuations, both in terms of native and alien species spread.

Although our results show that boardwalks can positively affect vegetation recovery in coastal dune ecosystems in a relatively short time, a number of caveats should be kept in mind. First, we cannot exclude that the dune vegetation was not influenced by the construction of the boardwalks themselves. The ruderal and exotic species, found especially in the dune grasslands, may have been transported and spread during the installation, contributing to a certain level of disturbance. Furthermore, all plots are located within 100 m of the boardwalks, where the disturbance is likely to be greater. In any case, the increase of focal species in richness and cover in the first years of vegetation monitoring is a positive indicator of recovery of the entire dune system. Nevertheless, longterm monitoring plans (probably implemented by capitalization programs) are necessary to understand more deeply the vegetation changes occurring after boardwalks installation. Moreover, larger area monitoring should be promoted in the future.

P11. Marche coastal area (IT)

The monitoring performed has shown a reduction in the quality of the coastal ecosystems, due to different reasons.

One of the main pressures affecting coastal ecosystems is coastal erosion consequent to climate change. Coastal systems of Marche Region are characterized by low sandy beaches. Residuals coastal biocenosis are endangered by flooding and inundation, that are more frequent and intense in reason of current climate change. European regulatory framework on flood risks (European directive 2007/60/EC) has imposed the perimeter of the areas subject to flooding systems. This information is essential for coastal ecosystem management, since allows to identify biocenosis directly threatened by flooding and to make coastal management compatible with natural ecosystem's conservation.

An analysis was performed to evaluate the inundation perimeters under climate change scenarios (return periods 20, 100 and 200 years) of given sites along the coast of the Marche Region. The analysed sites are: Pesaro, Fano, Montemarciano, Fermo e San Benedetto del Tronto. The numerical modelsin use and the applied procedure are illustrated in the D.4.2.1. Sediment characteristics have been taken into due account by varying the Manning coefficient, setting 0.020 s/m1/3 for the sites characterized by sandy and 0.035 s/m1/3 for those characterized by gravel beaches. The roughness of the emerged part of the domains has been modeled using a Manning coefficient equal to 0.070 s/m1/3. For some cases, namely Montemarciano and Fermo, the bathymetry obtained from the Lidar has been modified to better model the inundation perimeters since it created artificial "obstacles" for the water flow. For Montemarciano (RP20 and 100), the simulation has been repeated without and with the presence of a winter defense work along the beach. For Pesaro (RP100), an additional simulation representing the adaptation to future climatic conditions of the coastal defense barriers has also been run. For each site and return period, the results have been processed in QGIS to obtain the final vectorial files for the inundation perimeters and water depth maps. To evaluate the influence of the beach profile on the inundation, 1D simulations have been performed varying the bathymetry using once the winter profile and once the summer profile.

The analysis shows that for RP20 and RP100, the new inundation perimeters are similar to those computed in the ICMZ Plan, while for RP200 they differ from those of the ICMZ Plan. The differences are probably due to the different techniques used to retrieve the inundation perimeters used in the two works. In general, for RP20, results show loss of the beaches, while the inundation reaches buildings and streets for RP100 and RP200.

For Pesaro, the area to the south of the Harbour is highly vulnerable to the inundation, with water penetration up to 500 m for RP200. The inundation hits a large part of the urban area. The simulation with the adaptation of the coastal defense structures shows that: 1) for emerged barriers, the increase of their elevation produces a positive effect on the inundation perimeters; 2) for submerged barriers, despite the reduction of the wave height, the increase of their elevation causes a piling-up behind the structures that worsens the inundation.

In Fano, the inundation reaches around 200 m and 360 m from the shoreline, respectively for RP100 e RP200, affecting many buildings.

The steep slope of the Montemarciano beach limits the inundation. However, the inundation perimeter includes buildings and streets for RP100 and RP200, with penetrations up to 95 m. The winter defense work turned out to be effective for RP20 wave storms, while it is ineffective for RP100.

For Fermo, the area to the North of the domain is more vulnerable to the inundation, with penetrations up to 200 m for RP100 and RP200, including also buildings.

The inundation hits the Harbour of San Benedetto del Tronto and the area shoreward of it, with water penetrations up to almost 500 m for RP200. In the rest of the domain, the inundation affects the beaches and the structures located on them.

Results of 1D simulations show that, only in four out of fifteen cases the winter profile leads to worse inundations than the summer profile. For all other cases, there are no differences probably because the differences between the summer and winter profiles are very small.

The revision of perimeter will be updated in the ICMZ Plan where the measure for the management of coastal area are stated. This work has allowed to take in to account the identified vulnerability in the management of coastal ecosystems.

5 Follow-up actions foreseen

P1. Grado and Marano Lagoon and Gulf of Trieste (IT)

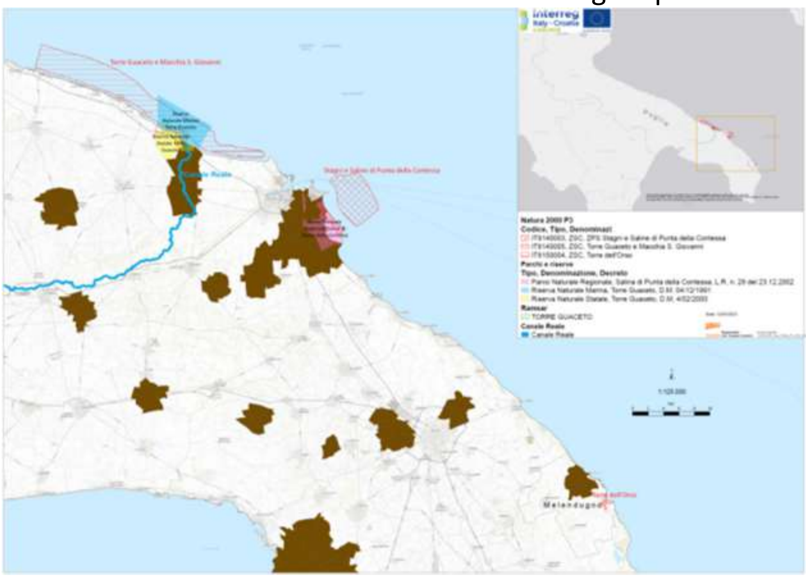
Any follow-up is not foreseen in the context of WP5 for the benthic rocky shore community in the Gulf of Trieste (Pilot Area 1). Some general considerations in order to monitoring and preserve this habitat are drawn in the following conclusion chapter.

P2. Transitional (e.g. Goro area and Bevano Mouth) and coastal areas in Emilia Romagna (IT)

Transitional waters and coastal lagoons have a wide variety of hydrological, biological and sedimentological gradients that make those areas heavily affected by temporal variability and high spatial heterogeneity (Basset et al., 2006; Pérez-Ruzafa & Marcos, 2012; Newton et al., 2014).
Such conditions demand a holistic monitoring approach to explore and describe environmental and ecological relationships in order to provide support to set new and effective monitoring plans.
P2 areas are regularly monitored by ARPAE, however sampling frequency and sampling stations adopted during CASCADE were not the same in every area.

P3. Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT)

The pilot site P3 is actually a huge area with specific differences among the three components, Torre Guaceto, Saline Punta della Contessa and Melendugno. Anyhow all the three components are affected by Land Sea Interaction, for instance all three components are designated as nitrate vulnerable zones as illustrated in the following map.



Besides the Nitrate the river basin influence the runoff and possible contamination from the upriver highlighting the strategic importance of ICZM for the P3. In particular for Torre Guaceto and Salina di Punta della Contessa in which lay also a MPA and Natura 2000 sites.

P4. Neretva river mouth (HR)

The participatory process clearly showed that the knowledge and awareness of the stakeholders about the problems of the pilot area improved. It was concluded that the stakeholder awareness work will have a positive long-term impact on the willingness of the local community to participate in policy processes and to lobby decision-makers to introduce the necessary changes for the benefit of the ecosystem and the local community itself.

This pilot area is important for biodiversity at local, regional, and global scales, but the pressures affecting biodiversity stability and condition are also diverse and complex. The listed pressures have a cumulative effect on the biodiversity condition of the pilot area, so any follow-up actions should consider ameliorating the various impacts together to achieve recovery of the area.

It is important to emphasise that these impacts often originate from different sectoral agencies and a joint approach is difficult. Given the data and experience currently available, we consider an awareness campaign for the stakeholders of the pilot area with the aim of empowering the local community to participate in the elaboration, adoption and modification of policies on local, regional and transboundary issues as the best possible option to address these issues.

P5. Coastal area in Veneto (IT)

Any follow-up is not foreseen in the context of WP5 for benthic and demersal communities at the Tegnùe di Chioggia (Pilot Area 5). General considerations regarding monitoring and conservation of this habitat are included within the following chapter.

P6. Miljašić Jaruga river mouth, Nin bay (HR)

Based on the foreseen impacts on this site, as one of the options for mitigating these effects described within the 5.2.2. report, the proposed variant is to not take the existing stones out of the sea, but to move them to a deeper location before the start of construction with the help of mechanization and divers, where they will not be under such a great influence during the construction of the new Miljašić jaruga sea shore. In that case, these individuals would have the possibility of staying at the existing installations in the sea and possibly later repopulating other areas, when the impact of the construction is no longer visible. At the same time, it is necessary to carefully select the location for the transfer of certain elements so that they do not have an impact on the sea route and other habitats and species, which is why the transfer itself is preceded by a biological survey in order to precisely determine the micro location for the transfer, with the consent of the competent authorities for the safety of navigation on the sea. A regular annual inspection of these localities is recommended in order to determine the processes of adaptation of these types of snails to the new conditions. Given that these rare species settled on an artificially constructed habitat in the past and found a permanent habitat there, it is to be expected that a similar structure of species will settle on newly constructed elements over a certain period of time. Therefore, it is recommended to place an element in the underwater part that will be pH neutral and have a rough surface structure and clear cavities and recesses that have been shown in similar situations around the world to be useful in promoting the acceptance of soil organisms, and the settlement of other benthic organisms, including snails.

Also, the possible impact of sedimentation will have less impact on the habitat of the sea flower *Cymodocea nodosa*, several small fields of which have been observed nearby. Nevertheless, this species is relatively resistant to short-term sedimentation processes and is regularly present in similar conditions such as harbors and marinas. Therefore, no significant impact of the process of construction of the new bank of the Miljašić ravine on this habitat is expected.

P7. Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC) (IT)

After restoration actions on Pilot 7 wetland, the vegetation dynamics evidenced a clear improvement in ecosystem quality, as evidenced by some bioindicator species (e.g. a gain of diagnostic native species and a reduction of ruderal and alien ones). An increase of cover of halophilous and thermophilous species was also registered.

Were then found variations on ecological features and species occurrence and abundance pattern across the different EU habitats conforming the brackish mosaic: salt meadows, halophilous scrubs and salt steppes (respectively 1410, 1420 and 1510*). Such changes are most likely related to an intertwining of environmental changes (restoration actions, climate change and coastal erosion).

It was observed, after the restoration action, a general improvement of the naturalness of the Biferno mouth with a successional process that led the salt marsh mosaic towards

typical paucispecific plant communities. Moreover, the results demonstrated that, after adequate hydraulic work and reduced human pressure, these fragile ecosystems could be able to recover typical vegetation in the Mid and Long Term.

In addition, the observed expansion of hypersaline communities may be also related to other environmental drivers as climate change (e.g. rise of local temperatures, the decline of summer precipitation) and coastal erosion that affected this section of Adriatic coast. These environmental changes likely exposed Biferno wetland to an increase on water salt concentration and to a greater influence of salt aerosol which favored the expansion of halophilous diagnostic species and the rarefaction and loss of ruderal and alien plant taxa.

The applied re-visitation approach and analysis of plants as bioindicators, represents a cost-effective monitoring procedure that matches the need of periodical reporting requested by the European HD. We hope re-visitation monitoring studies by vegetation plots will be implemented

over increasingly larger scales, in order to increase the current knowledge on vegetation dynamics after wetland restoration actions and identify the most effective approaches so as to manage and recover these fragile ecosystems.
During workshops and communication activities carried out on WP5, different solutions to restore coastal wetlands and procedures to include it in a frame of integrated coastal management plans were discussed.

P8. Northern-eastern Adriatic in Croatia (HR)

The monitoring in pilot 8, which is a Natura 2000 site,is going on. Comparing traditional methodologies and modern methods with the aim to describe biodiversity of the region. Special accent is given on newly introduced species, alien species and harmful species which can harm human activities on the sea.

P9. Cetina river mouth (HR)

The follow-up actions are based on the results and recommendation of the activities carried out through WP 3 and WP 4 of the CASCADE project, complemented with actions which result from the long-term work of the professional service in this area of Cetina estuary.

Table 20. List of actions for the conservation of Natura 2000 site Cetina estuary, including indication of priorities and associate institutions are presented in table below. Such representation of actions is following national *Guidelines for planning the management of protected areas and/or ecological network areas* (MZOE, 2018.)

No.	Action	Priority (1 - required, 2 - necessary or 3 - desirable)	Associates
1	Ensure continuous monitoring of all existing target habitat types (1110, 1140 and 1130) and species (sea lamprey *Petromyzon marinus*).	1	scientific and professional institutions, companies and associations
2	Ensure continuous monitoring of the little Neptune grass *Cymodocea nodosa.*	1	scientific and professional institutions, companies and associations
3	Encourage maintenance of the current favourable condition of the little Neptune grass *Cymodocea nodosa*	1	Split-Dalmatia County, City of Omiš, Dugi Rat Municipality, Croatian waters

No.	Action	Priority (1 - required, 2 - necessary or 3 - desirable)	Associates
4	Conduct further research for the little Neptune grass *Cymodocea nodosa* (impact of fishing practices, understanding the food chain, defining ecosystem services, etc.).	3	scientific and professional institutions, companies and associations
5	Carry out activities for the preservation and recovery of the Noble Pen shell (*Pinna nobilis*) population at Cetina estuary	1	Ministry of Economy and Sustainable Development, partners on the project of conservation of the Noble Pen shell in the Adriatic Sea
6	Conduct an integrated study of sediment dynamics with the aim of preserving the target habitat types (1110, 1140 and 1130) and species (sea lamprey *Petromyzon marinus*).	1	scientific and professional institutions, companies and associations
7	Conduct a research/study for the sea lamprey *Petromyzon marinus* in order to obtain reliable data and develop conservation measures for the Cetina estuary.	1	scientific and professional institutions, companies and associations
8	Ensure continuous monitoring of the cushion coral *Cladocora caespitosa.*	2 (will become 1 if reefs become priority habitat type)	scientific and professional institutions, companies and associations
9	Assess the scope, composition and state of preservation of rhodoliths (partly in the process through the national marine habitat mapping project).	3	scientific and professional institutions, companies and associations
10	Revise Natura 2000 habitat map of the Cetina estuary site (partly in the process through the national marine habitat mapping project).	1	Ministry of Economy and Sustainable Development
11	Take further measures to preserve the reefs of cushion coral *Cladocora caespitosa* and rhodoliths - expand the borders of the Cetina estuary site and the list of the target habitat types, i.e. include the target habitat type 1170 reefs.	1	Ministry of Economy and Sustainable Development
12	Monitor the impacts of climate change through indicator species (*Ballanophyllia europaea* and *Cladocora caespitosa*).	3	scientific and professional institutions, companies and associations

No.	Action	Priority (1 - required, 2 - necessary or 3 - desirable)	Associates
13	Monitor the impacts of climate change through devices (HOBO data loggers and multi-parameter probe Aquatroll 500 installed as part of the CASCADE project) including access to the T-MEDNet network.	2	Plovput ltd T-MEDNet
14	Encourage preservation of adequate ecological conditions in the sea under the influence of the Cetina River.	1	Split-Dalmatia County, City of Omiš, Dugi Rat Municipality, Croatian waters, Industries, HEP - Croatian Electricity Company, SDC Port Authority, Sports fishing association, etc.
15	Produce a Management Plan for the area of the ecological network Cetina estuary.	1	Ministry of Economy and Sustainable Development, all regional and local stakeholders concerned by the plan
16	Encourage revision of the *Ordinance defining the goals and conservation measures of target species and habitat types for conservation areas important for species and habitat types* for the purpose of including Cetina estuary site.	1	Ministry of Economy and Sustainable Development
17	Encourage Ministry, SD county and local authority to participate in public institutions in the procedures for granting concessions/concession approvals on maritime property.	2	Ministry of Economy and Sustainable Development, Split-Dalmatia County, City of Omiš, Dugi Rat Municipality
18	Participate in the creation of annual plans for the management of maritime domain.	1	Split-Dalmatia County, City of Omiš, Dugi Rat Municipality
19	In cooperation with other competent inspections and state bodies, strengthen supervision over the implementation of measures to preserve the ecological network, strictly protected species and rare and endangered habitat types.	1	State Inspectorate, Ministry of Economy and Sustainable Development
20	Participate in public debates on environmental impact studies and the creation of nature protection measures for projects with a possible negative impact on target habitats and species within the scope of this site.	1	Ministry of Economy and Sustainable Development, Split-Dalmatia County, City of Omiš, Dugi Rat Municipality, Private Investors

No.	Action	Priority (1 - required, 2 - necessary or 3 - desirable)	Associates
21	Cooperate with the competent authorities and draw up opinions in the procedures for assessing the acceptability of interventions for the ecological network, and cooperate with them in determining the conditions for nature protection in the procedures for issuing permits for interventions, issuing construction acts and granting concessions and concession approvals on maritime property.	1	Ministry of Economy and Sustainable Development, Split-Dalmatia County, City of Omiš, Dugi Rat Municipality
22	Monitor spatial and strategic plans and give opinions aimed at preserving the Natura 2000 site, strictly protected species and rare and endangered habitat types.	1	Split-Dalmatia County, City of Omiš, Dugi Rat Municipality, Institute for Spatial Planning
23	Cooperate with the local community in the interpretation and promotion of the Cetina estuary Natura 2000 site.	2	City of Omiš, Dugi Rat Municipality, Tourism Boards
24	In cooperation with educational institutions, design and implement educational activities for children on the preservation of the Cetina estuary Natura 2000 site.	2	Schools

P10. Torre del Cerrano, Pineto Abruzzo (IT)

The construction of boardwalks, at the light of our observations on the central Adriatic coast, can be considered an effective tool for mitigating the negative impact of bathing tourism and could allow a rapid, natural recovery of the psammophilous vegetation After restoration actions , the vegetation dynamics evidenced a clear improvement in ecosystem quality, as evidenced by some bioindicator species (e.g. a gain of diagnostic native species and a reduction of ruderal and alien ones).

In recent years, similar soft management infrastructures, as boardwalks and fenced areas, have been regularly included in the management plans of sites of conservation concern and are common practices in many coastal areas both with high conservation values and in degraded areas that need to be restored. Wooden boardwalks require little and inexpensive maintenance and have low environmental impact. Moreover, boardwalks may also contribute to raise public awareness about dune habitats' fragility.

However, based on our experience, particular attention should be paid to the design phase. To ensure the success of the installation, we recommend a preliminary geo-morphological study which takes into account the sediments deposition, the direction of the dominant winds. During workshops and communication activities carried out on WP5, different solutions to recover and manage coastal dune mosaic and procedures to include it in a frame of integrated coastal management plans were discussed.

P11. Marche coastal area (IT)

Any follow-up is not foreseen in the context of WP5 for Marche coastal area (Pilot Area 11)

6 Conclusion

On the whole the WP produced a huge and site specific datasets for the different Pilot areas and assessed the hazards, impacts and vulnerability which presented a wide range since the pilot areas span trough the Northern to Southern Adriatic shores including brackish lagoon, coastal areas with both rocky and sandy shores.
Despite the variety of different monitoring activities in none of 11 Pilot area were detected the effects of pandemic lockdown.
Detailed conclusion for each pilot areas are reported in the following chapter.

P1. Grado and Marano Lagoon and Gulf of Trieste (IT)

AMBI index seemed to be a good descriptor on the status of the meso/infralittoral macrozoobenthic community settled on the rocky shore in the Gulf of Trieste, in term of water quality, thank to the large number of taxa which could be assigned to ecological groups (EG) form the AMBI list.
The hydromorphological (HM) alteration sensu Orlando-Bonaca et al. (2012) represents a good descriptor to define the status of the biotope where community is settled.
The macrozoobenthic community on the rocky shore of the study area showed the typical species zonation of this environment and seemed to be in an undisturbed/slightly disturbed condition on the basis of AMBI index.
The HM index revealed that 75% of the sampling sites were in pristine/slightly altered conditions, whereas 25% were moderately altered.
The monitoring and the methodology applied on rocky shore invertebrates of the Gulf of Trieste could be adopted also in other Adriatic areas.
The brown algae *Fucus virsoides* resulted to be absent in the whole study area, but restoration experiments in protected areas of the Gulf of Trieste are already underway.
The giant goby *Gobius cobitis* resulted as present in most of the study area, although a research on the status of this species in the whole northern Adriatic should be done.
The monitoring and preservation of this habitat is needed because the arising sea level is seriously threatening the littoral ecosystem.

P2. Transitional (e.g. Goro area and Bevano Mouth) and coastal areas in Emilia Romagna (IT)

All the environments considered in this chapter, belonging to the pilot area P2, were regularly monitored by Regione Emilia-Romagna, together with additional basins which are also included in the transition water system of the Region. This report contains mainly the results and considerations of recent studies performed under WP3/WP4/WP5 of CASCADE project by different partners.

Concerning phytoplankton monitoring, water analysis could be implemented by also quantifying other inorganic nutrients that can boost and support algal blooms such as urea, ammonium, silica and iron. Additionally, picophytoplankton analysis should be included as well in the monitoring plan, especially in Lago delle Nazioni where small species are increasingly present; in this context coupling traditional and innovative techniques (e.g., eDNA-metabarcoding) would provide a better understanding of phytoplankton dynamics and composition and new data to enhance monitoring tools for protection and management of the coastal and lagoon marine ecosystem.

P3. Torre Guaceto - Canale Reale, Punta della Contessa, Melendugno in Puglia (IT)

UniSalento collected existing data on chemical parameters in Pilot Area 3, including surface water body monitoring data from Arpa Puglia and the ECODO-NET INTERREG IIIA/Greece-Italy project. Additional data were obtained from research papers focusing on the impact of heavy metals on bacterial activities in coastal marine sediments and the ecological assessment of a marine coastal area affected by a power plant water discharge.
The collected water and sediment samples were analyzed for heavy metals, chlorinated pesticides (which were below the limit of quantification), and microplastics. No measurable effects clearly attributed to the pandemic lockdown were observed in the chemical measurements.

UniSalento also used amphipods (Gammarus insensibilis species) as bioindicators to monitor the status of the transitional water ecosystem in Pilot 3. Enzymatic activity measurements were conducted to assess metabolic and antioxidant response systems in the amphipods. The results did not show any measurable effects clearly related to the pandemic lockdown.

In the Torre Guaceto pilot site, CMCC installed a fixed monitoring system using low-cost measurement instruments integrated into a delimitation buoy. The system can acquire various parameters with a frequency of 10 minutes, and the data is transmitted to a server in .csv format. Examples of data collected during the initial installation period were provided. CMCC conducted also an extended survey in the Torre Guaceto Marine Protected Area in July 2021, consisting of 20 stations where a multiparametric probe was used to acquire vertical profiles of parameters such as depth, temperature, conductivity (salinity, density), dissolved oxygen, pH, chlorophyll a, and turbidity. The acquired data will be utilized for numerical model validation, and results processed by Ocean Data View software were presented.

P4. Neretva river mouth (HR)

Future restoration efforts will depend on changes in local and transboundary policies and their long-term effects on the condition of the pilot area. The results of a multidisciplinary integrated analysis of existing data and the results of existing studies, as well as the results of the research campaign conducted as part of the project, should include the impacts of all relevant factors studied on biodiversity as well as on local society, and mitigation methods should be determined.

It is of utmost importance that the results can be used in the future for the needs of nature conservation, tourism and economy, flood protection in agriculture and transboundary issues.

P5. Coastal area in Veneto (IT)

Hard substrata assemblages distribution shown consistency with previous records in the area, and highlighted differences among sampled stations. Further assessment of spatial and temporal gradients of sediment resuspension and turbidity originated from the rivers and from trawling activities seems to be of interest.

ROV campaigns highlighted the accumulation of marine litter in the area (in accordance to previous reports, Melli et al., 2017). Monitoring should be continued to evaluate the extent of materials accumulated.

Fish community in the area shown a substantial decrease with respect to previous records. Further investigations should focus on assessing potential effects of changes due to increased water temperature and/or increased fishing pressure.

Acoustic campaigns performed show an interesting potential to spatially extend the results obtained by scientific fishing campaigns. However, further cross-validation between the results obtained with the two methodologies should be performed, based on the collection of synoptic samples.

Modelling activities performed reported a high sensitivity of food web indicators to changes in water temperature and primary production phenology in the area. Further model testing is required, by extending the set of scenarios considered (i.e. changes in fishery pressure in the area) and including the estimation of additional ecological network analysis indicators (e.g. Finn cyclic index). Further development of modelling activities in the area should also include an uncertainty analysis of the results.

P6. Miljašić Jaruga river mouth, Nin bay (HR)

The Miljašić jaruga micro-site stands out in the area of the entire Nin lagoon with its high biodiversity and the presence of rare species of snails and the occasional presence of habitats of the sea grass *Cymodocea nodosa*. Also, several individuals of endangered bivalve *Pinna nobilis* have been reported to still exist in the surrounding area. The planned construction interventions in this locality were assessed as necessary to prevent the process of coastal erosion, primarily through floods and the influence of wind. The current condition of the constructed coast is unsatisfactory and cannot provide safety in the event of large amounts of water or strong wind and waves. However, most of the observed species only occasionally inhabit this area, and therefore no significant impacts on them are expected during and in a certain time after the construction itself. A more significant impact is possible on the population of rare gastropods, which is why task 5.2.1. there are proposed measures in more details, aimed to mitigate this effect. The idea is to move the stone elements under the sea to a closer location that will not be affected by the construction. This would enable survival of the mentioned organisms in similar conditions as they had before, and eventual repopulation of the newly built habitat, which is why it is also suggested to use building elements that encourage the settlement of soil and other marine organisms. Also, monitoring program for the bivalve *P. nobilis* needs to continue as this area is one of the rare sites in the whole Mediterranean Sea to report several live individuals after the outbreak of the mass mortality event (MME) that caused a severe reduction of this species.

P7. Coastal area in Molise (Biferno river mouth, Campomarino Coast and Bonifica Ramitelli SAC) (IT)

Coastal wetlands are biodiversity hotspots, highly threatened, and for which restoration actions have been widely implemented. Systematic monitoring of biodiversity after restoration actions on Mediterranean salt marshes vegetation needs further attention. Have been analyzed temporal changes in plant species composition and ecology in a restored brackish wetland on the Adriatic coast (Central Italy) by a re-visitation study of 33 historical plots (year 2010), newly collected after 10 years (2021), across a brackish mosaic composed by salt meadows, halophilous scrubs and salt steppes referable to three habitats of conservation concern in Europe (EU codes: 1410, 1420 and 1510*). Changes in species richness and cover, in the ecological characteristics of the mosaic and each habitat type were tested by comparing some ecological groups (e.g. diagnostic, alien and ruderal species) and Ellenberg bio-indicator values by a Mann-Whitney test. Similarity percentage procedure for identifying which species indicate temporal changes was also performed. After restoration, we observed a general improvement of the environmental quality of the brackish mosaic with the establishment of typical pauci-specific plant communities, a significant recovery of diagnostic species covers and a reduction of ruderal and alien ones. It was also registered an increase in Ellenberg salinity and temperature values likely related also to coastal erosion and climatic change. The results of our study suggest that vegetation dynamics could be used to monitor coastal restoration trajectory in the Mid- and Long-Term local interventions.

The obtained results and the effectiveness of implementing restoration actions on coastal brackish mosaics were discussed during several workshops and communication activities carried out during the project and in particular on WP5 working package

P8. Northern-eastern Adriatic in Croatia (HR)

Climate change has complex effects on biodiversity and ecosystem functioning. Warmer climates can lead to both a loss (Tomas et al. 2004) and an increase in biodiversity (García Molinos 2016). At the local scale, the effect of increased temperature on species richness shows mixed trends, with studies finding declines (Cardinale 2018) increases (García Molinos 2016), or no discernible trend. The effects of increased thermal variability also remain unclear: positive, negative, or no effects on richness have been reported (Burgmer 2011, Gerhard, 2019). Furthermore, climate change can alter ecosystem functioning, either directly or indirectly through changes in biodiversity (Burgmer 2011,). The combined acceleration of climate change and biodiversity loss necessitates understanding how ecosystem functions and services will be affected.

The 21st century has seen an acceleration of anthropogenic climate change and biodiversity loss, with both stressors deemed to affect ecosystem functioning. However, we know little about the interactive effects of both stressors and in particular about the interaction of increased climatic variability and biodiversity loss on ecosystem functioning. This should be remedied because larger climatic variability is one of the main features of climate change (Bestion 2021).

Bestion (2021) and coworkers demonstrated that temperature fluctuations led to changes in the importance of biodiversity for ecosystem functioning. Species-rich communities maintained their ecosystem functioning with increased fluctuation as they contained species able to resist the thermally fluctuating environments, while this was on average not the case in species-poor communities. Our results highlight the importance of biodiversity for maintaining ecosystem functions and services in the context of increased climatic variability under climate change.

P9. Cetina river mouth (HR)

The CASCADE project enabled Public Institution Sea and Karst to consolidate all existing data and information on the state of the Cetina estuary Natura 2000 site in one place. Furthermore, thanks to the project, field collection of additional data on the condition of key habitats and species was carried out for the first time, and analyses were carried out that resulted in recommendations for their conservation. Such an approach aided Public Institution Sea and Karst to formulate the follow-up actions, among which it is certainly important to highlight: the expansion of the borders of the Natura 2000 Cetina estuary site and the list of the target habitat types, i.e. include the target habitat type 1170 reefs; preparation of the Management Plan for the site; as well as to encourage the revision of the Ordinance defining the goals and conservation measures of target species and habitat types for the site. Furthermore, and to conclude, it is necessary to ensure funds that will enable implementation of all listed follow-up actions especially those having priority 1 and 2. Such funds have already been provided for the monitoring of the Noble Pen shell through a national Project for the preservation of the Noble Pen shell in the Adriatic Sea, which is co-financed by the Environmental Protection and Energy Efficiency Fund.

P10. Torre del Cerrano, Pineto Abruzzo (IT)

As suggested by previous studies a more valid conservation or restoration plan could include a number of different and integrated nature conservation and management strategies, as well as dissemination activities (implemented on WP5).

Actually, there is often a general lack of knowledge among residents and politicians about how vegetation can stabilize dunes and protect the beach by sea erosion, how plants can be damaged by trampling and, thus, why management initiatives, such as boardwalks, dune fence and planting events, are valuable.

Although limiting dune access and trampling is important to support conservation actions in Mediterranean coastal areas affected by mass tourism, this should be coupled with the promotion of a nature-aware tourism, where informed citizens are encouraged to monitor and conserve biodiversity as part of their own natural heritage as we have done during CASCADE with the citizen science campaigns supported by the dedicated wild coast cascade platform (https://www.inaturalist.org/projects/wild-coast-cascade). Environmental researchers have the big responsibility publicizing the importance of nature conservation through a focused dissemination in line with socioeconomic needs. Only through dialogue and knowledge transmission can we aspire to an integrated environmental management that envisages citizens as the leader of their own future (Directive 2008/56/EC).

The obtained results and the need of implementing restoration actions on coastal brackish mosaics were discussed during several workshops and communication activities carried out during the project and in particular on WP5 working package.

P11. Marche coastal area (IT)

The activities carried out provided valuable insights into the emerged and submerged biocenosis along the coastline of the Marche region. The analysis of emerged biocenosis focused on updating dune vegetation data and identifying coastal habitats, including the presence of *Charadrius alexandrinus* (Kentish plover) and potential nesting sites. The investigation revealed the presence of different habitats, such as 1210 annual vegetation of marine deposit lines, 2110 mobile embryonic dunes, and 2110 shifting dunes with *Ammophila arenaria*.

The analysis of submerged biocenosis involved the use of advanced technology, including ROV with high-definition cameras and positioning systems, to survey the seabed and identify underwater biocenoses and habitats. Two main habitats were identified: 1170 reefs characterized by rocky substrates and among the other species the presence of *Sabella spallanzanii* polychaetes; 1110 sandbanks with a weak permanent sea water cover, which included species such as *Liocarcinus vernalis* crustaceans and *Sabellaria* bioconstructive polychaetes.

It is worth noting that the reduced human presence along the coast during the lockdown may have indirectly affected the emerged biocenosis by allowing them to recover or reduce the anthropogenic impacts they typically face.

Overall, the activities carried out in WP3 and WP4 provided important data on the coastal ecosystems of the Marche region, highlighting the pressures, vulnerabilities, and potential impacts on these ecosystems. This information can contribute to the effective management and conservation of coastal areas and their valuable biocenosis.

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